Section

1

Introduction

Chapter 1

Human Health and Clinical Diagnosis in Developing Countries

Kanai L Mukherjee, Swarajit Ghosh and Chhotelaal Pande

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Human Body in Health and Disease

Our body is an incredible organic machine. It maintains its balance (called **homeostasis**) under normal healthy conditions. The **World Health Organization** (WHO) defines this as—"A complete state of physical, mental and social well-being, and not merely the absence of any disease or infirmity." When imbalanced, nature has its own means to correct the imbalance by itself. With this evolved organization of our body, we have survived for several million years. This reminds us of the Hindu scripture—*Purna madaha Purna midam*—that points to the perfection of our Creator to protect His creation.

However, sometimes we fail to understand why homeostasis is disturbed under certain pathologic conditions; and under such circumstances the individual becomes a patient or a sick person. In order to bring the patient back to normal life, the cause of illness must be known. This search for the cause of illness is initiated by a professional, the 'physician', who is trained in this line. A physician's job is to diagnose the cause and take appropriate therapeutic measures to set things right. The process of investigation is called 'clinical diagnoses'. This is comparable to criminal investigation performed by a detective.

Diagnosis of Ailment

The first step of clinical investigation is a thorough physical examination of the patient along with a full account of the history of his illness. This procedure is still the gold standard for all health practitioners. Many traditional medical approaches in developing countries still count heavily on a careful physical examination of the patient focussing on their sixth sense to understand the cause of illness. This art of diagnosis, without any accessory aids, is gradually disappearing as we rely more and more on tools and gadgets of clinical diagnosis.

Realistically speaking, physicians, be it in a developing country or in an advanced country, are getting less and less time to examine the patient. In developing countries, it is the staggering rise in population, while in advanced countries, physicians prefer to generate more money out of their working hours. Therefore, they refer the patient to the laboratory to acquire further information that facilitates in rapid diagnosis.

Role of Clinical Laboratories

These days, clinical laboratories are playing a bigger role in providing accurate diagnosis. It is said that 80% of clinical diagnosis in the USA is based on laboratory reports. In developing countries, on the other hand, the laboratory might not be advanced, but wherever it is possible, they are taking full advantage of scientific progress, keeping in view their own financial constraints. The World Health Organization publications are of great value in improving the situation.

Medical Care in India

Medical care in India is a blend of old and new practices. The traditional systems of medicine, such as *Ayurveda, Homeopathy, Unani* and *Traditional Chinese Medicine* which are often down-graded by the term 'unscientific medicine'—find their own niche along with modern technology like CT scan (computerized tomography). The most important deciding factor in making the choice between the old and new tradition is undoubtedly the patient's economic condition. However, it is not unusual to see a physician who practices modern medicine by profession takes his only son to a homeopath for treatment when modern medicine fails. It is unfortunate that traditional medicine (such as *Ayurveda*) is today fossilized. Prejudice against any system limits our quest to learn the mystery of its healing power. Paying respect to the past, laboratory personnel (including the pathologist) should constantly strive to keep abreast with the current information and apply as much of it in practice as their limitations allow.

India is unique with respect to healthcare. It cannot be classified either with underdeveloped countries or with advanced countries. The Government of India does not have enough funds to offer 'free medicine for all', nor do the people have money to buy health insurance to protect themselves.

It is well documented that the doctor–patient ratio in India is about 1:1666 (*World Health Statistics* 2007, World Health Organization). Doctors are scarce in rural areas; money is lacking and so also all that goes with it in the form of drugs, equipment and facilities. The patients

are deprived in terms of nutrition, education, transport and housing facilities. Urbanization has taken place at a formidable rate and slums have increased, unemployment and birth rates are on the rise. In spite of the existing handicaps, health planners can bring about positive changes that will improve healthcare for the majority on a long-term basis.

Considering the existing situation, medical care in India must be adapted to the needs of 'intermediate technology' that will provide services to the people close to their home. Minimum services should be organized. The training of the auxiliary comes first. An auxiliary is a technical worker in a certain field (midwives, nurses, medical laboratory technicians and others) with less than full professional qualification. A Primary Health Centre (PHC) with good auxiliary staff is better than a hospital with poor staff. In terms of overall health strategy, the needs of people can be best met by giving them well-trained auxiliaries, modestly equipped health centres and facilities for preventive medicine.

The statement above may contradict the image depicted in many foreign newspapers—the best place to undergo heart surgery is Delhi, India. Many insurance companies are choosing India for long-term care of their clients. Many universities in the United States are planning to open hospitals in India for low-cost care of their patients. Outside the hospital facilities catering to 'foreign clients' lie India's mass fighting to survive. This book is written to serve them.

Apart from considering the unaffordable hospital care for a common Indian, just think about the gloomy picture of huge number of medicinal drugs that India is producing and exporting at a price competitive in the world market. This should make us proud of our progress in the field of healthcare. However, the reality is different when one looks under the cover. The illiterate common man of India is a good subject for trying 'experimental drugs'. In addition, global manufacturers love to open industries on Indian soil. There are considerably fewer regulatory restrictions and plenty of skilled labour looking for a job. As a result, the profit-making industrial world can exploit India, making it an open dumping ground for industrial wastes. Now we have to wait and see what happens to nuclear wastes.

Medical Statistics of India

As a member of the healthcare team, Medical Laboratory Technicians should be aware of the limitations that India is currently facing which affects its population:

- India has a life expectancy of 64/67 years (m/f), and an infant mortality rate of 46 per 1000 live births. Approximately 1.72 million children die each year before turning one. Reduced funding for immunization leaves only 43.5% of the young fully immunized.
- 42% of India's children below the age of three are malnourished; malnutrition impedes the social and cognitive development of a child, reducing his educational attainment and income as an adult.
- Diarrheal diseases are the primary cause of early childhood mortality. India also has the world's highest incidence of Rabies.
- Diseases such as dengue fever, hepatitis, tuberculosis, malaria and pneumonia contin ue to plague India due to increased resistance to drugs. In 2011, India developed a *totally drug-resistant* form of tuberculosis.
- Infrastructure like hospitals, roads, water and sanitation are lacking in rural areas. Only 25% of total population of India have drinking water on their premises. Groundwater pollution, excessive arsenic and fluoride in drinking water pose a major threat to India's health.
- HIV/AIDS in India is ranked 3rd highest among countries with HIV-infected patients.
- Health issues confronted by rural people are many and diverse—from severe malaria to uncontrolled diabetes, from a badly infected wound to cancer. Because of limited

government resources, much of the healthcare provided comes from non-profit organizations located outside India.

- Although India has a universal healthcare system (public health sector) but parallel to, and indeed more popular than it, is the private medical sector. Government hospitals are often understaffed with poor facilities.
- All major cities and medium-sized urban centres have private hospitals that provide an excellent standard of care. Some of these private hospitals (Apollo, Fortis, Manipal and Max) offer a high standard of care that is at the same level as North American and European countries.
- All types of prescription medicines and healthcare products are available in India at a very low cost. Doctors provide prescriptions for certain medications but some pharmacies do not always ask for them.

Healthcare in India

The healthcare scenario in India is extremely complex. While there have been significant reductions in fertility and mortality, these gains have been offset by the increasing burden of communicable and non-communicable diseases. Children under-nutrition levels have remained persistently high and there is an ever enlarging gulf in the health status of the rich and the poor. There is a great disparity in the quality of services between rural and urban India. On one hand, India produces one of the largest numbers of doctors and is one of the largest producers and exporters of drugs, while there is a severe shortage of financial and human resources especially evident in rural areas. Medical services are disparate and fragmented and have a long way to go to meet the ideal of health for all. Support services like clinical laboratories are largely unregulated and a significant majority of these setups lie in the unorganized sector. While there have been some relatively recent policy initiatives to promote the standard of care provision in underserved areas, support services like clinical laboratories are inequitably distributed and lag in reach and standardization.

To put the setting of clinical laboratories in context, one would need to have an idea of the structure of public health services in India. The management of health services is mainly housed in the public sector and private for profit sector. It is the mandate of the public sector to provide healthcare services to the entire population with a specific emphasis on those who cannot afford to pay for care. The public health system is organized in multiple tiers starting at sub-centres at 5000 population levels, Primary Health Centres (PHC) at 30,000 population, Community Health Centres at 100,000 population levels, multispecialty district level hospitals, medical colleges, paramedical training institutions, laboratories and tertiary hospitals. PHCs are meant to be the cornerstone of rural health services and yet have failed to deliver adequate services. The reasons for this are manifold and include poor management, lack of infrastructure and very inadequate resource prioritization. In many regions, laboratory services are extremely rudimentary and have failed to equip the PHC with the capability to render adequate medical care. The laboratories in many facilities can only examine for malarial parasites and others can test only for mycobacterium TB.

In 2005, the National Rural Health Mission was launched to strengthen public health services in regions that had poor health indicators and/or inadequate infrastructure. This mission envisaged an upgradation of medical care services as well as support services like laboratories. Following this, the Indian Public Health Standards (IPHS) were published in 2007 to provide guidelines that would ensure a minimum standard of care and laboratory services nationwide. In order to address the problem of inadequate laboratories in the PHCs, states like Bihar and West Bengal have since then entered into public private partnerships to provide laboratory services. Despite efforts to improve care, studies have indicated that the

confidence in public health measures as well as laboratory quality has been steadily eroding in rural India.

Deepening the healthcare conundrum is the phenomenon of corporatization of healthcare resulting in a mushrooming of medical laboratories in urban and semi-urban India. Irrespective of this emergence, India does not have mandatory accreditation for medical laboratories. The only governmental accreditation agency that can assure good practice on a country wide scale is the National Accreditation Board for Testing and Calibration of Laboratories (NABL) and all laboratories are required to register themselves with the health departments of respective states only. Rough estimates show that there are some one lakh medical diagnostic laboratories in the country. Of these, 80% are small, 18% are mid-sized and only 2% are big laboratories. Enquiries reveal that only 450 laboratories (0.45%) are accredited by the NABL.

With an expanding middle class, healthcare demands have shot up in certain sections of the country resulting in an increased demand for diagnostics. Unlike in the developed world, India still has a long way to go to establish rigorous accreditation, registration and quality control mechanisms that would ensure accurate, reliable and comprehensive delivery of test results to all sections of the population in a timely manner. That being said, medical laboratories are a critical part of the healthcare system and with increasing health awareness and mounting disease burdens, setting up an evidence based regulatory and supervisory body that can oversee the continuing development of a corpus of standardized and accredited clinical laboratories country-wide is the need of the hour.

Clinical Laboratories and Laboratory Personnel in India

Clinical laboratories stand as the cornerstones of **modern medicine**. Clinical laboratory tests have become a key element in most diagnostic procedures. The heart of a medical laboratory is the laboratory technician. Although the pathologist, a medical school graduate, is responsible for activities of the medical laboratory, he is rarely involved with the bench work. The technician who works behind the scenes provides crucial data submitted by the laboratory for diagnosis. The authors are of strong belief that the effectiveness of laboratory medicine in patient care largely depends on the education, training and ethics of the technician.

Kinds of Laboratories

There are four kinds of laboratories involved in performing various laboratory diagnostic tests. These laboratories may deal with **outpatients** (ambulatory) or **inpatients** (in hospital). The outpatient laboratories generally perform low complexity tests that are designed as simple but accurate and require very little interpretation to deliver reproducible results. Table 1.1 gives a general comparison between tests done in a physician's office and in a hospital-based laboratory.

a. ***Physician's Office Laboratory (POL)*** These laboratories are attached to a physician's office that deals with outpatients.
b. ***Hospital Laboratories*** They are dedicated to serve the needs of an institution where the patients are admitted (inpatient). They deal with routine as well as emergency tests.
c. ***Private Laboratories for Routine Tests*** They perform all the tests done in a physician's office and in hospitals. They do not get in touch with the patients unless called for giving specimen (blood, urine, sputum etc.).
d. ***Reference Laboratories*** They perform specialized tests but not in a physician office, hospital laboratories or routine private laboratories. They never get in touch with the patients. Specimens collected by physician's office or in hospital, as guided by the reference laboratories, arrive for analysis. Most of them require special skills to interpret the results.

TABLE 1.1 *Examples of tests performed in physician's office laboratories and hospital laboratories*

Physician Office Laboratories	Hospital (or Private) Laboratories
• Rapid microbiology testing for the presence of roup of *Streptococcus* and influenza • Urine analysis • Pregnancy testing • Tests such as those for mononucleosis, Helicobacter pylori, and HIV • Coagulation test to monitor patients who are taking anticoagulants • Glucose levels and other tests (e.g., A_1C) used to monitor diabetic patients • Faecal occult blood tests for the presence of blood in stool • Cholesterol tests	• Electrolytes • Kidney function tests • Liver function tests • Blood typing and cross-matches for transfusion • Identification of microorganisms and antibiotic sensitivity testing • Urine analysis • Coagulation testing • Cardiac enzyme assays • Complete blood count (CBC) and other haematology testing

Apart from the aforesaid four major classifications of laboratories, hospital laboratories or attending physicians may also adopt another method for testing samples, called **point-of-care testing or POCT**. Point-of-care tests are actually performed at the patient's bedside rather than in the laboratory, using a portable instrument that gives immediate results. These tests may be performed by laboratory personnel or in some situations by other hospital employees (nurse or physician's aid) who have been trained to perform the testing. This book will focus on the diagnostic laboratory tests routinely performed in various conditions.

Quality Control and Quality Assessment in a Laboratory

A laboratory report must be reliable or else it will misguide the physician and risks the life of the patient. This calls for not only the **Quality Control (QC)** of laboratory results but also includes specimen handling and reporting. Thus, reputed laboratories run **Quality Assurance (QA)** programs in order to assure the results reported to the physician. In recent years, the term Quality Assurance has been replaced by the term **Quality System (QS)** which is designated to refer to all policies, procedures, and processes needed to achieve quality testing. The ultimate purpose of quality programs is to provide quality healthcare that is safe, effective, timely, equitable, and patient-centred. Quality programs help achieve these goals by ensuring performance excellence and reliable laboratory test results.

The results reported in developing countries depend largely on the efficiency of the laboratory technicians than in advanced countries where automation has replaced human deficiencies. The following statement proves this point '... one half of all biochemical results from 60 laboratories (of India) were incorrect by 15% or more and many results were clinically misleading' (Improvement in laboratory performance by an inter-laboratory quality control programme, by P.G. Hill and A.S. Kanagasabapathy, *Indian* J. Med. Res. 69: p. 853, 1979). Hence, adoption of Quality Control programs in the laboratories of developing countries should be mandatory. This will be further discussed in later chapters of the book.

The following recommendations are made by the present authors to improve the situation:

- Better education of technicians supplemented by opportunities towards updating of current information.
- Follow standard procedures that can be adapted to local conditions, written in a language that the technician can understand and amenable to duplication without error.
- Regulations imposed on laboratories by the government and professional organizations so that their reports are dependable for diagnosis.

Medical Laboratory Services in Developing Countries

Here are some of the newspaper reports regarding medical laboratory services in developing countries:

- *In some developing nations, the lack of reliable, accessible medical laboratory testing is getting wide coverage in the local press. This is true of recent events involving pathology laboratories located in India, Nepal and Sri Lanka.*
- *Clinical Pathology (CP) in Nepal is still in primitive stage. Mostly all the CP tests are done manually. In many health institutions, even basic CP test facilities are not available leading to an empirical treatment. Even the so-called big medical centres are lacking many important CP test facilities forcing the patients (those who can afford) to proceed to foreign countries for investigation and treatment of their health problem.*
- *In India, the convergence of two trends is spurring government action. Both patients and their physicians are losing confidence in the reliability of medical laboratory test results.*
- *There is a steady flow of news stories in Nepal and Sri Lanka about how inaccurate medical laboratory test results are causing great sufferings and hardships.*
- *A Nepal physician reported that, "people in his country often have to travel from place to place at great expense, but are unable to get an accurate diagnosis".*
- *In Nepal, the number of patients visiting hospitals with conflicting diagnosis based on inaccurate diagnostic test results has increased appallingly in the last couple of years.*
- *In India, the (clinical laboratory) situation has escalated to the point where government agencies have had to take action, according to a recent story published in The Times of India.*
- *Private laboratory owners are "at war" with policy makers and government agencies. Health officials in Thiruvananthapuram, Kerala, in south-west India have been conducting raids on private clinical laboratories.*
- *The raids were in response from a growing number of complaints by patients about inaccurate medical laboratory test results and price-gouging. Officials issued closure notices to 22 laboratories in that region. Owners of these private pathology laboratories contend that the government's raids are illegal. "The government has not issued any guideline or policy for operating medical laboratories in the state," stated one of the medical laboratory technologists (MLT).*
- *Nepal study confirms majority of laboratories fail to meet government criteria. It showed that 84% of the pathology labs in and around Kathmandu Valley did not meet core governmental standards.*
- *"We received a considerable number of complaints from the public, with a majority on the accuracy of the medical reports," stated Dr. MG.P. Samarasinghe, Deputy Director General (Laboratory Services) in the Sri Lanka Health Ministry.*

Training of Laboratory Personnel

By definition, medical laboratory technicians (MLTs) are workhorses of a laboratory who receive one to two years of certification training after higher secondary school which is two years of academic education after high school. Medical technologists (MTs), on the other hand, have a Bachelor of Science degree and receive one to two years of undergraduate training in a university. Medical technologists hold supervisory responsibilities and are better paid. The duration of one to two years of technical training in case of both the MLT and MT varies from institution to institution; in most cases it is two years. The MLT and MT are both grouped under the broader group of health auxiliaries.

The teaching of laboratory personnel should be 'competency-based'. Any qualifying examination should focus on 'workable knowledge' and not 'academic knowledge'. Thus, an in-depth question on diagnosis appears to be redundant for a technologist but is very important for a medical student. On the other hand, setting of proper speed of the centrifuge to obtain urinary

sediment is important for a technician but not for a medical student. The World Health Organization has published a guideline to attain this goal. *(Training of Medical Laboratory Technicians: A Handbook for Tutors,* by Alex McMinn and Graham J. Russell. WHO, Geneva, 1975). Thus, a competency-based curriculum has been developed *(Competency-based Curriculum Development in Medical Education: An Introduction,* by W.C. McGaghie, G.E. Miller, A.W. Sajid and T.V. Telder. WHO, Geneva, 1978; Public Health Papers No. 68). The framework of competency-based education for technical personnel working in a clinical laboratory requires a basic understanding of anatomy and physiology of the body under healthy conditions and its abnormalities in diseased conditions, specimen collection and processing for laboratory diagnosis of the pathologic state, mastery in performing various diagnostic tests, calculation of results, adoption of quality control measures for the reliability of laboratory findings and appropriate methods of communicating results to the physician. A sound knowledge of principles of the test helps in troubleshooting while reliability of results comes from an awareness of quality control and its implementation.

Continuing Education of Laboratory Personnel

Continuing education is as vital as scientific achievement. Advances are constantly occurring and our knowledge should never stagnate. A profession like that of medical laboratory technology requires constant updating of knowledge. As technicians work for an average period of 40 years after school, the information provided by the school most often becomes obsolete by the time the technician reaches the prime of his/ her professional career. In order to meet this challenge of constant change in medical laboratory science, professional bodies should offer workshops for technicians so that they can be proficient and render better services. All laboratory personnel must realize that they function as a team and the technician's responsibility for patient management and healthcare is of prime importance.

In looking ahead, it is almost certain that the demand for medical laboratory technicians will grow and hence new employees should be better equipped with current information rather than be on-the-job trained (OJT).

Professional Outlook

Medical laboratory technicians must cultivate a professional outlook in performing their duties. Theirs is not just a job to be performed; it determines the life of an individual who might be the only bread earner of the family or the only child of his parents. The professional attitude is self-imposed and also self-rewarding.

Some professional qualities include the following—both for the physician and the technician:

- Dedication to high standards of performance.
- A feeling of personal responsibility; the awareness that others are dependent on his performance.
- A drive to continuously improve and update personal skills and knowledge.
- Pride and satisfaction in his work.
- Commitment towards the profession with interest going beyond the immediate job, to participate in programmes aimed at raising the level of competence of the entire profession.

Working Environment

While the work of a medical laboratory technician is in the laboratory, he/she is not isolated from others nor glued to bench work. He is surrounded by his/her colleagues and has to cultivate and maintain the discipline of working under a supervisor and pathologist. The laboratory organization has a definite role in serving the community; however, the ego of status should not jeopardize smooth human relations.

Patient contact within the laboratory is minimal, except for drawing blood or other specimens. It is important that the appearance of a technician should be such that the patient can build up confidence on his/ her performance; personal appearance, hair style, uniform, shoes and cleanliness are important. None of these should be such as to provide a non-professional impression.

Ethics and Laws of Laboratory Operation

Although legal and ethical regulations are relatively relaxed in developing countries, they may not be too far removed when the laboratory has to consider patient's right to information, properly signed consent forms, explanations and instructions regarding chain-of-custody requirements and the need for explaining risks as well as benefits of tests before the test is performed. Respect for the dignity of an individual reflects basic ethical considerations. Patients and family have a right to consent, to question, to request other opinions and to refuse diagnostic tests. Conversely, caregivers have the right to know the diagnoses of patients they care for so that they can minimize risks to themselves.

One has to bear in mind that better and stronger laws do not make a good medical laboratory technologist. Like other jobs, this has its standard of professional performance, based on moral obligations toward our fellow human beings. Such rules of conduct, personal and professional, are called **ethics**. The most important consideration of professional ethics is **honesty**. The data provided by a technician should be reliable. The tragedy lies in the fact that many technicians do not know that 'they do not know'. Hence, running of proper **quality control measures** is crucial and so also is the use of revised procedures. Confidentiality of the patient must be maintained, the place of work must be neat and clean, equipment should be properly checked at regular intervals and any breakdowns must be immediately reported to the supervisor.

Status of Medical Laboratories in Developing Countries

In developing countries, infectious diseases remain the leading cause of morbidity and mortality, primarily because known measures of laboratory-assisted diagnosis; control and therapy are not applied. The descriptions in the following chapters of sound laboratory methodologies, appropriate to various diseases, are most suitable for India and other developing countries. Our goal is to provide clinical laboratory services to rural areas of these countries, where the majority of the population live. Automation, in a limited sense, is highly suitable in cosmopolitan and other centres. Some manufacturers have studied the needs of developing countries and have proved to be highly successful in introducing discrete batch analysers into the market. These are becoming increasingly popular and the quality of results has improved sharply. Because of limitations of automated systems in developing countries, it is desirable that a standard procedure manual is published. This book attempts to fulfil/ attain this goal.

Clinician's Role in Laboratory Diagnoses

In this era of high technology, healthcare delivery involves many different disciplines and specialities. This includes diagnostic evaluation and diagnostic services. Laboratory and diagnostic tests are tools to gain additional information regarding the patient. By themselves, these tests are not therapeutic; however, when used in conjunction with a thorough history and physical examination, these tests might confirm a diagnosis or provide valuable information regarding a patient's status and response to therapy, which might not be apparent from the history and physical examination alone.

Laboratory testing begins before birth and frequently continues after death. A competent and experienced clinician only asks for relevant tests to diagnose an illness or use the test as

a follow-up of treatment. Thus, a fall in haematocrit indicates anaemia and its rise after treatment might be an indication of successful therapy. A few examples are given here (Table 1.2).

TABLE 1.2 *Examples of diagnostic tests requested by a physician for monitoring or identifying the pathological state of a patient*

Diagnostic test	**Indication**
Stool occult blood	Yearly screening after 45 years of age
Liver enzyme levels	Monitoring patient on hepatotoxic drugs, establish baseline values
Serum amylase	Abdominal pain and suspected pancreatitis
Haematocrit and haemoglobin	Detection of anaemia and abnormal bleeding; combine this with CBC results
Fasting blood glucose	Diabetes—diagnosis and monitoring
Urinalysis	Urinary tract infection, pregnancy in women, bleeding in urinary tract

The clinician must also have updated information about tests that have replaced older ones; this helps in appropriately diagnosing within the parameters of clinician's professional standards.

Patient is the Focal Point

As a technician takes all the precautionary measures to obtain a reliable specimen, equally important is the preparation of the patient. This is especially true in developing countries. Give simple, accurate, precise instructions according to the patient's level of understanding. For example, the patient needs to know when and what to eat and drink, or for how long to fast. Encourage dialogue about fears and apprehensions. Encourage questions and verbalization of feeling, fears and concerns. Do not dismiss, minimize or invalidate the patient's anxiety through trivial remarks. Walking a patient through the procedure using imagery and relaxation techniques may help them to cope with anxieties. Never underestimate the value of a caring patient. Whenever possible educate the patient and family regarding the testing process and what will be expected from them.

Importance of Communication with Patients

Considering the time constraints, the importance of communicating effectively cannot be emphasized enough. Effective communication is the key to achieving desired outcomes, preventing misunderstanding and errors and helping patients feel secure and connected to the diagnostic process.

Sometimes healthcare professionals, who deal constantly with illness and depression, tend to forget that patients are people just like us. These individuals present with their perceptions, worries and anxieties regarding the diagnostic process and what their illness means to them and their loved ones, what strategies they use for coping, what resources are available for their use and what other knowledge they have about themselves. We should be willing to 'take on the mind' of another—that is, to identify with the patient's point of view as much as possible and to show empathy. This 'human touch' makes us different from other animals.

Commonly Requested Laboratory Tests in India and Other Developing Countries

The authors agree with the fact that India has advanced considerably during the past few decades. Some private laboratories might even surpass the standards of the West. There

is an increasing trend in the West to outsource treatment to India and thus saving huge amounts of money. This is especially true for those who are uninsured. These developments, however, are occurring only in the private sector and not in rural areas or in the government, which truly serve the Indians of the soil. These westernized hospitals in India are located in isolated urban areas and do not provide any service to the poor and needy Indians. As this book is dedicated to meet the needs of those laboratories, far from automation, the list of tests are thus limited and are ones that might be performed manually with limited facilities (Table 1.3) or by automated procedures in bigger laboratories. Publications of the World Health Organization have been very helpful in choosing an appropriate list of tests. In the following pages we will try to understand the clinical significance and procedures of these routine tests.

TABLE 1.3 *Commonly requested tests in metropolitan laboratories in India (alphabetically arranged)*

S.No	Test Name
1	Alkaline phosphatase
2	Albumin
3	Bilirubin
4	Blood Group (ABO and Rh)
5	Blood sugar
6	BUN
7	Creatinine
8	Calcium
9	Cholesterol (Total)
10	CRP—C-Reactive Protein
11	Culture and sensitivity
12	C-peptide
13	DHT—Di Hydro Testosterone
14	1,25-Dihydroxy Vitamin D
15	ESR
16	Electrolyte
17	Faeces routine, occult blood
18	FSH—Follicle Stimulating Hormone
19	Folic acid
20	GGT (Gamma Glutamyl Transferase)
21	Haemogram
22	Haemoglobin
23	HbAlc-Glycated haemoglobin, blood
24	HCG-Beta subunit, serum by CM1A
25	HCV—Total AB to Hepatitis C Virus, serum by CM1A
26	25-Hydroxy (OH) Vitamin D
27	17-Hydroxy Progesterone
28	Iron

(Contd.)

S.No	Test Name
29	LH—Luteinizing Hormone (specific)
30	Lipid profile
31	Mantoux
32	Malarial parasite
33	Malarial antigen test
34	Phosphorous
35	Protein electrophoresis
36	Prothrombin
37	Prothrombin Time
38	Progesterone
39	PSA (Prostate specific antigen)
40	Proteins in serum
41	PTH (intact molecule)
42	RA-Rheumatoid arthritis
43	Rubella - IgM antibodies
44	Renal function test
45	SGOT (AST)
46	SGPT (ALT)
47	Thalassemia studies
48	Quadruple test (Maternal-Down's Syndrome)
49	TSH
50	Toxoplasma-IgM
51	Testosterone
52	Urine routine examination
53	Uric acid
54	VDRL, (RPR) serum
55	Vitamin B12
56	Widal

Review Questions

1. What is homeostasis and how does it differ from the pathologic state of the body?
2. What do you mean by diagnosis? How is this comparable to criminal investigation? How does a clinical laboratory help in the process of this investigation?
3. What is health insurance? How does it differ from governmental healthcare plan?
4. What is competency-based education? How does it differ from the classical (fundamental) education? Can the competency-based education be combined with classical education?
5. Identify the problems of medical laboratories in India. What are your suggestions to improve the situation?
6. What are the differences between MLTs and MTs in terms of education, training and job performances?
7. What is the significance of continuing education for clinical laboratory personnel?

8. What is quality control? Why is it important to introduce quality control measures in the clinical laboratory operations?
9. Justify or refute the following statement:
   'Indiscriminate training of laboratory personnel without appropriate educational background has resulted in poor quality of laboratory results in developing countries.'
10. What are the roles of a physician and a pathologist?
11. Why is it important to share information with the patient?
12. 'India has made as good a progress in Laboratory Technology as the advanced countries'. Do you agree with the above statement? Justify.
13. List the sources of error in laboratory findings.
14. Does the abnormal value in tests always indicate a pathologic state?
15. Why do physicians follow the trend of a laboratory result rather than its absolute value?
16. What precautionary measures should your laboratory take in order to prevent the spread of infection from the laboratory?
17. What do you mean by 'biohazard'? How does this affect a laboratory?
18. What steps would you take to improve your relationship with the patient?
19. If the laboratory technician is able to diagnose the ailment, should he/she report to the patient directly?
20. Which is the most common specimen submitted to the haematology laboratory?
21. Name the tests requested for assessing liver function.
22. Which laboratory does the prothrombin test?
23. What is the significance of Quality Control of laboratory findings? How is Quality Control different from Quality Assurance program?

Chapter 2

Introduction to Clinical Laboratories

Ashoke Khanwalkar and Chhotelaal Pande

Chapter Outline

Introduction to Clinical Laboratories

The clinical laboratories play a vital role in the healthcare delivery system. Laboratory testing enables the healthcare provider to obtain information inaccessible through the patient history or physical examination. Physicians may order laboratory tests for one or more of the following reasons (Figure 2.1):

- ***To assign a diagnosis and prognosis:*** Laboratory testing may be used to help narrow a differential diagnosis when the patient's signs and symptoms are characteristic of multiple disease states. It may also be used confirm a clinical diagnosis like diabetes or myocardial infarction. The information may also be used to offer the patient a prognosis for his/her suspected disease. While **diagnosis** refers to the identification and characterization of the disease affecting the patient, the **prognosis** refers to expectations regarding personal outcomes for the patient, including but not limited to survival, life expectancy, quality of life, functional capacity, and so on.
- ***To develop a treatment strategy:*** A confirmed diagnosis helps the physician to establish an effective treatment strategy for the patient. Depending on the findings of laboratory testing, the physician may encourage behavioural modifications, prescribe a medication, and so on.

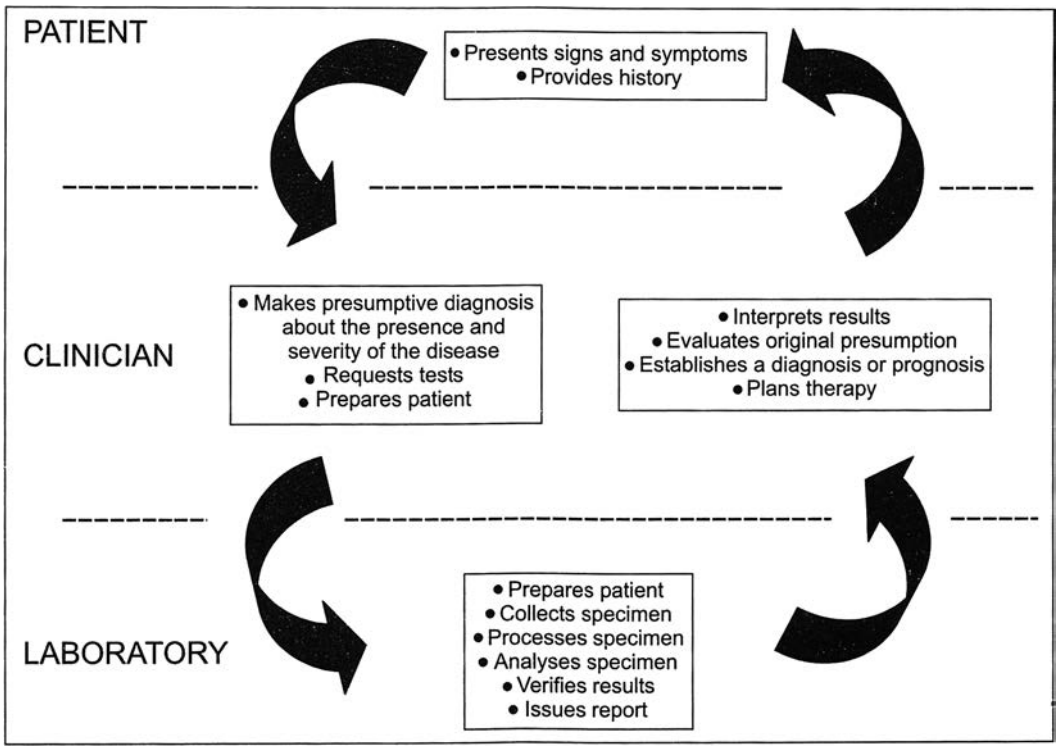

FIGURE 2.1 *Relationship among patient-physician-laboratory (The cycle progresses in a counter clockwise direction)*

- ***For prevention or early detection of disease:*** Screening tests can help in the early detection of a disease that is asymptomatic in the early stage. Routine screening may detect a disease process in an early stage, allowing timely initiation of treatment to avoid complications and keep the patient healthy.
- ***For on-going assessment and follow-up of treatment:*** Treatment efficacy must be closely monitored. In some cases, signs and symptoms may not fully characterize response to therapy. In such cases, laboratory testing may be helpful to further assess the status of the patient and disease, or to assess for complications of therapy (e.g., hepatotoxicity of a medication).

ORGANIZATION OF CLINICAL LABORATORIES

Organizational Structure

A clinical laboratory must have a clearly defined organizational structure in order to perform its job efficiently. The laboratory may either be attached to a hospital or operate independently. Every hospital must have a clinical laboratory to respond to **emergencies** as well as **aid surgical decisions.** The hospital classifies the clinical laboratory as a part of its special diagnostic services.

In all circumstances, a pathologist oversees the laboratory and hence is responsible for the operation of its various components. The component laboratories, however, may function independently under a supervisor, although the pathologist still acts as the decision maker (Figure 2.1). The authors, through experience with Indian hospitals, have found that pathologists primarily spend their time in the microscopic examination of body tissues, and therefore the subjective decision of the pathologist remains crucial. Other laborator workers

follow standardized procedures and keep meticulous records, and hence their decision process is perhaps more objective.

The **pathologist**, as the leader of the laboratory, has a medical degree with specialized training. The bench workers, on the other hand, may be either technologists or technicians. While all bench workers are college graduates, a technologist typically has a higher degree, either a Masters (MS) or a PhD, and has also received higher-level laboratory training in order to assume a supervisory role.

Technologists must hold at least a baccalaureate degree, if not an MS or PhD, and complete a minimum of three years of technical training offered by a university. Each technologist is responsible for all activities within his component laboratory section, including work distribution, problem-solving, and daily decision-making. They deal with external agencies and represent the pathologist when needed. They complete all paperwork, order supplies, purchase and maintain equipment, maintain compliance with safety regulations, and perform other miscellaneous activities. They receive higher pay than technicians. As supervisors, they sign off on test reports prepared by the technicians. The pathologist may choose to discuss results with the technologist before signing the report and sending it to the physician. This discussion has been facilitated by the introduction of computers into modern laboratories. The final report, after receiving the signature of the pathologist, then goes to the clinical physician. Only the pathologist or the laboratory supervisor may release confidential laboratory findings regarding the patient to the physician.

The **technicians**, as bench workers, are workhorses of the laboratory. They have a higher secondary degree or a baccalaureate science degree (BS) and have typically received one or two years of laboratory training at a technical school. Some technicians are on-the-job trained (OJT), while others are high school graduates with one or two years of technical training in laboratory performance. The technician performs tests, records results in a laboratory record book and sends results with his dated signature to his supervisor, the technologist, for his dated counter-signature. Laboratories typically strive to maintain low costs and maximize worker efficiency. Hence, a technician must know more than one field in order to keep up with laboratory operations. Keep in mind that salaries constitute the highest expense of any laboratory.

Other than technicians, the laboratory workforce includes several non-technical personnel who participate in less technical activities, such as receiving and processing of specimens, sterilization, autoclaving, disinfections, distillation, preparation of swabs, and so on. Laboratories of developing countries rarely enjoy ideal circumstances as workers are mostly OJT. In rural laboratories, the pathologist may not always be available on site and therefore the laboratory runs with two personnel: one with a high school diploma, trained in laboratory operations, and the other with minimal education, who does all the non-technical work.

In order to function properly, a laboratory is divided into several components. These artificial divisions are made for convenience and hence can be drawn differently depending on the laboratory workload and the number of employees. The laboratories typically strive to maintain low costs and worker efficiency. Hence, a technician must know more than one field in order to keep up with laboratory operations. Keep in mind that workers' salaries constitute the highest expense of any laboratory.

In short, administrators of clinical laboratories must bear in mind that efficient operation and effective delivery of laboratory services are dependent on modern equipment, well-trained staff, a well-designed physical environment, and a good management team (Figure 2.2).

Information Flow within a Clinical Laboratory

A clinical laboratory has several departments and each has a specific role. A smooth flow of information between departments ensures the total operation of the clinical laboratory and the test results are as useful as possible to the healthcare provider in order to enhance patient care. Specimen collection, processing, and testing must properly utilize the required

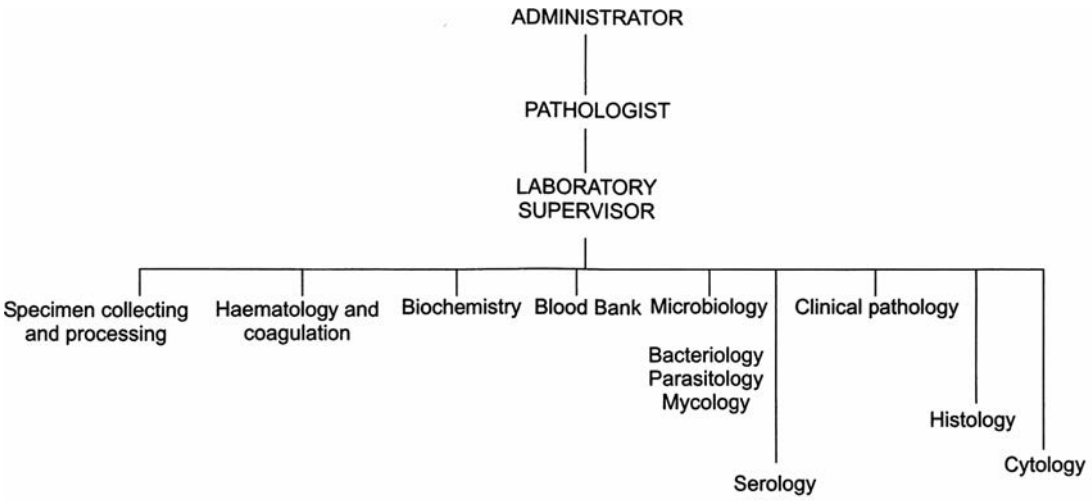

FIGURE 2.2 *Organizational structure of clinical laboratories*

paperwork and database systems. This information exchange primarily involves a **laboratory requisition**, a **laboratory directory**, a **computer database**, and a **laboratory report.** These will be elaborated further in the following sections.

Functional Components

In order to function properly, a laboratory is divided into several component sections. The most common functional components of clinical laboratories are shown in Figure 2.3. These include the Specimen Collection Area, Haematology, Coagulation, Biochemistry, Blood Bank, Microbiology, Serology, Clinical Pathology and Histology, among others. These divisions are made for convenience and hence can be drawn differently depending on the laboratory workload and the number of employees—smaller laboratories may combine several of the above components to improve efficiency. Thus, Haematology may be combined with Coagulation, Microbiology with Serology, Biochemistry with Clinical Pathology, and so on. Again, these components are artificial divisions made for convenience and hence can be grouped differently depending on the laboratory situation.

The departments where examples of various laboratory tests are requested and the specimens needed to perform those tests are shown in Table 2.1.

Figures 2.4 and 2.5 illustrate two possible laboratory floor plans, one attached to a hospital and the other attached to a physician's office.

In regards to flow, a patient typically submits the request slip (Figure 2.6) provided by the physician during the clinical encounter at the registration window. The registration desk records demographic details, including the patient's name, date of birth, referring physician, and laboratory tests to be performed, much of which are available from the request slip. The desk then assigns a record number, often known as the accession number, for referral and follow-up. In modern laboratories, bar coding has facilitated this process considerably. The request slip is also often used for the reporting of results. This will be further discussed in Chapter 5, which deals with the collection of specimens. The report can be sent using detailed directions on the request slip or through the use of electronic media. Electronic communication is always faster and the time saved may prove crucial.

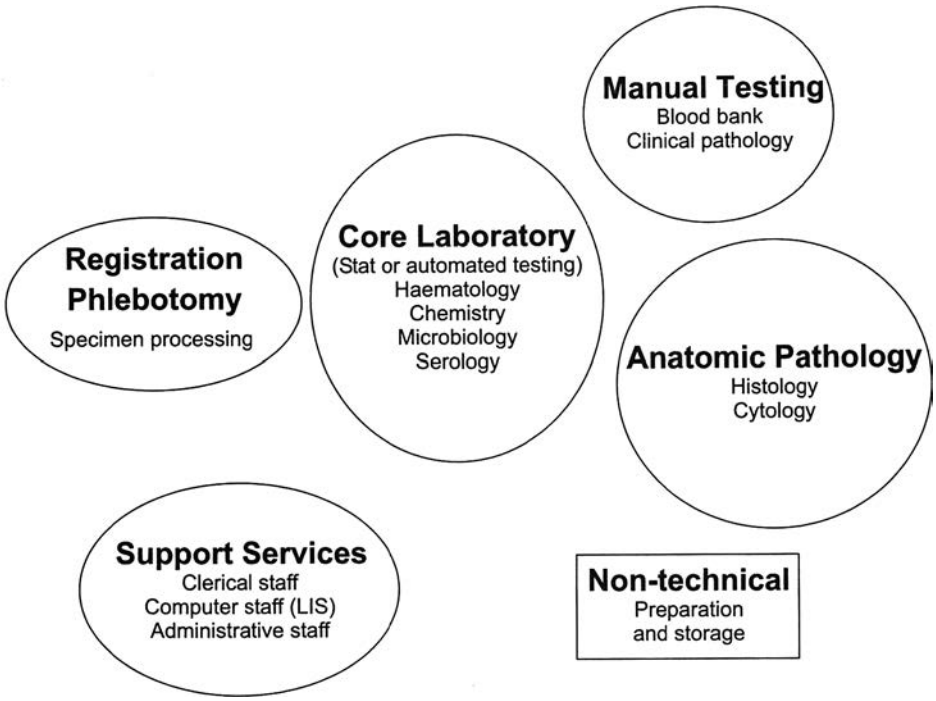

FIGURE 2.3 *Functional components of a clinical laboratory*

TABLE 2.1 *Example test requested and specimen required in different laboratory departments*

Department	Specimen Required	Example Test Request
Haematology	Whole blood	Haemoglobin, Haematocrit, RBC count, WBC count, RBC indices (MCV, MCHC, reticulocytes), Platelet count, DLC (Differential Leukocyte Count), ESR, CRP
Coagulation	Whole blood	Bleeding time, Prothrombin time (PT), Activated partial prothrombin time (APTT), FDP assay
Blood Bank	Whole blood and clotted blood	Type and Screen/Type and Cross (blood typing, compatibility testing, identification of incompatible antibody)
Biochemistry	Serum	*Routine Biochemical Tests* Glucose-Fasting, PP (Postprandial or after meal) Lipid Profile-Triglycerides, Total Cholesterol, HDL, LDL *BMP (Basic Metabolic Panel)* Sodium, Potassium, Chloride, Bicarbonate, BUN, Creatinine *LFT (Liver Function Test)* SGPT/ALT, SGOT/AST, Alkaline phosphate, Bilirubin (total), Bilirubin (direct)
Clinical Pathology	Urine	Colour, SG (specific gravity), pH, Protein, Glucose, Ketones, Bilirubin, Urobilinogen, Leukocyte Esterase, Nitrites Cells—RBC, WBC, Epithelial cells, Crystals, Casts
Clinical Pathology	Stool	Occult blood
Microbiology	Various specimens, e.g., throat swab, wound swab, blood, urine, spinal fluid, sputum, stool, etc.	Identification of infectious agent—Gram stain, Bacterial culture, Fungal culture, Monospot test, Urine antigen test, Viral panel

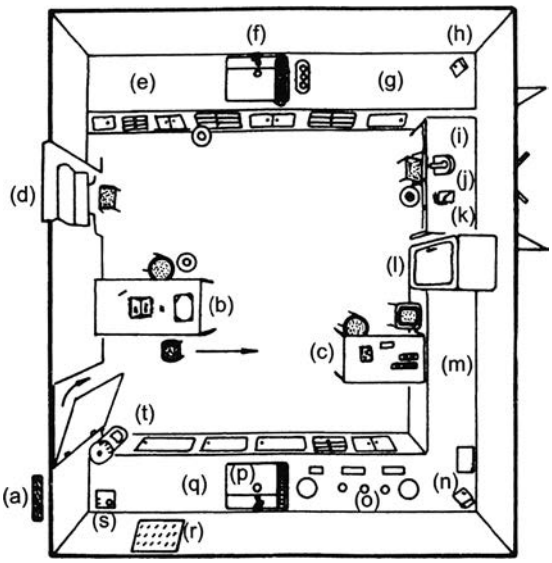

FIGURE 2.4 *Example plan for laboratory attached to a hospital—(a) waiting area, (b) registration desk, (c) blood drawing, (d) specimen receiving window, (e) urine analysis area, (f) sink with staining racks, (g) histology area, (h) oven, (i) haematology area, (j) microscope, (k) record book, (1) refrigerator, (m) biochemistry area, (n) water-bath and incubator, (o) microbiology area, (p) sink with staining rack, (s) deionizer, and (t) sterilizer*

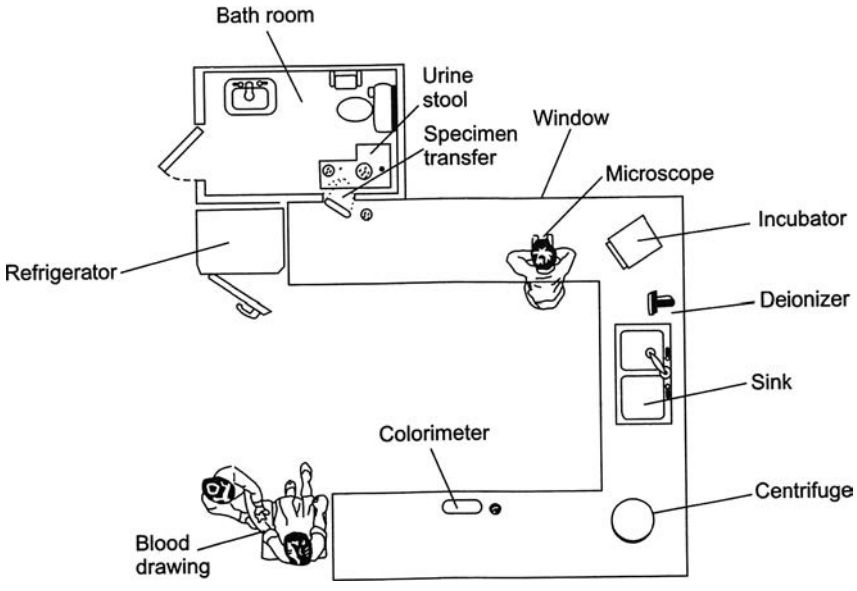

FIGURE 2.5 *Example plan for laboratory attached to a physician's office.*

Letter head with the name of the physician (or the name of the hospital)

Date: _____

Requesting Physician: _____ Provisional diagnosis: _____

Specimens: _____

For inpatient (IP):

Hospital: _____ Ward: _____ Bed: _____

Patient's Name: _____ Sex: _____ Age: _____

Address: _____

For outpatient (OP):

Patient's Name: _____ Sex: _____ Age: _____

Address: _____

Test(s) requested:

Test requested	Laboratory report	Normal value
_____	_____	_____
_____	_____	_____
_____	_____	_____

Specimen collected by: _____ On (date): _____ Time: _____ (A.M./P.M.)

Specimen accession No.: _____

(given by the receiving clerk and entered in the log-book)

Date and time of submitting report: _____

Signature of supervisor: _____ Date: _____

FIGURE 2.6 *Sample request slip*

The patient then proceeds to the specimen collection area, in which either blood or urine may be sampled. For blood tests, the blood sample is drawn by a **phlebotomist.** These individuals typically acquire on-the-job training and improve their blood drawing skills through experience. They may perform simple point-of-care blood tests for rapid diagnostic studies, but often the technician processes test results, records them in the laboratory register, and submits the official signed, dated report to the technologist/supervisor, noting the time of completion.

The introduction of **bar codes** to identify specimens has been a big step towards automation. Automated instruments are now designed for larger laboratories with high workloads, as well as for small physicians' offices where results are instantly delivered. This quick turnover supported by automation is beneficial for both physicians and patients, and represents an important technological advancement.

Departments of Clinical Laboratories

This book focuses primarily on hospital-based and private laboratory operations. As previously discussed, these are usually departmentalized into functional components for efficiency. In a large laboratory there may be several supervisors managing different sections, whereas in small laboratories there may be a single supervisor managing all sections.

- ***Specimen collection and processing:*** This is the area of laboratory where specimens (e.g., blood, urine, and stool) are collected or received from external sources. Specimens are sorted, accessioned into the computer system of the laboratory, and appropriately labelled for transport to their respective department for processing and testing. If a specimen is already assigned an identification number at the point of collection, it is noted (or scanned). If specimens must be referred to an external reference laboratory, the processing department prepares the specimen according to strict guidelines provided by the reference laboratories.
- ***Haematology:*** Performs quantitative (blood counts) and qualitative (blood smear) assessment of the cellular components of blood. Quantitative assessment may be done manually or through the use of an automated blood counter to determine the number and differential count of blood cells. Qualitative assessment may be performed through blood smears on a slide for microscopic examination of cell morphology. The results help the physician to diagnose blood-related disorders, such as anaemia and leukaemia. This department also often includes the **coagulation laboratory** in its operation. Plasma specimens are analysed for investigation of clotting time and quantification of various clotting factors to help identify the underlying cause of bleeding or thrombotic disorders.
- ***Serology:*** Assesses the presence of antigens or antibodies on cells or in the liquid (plasma) portion of the blood. Analysis aids in blood typing and antibody screens for cross matching prior to transfusions (often through the **blood banking** department), as well as to determine the aetiology of infectious agents. The identification of abnormal antibodies in the patient's serum can help identifying pathogenic microorganisms, and viral infections are often diagnosed by serodiagnostic methods.
- ***Clinical Chemistry:*** Analyses the dissolved substances circulating in the blood, many of which change as a result of disease processes. The specimens submitted are typically serum. Most testing has become automated, and many of the tests are performed as panels or groups of tests. In recent years, smaller portable machines have been introduced into the market and are becoming popular in rural India. However, some rural hospitals still rely on manual testing.
- ***Urinalysis:*** Characterizes the physical appearance, chemical contents, and microscopic analysis of urine.
- ***Microbiology:*** Identifies pathogenic microorganisms to establish the cause of an infectious disease. These studies are broadly divided into three groups - bacteriology (bacteria), mycology (fungal infections), and parasitology (parasites). The bacteriology department also performs antibiotic sensitivity testing to help guide proper therapy. This is often the most active department in the clinical laboratories of developing countries.
- ***Clinical Pathology:*** Studies male fluids (urine, semen, spinal fluid) and tissue for the presence of disease. It is typically the umbrella for the departments of **Cytology** and **Histology.**
  - ***Cytology:*** Examines specimens for the presence of abnormal cells, e.g., Pap smear, pleural fluid analysis, CSF (cerebrospinal fluid) analysis.
  - ***Histology/Pathology:*** Examines tissue, often obtained via biopsy or during surgery, to determine the presence, cause, or extent of a disease.

Ethics and Laboratory Medicine

Ethical issues have been given limited attention by professionals in laboratory medicine. Professional ethics is the moral bond that links a profession, the people it serves, and society. Understanding the complexities of an individual and common good is essential for full professional participation in major issues in healthcare. The ethical attitudes laboratory personnel display influence the kind of people who choose to work in their profession. More open discussion about ethics is necessary in this professional literature. In most countries there are high moral and professional standards of behaviour for clinical staff and laboratory personnel. Every laboratory worker handling clinical materials must adhere to these standards (see *Manual of Basic Techniques for a Health Laboratory*, 2nd Edition, World Health Organization, Geneva, 2003).

What are ethics?

- Ethics are the moral guide that governs the professional and personal conduct of all regulated members of an institution and communicates to the public; the principles by which professional performance is adjudicated.
- They are the inspirational and value-oriented guidelines expressing the most honourable ideals to which professionals should aspire.
- Ethics give precise guidance and direction for action in concrete situation, and they require ethical reasoning too.

Why do we need to have a well-developed sense of ethics?

A well-developed sense of ethics improves our image as professionals. There are some obvious ethics, a public obligation, in the practice of medicine. Professional ethics should give voice to the moral bond that links profession, the individuals it serves, and the society as a whole. Ethical standards are the key elements of public trust in any profession. *"They give professionalism its moral dimension and transform the career of selling services into the calling of providing services".* We have not been doing well enough to emphasize ethics in medical laboratories on account of which laboratory reports in developing countries are far from satisfactory.

Ethical Principles of Medical Technologists

- Put yourself in the shoes of the patient. Never do such things that you do not want others to do to yourself. The ethical attitudes we display influence the kind of people surrounding us.
- Ethics is the moral police who is always there to keep us uneasy when we make wrong decisions, and commends us when we make the right ones.
- Ethics are applied for the best interest of the patients. It is based on *trusted relationship* with the patient. For example, false positive results are as deadly as false negative results.
- Satisfactory laboratory performance is attained through **quality assurance** which mandates maximal contributions to benefit patients and to assist healthcare providers in an effective, efficient, and economic manner. Although both accuracy and precision have always been prerequisites for good laboratory practices, timeliness and promptness, or 'turnaround time' (TAT), of the resulting report is equally critical to overall excellence in patient care.
- Accurate record keeping of the results is essential in order to facilitate follow up.

- Laboratory results are as good as the specimens collected. Hence, all care must be taken in specimen collection, its transportation, storage and retention.
- Professional attitude and behaviour of medical technologists should convey that they are honest, dependable, and equitable.
- Medical laboratory technologists must stay abreast with new developments and updates in methods.
- Medical laboratory technologists are accountable for their actions.
- The clinical laboratory operates with a sense of team spirit. During the course of their work, medical technologists learn a considerable amount of sensitive information about patients and their illnesses. As clinical staff, laboratory workers must regard this information as strictly confidential; only the clinical staff who request the examination should receive the reports. Preserve the dignity and privacy of others. Uphold and maintain the dignity and respect of the profession.

Automation in Clinical Laboratories

As discussed previously, the automation of clinical laboratory equipment has revolutionized the operation of diagnostic laboratories. The change is especially apparent in the area of biochemistry. Some laboratory components such as microbiology are semi-automated, while others are still performed manually. Nevertheless, laboratories in developing countries continue to advance. The private sector has established highly automated laboratory assays for urban settings, thereby minimizing the turnover time. The results of automated processes are reliable, fast, and repeatable. Human errors are likely to occur with manual operations—fatigue, competency, and the attitude of an individual are liable to influence results. Nevertheless, in spite of its many advantages, automation can lead to serious breakdowns due to mechanical failure or an interrupted power supply. Moreover, the necessary skill to maintain the automated system is still limited in developing countries. Thus, manufacturers are forced to supply simpler equipment with a limited number of routine tests that can use battery or generator power.

It is not unusual to see small automated laboratories even in small Indian villages, as long as electricity is available. In this case, the laboratory technician's skill lies in the operation and maintenance of the equipment rather than the scientific skill required for performing a clinical test. Thus, a motor mechanic may be better suited for the automated system than a science graduate from a university. Even so, the laboratory needs a supervisor to communicate with the outside world as well as to tackle problem solving.

Cost containment in laboratory operations is possible through reducing waste and enhancing efficiency. Many laboratories restructure their components by putting them into various groups, such as combining chemistry with haematology ('chematology'), where both components are highly automated. Similarly, clinical pathology (i.e., body fluids), microbiology, and immunology are often combined. Furthermore, technicians are usually trained in more than one field and therefore they can easily switch positions if needed. In addition, because of increased automation, even less qualified individuals with good insight into troubleshooting machine operations can help to contain costs.

In the effort to modernize a clinical laboratory in a developing country, the following criteria should be taken into consideration: usefulness, cost, accuracy, ease of maintenance, ability to self-calibrate, quality control functions, reporting capabilities and safety. The instrumentation should be especially durable, weather resistant, simple to use, cost-effective and capable of rapid throughput or turnaround time (TAT).

Laboratory Information System

It is widely accepted that about 70% of the information used in the management of patients comes from the clinical and anatomical pathology laboratories *(Informatics for the*

Clinical Laboratory: A practical guide, Daniel F. Cowan, Ed. Springer-Verlag, New York, Inc., 2003). The primary objective of clinical laboratories is to provide the highest possible quality of service to patients and to those who care for patients. High-quality service encompasses accurate and precise analysis; timely, clear and concise reporting; and service delivery to a location in a format most valuable to the service user within the shortest TAT.

Today's pathologist is expected to do more than simply read a slide and make the correct diagnosis; rather, the management ofthat information is critically important.

Information Technology (IT) is an essential link between all segments of integrated medical delivery systems, acting both as a tool to convert data into useful information and to determine clinical efficacy. Clear communication between the pathologist and the clinician is vital for quality healthcare. Even though the primary mode of communication has been the pathology report, we may have to rethink the way in which we report in an attempt to provide the clinician (and possibly the patient) with integrated and useful information for each particular patient, instead of simply the data. The pathologist, with sufficient knowledge of information systems, is in a unique position to become an expert in information management.

Information Sharing

Computers are now available in many laboratories of developing countries. It traditionally has taken significant time to move the test request from the physician to the laboratory and back to the source. This has been greatly facilitated through the use of personal computers (Figure 2.7). With the use of modem and broadband Internet, intercommunication between the laboratory and physician's office has been minimized, and furthermore there is no loss of information in this process of communication. Of course, reliability of both the computer and the electrical supply are essential, but the situation continues to improve with time.

The **laboratory information system** (LIS) provides different services for the laboratory itself as well as for clinical services. Order and result entry, inquiry, inventory control, and time management are the most common laboratory services obtained from the LIS. Reporting and patient institutional billing are the most used clinical services.

Advantages of laboratory information systems include:

- Elimination of charting errors
- Improved efficiency
- Automatic identification of abnormal or unusual test results
- Matching of patient specimens to test results
- Prevention of unauthorized testing and reporting

Barcode and Barcode Scanners

Computerization in laboratories and hospitals has reduced errors in specimen identification and tracking. Specimens are labelled with printed barcoded labels that match barcoded test requisitions and patient identification bracelets (Figure 2.8). The same code is used at the time of patient admission followed by laboratories. To retrieve information, the barcode needs to be scanned.

Data can be entered directly into the computer system using barcode scanners. Even small laboratories have a system for pre-printing barcoded specimen labels with patient data.

While communication between the pathologist and the clinician is critical, the information is useless if not delivered on time or if lost during its transfer. Despite the usual assumption that modern organizations ought to be computerized, it is a useful exercise to formally list the

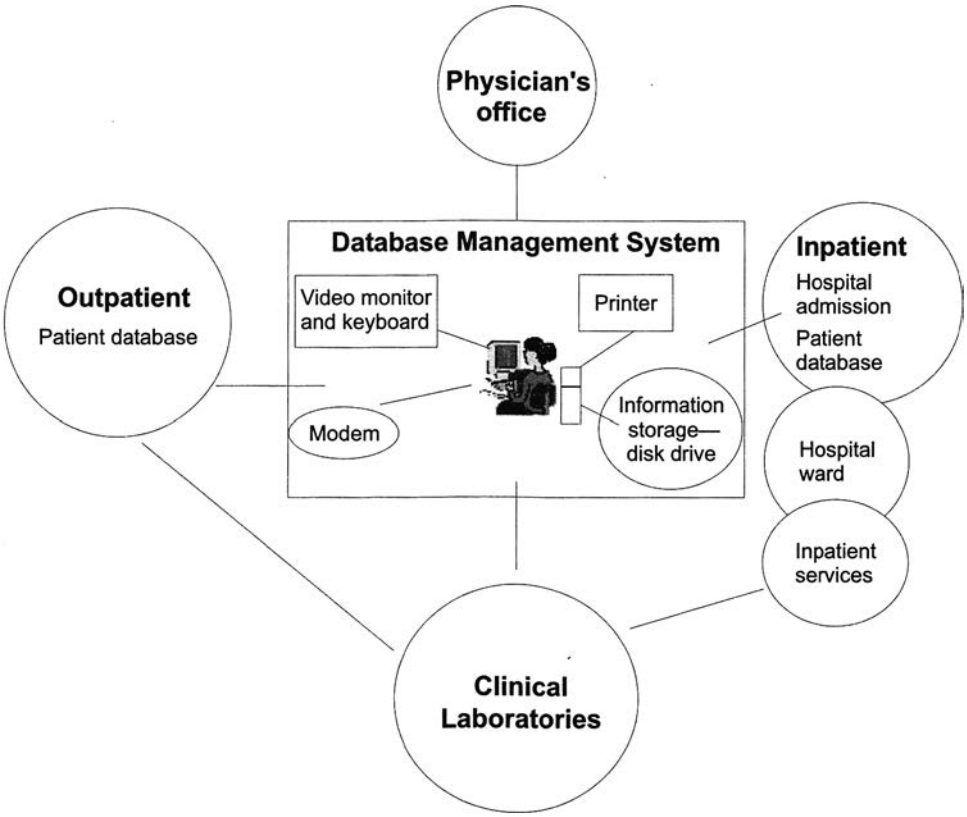

FIGURE 2.7 *Information sharing among patient, physician, hospital healthcare team, and the laboratory*

advantages and disadvantages of an LIS. The development of a list may avoid the naïve expectation that an LIS will solve all laboratory problems *(Clinical Laboratory Medicine,* 2nd edition, Kenneth D McClatchey, Ed., Lippincott Williams and Wilkins, Baltimore, 2002). It may invite many other problems inherent to developing countries, with their poor support for technology and lack of a steady power supply.

Strengths

Computers are able to consistently perform the same task as many times as needed.

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FIGURE 2.8 *A typical of barcode used by hospitals and laboratories for tracking information shared by different individuals and departments.*

Weaknesses

Computers can do only what they are programmed to do. Hence, they cannot perform anything on their own; commands must be fed into computers for execution.

Benefits with human-machine interface

Laboratory professionals acquire 'perfect memory'. The computer's ability to perform repetitive tasks relieves the laboratory staff from the onerous aspects of work. Laboratory professionals are thus able to deal with the exceptions and tasks for which they are trained.

In short, interrelated components of information system work together to collect, process, store, and disseminate information to support decision-making, coordination, control analysis, and visualization in an organization. It cannot be overemphasized that the information system transcends the computer and one must be ready to occasionally conduct manual procedures and perform tasks without computer support. The computer and its programs support the information system; they are not the system itself.

Review Questions

1. What is the difference between prognosis and diagnosis?
2. Who is responsible for the operations of a clinical laboratory?
3. Clarify the difference between a technician and a technologist?
4. State the functions of the following clinical laboratories: Haematology, Blood banking, Immunology, Microbiology, Clinical pathology and Histology
5. What is phlebotomy? What training is needed to become a proficient phlebotomist?
6. How is Histology different from Cytology?
7. What is a barcode? How does it help in the clinical laboratory operations?
8. A swab specimen taken from a wound came from the ward. Which department should receive this specimen?
9. What is biopsy material? Which department of the laboratory receives biopsy specimens?
10. What is differential diagnosis? How is it different from confirmed diagnosis?

Chapter 3

Laboratory Safety and First Aid

Monisha Bhatia

Chapter Outline

- Clinical Laboratory Environment
- Laboratory Safety Policies
  - Safe laboratory design principles
  - Work area
  - Patient and visitor safety
  - Common causes of accidents
  - Laboratory accidents and safety precautions
  - Control of hazardous energy
- Biohazard and Safety Precautions
  - Waste disposal
- Radiation Hazard
- Fire Hazard and Explosion
- Specialized Equipment
  - Compressed gases
- Laboratory Hygiene and Housekeeping
- Personal Safety of Laboratory Workers
  - Work habit
- Warning Signs
- Accident Record and Training
- First Aid Kits and Procedure
  - Bleeding
  - Poisoning
- Poisoning with Strong Acids and Caustic Alkalis
  - Burns
  - Eye accidents
  - Miscellaneous
- Guide to Standard Precautions
- Review Questions

Clinical Laboratory Environment

Clinical laboratories are hazardous environments. Dangerous chemicals, specimens, equipment, and technology surround the laboratory personnel. The chemicals might be toxic and infectious, equipment may malfunction, and any technology might be misunderstood. Many laboratory procedures expose the worker to the risk of injury. However, every laboratory hazard can be managed to reduce this risk. No clinical laboratory can afford to operate without a safety programme, and the best laboratories will build safety into the culture of the laboratory. The safety programme of the laboratory must be organized, well documented, and supervised, preferably by an external agency. "**Better safe than sorry**" is the golden rule of laboratory safety.

Laboratory Safety Policies

The clinical laboratories must maintain a document with safety regulations. The *"Laboratory Biosafety Manual"* published by World Health Organization (2nd Edition, 2003; available from WHO, 1211 Geneva 27, Switzerland, or via Internet at http://www.who.int/gpv-documents/) is a good guideline. It is ideal to get this document or guidance from an outside agency that specializes in safety regulations and training, as it may be difficult for laboratory personnel and leadership to spot some subtle potential problems. All laboratory personnel must be made aware of safety regulations as documented. This chapter will deal with general safety, while safety regulations of microbiological laboratories will be discussed in detail in Chapter 19.

Hazard containment is the use of routine control processes to keep the incidence of accident and injury within reasonable expectations. Compliance with appropriate and established safety policies of the facility should limit risks that are associated with clinical laboratory practice. This begins with a hazard assessment to determine what potential dangers require containment. Hazard assessments should be followed by immediate implementation of strategies to reduce risks of accidents. After an accident occurs, it must be reported, investigated, and precautionary measures should be implemented to prevent the same error from occurring later. Therefore, a hazard minimization programme is a continuous and regular effort that seeks to reduce risk to the lowest level as can reasonably be attained. Laboratory leadership must take responsibility for implementing a comprehensive culture of safety that includes these practices, and continually remind workers of the importance of safety in all laboratory activities.

Safe Laboratory Design Principles

Hazard containment programmes may begin at the design stage of the laboratory. Electrical design, plumbing, building of biosafety cabinets, placement of containers for disposing needles, lancets and gloves, designating a safe place for placing gas tanks held in chain, and various other fixtures to avoid accidents might be included. Wherever possible, engineering control is preferable to any approach that requires individual workers to constantly remember safety procedures.

WHO has recommended the following laboratory designs and facilities for basic laboratories:

- The laboratory should be separated from the areas that are open to unrestricted traffic flow within the building.
- Entry for personnel must be through a vestibule or double-door entry.
- The surface walls, floors and ceilings should be water-resistant and easy to clean. Openings in these surfaces (e.g., service pipes) should be sealed to facilitate decontamination of room(s).

- The laboratory room must be sealable for decontamination. Air-ducting systems must be constructed to permit gaseous decontamination.
- Windows must be closed, sealed and break-resistant.
- There must be ventilation system that establishes a directional airflow from access spaces into the laboratory room. Staff, at all the times, must ensure that proper directional airflow into the laboratory room is maintained.
- A negative pressure should be maintained in the controlled air system of the laboratory. The supply and exhaust air must pass through high-efficiency particulate air (HEPA) filters. The exhaust air from the laboratory must be discharged to the outside of the building, after it passes through a series of HEPA filters, such that it is dispersed away from occupied buildings and air intakes.
- Anti-backflow devices must be fitted to the water supply.
- Effluents should be decontaminated before being discharged to the sanitary sewer.
- Biological Safety Cabinets (BSCs) must be operated with negative airflow filter. BSCs are designed to protect the operator, the laboratory environment and work materials from exposure to infectious aerosols and splashes that may be generated when manipulating materials containing infectious agents, such as primary cultures, stocks and diagnostic specimens. A simple design of a BSC is shown in Figure 3.1.

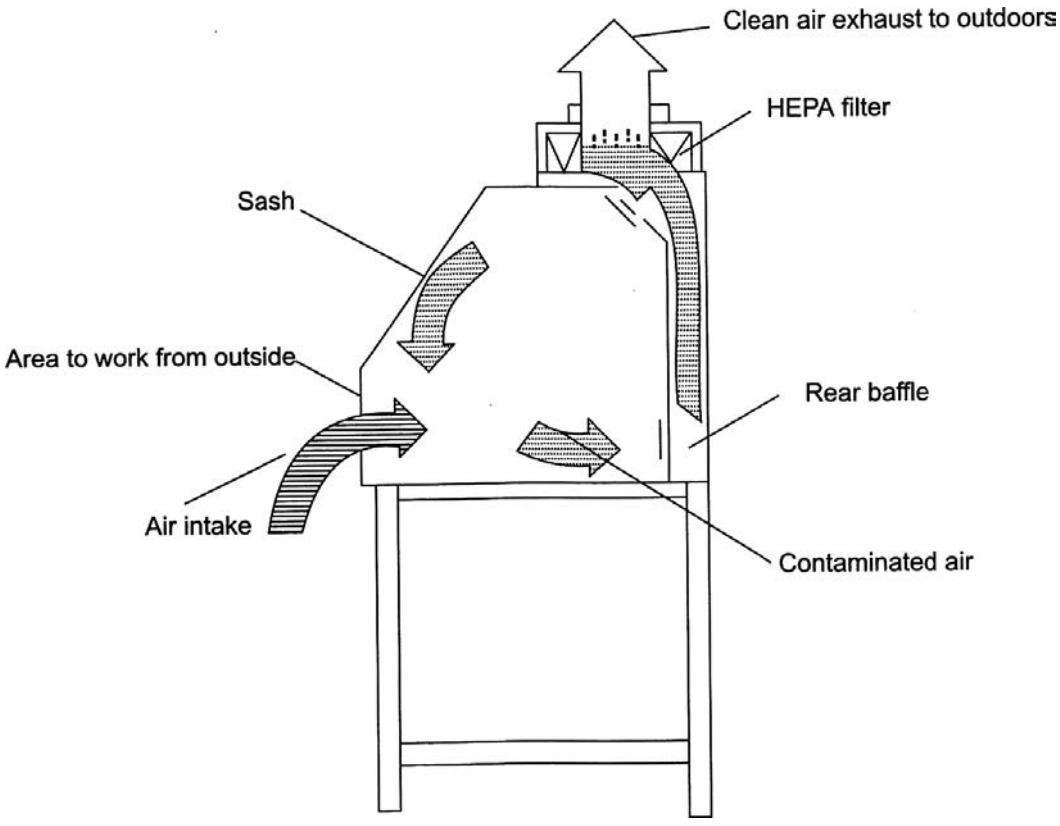

FIGURE 3.1 *Biological Safety Cabinet (BSC) class I*

Work Area

The laboratory should allot sufficient bench space in which all work processes can be performed safely. Similarly, equipment should be arranged on the bench to maximize working space and minimize accidents. For example, reaching across hot plates or brushing past caustic containers as a routine might invite an accident. Power cords or hoses that cross walkways constitute unsafe laboratory design or management. Lighting must be bright enough to illuminate all areas of the laboratory. Safety supplies such as gloves, aprons, and others must be kept in adequate supply and at convenient locations to make compliance with safety practices easy.

The laboratory should be designed to accommodate special needs of *handicapped workers.* Ramps, wide aisles as well as lowered work surfaces, and storage areas are necessary for wheelchair-bound workers.

Airflow in the laboratory must be engineered to suit the laboratory's activities. Fume hoods and biosafety cabinets should be available, and storage of volatile chemicals should be clearly indicated. Operations on open benches that generate occasional or low-level fumes should be positioned such that fumes are drawn away from workstations and not across them.

Patient and Visitor Safety

Access to hazardous areas should be limited to individuals who have been trained to avoid injury. Patients and visitors should not be allowed to come to the work areas. The laboratory design should include a reception area having waiting room that protects untrained individuals from any appliance, fume, or aerosol that might injure them.

The phlebotomy area should be designed with patient safety in mind. The patient arriving in the laboratory for blood specimen collection should be escorted to a comfortable chair that includes an armrest and a passive restraint system to protect any patient who loses consciousness from falls. If possible, they should be offered a bed, reclining chair, or other comfortable flat surface to lie down for the procedure. If the patient is supine, assist with lifting their legs in the event of loss of consciousness. Patients should be offered fluids before the procedure.

Common Causes of Accidents

All accidents have some cause and most can be prevented. Some basic causes include failure to follow instructions, defective engineering design of the laboratory, careless work, failure to use protective devices, compromised mental status of the worker (such as fatigue, overwork, intoxication, illness, poor sleep) and so on.

Every laboratory worker should become familiar with common types of accidents. One should bear in mind that the documented safety regulations must be followed routinely to be effective. The single most important cause of any accident is carelessness that can arise from lack of knowledge, absentmindedness, or overconfidence. Few of the major types of accidents are illustrated in Figure 3.2.

Laboratory Accidents and Safety Precautions

Laboratory accidents are broadly classified into six groups—physical injuries, use of specialized equipment, electric shock, exposure to dangerous chemicals, exposure to radioactive chemicals, fire, and biological hazards.

FIGURE 3.2 *Common types of laboratory accidents*

Physical safety

Physical injuries can result from many accidents, like falling on a wet floor, cutting oneself with broken glass, getting long hair caught in an equipment, improper lifting of heavy objects and so on. A sensible approach to avoid such injuries is to prevent them from happening. Therefore, always keep the floor dry, use glassware with care, and use towels while inserting glass tubing into stoppers; long hair should be tied back; keep the back straight and bend knees while lifting heavy objects.

Clutter can lead to slips/trips/falls and other possible injuries. In addition to being a slip hazard, continually wet surfaces promote the growth of mould, fungi, and bacteria that can cause infections. Following are some of the recommended precautionary measures:

- In case of wet processes, maintain proper drainage and provide false floors, platforms, mats, or other dry standing places wherever practicable, or provide appropriate waterproof footgear.
- Provide floor plugs for equipment so that power cords need not run across pathways.
- Ensure that spills are reported and cleaned up immediately.
- Eliminate cluttered or obstructed work areas.
- Use prudent housekeeping procedures such as using caution signs, cleaning only one side of a passageway at a time, and provide good lighting for all halls and stairwells to help reduce accidents, especially during the night hours.
- Instruct workers to use the handrail on stairs, avoid undue speed, and maintain an unobstructed view of the stairs ahead of them even if that means requesting help to manage a bulky load.
- Eliminate uneven floor surfaces.
- Promote safe work practices, even in cramped work spaces.
- Avoid awkward positions, and use equipment that makes lifting easier.

Phlebotomists are more likely than any other hospital worker to be injured by ***needle stick***. Blood collection devices such as butterfly needles and needles with caps minimize the risk of needle stick injury and should be used in preference to exposed needles. These devices, however, might not be common in the developing countries.

As a general rule, used, *exposed* needles are never to be broken or recapped. Clipping or breaking of needles can spatter blood. Unless there are special devices available, the simplest option is to drop the entire collection device into a waste container that is reserved for sharps. Containers for sharps should never be more than two-thirds full to avoid the temptation to force one more needle into the container.

Electric safety

Electrical hazards are among the most difficult to detect in clinical laboratories. WHO *(Laboratory Biosafety Manual*, 2003, WHO, Geneva, Switzerland) has listed the following as the most common causes of electrical accidents, which the laboratory staff should be made aware of:

- Wet or moist surfaces near electrical equipment
- Long flexible electrical connecting cables
- Poor insulation on cables
- Overloading of circuits by use of adapters
- Sparking equipment near flammable substances and vapours
- Electrical equipment left switched on and unattended

Shock hazards mostly originate from improper functioning equipment. It can be especially dangerous in countries where equipment operates at 220 V, and an electric shock can be fatal. The laboratory electrical wiring should be regularly inspected. The laboratory should be surveyed at least once a year as part of an electrical safety inspection. All new instruments should be thoroughly inspected before being placed into service. A qualified electrician should check instruments for current leakage with the ground open. Adhere to manufacturer's specifications to ascertain allowable current for the test. Do not place the electrical equipment in areas wherein ignitable vapours might accumulate. Keep the flammable liquid in a special storage vault away from any electric cables or devices.

Use rubber sheaths, waterproof cords and connecting wires for all equipment. Extension cords should not be used except as a temporary measure. Replace them with a permanent connection as soon as possible. Be certain that the equipment is properly grounded to the plumbing or to any special wall outlets. All connecting wires must be matched with the apparatus or heating unit in use. Do not attach a two- or three-way outlet to a single outlet

because this might overload the wire and result in fire. Replace any damaged wires, plugs and broken equipment as quickly as possible. On inspection, immediately correct for loose screws, improper connections, splicing, frayed cords, corroded or dirty switch contacts and similar imperfections. Always use reliable insulators, like high quality electrical tape, for covering any open wire.

Under no circumstances should hands be placed inside an electrical instrument when the current is on. Keep the electrical cord as short as possible and out of areas where it might come in contact with other fluid. All laboratory personnel should be aware of the location of fuse boxes or circuit breakers for different outlets and, if possible, must know how to replace the fuse. If the electrical equipment fails to function properly, particularly when smoke is visible or sparks are seen, immediately disconnect the apparatus and notify the maintenance department or the dealer. In case of electrical fire, use only a carbon dioxide fire extinguisher. Make sure that adequate fire extinguishers are available at clearly designated locations throughout the laboratory, and make sure that personnel understand how to use the extinguisher. *Never throw water on an electrical fire* as this could raise more sparks.

The safety training programme should anticipate electrical accidents. All personnel should know that the first response is to disconnect power to the sparking or burning equipment if it can be done without personal risk. This is especially important for individuals working in the vicinity of high-voltage devices such as those used for electrophoresis.

Chemical hazards

The smaller the quantity of hazardous materials a laboratory has in hand, the better it is. Hazardous laboratory chemicals can be grouped as ignitable, corrosive, unstable or toxic, but all chemicals in the laboratory must be considered dangerous. The characteristics of many chemicals belong to more than one class. Containment strategies are based on the nature of hazards involved. Safe storage, work practices, and disposal of chemicals can protect employees and the environment from hazards of chemicals that they are using. Exposure to fumes must be kept within permissible limits. There must be special control measures to minimize exposure, monitoring programs for the routine exposure of the technician, and provision for medical attention when dangerous exposures have occurred.

Accidental ingestion and inhalation of toxic fumes may occur if protective measures are ignored. Highly flammable chemicals such as ether, which has a flash point of –45°C, must be stored in safety vault or metal containers. Do not store flammable chemicals (ether, acetone, alcohol, xylol, etc.) in refrigerators as this may lead to explosion if there is an electrical spark. Small quantities should be placed in clearly labelled bottles for daily use. However, it is a good practice to return flammable chemicals to the storeroom or cold room after the day's work. While using these inflammable chemicals in the laboratory, utilize the vented safety cabinet or hood for work. Do not use rubber stoppers for volatile organic solvents as such solvents may corrode rubber; use cork-lined or wax-lined screw caps. For the disposal of small quantities of ether, soak paper towels or rags in it and place them in a hood with airflow to evaporate ether. Under no circumstances should ether be poured down the sink. In case of accidental spills of inflammable chemicals, Bunsen burners and electrical devices should be turned off until the odour disperses. Inhalation of toxic fumes or volatile organic solvents can cause breathing difficulties or asphyxia. This often happens in laboratories with improper ventilation. Anyone who suffers from an episode of asphyxia should be assisted by loosening any constriction on their airway, such as a necktie, and try to provide him/her with fresh air by fanning and removing the offending volatile substance.

Strong acids (pH lower than 2) and alkalis (pH higher than 12) are corrosive compounds. Always store them in safe cabinets or containers as close to the floor as possible with warning sign on the bottle. A catchments basin on the floor near the point of use and the regular use of

protective bottle carriers minimize the contact with a spilled chemical. Personnel must wear proper equipment while handling corrosives—this includes apron, corrosion-resistant gloves and laboratory-grade eye protection. Vapours of corrosives are often highly irritating, and hence pouring and pipetting of concentrated corrosive liquids within a chemical fume hood are highly recommended. The area of the laboratory in which corrosives are used must have an adequate source of water, such as an emergency shower and eyewash station for rapid decontamination.

Some important safety regulations in handling chemicals include the following:

- Manufacturer's instructions for unpacking and storing of chemicals should always be followed.
- Use laboratory carts for moving large containers; move them slowly and carefully. Do not carry too many small containers at once by hand; instead use plastic buckets.
- When unpacking or unloading the chemical from its container, do not touch the chemical. Use a spatula or spoon.
- While handling corrosive chemicals (such as strong acids and alkalis), wear aprons and rubber gloves. Wear insulated gloves while handling dry ice.
- Never draw liquid chemicals by mouth pipette; use automated devices only (Figure 3.4).
- Follow recommended procedures for the disposal of chemicals. Most chemicals, except solutions with cyanide, are discarded during and after use into the sink followed by sufficient amount of running water. Trapped acid in the sink might produce dangerous toxic gases such as hydrogen cyanide. Discarding with continuous running water may be safer.

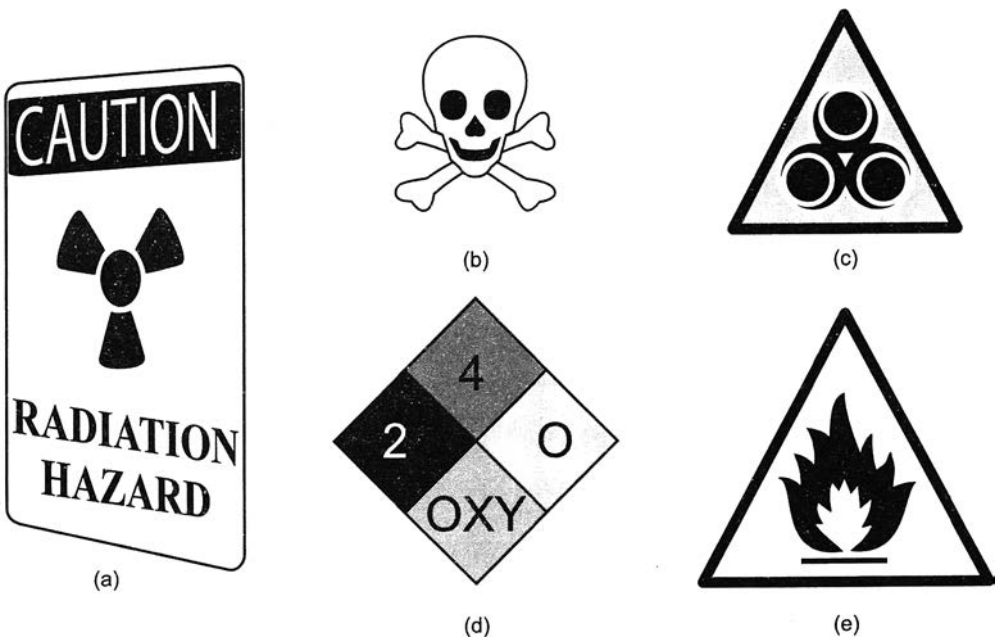

FIGURE 3.3 *Common signs of emergency: (a) Radiation hazard, (b) Poison, (c) Biohazard, (d) Hazardous chemicals, (e) Flammable*

Control of Hazardous Energy

Workers performing service or maintenance on equipment may be exposed to injuries from unexpected energization, start-up of the equipment, or release of stored energy in the equipment. Safety instructions require the adoption and implementation of practices

and procedures to shut down equipment, isolate it from its energy source(s), and prevent the release of potentially hazardous energy while maintenance and servicing activities are being performed. The primary purpose is to prevent hazardous exposure to personnel and possible equipment damage. The procedures apply to the shutdown of all potential energy sources associated with the equipment. These could include pressure, flow of fluids and gases, electrical power, and radiation. This standard covers the servicing and maintenance of machines and equipment in which "unexpected" energization or start-up of the machines/equipment, or release of stored energy could cause injury to workers. Employers must adopt such procedural solutions for controlling hazards to ensure worker safety during maintenance. However, such procedures are effective only if strictly enforced. Employers must, therefore, be committed to strict implementation of such procedures.

Chemical spills

Most manufacturers of laboratory chemicals issue charts describing methods for dealing with spillages. The spillage chart is also available commercially. Display these charts in a prominent position in the laboratory.

The following equipment should also be provided to deal with spillage.

- Protective clothing, e.g., heavy-duty rubber gloves, overshoes or rubber boots, respirators
- Scoops and dustpans
- Forceps for picking up broken glass
- Mops, cloths and paper towels
- Buckets
- Soda ash (sodium carbonate) or sodium bicarbonate for neutralizing acids
- Sand for absorbing spilled alkali solvents
- Non-flammable detergent

Note Neutralize acids and corrosive chemicals with soda ash (sodium carbonate) or sodium bicarbonate. Cover alkali spills with dry sand before removing.

Biohazard and Safety Precaution

All clinical specimens are potential sources of infection. Human blood and body fluids may carry viruses such as hepatitis B virus (HBV) and human immunodeficiency virus (HIV) or acquired immunodeficiency syndrome (AIDS). Control of these hazards is a concern throughout the healthcare industry. Workers in microbiology laboratories face additional risks of pathogens that are isolated and cultured. Any careless handling might lead to serious laboratory-acquired infection. Therefore, infection control policy is a vital part of everyday laboratory routine and is designed to protect the laboratory employees, hospital and community environment. Infection control can be grouped under four distinct categories:

1. Specimen collection at the collection site
2. Specimen transport to the laboratory
3. Specimen processing in the laboratory
4. Clean-up and disposal of specimens

During *specimen collection,* prevent self-injury. Follow basic laboratory rales for personal protection such as use of gloves, wearing an apron, washing of hands before and after procedures, and so on. Clearly label containers with the name of the patient, date of collection, source of the specimen and time of collection. The outside of the container should be wiped clean with alcohol wipes.

For *transportation*, put the specimen, whenever possible, in a sealed plastic bag. Leaky containers arriving from the hospital ward should be carefully handled. Some precious specimens, such as spinal fluid, require special care. Wear disposable gloves while handling the specimen.

Working inside a BSC during the specimen transfer is recommended. Use facemask and goggles during the transfer process. If a leaky or spilled specimen arrives in the microbiology laboratory, place the container on several layers of waste paper or newspaper and thoroughly disinfect the outer surface. After processing the specimen for laboratory testing, clean the work area thoroughly, wash hands with phenolic soap and autoclave the waste paper.

Specimen processing in a microbiology laboratory requires skill and care. The laboratory should display the sign "**Biological Hazard Area**" close to the designated area where biospecimens are handled. Microbiology laboratory principally handles pathogenic microbes. Mouth pipetting is prohibited. Technicians must wear disposable gloves while working in the microbiology laboratory. Aerosolization during flame sterilization of loops and needles is a

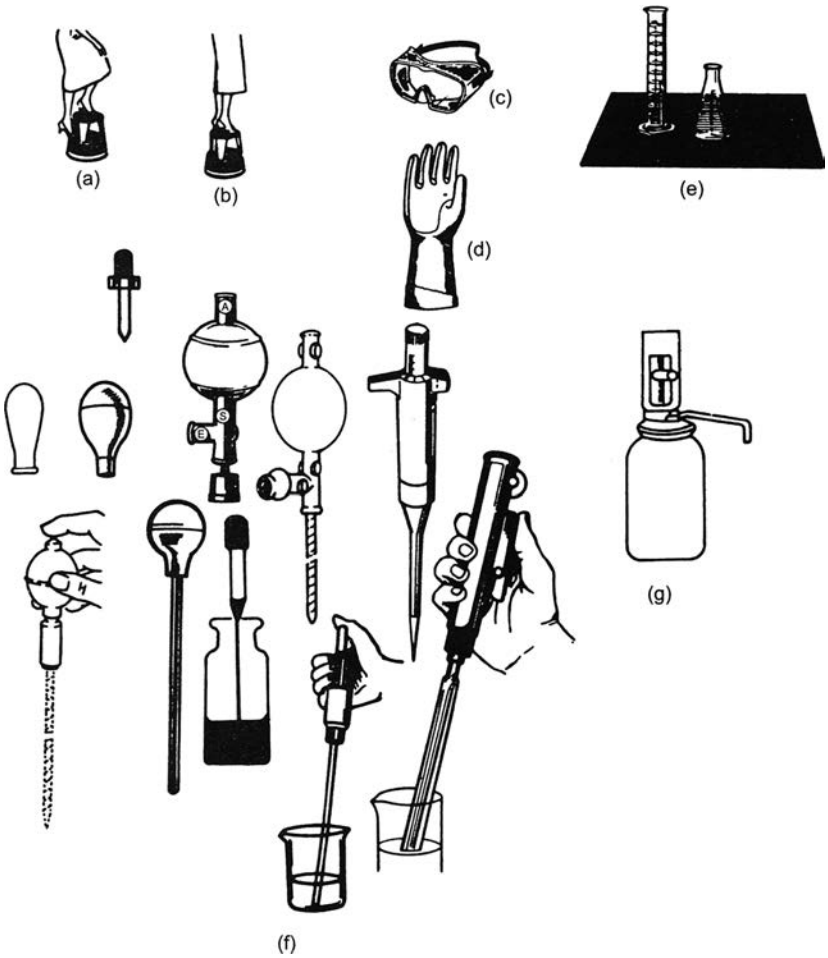

FIGURE 3.4 *Commonly recommended safety measures: (a, b) Step stool to reach objects at a higher level, (c) Goggles for eye protection, (d) Rubber gloves to avoid contact with poisonous chemicals, (e) Rubber mat to protect glassware from breakage, (f) Various types of rubber bulbs and pneumatic pipetting devices to avoid mouth pipetting*

source of biological hazard. If the needle or loop is moist, dry it from above the flame and then insert it into the hot part of the flame for "red hot" sterilization. Inadvertent motions may also cause aerosol formation, so move any contaminated needles or loops directly from place to place. Containment is possible with careful technique and by performing all

operations that predictably generate aerosols (such as centrifugation and sonication) within a BSC. If available, use a micro-incinerator to prevent aerosol dispersal of infectious materials. Gas and electrically heated micro-incinerators have borosilicate glass or ceramic shields that minimize the spatter and dispersal of infected materials when transfer loops are sterilized. Disposable transfer loops are not yet common in laboratories of developing countries.

Disposing of specimens requires decontamination first. ***Decontamination*** is a process by which infectivity of a substance or material is reduced to a safe (non-infectious) level. ***Sterilization*** destroys all organisms; however, disinfectant is effective against selected micro-organisms. **Disinfectants** might not be effective against bacterial spores or mycobacterium causing tuberculosis. The effective action of all disinfectants requires adequate contact time; therefore, the manufacturer's instructions should always be followed. Sanitizers and antiseptic procedures are not relied on as laboratory disinfectants. The most commonly used disinfectant is 5% phenol. A freshly diluted (1:10) household bleach solution, which is 5.25% aqueous sodium hypochlorite, can serve as a disinfectant. Glutaraldehyde can be used for decontaminating surfaces. One must, however, remember that all these disinfectants are toxic and should be handled with care. These should only be used in areas of adequate ventilation or with the aid of a chemical fume mask.

Decontamination of laboratory instruments and equipment that come into contact with specimens should be a regular event. Blood smears and tissue samples are first removed with disinfectant followed by cleaning with detergent and water. This is more effective than using only disinfectant. Most laboratories in developing countries do not use disposable laboratory wares. Glassware should be disinfected first and then autoclaved and dried before they are put back into drawers. Pipettes should be dried in high-temperature oven (100°C). Use insulated gloves for handling hot containers.

Alcohols, iodine, and iodophors are commonly used as skin ***antiseptics.*** Alcohol is more effective when it is used in diluted form with water (70% aqueous solution, v/v) or with formaldehyde (70% alcohol mixed with formaldehyde solution, v/v). Keep in mind that alcohol is volatile and inflammable. Iodine and iodophors are more effective but can stain fabrics and environmental surfaces and therefore are often unsuitable for laboratory use. Povidone-iodine is a reliable and safe surgical scrub and preoperative skin antiseptic. However, iodine should not be used on aluminium or copper.

In short, proper use of germicides and autoclaves will contribute to workplace safety while reducing the risk from infectious agents. The number of germicidal chemicals to be used should be limited, not only for economic reasons and better inventory control but also to avoid the loading of environment with potentially harmful chemicals.

Immunization of laboratory workers is recommended due to the risk of transmission from biospecimens. Vaccines or treatment for immunoprophylaxis are available for workers who are regularly exposed to specific agents of virulence such as Hepatitis and AIDS.

Waste Disposal

Disposal of specimens should obey government regulations. Discard all cotton swabs and small containers with unused specimens in a 5% phenol solution or bleach. Most unused specimens, after treating with 5% phenol for two hours, can be discarded in the drain as long as the drain is connected to a sanitary sewer system. The containers can be recovered for reuse after autoclaving for 30 min at 121°C. Never throw any specimen in the sink. They can, however, be disposed off in a sewer and flushed thoroughly. This is the standard procedure for discarding urine, stool, blood tissue filtrates and various body fluids. Some laboratories make a special waste hopper for this purpose. Highly infective specimens can be covered with water and autoclaved before discarding. This also applies to infectious liquid media. Solid wastes that can be burned are incinerated or covered with water and sterilized. Sputum specimens from

suspected tuberculosis cases must be autoclaved for 40 min along with the container before discarding materials in the drain. Fixing and staining of blood, sputum and faecal samples for microscopy does not necessarily kill all organisms or viruses on the smears. These items should be handled with forceps, stored appropriately and decontaminated and/or autoclaved before disposal. *Never dispose off biohazard wastes with regular trash*. Adequate trash containers must be available for biohazardous waste. The plastic bag used for collecting biohazardous waste should have a different colour so that it does not get mixed up with regular trash. Special personnel should perform incineration at an isolated area.

Radiation Hazard

Ionizing radiations can be one of the most toxic sources of exposure to personnel in the laboratory. Extended exposure can cause irreversible damage to the body. Sources of radiation hazards in a laboratory include both medical devices and reagent materials. The radioactive substances found in laboratories are generally limited to tracer chemicals that are of insignificant hazard unless ingested or absorbed. The same good laboratory techniques that effectively contain infectious hazards will limit personal exposure to soluble radioactive substances. Pregnant women must not work with radioactive substances. Technicians are often careless because radioactive rays are undetectable by human senses. Technicians must always wear radiation dosimeters while handling radioactive chemicals and these should be screened at regular intervals by laboratory management to limit the exposure to radiation. The technician should be replaced temporarily in case of exposure beyond safe limits. Work areas must be checked regularly for contaminating radiation.

Gloves should be worn when handling radioactive materials and hands should be washed after removing gloves. Any manipulation that might lead to hand-to-mouth or hand-to-mucous membrane transfer must be prohibited while handling the radioactive substances. Workbenches should always be covered with absorbent paper towels, which should be changed at the end of each shift (or after overt contamination) and properly discarded.

During *spills and clean-ups* of radioactive materials, personnel engaged in decontamination must wear gowns or coats, aprons, gloves and eye protection. Disposable items are strongly preferred over clothing that requires laundering. The contaminated area should be scrubbed with water and detergent (preferably using a cleaning compound designed for radiation decontamination). Check the level of radioactivity in contaminated areas. All disposable materials involved in the clean-up must be discarded as radioactive waste unless survey measurements have indicated the amount of radiation remaining in the materials to be less than twice the background. Document all the incidents of spill and decontamination.

Warning signs that indicate the presence of radioactive materials should be posted in all areas where radioactive chemicals are stored, used, or discarded (Figure 3.3). In general, radioactive waste stored for some period returns to non-radioactive harmless ground state, which can then be discarded as any other waste when the radioactivity is low. The radioactivity must be carefully checked with a meter before discarding.

Fire Hazard and Explosion

Large laboratory fires are rare. Small bench-top fires in laboratory spaces are not uncommon. However, the risk of severe injury or death is significant because fuel load and hazard levels in labs are typically very high. Laboratories, especially those using solvents in any quantity, have the potential for flash fires, explosion, rapid spread of fire, and high toxicity of products of combustion (heat, smoke, and flame).

Causes of fire

Common **causes of fire** in laboratories are:

- Electrical overloading
- Poor electrical maintenance
- Excessive long gas tubing and electricity leads
- Equipment left switched on unnecessarily
- Unattended open flames
- Deteriorated gas tubing
- Misuse of matches
- Carelessness with flammable materials
- Flammable and explosive chemicals stored in ordinary refrigerators

Preventive measures

The laboratory must keep constant **vigilance to prevent fire.** There are numerous flammable objects inside a laboratory (e.g., paper, cotton, cloth, inflammable chemicals). Hazardous areas with flammable materials must be marked with appropriate signs (Figure 3.3). In dealing with laboratory fire, all containers of infectious materials should be placed into autoclaves, incubators, refrigerators, or freezers for containment.

The following **preventive measures** against fire are recommended:

- ***Plan work:*** Have a written emergency plan for your space and/or operation. All laboratory personnel must know the location of the fire extinguisher and fire escaping routes.
- ***Minimize materials:*** Use only the minimum quantities of chemicals/reagents necessary for work in progress. Not only does this minimize fire risk, it reduces costs and waste as well.
- ***Observe proper housekeeping:*** Keep work areas uncluttered and clean them frequently. Put unneeded materials back in storage promptly. Keep aisles, doors, and access to emergency equipment unobstructed at all times. Put **NO SMOKING** signs in areas where flammable compounds are handled.
- Do not throw burning matches into a wastebasket; throw them into a non-flammable metal container.
- Observe restrictions on equipment (i.e., keeping solvents only in an explosion-proof refrigerator).
- Keep barriers in place (shields, hood doors, lab doors, etc.).
- Wear proper clothing and personal protective equipment.
- Avoid working alone.
- Store solvents properly in approved flammable liquid storage cabinets.
- Shut doors behind you when evacuating.
- Limit **open flames** use under fume hoods only and attend them constantly. Keep combustibles away from open flames.
- Do not heat solvents using hot plates.
- Leakage of gas or propane from the gas cylinder may cause an explosion. After installing a new gas cylinder, always check joints for leakage.
- Turn off the flame before leaving the laboratory any time. Never keep an open flame near flammable liquids. If the flammable liquid is on fire inside the container, quickly cover the container with non-flammable materials such as glass or a metal plate.
- Remember the **RACE** rale in case of a fire.
  R–Rescue/remove all occupants,
  A–Activate the alarm system
  C–Confine the fire by closing doors
  E–Evacuate/Extinguish

Emergency procedures

Employers should ensure that workers are trained in the following **emergency procedures:**

- ***Know what to do:*** You tend to do under stress what you have practiced or pre-planned. Therefore, planning, practice and drills are essential. Employers must train and exercise the emergency plan.
- ***Know where things are:*** Workers must be aware of the nearest fire extinguisher, fire alarm box, exit(s), telephone, emergency shower/eyewash, and first-aid kit, etc.
- Check the fire alarm and practice fire exit drills at regular intervals.
- Be aware that emergencies are rarely "clean" and will often involve more than one type of problem. For example, an explosion may generate medical, fire, and contamination emergencies simultaneously.
- Learn to use the emergency equipment provided.

Fire extinguisher

The two most common types of **fire extinguishers** in a chemical laboratory are pressurized dry chemical (Type BC or ABC) and carbon dioxide type. In addition, you may also have a specialized Class D dry powder extinguisher for use on flammable metal fires. Water-filled extinguishers are not acceptable for laboratory use. Employers should train workers to remember the "PASS" rale for fire extinguishers. PASS summarizes the operation of a fire extinguisher as follows:

P–Pull the pin
A–Aim extinguisher nozzle at the base of the fire
S–Squeeze the trigger while holding the extinguisher upright
S–Sweep the extinguisher from side to side; cover the fire with the spray

Check the **fire extinguisher** at least once a year. Recharge it immediately, if necessary. Keep the portable fire extinguisher within reach. Keep a bucket of sand with a scoop and a fire blanket readily available. In catse of fire accident in the laboratory, close all doors and windows and prevent draft. For a small blaze, use sand and a fire blanket to put out the fire. A fire extinguisher is used for a larger blaze. Do not use water on an electric fire or a fire caused by grease, oil or gasoline. Turn off the electric current and use only a carbon dioxide fire extinguisher which does not conduct electric current. Water or sod-type extinguishers may conduct electricity and should not be used for electrical fires. Sand is used to smother the fire on grease or oil. While escaping, it is safest to crawl and stay close to the floor. Cover mouth and nose with a damp cloth if possible.

Clothing fire

Employers should train workers on appropriate procedures in the event of a clothing fire.

- If the floor is not on fire, **STOP, DROP** and **ROLL,** to extinguish the flames or use a fire blanket or a safety shower if not contraindicated (i.e., there are no chemicals or electricity involved).
- If a co-worker's clothing catches fire and he/she runs down the hallway in panic, tackle him/her and smother the flames as quickly as possible, using appropriate means that are available (e.g., fire blanket, fire extinguisher).

Specialized Equipment

Hazards might accompany any new item of laboratory equipment. Items that have moving parts, thermal elements, pressurized components, and others, may be of particular concern. Most manufacturers provide recommendations for workers' protection.

Centrifuge is a common source of mechanical hazard. Hair or clothing can become entangled in the mechanism if the centrifuge is allowed to operate with its cover open. Unbalanced cups and broken glass can be especially dangerous during its working. Spinning rotors must never be slowed down or stopped by manual means. Always close the centrifuge while handling infectious materials. Breakage of centrifuge tube within the centrifuge head is a source of biohazard. Whenever possible use plastic tubes. However, this might not always be possible; for example, faecal materials are processed with the use of ether, which attacks plastic. While using glass centrifuge tubes, if there is a breakage during spinning, remove the fluid and the broken glassware. Wear rubber gloves (not disposable plastic gloves) during the cleaning process. Apply several layers of absorbent paper such as newspaper to soak the liquid and then clean with a disinfectant (5% phenol). In the event of a spill, remove cups holding the centrifuge tube and soak them in the disinfectant solution for two hours before reusing.

Heat is the most common among several physical agents used for decontamination of pathogens. "Dry" heat, which is totally non-corrosive, is used to process many items of laboratory ware that can withstand temperatures of 160°C or higher for 2–4 h. Burning or incineration is also a form of dry heat. "Moist" heat is most effective in the form of autoclaving. Boiling does not necessarily kill all micro-organisms and/or pathogens; however, it can be used as the minimum means of disinfection where other methods (chemical disinfection or decontamination, autoclaving) are not applicable or available.

Autoclaves provide steam sterilization. Saturated steam under pressure (autoclaving) is the most effective and reliable means of sterilizing laboratory materials. In most purposes, the autoclave is run for 15 min at 121°C. Most commonly used autoclave is the gravity displacement autoclave, which is described in Chapter 4. Here the steam enters under pressure and displaces the heavier air downwards through the valve. This machine is fitted with HEPA filter. Always loosely pack materials inside the chamber for easy steam penetration and air removal. Bags should allow the steam to reach their contents. Only trained and knowledgeable individuals should operate the autoclaves. Autoclaving presents risks of scalding and explosion due to high pressure and temperature inside the autoclave. The installation and fitting should be examined periodically. Always wear heat-resistant gloves, aprons and face protection before unloading the autoclave chamber.

Use of Biological Safety Cabinets (BSCs) is mandatory for **microbiology laboratories**. The cabinet must not be used unless it is working. The simplest design of the cabinet is shown in Figure 3.1. Some of the precautionary measures to be taken while using BSCs are given below:

- The glass-viewing panel must not be opened when the cabinet is in use.
- Apparatus and materials in the cabinet must be kept to a minimum quantity. Air circulation at the rear plenum must not be blocked. Materials should be surface-decontaminated before placing them inside the working area of the cabinet.
- Bunsen burners must not be used in the cabinet. The heat produced will distort the airflow and might damage the filters. An electric micro-incinerator is permissible; however, sterile disposal transfer loops are better.
- All work must be carried out in the middle or rear part of the working surface and be visible through the viewing panel.
- Traffic behind the operator should be minimized.
- The operator should not disturb the airflow by repeated removal and reintroduction of his/her arms.
- The surface of the BSC should be wiped using an appropriate disinfectant after completion of work and at the end of the day.
- The cabinet fan should be run for at least 5 min before beginning of work and after completion of work in the cabinet.

Decontamination of BSC is done with paraformaldehyde gas. To decontaminate, the disinfectant is placed inside the cabinet on an electric hot plate or fry pan on low heat. A second hot plate or fry pan with 10% (w/v) of aqueous ammonium carbonate solution is placed on the side with similar settings to be turned on later. The hot plate or fry pan with ammonium carbonate should have a cover over it which can be removed from outside (e.g., it could be attached to a strong cord that can be pulled from outside the cabinet). This will minimize premature neutralization of formaldehyde gas. If the relative humidity is below 70%, an open container of hot water should also be placed inside the cabinet before the front closure is sealed in place with strong tape (e.g., using a duct tape). If there is no front closure, heavy gauge plastic sheeting is taped over the front to make sure that the gas cannot seep into the room. The switch for the paraformaldehyde pan is turned on, and then one hour later or when the paraformaldehyde has completely vaporized, it is turned off. The cabinet is left undisturbed overnight. The second pan is turned on after the cover is removed and ammonium bicarbonate is allowed to vaporize. Meanwhile, the pan is turned off and the cabinet is turned on; ammonium bicarbonate gas is allowed to circulate for one hour. The front closure or plastic sheeting and the cabinet used can then be removed.

There are several other appliances in a laboratory which may create hazardous conditions. However, no piece of equipment should be placed into service until the special hazards have been defined, appropriate engineering controls are installed, requisite personal protective equipment are provided and personnel been appropriately trained.

Compressed Gases

Compressed gases used in a laboratory and elsewhere create a special hazardous condition because of the potential of rupture of the container. Many are also toxic or ignitable. The breakage of the regulating valve of a compressed gas cylinder can propel the cylinder like a missile. A heated cylinder will explode when its ability to contain the growing internal pressure is exceeded.

All but especially the smaller cylinders should be secured upright. Each cylinder should be clearly labelled. Additional temporary labels that read “in use” or “empty” should be applied as appropriate for clear communication. Cylinders should be secured to handcarts before being moved from place to place. They should never be dragged or rolled.

The gas cylinders and piped systems have pressure-regulating devices. These valve systems are designed separately for each family of gases and must not be interchanged. Valve safety covers should not be removed until pressure regulators are attached. Threaded fittings must be tightened carefully with properly sized wrench. Cylinders and connections should always be tested with a soap solution for leaks after attaching, adjusting, or disconnecting the system. Valves should never be lubricated.

The typical valve system has two components: high-pressure valve at or near the tank to release the contents, and a low-pressure valve at the point of use to regulate flow. Valves should be opened slowly and cautiously and personnel should stand to the side of the gauge as a precaution against blowout. Valves should be maintained in a closed position when the cylinder is not in use.

Flammable gases must be used with special caution. Supplies should be minimal. Only cylinders in active use should remain in the work area. The storage area should be a secured room or enclosure reserved exclusively for that purpose. The location should be away from combustible material, elevators, stairs and passageways. Oxidizing gases must be separated from flammable gases. Sources of ignition should be carefully insulated from the emplacement of cylinders. Empty cylinders awaiting return to the vendor must be treated carefully because considerable pressure may remain within.

Laboratory Hygiene and Housekeeping

Cleanliness and good housekeeping are attributes of the best laboratories. Proper storage of laboratory implements when not in use keeps work areas clear to facilitate disinfection, decontamination, and hazard containment. Cluttered work areas or halls are a nuisance and can lead to falls or other injuries. Accumulation of chairs, equipment and boxes that block exits or otherwise interfere with emergency egress must be forbidden. Escape routes in case of fire should remain completely unobstructed. Emergency equipment (such as portable fire extinguishers and eye wash fixtures) and first aid boxes should be easily approachable and ensured at all times. Floors, walls, cabinetry, freezers, and refrigerators should be cleaned on a regular schedule. Incubators and water baths should be cleaned after the day's work. In case of spills, clean them immediately, especially for infectious materials. In general, all clean-ups of spills should follow decontamination of the area with a disinfectant.

Hand-washing is one of the most effective means to minimize personal exposure. Thorough washing with good detergent removes external bacteria and chemicals before they can be ingested or absorbed. Hands should be washed frequently throughout the day. Washing is especially important before eating, leaving the laboratory, applying cosmetics, and using lavatory facilities. In most situations, thorough washing of hands with ordinary soap and water is sufficient to decontaminate them; however, the use of germicidal soaps is recommended in high-risk situations. Hands should be thoroughly lathered with soap up to the wrist, using friction, for at least 10 s, rinsed in clean water and dried using a clean paper towel. Cloth towels must be regularly laundered if they are used. If available, warm-air hand dryers are recommended. A well-designed hand-washing sink is operable with elbow blades/foot pedals to control water flow without using contaminated hands. When not available, a towel should be used to turn off faucet handles to avoid decontaminating washed hands. Paper towels can be disposed off with general refuse. Alcohol-based hand-rubs should be used to decontaminate lightly soiled hands when proper hand-washing is not available or convenient.

Eating, drinking and manipulating items that might contact mucus membranes (e.g., contact lenses, cosmetics and lip balm) within technical work areas should be prohibited. Food should never be stored in refrigerators used for storage of reagents and specimens. Dining areas and refrigerators are to be reserved exclusively for food items.

Personal Safety of Laboratory Workers

Proper attire is a must for all laboratory workers. It minimizes exposure to infectious and chemical agents. Use of laboratory coat is a common practice that need not be taken off while working within the facilities. Clean attire gives a professional look to the laboratory personnel and reduces patient's anxiety. At the same time, it also protects laboratory workers from infectious hazards. Wearing a long-sleeved, buttoned laboratory coat and disposable gloves is an ideal habit for personal safety. Wearing fluid-resistant apron, eyeglasses or masks are additional protections under special circumstances.

Laboratory workers must use *rubber-soled shoes* for effective traction and decrease the shock hazard precipitated by an electrical accident. Shoe covers made from appropriate resistant materials are essential if splashing is a risk. It is a good practice to store uncontaminated personal belongings separately and not to carry infectious materials home after work. When in doubt, wash clothes immediately. Single-use gear (plastic disposable wares) should be discarded in proper marked containers. Reusable laboratory coats and gowns should be placed in leak-resistant bags to be laundered in a manner that ensures decontamination. To contain hazards as much as possible, the laundering of contaminated clothing at home should be prohibited. The laboratory should have access to facilities that offer specialized washing of contaminated clothing.

Hazard containment applies to hairstyle and personal adornments. *Hair* should be worn behind the head and off the shoulders to preclude its contact with contaminated surfaces. Disposable hair covers may be indicated for some activities. Hair, beard and jewellery should not be allowed to overhang into contaminated areas or risk entanglement in moving equipment.

Use of *gloves* is the most commonly used personal protective device against noxious substances and dangerous materials. Gloves should fit as comfortable as possible. Glove length must be sufficient to extend over the cuffs of the sleeves to protect wrists. Heavy rubber gloves are designed for chemical protection. Choose a material known to resist specific chemicals to which the worker will be exposed. Thin latex or vinyl gloves provide short-term protection against dusts and contaminated aqueous solutions; minute and insensible defects can develop easily in such gloves, and viruses can penetrate even intact gloves. Such gloves must be changed and discarded regularly to be effective. Thin gloves are designed to be single-use devices and must be replaced immediately when torn or contaminated. Some people, including laboratory workers and patients, might be allergic to latex, and in such cases vinyl plastic may be the alternative. Insulated gloves are to be used while handling items at blistering temperatures, handling corrosive substances, or while working in frigid conditions.

Eye and face protection requires glasses made of high-impact plastic; those with imperforate side shields are standard. Simple safety glasses offer a minimal and usually inadequate level of protection. Goggles, which fit snugly around the face, are preferred and should be combined with masks that protect the mouth and nose during certain activities. A shatterproof facemask gives more complete protection. Under special circumstances, working inside a hood while manipulating from outside is safe. Keep the airflow on if there is a chance of splattering.

Individuals who wear *contact lenses* must take special precautions. Toxic, irritating, or infectious fluids into eyes present a special hazard for contact lens wearer as these substances may become lodged under the lens. Ask an eye doctor for details. Some laboratories prohibit the use of contact lens in the laboratory but goggles or face shields provide an acceptable level of protection. One must remember that people without contact lens or glasses are also at a risk for eye injury; the safest strategy is to require the wearing of full facial protection for all employees engaged in tasks that chance splashing or spraying.

Respiratory protection with the use of gas mask is done to prevent inhalation of fumes, dusts or aerosols that might be harmful. For the prevention of dust, dust masks made of cloth or paper devices are quite effective in preventing particulates to enter the body. Fume masks, however, cover half of the face and employ replaceable cartridges that contain absorbent chemicals. The choice between mask and respirator, and the type of respirator will depend on the type of hazard. *Respirators* are available with interchangeable filters for protection against gases, vapours, particulates and micro-organisms. Note that no filter other than a HEPA (High Efficiency Particulate Air) filter will provide protection against micro-organisms, and it is imperative that the filter be fitted in the correct type of respirator. To achieve optimal protection, respirators should be individually fitted to the operator's face and tested. Fully self-contained respirators with an integral air supply provide full protection. In order to remove fumes from the entire laboratory, fans of fume hoods are run at highest speed when fumes are sucked into the hood and thrown out from the roof.

Always use mechanical devices for *pipetting* tasks. The pipetting of fluids by mouth must be strictly prohibited throughout the laboratory. Even harmless fluids such as water should be pipetted mechanically for consistency. Some of the commonly used devices for measuring the volume of fluids are given in Figure 3.4.

Work Habit

Work habit is crucial in avoiding accidents. The efficiency of a laboratory worker can be judged by the way the technician keeps his/her working area (Figure 3.5). Always keep your work station organized and neat so as to minimize any risk of accident.

FIGURE 3.5 *The working habit of a bad technologist (a) is distinctly different from that of a good technologist (b)*

Warning Signs

Several systems of signs have been devised to warn individuals of hazards and potential injury. Figure 3.3 displays some common signs used in laboratories. Use of standardized symbols is more helpful.

The labels on reagent vessels should clearly delineate hazards of contents and their adverse effects. Commercially labelled reagent containers often display additional information such as advice on first aid and spill clean-up. In case secondary containers are used, they must be labelled with key safety information. Warning labels should never be removed or obscured unless a revised label is applied immediately. Unlabelled containers should remain unopened and discarded as an unknown chemical is presumed to be hazardous.

It is a good practice to indicate the date on the label when each container was opened. Such a practice is also a safety precaution when dealing with chemicals for which the hazard level increases upon exposure to air (e.g., diethyl ether). Many chemicals deteriorate upon exposure to air. The date of opening indicates how far the aging process might have proceeded.

Accident Record and Training

All laboratory accidents must be recorded in the laboratory record book. It must indicate the date, time, place, and cause of the accident. It should also note all individuals involved in the accident or its aftermath. Any accident must be assessed for preventive strategies, and changes should be implemented without delay. Laboratory safety policies must be well documented and available to all employees. The policy should include each area of the laboratory and its specific hazardous situations. Hence, the new employee, through safety documents, will be able to comprehend hazards associated with his/her job and the associated precautions. The documentation must be updated from time to time and must be reviewed after each accident. Periodic refresher briefings of existing safety policies for all employees are strongly recommended. This applies to chemical handling, infectious exposures, waste disposal, emergency preparedness and review of fire escape routes. All personnel must have the information and skills to control hazards in their environment. New employees must

understand the basic training programs on laboratory safety. It may be wise to include in the safety training for First Aid Supplies as well. While offering the training, the material should be presented in a manner consistent with the educational level, literacy, and language background of the technician to whom it is presented. Whenever any changes are made to the laboratory policies or procedures, all personnel should be updated on the changes and any changes to the safety protocol.

Most safety training will be in a lecture format, although hands-on training is preferable. The operation of portable fire extinguisher, use and storage of gas tanks and spill containment are best presented in safety workshops. Some of the most effective programs are those that encourage workers to suggest safer alternatives to current practice. Following any workshop, there should be a session of evaluation in order to assess the effectiveness of the training. This may include multiple-choice questions in written format or simple "fill in the blanks" format.

Finally, keep in mind that a successful safety programme is the result of combined efforts of everyone in the laboratory. Each individual in the laboratory should contribute to the total effort. The facility administration must make a statement of commitment to the goal of a safe workplace. Job descriptions should be concrete with regards to employees' responsibilities to comply with safety policies.

First Aid Kits and Procedures

It is always wise to be prepared for any possible accident in a laboratory. A fire extinguisher, sand bucket, first-aid kit are all parts of this preparedness. A first-aid kit is necessary for facing any personal injury and should be placed in a conspicuous location in the laboratory. It should be designated with a red cross on a white background or some other easily recognizable symbol. It should never be kept in a locked cupboard. All personal injuries must be reported and recorded for future reference.

The first-aid kit should have the following materials:

- Sterile cotton or cotton wool and gauze
- Medicinal adhesive tapes
- Roller bandages of various widths
- A pair of scissors
- Ammonia
- Safety pins
- Sodium carbonate (aqueous, 5% solution)
- Sodium carbonate (aqueous, 2% solution) in an eyedropper bottle
- Acetic acid (aqueous, 5% solution)
- Boric acid (saturated) in an eyedropper bottle
- Soap powder solution (5 g per litre of water) in a small bottle

Laboratory workers must be aware of the emergency and first-aid procedures to be given to an accident victim. Some of the emergency procedures and first aid, in case of an accident, are illustrated in Figure 3.6.

It is important in all emergencies that one remains cool, calm, and collected. First-aid training should be provided to all laboratory personnel so as to avoid the panic of "What to do?" Call for help or emergency transport immediately if the situation requires it. Deal with the most serious injury or condition first. The most urgent medical emergencies, which require prompt action to save life, are severe bleeding, asphyxia (difficulty in breathing) and poisoning. Shock might accompany any one of these conditions and is due to the failure of blood circulation. It may vary from a mild form resembling fainting, to a severe form that might be fatal. The signs of shock include the following: pale face, vacant look, skin cold and clammy

with rapid and shallow breathing. Wrap the victim in a blanket if he is feeling cold and provide warm drinks. Relieve his pain as much as possible. Try not to move the victim, but if it is essential, be especially careful to support or protect injured limbs and to handle them gently.

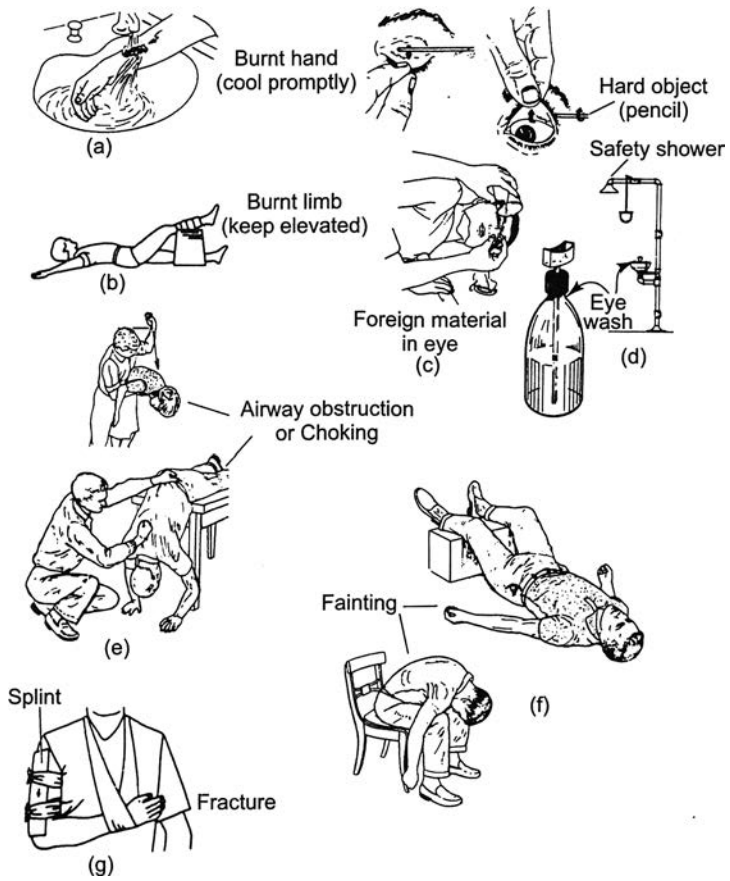

FIGURE 3.6 *Some emergency procedures and first aid in case of accidents: (a) Promptly cool down the burnt spot, (b) Keep the burnt limb raised, (c) A foreign object in eye can be removed by rolling the eyelid over a stick, remove the object with a soft cloth and later washing off the eye, (d) The laboratory should have eye-washing devices, (e) First aid for airway obstruction requires back thrust, (f) Fainting victims should be allowed to rest in a certain position where they will not be likely to fall and where their blood flow can return to their heads, (g) Splint should be applied in case of fracture*

Apply splint in case there is a breakage of bone before moving, but it is preferable to wait for trained emergency medical technicians (Figure 3.6).

Physical injury of the skin might result from corrosive chemicals or burns, splashing of chemicals into eyes, injury due to broken glass, or a foreign body lodged in the eye or ear (Figures 3.6 and 3.7). These all require immediate attention and may lead to serious complications if left unattended. They are, however, not considered as life threatening unless they are severe. In the following pages we will discuss some of the common accidents and the first aid to be administered.

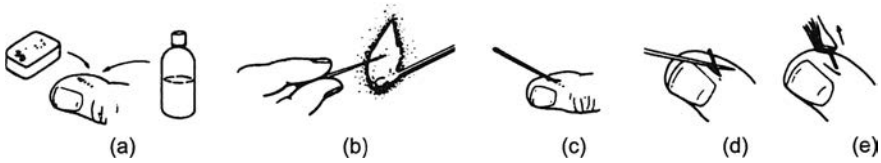

FIGURE 3.7 *Removing of a splinter: (a) Disinfect the area with the splinter, (b) Flame sterilize a sewing needle, (c) Insert the needle through the skin, (d) Insert until the splinter is lifted out, (e) Pull out the splinter with a pair of forceps*

Bleeding

Bleeding can be external, which is visible, or it might be internal. Try to stop external bleeding by applying pressure directly over the external bleeding spot. Place a thick pad of gauze or other clean cloth over the wound and bandage it snugly in place with strips of cloth or adhesive tape. Apply hand pressure, if necessary, over the compress (pad). If nothing is quickly available, use your hand to apply pressure over the wound until a pressure dressing can be obtained. If the bleeding is from an arm or leg, elevation of the limb will help to control bleeding (Figure 3.6). You may use a tourniquet (constriction of the blood vessel by tying), but use only as a last resort for severe bleeding as this may lead to complications if prolonged. Any fairly wide flat band, long enough to go twice around the limb can serve as a tourniquet. Never use cord, rope or wire. Apply the tourniquet between wound and heart, placed as close as possible to the wound.

Laboratory workers must be given training in tying a tourniquet, giving artificial respiration and dressing wounds. First aid stops with the checking of bleeding and application of sterile pads over the wound. The doctor must attend to severe bleeding and serious wounds. Small injuries (cuts, burns, and scratches) are, however, handled within the laboratory. While attending to the wound, one should remember to check every break in the skin, especially in the event of glass breakage, clean the wound and disinfect it before putting on the bandage. Iodine solution and mercurochrome (Merbromin) are widely used disinfectants and are commercially available. Adhesive bandages may be sufficient for covering small cuts and scratches. Use a sterile cloth pad or compress over a deep wound and apply pressure over the gauze pad. If bleeding continues, consult a doctor.

If a sharp object, which is contaminated, causes bleeding, allow the blood to flow out or squeeze hard to make it bleed for at least one minute. Then disinfect the wound, both inside and outside, with tincture of iodine (tincture of surgical antiseptic or mercurochrome can also be used). Wash the iodine off thoroughly with soap water if tolerable. Disinfect again with iodine and then apply a dressing. Inform the pathologist/ physician regarding the incident and the infectious agent handled by the technician so that an antitoxin can be administered.

Poisoning

Accidental swallowing of a poisonous material is also an emergency. Poisoning can also be caused by inhalation of toxic vapours or gases, e.g., chloroform. Prohibition of mouth pipetting and sufficient ventilation of the laboratory will reduce such accidents.

The signs and symptoms of poisoning vary with the poison taken. Nausea, vomiting, pain in the stomach, cramps, diarrhoea, collapse and convulsions are some of the possible immediate effects. Always remember that in case of poisoning, every moment delayed results in greater poison absorption. Hence, act promptly: call the local poison control centre for specific instructions regarding ridding poison from the body. Inform the physician about the toxic substance involved. If a toxic substance is inhaled, the victim may be placed in the open air

while waiting for the physician. If the poisoning is due to swallowing of a toxic substance, dilute the poison under all circumstances, whether the nature of the poison is known or not. Induce vomiting in order to wash out the poison from the stomach, but only if directed to do so by a medical professional. This is accomplished by giving a substance that causes vomiting (an emetic) or by manoeuvres such as pressing fingers at the back of the throat. Do not induce vomiting in case of poisoning due to strong acids or caustic alkalis. Give an appropriate antidote to neutralize the poison. Labelling of bottles with instructions followed in the event of poisoning should be undertaken if the laboratory anticipates that it will not be able to contact a poison control centre in time.

Poisoning with Strong Acids and Caustic Alkalis

Hydrochloric acid, sulphuric acid and nitric acid are some of the strong acids, while ammonia, caustic soda (sodium hydroxide) and potash lye (potassium hydroxide) are some of the strong alkalis that may leave the victim with stained or blistered skin indicating possible chemical burns. The accident is caused when the corrosive chemical comes in contact with the skin or is splashed into the eye. Immediately strip off his/her clothes, which have come in contact with the chemical and flood the skin with large quantities of clean water (Figure 3.6). If there has been any delay in giving first aid, do not use water; get medical aid at once. It is important to note that alkali burns are as serious as, often more serious than, acid burns.

Burns

Flames, hot liquids, splashing of inflammable solvents, explosions and hot plates cause the most common burns. In case of minor burns, only the surface of the skin is affected, whereas deeper tissues will be affected in case of an extensive burn. Reddened, unbroken skin or surface blisters characterize minor burns. Do not tear off blisters that form over burns. Immediately after a minor burn, plunge the affected parts into cold water. After this, apply sterile petrolatum, burn ointment, olive oil or mineral oil, and cover the burnt area loosely with a dry gauze dressing. If the wound is exposed, apply mercurochrome (Merbromin or tincture of iodine).

Severe burns require immediate medical aid. If the victim is on fire, roll him in a fire blanket to smother the flames. Lay the victim on the ground until emergency care is available. Do not remove his clothing. Tie a piece of gauze or any clean cloth over the mouth and nose to serve as a mask. If cloth is not available, keep the mouth closed. Contamination with germs from the mouth and nose is responsible for most infections due to serious burns. Cover the burnt area with a thick layer of sterile gauze and bandage it firmly in place. If the limb is burnt, keep it raised and keep the victim comfortably warm.

Eye Accidents

Eye injuries are perhaps the most common and most debilitating accident in the laboratory. Always wear goggles if dangerous corrosive chemicals (acids or alkalis) are handled. If the chemical enters the eye, immediately wash out the chemical with clean water for at least 15 min (Figure 3.6). There are two ways to wash—spray from a wash bottle or rubber bulb; squirt the water into the corner of the eye near the nose. Alternatively, hold the eye under the running tap and move head slightly from side to side. After thorough washing, put a few drops of 2% aqueous sodium bicarbonate into the eye in case of acid-splash or several drops of saturated boric acid solution in case of alkali splash. Do not use water if there has been any delay in giving first aid. Get medical help at once.

Caution Avoid using plastic bottle eyewashes as they flush for less than one minute and, unless changed daily, may have growth of bacteria.

A foreign body such as cinder or particles of dust may become lodged in the eye and cause distress. Do not try to remove the speck if it is on the iris (dark portion of the eye). Only an eye doctor (ophthalmologist) should attend to this. First aid is confined to removing particles on the white portion of the eye and objects close to the edge of the eyelids. Be very careful while doing this. Instruct the victim not to rub the eye. Pull the lower lid down gently and look for the dirt. If visible, remove the speck with a sterile cotton swab or a wisp of cotton moistened with water. If the speck is on the inner lining of the upper eyelid, grasp the lashes of the upper eyelid gently, have the victim look up, and pull the eyelid forward and down over the lower lid. This may dislodge the particle so that the tears can wash it out. If this fails, flush the eye with sterile water or a solution of baking soda (2% aqueous solution of sodium bicarbonate) dropped from an eyedropper. In case of persistent irritation, refer the victim to an ophthalmologist or emergency department.

If the eye is wounded by a hard object, loosely bandage the eyes and get medical aid at once. Keep the victim lying down until the physician arrives.

Miscellaneous

Skin puncture by broken glass or splinter might not be life threatening but causes considerable inconvenience. To remove a foreign object, first disinfect the injured portion of the skin, remove the object carefully (Figure 3.7) and disinfect again.

First aid for an *object lodged in the ear* involves addition of oil into the ear having the object. Later when the head is tilted, the object may flow out with the oil.

Airway obstruction leads to *respiratory emergencies*, which is indicated by the universal sign of choking (Figure 3.8). Application of back blow after leaning the head forward or thrust on the abdomen or chest can help propel the object out.

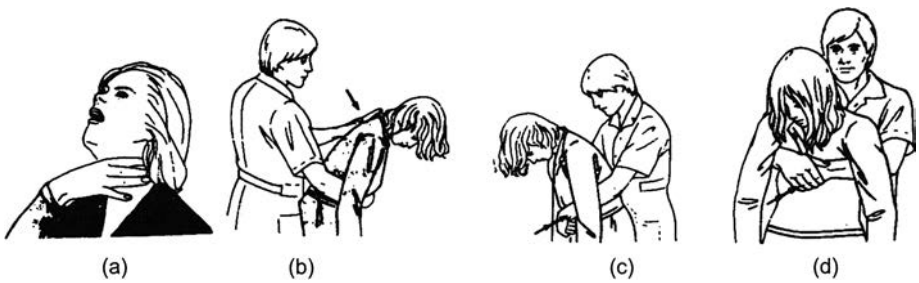

FIGURE 3.8 *Respiratory emergencies: (a) Universal distress signal is choking, (b) For air blockage in throat, give back blow in standing position, (c) and (d) Abdominal thrust or chest thrust are the alternative procedures which should be done in the standing position*

Asphyxia is difficulty in breathing that leads to fainting. In case of severe asphyxia, artificial respiration should be given. Fainting usually results from slight injuries, exposure to overheated rooms, want of food, exhaustion and fatigue. The person about to faint feels dizzy and weak, turns pale and falls unconscious on the floor or on the chair. If you notice that a person is going to faint, offer them a seat, water, and fresh air until they appear to recover. If someone faints, you can revive him by taking the following steps in sequence:

- Bend his head down between his knees, if he is sitting on a chair (Figure 3.6).
- If he is on the floor, lay him flat on his back and lower his head by shoving a folded coat or blanket under his hips or by raising his feet and legs (Figure 3.6):

In either condition, loosen all clothing around the neck (e.g., necktie or tight collar) and waist (e.g., belt, tight-fitting pant). Open the windows or fan him, particularly if a strong chemical appeared to induce the fainting. If the temperature is warm or elevated, make efforts

to reduce the temperature. Hold smelling salts or handkerchief containing a few drops of ammonia under his nose every minute or two. When consciousness retums, the person should continue to lie quietly for a while before getting up. If the faint lasts more than a few minutes, call a physician. In case of severe asphyxia, artificial respiration should be given.

Guide to Standard Precautions

Following are some of the most important common precautions suggested to laboratory personnel that should be followed at all times:

- Wash hands before putting on gloves and immediately after removing them.
- Wear gloves while handling any kind of specimen or other potential infectious materials.
- Wear mask and eye protection or face shield while taking part in activities that are likely to generate splashes or sprays of blood, body fluids, secretions, or excretions.
- Wear fluid resistant laboratory coats.
- Prevent needle-stick injuries by using safety needles and needle safety devices. Discard all such devices in appropriate containers.
- Do not manipulate used needles. Dispose them without trying to recap unless they have a hinged attached cap.
- Promptly disinfect the bench after use.

Review Questions

1. What are the primary causes of accidents in a clinical laboratory and how can these be prevented?
2. State the uses of each of the things kept in a first-aid box.
3. How would you handle a person suffering from asphyxia?
4. Why it is not recommended to store ether in the refrigerator?
5. What is HEPA filter? How is it different from other filters?
6. Describe the construction and operation of a Biological Safety Cabinet (BSC). How would you decontaminate a BSC?
7. What steps should be taken to remove an object lodged in the ear?
8. Imagine a situation that you have broken the centrifuge tube while working with the centrifuge. How would you proceed with cleaning and decontaminating?
9. Why, where and how do you take precautions against radioactive substances?
10. Suppose you have accidently dropped a bottle of concentrated hydrochloric acid while carrying it, and the acid is completely spilled on the floor. What precautions you should have taken to avoid the accident and what should you do now?
11. What is the role of an autoclave in a laboratory? How does it work?
12. You have cut your finger and it is bleeding profusely. How would you handle the situation?
13. Why did micro-incinerators replace Bunsen burners in the microbiology laboratories?
14. What are the causes of electrical shock? What precautionary measures would you recommend to avoid it?
15. What steps would you take if you see that someone has fainted on the floor?
16. How would you dispose off your used needles?
17. What type of clothing should the laboratory workers wear?
18. Why must safety rules be strictly observed?
19. Give two examples of chemical hazards and describe how each might be avoided or managed.
20. Give two examples of physical hazards and describe how each might be avoided or managed.

Chapter 4

Introduction to Laboratory Equipment and Basic Laboratory Operations

Kanai L Mukherjee and Piyali Basu

Chapter Outline

- Overview
- Identification and Use of Common Laboratory Glassware and Equipment
- Use and Care of Laboratory Glassware and Plastic Ware
- Techniques of Simple Laboratory Operation
  - Use of volumetric glassware
  - Heating
  - Maintenance of glassware
  - Filtration
  - Pipetting
  - Use of Vernier scale
  - Titration
- Storage, Handling and Preparation of Laboratory Reagents
  - Storage of reagents
  - Handling of reagents
  - Preparation of laboratory reagents
- Techniques for Heating Liquid in a Test Tube
- Graphical Presentation of Data
- Use and Care of Common Laboratory Instruments
  - General Comments for the Use of Instruments
  - Microscope
  - Centrifuges
  - Balances
  - Colorimeters and spectrophotometers
  - Refrigerators
- Laboratory Water
  - Obtaining clean water
- Water for Human Consumption
  - Sampling for laboratory testing
  - Procedure of laboratory testing
- Common Laboratory Equipment
  - Mixer

- Liquid dispenser
- Diluter
- Filter pump

• Special Laboratory Equipment
- Incubator
- Water baths
- Equipment for sterilization
- Laboratory incinerator

• Review Questions

Overview

A striking feature of any clinical laboratory is the large display of glassware, plastic lab ware, equipment and apparatus, which are in constant use. The clinical laboratory technician working in this setting must learn the use and care of these things. In addition, a mastery of some of the basic laboratory techniques is essential. In this chapter you will be introduced to physical components of a laboratory, and to the use and care of common laboratory equipment.

Identification and Use of Common Laboratory Glassware and Equipment

Some commonly used glassware and other equipment in a laboratory are illustrated in Figure 4.1. Technicians working in a clinical laboratory should be able to identify them and know their uses. Catalogues from suppliers of scientific goods are helpful in getting acquainted with laboratory equipment. The Google search engine (http://google.com/) has made the search process a lot easier through the Internet.

Test tubes are used to heat and hold reagents for observing chemical reactions. A test tube may have a rim, which facilitates pouring fluid out, but the rim frequently chips and breaks, and hence test tubes without a rim are more popular. In a microbiology laboratory, only rimless test tubes are used so that the cotton plug can cover the edge in order to avoid the lodging of contaminants on the edge. Small size test tubes (5 mL) are used in coagulation laboratory, serology laboratory and in blood bank. Test tube racks hold test tubes in upright position (Figure 4.1). These are made of metal or plastic; racks used in a water bath must be made of stainless steel or plastic (or rubberized metal), so that they do not rust. Test tube holders are used during heating of test tubes. A centrifuge tube looks like a test tube, but it has a tapered bottom and is used in centrifugation. The tapered bottom of the centrifuge tube helps to hold the solid sediment so that the supernatant fluid can be easily separated. Centrifuge tubes are usually of 15-mL capacity and may be graduated. Cuvettes are special kinds of test tubes, usually rectangular in shape, which hold solutions intended for photometric readings in a colorimeter. Cuvettes are costly and should be used very carefully. Cuvettes should not get scratched and hence are placed only in plastic racks.

Funnel is used during filtration. During this process, the funnel is supported by a ring stand. A separating funnel is a special funnel, usually pear-shaped or cylindrical, which has a fitted ground-glass stopper and a stopcock. It is used in separating two immiscible fluids. The heavier liquid at the bottom of the flask is removed through the opening of the stopcock.

Volumetric glassware includes graduated cylinders, pipettes, burettes and volumetric flasks. Volumetric glassware is used in measuring the volume of a liquid accurately. The Pasteur pipette is non-volumetric glassware used in transferring liquids and it does not require

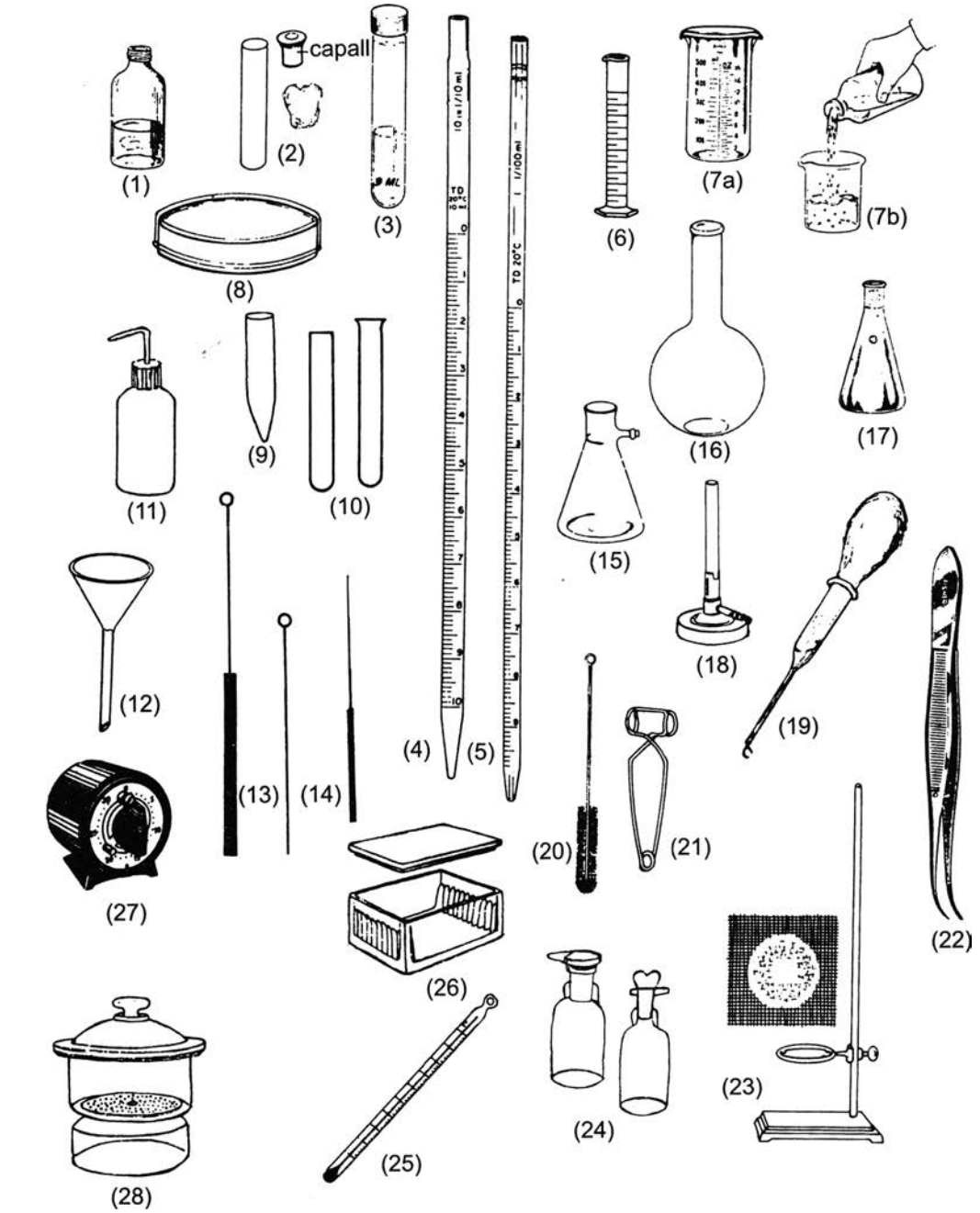

Figure 4.1 *(Contd.)*

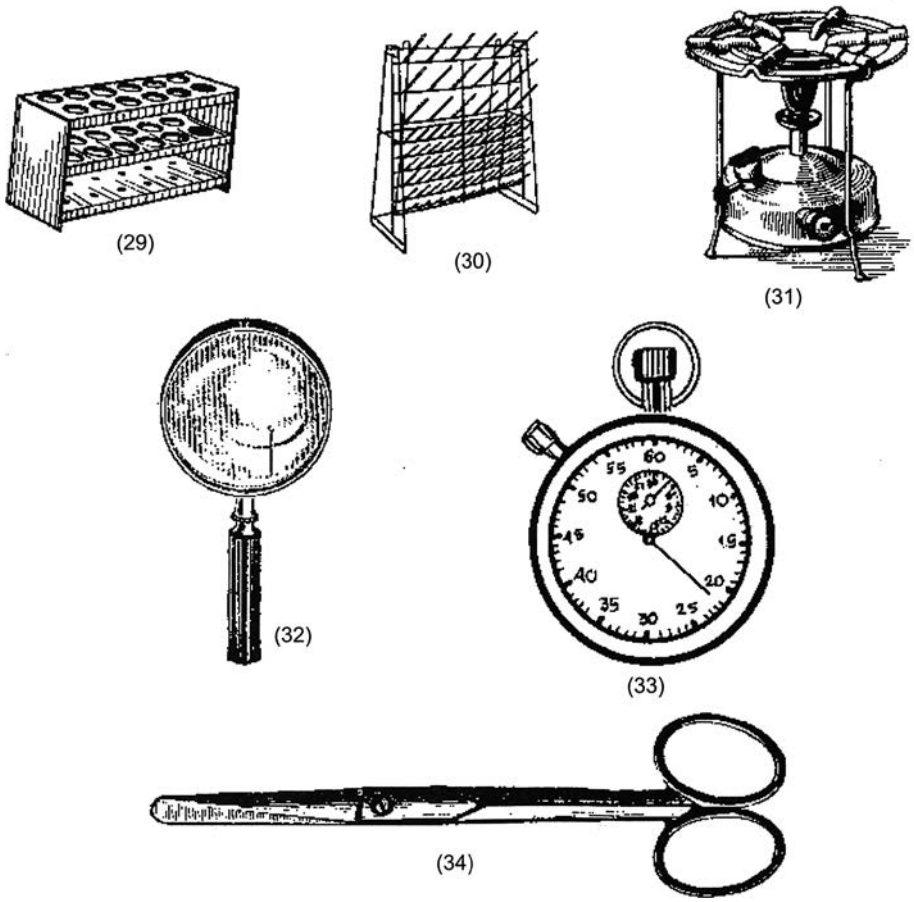

Figure 4.1 *Identification of common laboratory equipment: (1) Reagent bottle, (2) Culture tube and cap or cotton plug, (3) Screw-capped test tube, (4) Graduated pipette, (5) Serological pipette, (6) Graduated cylinder, (7a, 7b) Beaker, (8) Petri dish, (9) Centrifuge tube, (10) Test tube with or without rim , (11) Wash bottle, (12) Funnel, (13) Wire loop, (14) Needle, (IS) Suction funnel, (16) Flat bottom flask, (17) Erlenmeyer or conical flask, (18) Bunsen burner, (19) Pasteur pipette, (20) Test tube brush, (21) Test tube holder, (22) Forceps, (23) Wire gauze and burner stand, (24) Dropper bottle, (25) Thermometer, (26) Staining rack, (27) Alarm clock, (28) Desiccator, (29) Test tube rack, (30) Drying rack, (31) Primus stove, (32) Hand lens, (33) Stopwatch, (34) Scissors*

mouth suction. It has a long-drawn-out tip with a rubber bulb or teat to create suction. The Pasteur pipette is often made in the laboratory itself. Eyedroppers or medicine droppers can be used in place of Pasteur pipettes. A dropping bottle holds frequently used small quantities of reagents (like stains). It is fitted with a grooved, ground-glass stopper, which permits fluids to flow in a drop wise fashion. Its usual capacity is 50 mL. Dropping bottles can be clear or can be brown coloured to hold reagents which are affected by light.

There are other kinds of non-volumetric glassware, which have various uses. A **reagent bottle** is a glass container which holds reagent solutions. Flasks are made of different shapes. The **Erlenmeyer flask** is a conical flask, more commonly used as a laboratory container for holding fluids, media and preparing solutions. It may be graduated which is not as accurate as volumetric glassware. The round-shaped flat bottom flask with a long neck is specifically used for preparing solutions that require heating and shaking. The long neck helps in holding

it. The round-shaped round bottom flask is used only in distillation. The **beaker** is used for heating liquid and for preparing reagent solutions. It has many other uses. It may have graduations for estimating the volume of fluid held by the container.

There are several other miscellaneous items of laboratory equipment used under various conditions. The **wash bottle** is used for dispensing liquids. It is made of soft plastic so that water can be squirted out when the bottle is squeezed. Old-fashioned wash bottles are of glass in which the liquid is dispensed by blowing in air through a glass tube. **Petri dishes,** made of glass, are used in the aerobic culture of microbes. These are round in shape. The culture medium is placed in the bottom half and the cover snugly fits on it. A stirring rod is used for dissolving solute in preparing solutions. For uniform heating on a **Bunsen burner**, wire gauze with asbestos centre is placed on the tripod stand during the heating process. The asbestos has now been replaced with other materials because asbestos is considered carcinogenic.

Tongs are made of metal and are used to hold hot or cold objects. **Triangular file** and glass tubing scorer are used for cutting glass tubing. The **thermometer** measures temperature. A utility clamp is used to hold the burette during titration.

A **desiccator** is used for keeping reagents in a dehydrated condition. An appropriate desiccant is placed in the lower section of the desiccator chamber. Some desiccators have arrangements for creating a vacuum by sucking the air out through the stopper attached to the lid. The lid must be properly greased on the rim so that the chamber is properly sealed. The desiccant must be in an active state. Revive the desiccant (calcium chloride and anhydrous calcium sulphate, both commercial grades) by heating at 100°C for several hours. Presence of an indicator crystal in the desiccator helps to determine whether the desiccant is hydrated (pink) or dehydrated (blue). Replace the desiccant when too old.

Use and Care of Laboratory Glassware and Plastic Ware

While working in a laboratory, the technician must get acquainted with the types of glassware handled in the laboratory and use them appropriately. Improper use of glassware might lead to their breakage.

There are basically two qualities of glassware. **Borosilicate glassware** is heat and chemical resistant. It can also stand mechanical stress and does not break due to sudden change of temperature (thermal shock). This is ideal for beakers and other glassware, which are subjected to heating. They are also expensive. The other type is soda lime glass, which is less resistant to mechanical and thermal shocks. It has a high alkali composition and free soda is present on the walls of soda lime glass which must be neutralized before use. It is cheaper than borosilicate glass and is ideal for storage of reagents. Prolonged storage might, however, result in alkali contamination. **Soda lime glass** is easy to bend by heat and is used in preparing certain items of laboratory glassware, e.g., in bending tubes or making Pasteur pipettes.

Plastic ware is now often seen in laboratories in place of glassware. It has the greatest advantage of being unbreakable, non-toxic and resistant to corrosion (Figure 4.2). These, however, cannot stand heat and with prolonged use, they look dirty. Plastic ware also reacts with some chemicals. Plastic reagent bottles are very popular, but small amounts of reagents should not be stored in plastic bottles, since the plastic wall is permeable to water vapour and evaporation occurs through the plastic. Some plastics also absorb dyes and stains. They are very useful in preparing wash bottles as the soft plastic is flexible and can be squeezed. Ether, acetone and such organic solvents should not be kept in plastic containers as the solvent dissolves the plastic. Recently introduced plastic micro test plates and micro titre plates (Figure 4.2) have become very popular for observing haemagglutination reactions.

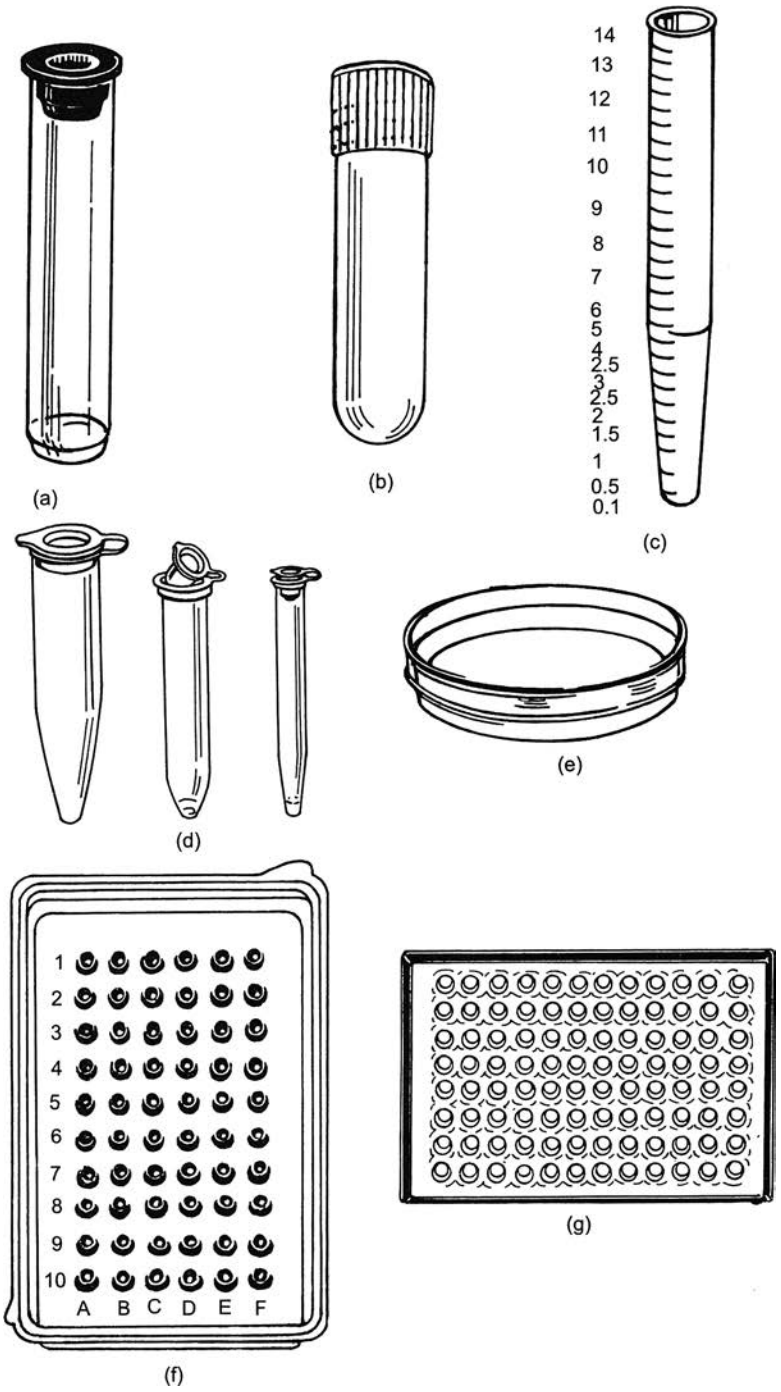

Figure 4.2 *Some plastic ware used in laboratories (figures provided by Bioscience Corporation, Mumbai, India): (a) Test tubes, (b) Storage vials, (c) Graduated centrifuge tube, (d) Ungraduated centrifuge tubes with cap, (e) Petri dishes, (f) Microtest plate, (g) Microtitre plate*

Laboratory glassware is classified as **general-purpose glassware** and **volumetric glassware.** The general-purpose glassware has uses other than the measurement of fluid volume, such as containers and receivers. Glassware such as beakers, Erlenmeyer flasks, test tubes, funnels, reagent bottles and others fall in this group and are available in different sizes. Some of these (e.g., beakers) may be graduated but the graduation is not accurate.

Reagent bottles hold reagent solutions. They are either clear or coloured and are usually made of soft glass. All reagent bottles must have tight-fitting caps or glass stoppers. The latter are expensive but more efficient as they do not react with any chemical. While storing empty reagent bottles, insert a thin strip of paper between the ground joint surfaces to prevent sticking. Do not store alkali in glass-stoppered bottles, as the stopper freezes at the joint. Bakelite or plastic caps are preferred over metal caps. Both should have an inner lining which can be made inert by coating with wax or plastic.

Helpful tips to release a frozen joint: If a ground joint sticks, separation can generally be achieved by carefully rocking the cone in the socket or gently tapping the socket flange on a wooden surface or by heating the socket (the neck of the bottle) with a localized flame. Do not heat the cone or the stopper. The use of penetrating oil often proves useful in aiding separation.

Plastic bulb pipettes are cheap and very useful for transferring volumes of liquid such as serum. They are available with different tips and usually have no calibration. They can be reused after disinfection and washing but cannot be autoclaved.

Techniques of Simple Laboratory Operation

Under this section we will discuss a few essential laboratory techniques. These include use of various heating equipment, handling and cleaning of glassware, techniques of filtration, preparation of reagent solutions, pipetting and titration. In addition, we will discuss about the graphic presentation of data and drawing of a calibration curve.

Use of Volumetric Glassware

Volumetric glassware is intended to hold, deliver or contain definite volumes of liquid (Figure 4.3). Permanently etched lines called **calibration marks** indicate volumes held or contained. Volumetric glassware is calibrated to a single volume with one marking or it can be graduated with multiple markings. Good quality volumetric glassware will have a continuous band of calibration marks. The continuous band can be seen from all directions and also helps in matching the liquid meniscus in line with the calibration point. The eye level is kept in such a way that the band appears as a single line and the bottom of the meniscus of a transparent liquid (e.g., water) touches the calibration mark. In case of a non-transparent liquid (e.g., mercury or deeply coloured fluid), where the bottom of the meniscus is not visible, the top of the meniscus is matched to the calibration mark. Before handling volumetric glassware, a technician must first find out the value of each graduation. For example, if there are 10 divisions between 0 and 1 mL, each graduation is equivalent to 0.1 mL; if there are only 5 divisions, each division is equivalent to 0.2 mL. On the other hand, if there are 20 divisions between 1 mL marks, each division will be equivalent to 0.05 mL. Hence, before recording the value, it is important that the user carefully look at the graduation marking and find out how much volume it indicates.

A **graduated cylinder** is a long cylindrical piece of glassware with calibration markings on it in an ascending order (zero at the bottom). It is a handy volumetric device which is used to measure volumes of liquids when a high degree of accuracy is not essential. The wide surface of the fluid at the measuring point reduces its precision. Use of a graduated cylinder is illustrated in Figure 4.4. Graduated cylinders are available in various sizes. These may be provided with a plastic collar that prevents breakage when the cylinder drops on the table from a standing position.

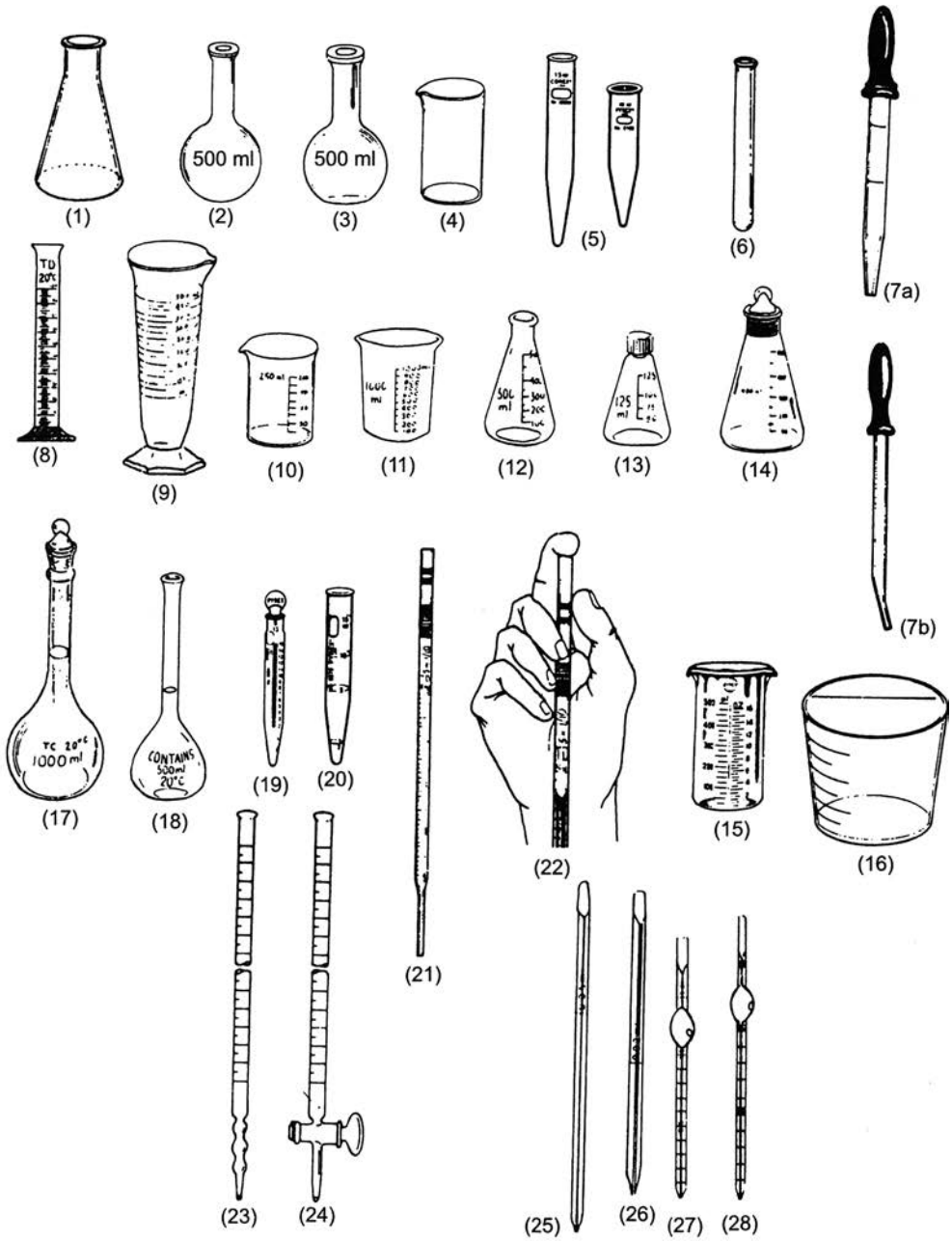

FIGURE 4.3 *Identification of non-volumetric and volumetric glassware.*

Non-volumetric glassware: (I) Erlenmeyer flask, (2) Round bottom flask, (3) Flat bottom flask, (4) Beaker, (5) Centrifuge tubes, (6) Test tube Semi-volumetric glassware: (7a and 7b) Medicine droppers with and without calibration mark, (8) Graduated cylinder, (9) Graduated specimen glass, (10 and 11) Beakers, (12–14) Conical flasks, (15) Graduated beaker with double beaks, (16) Graduated glass Volumetric glassware: (17 and 18) Volumetric flasks, (19 and 20) Graduated centrifuge tubes, (21 and 22) Graduated serological pipettes, (23 and 24) Burettes, (25 and 26) Micropipettes, (27 and 28) Diluting or Thoma pipettes

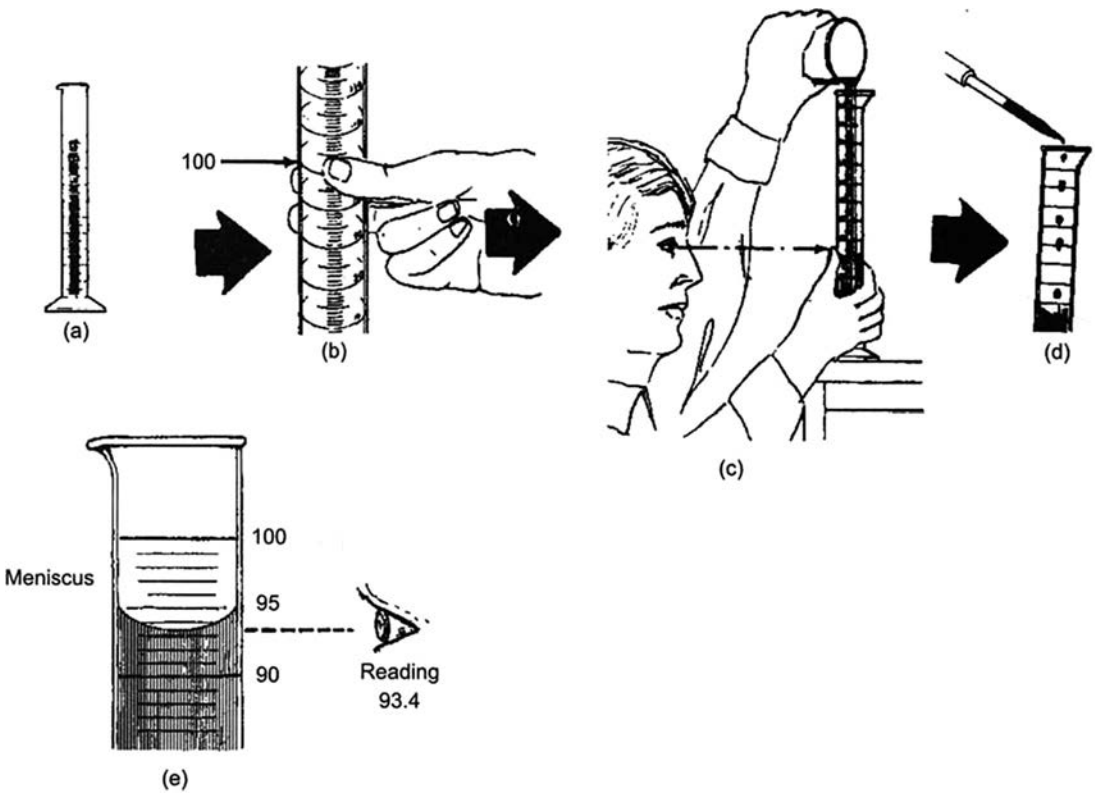

FIGURE 4.4 *Use of graduated cylinder: (a and b) Mark the volume to be measured, (c) Fill the cylinder close to the mark, (d) Finally use a dropper to bring the liquid to the calibration mark*

Note: (e) Always keep the eye at the same level with the meniscus and read the volume from the line of the meniscus.

Pipettes are used for measuring liquid volume with high precision (Figure 4.5). They are primarily used in transferring fluid. The narrow measuring surface of the pipette makes the measurement of volume more accurate with the pipette than the graduated cylinder. The tip of the pipette is usually tapered and has an opening that is critical in controlling drainage time. Drainage time is important for the accuracy of the volume being dispensed. The pipette is filled by suction provided by the mouth, a rubber bulb or a mechanical device at one end designed for this purpose (Figure 3.4). Mouth pipetting must be discouraged in clinical laboratories. Numerous safety devices are now available in the market that replaces mouth pipetting. Some pipettes meet special needs, for example, Oswald pipette that is used to deliver viscous fluids (Figure 4.6). This will be discussed later.

Pipettes are broadly classified as: (a) **to-deliver** (TD) pipette, and (b) **to-contain** (TC) pipette. The to-deliver (TD) pipette is calibrated to deliver a single volume of fluid (as marked on the volumetric pipettes) or fractional volumes (as marked on the graduated pipette).

Volumetric pipettes are calibrated to deliver definite volumes of liquids by drainage (Figure 4.5). All measurements must be made at room temperature. The liquid must be allowed to drain freely from the calibration mark to the tip and the last drop held at the tip should not be blown out. Retention of this drop has been considered in the calibration of the pipette. The residual drop of the fluid is discarded. Volumetric pipettes are of high precision for biochemical work.

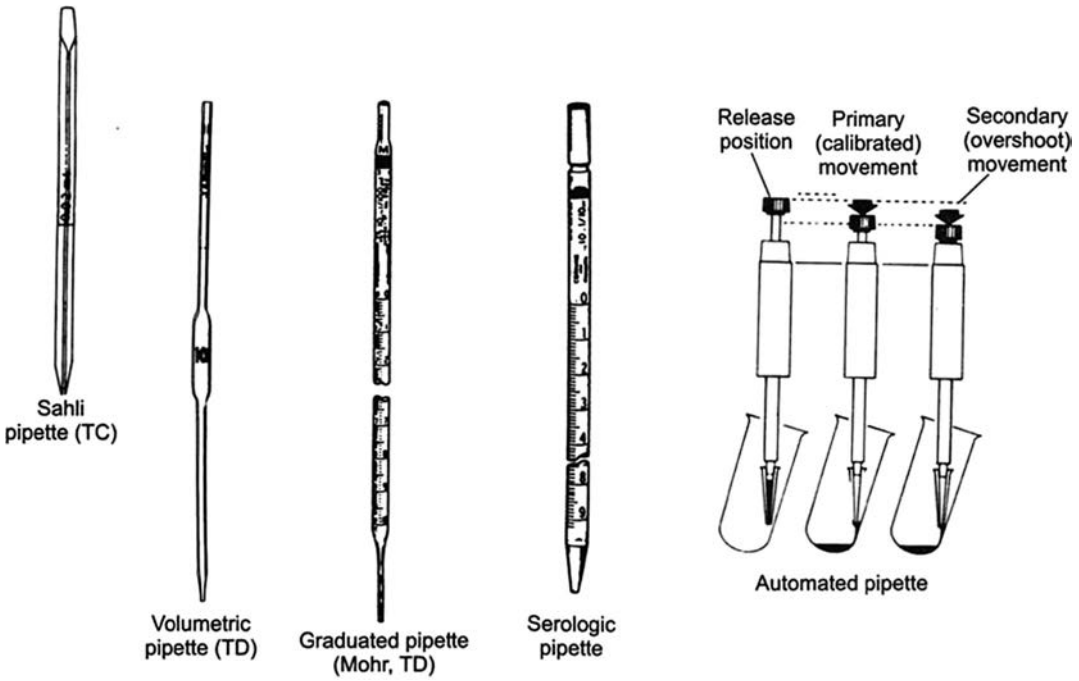

FIGURE 4.5 *Commonly used pipettes in clinical laboratories. Movement of the piston in an automated pipette (Oxford type) is illustrated.*

To-contain pipettes (TC) are among the most precise pipettes used in laboratories (Figure 4.5). Each TC pipette is calibrated to contain a specified volume of liquid. Usually they hold small quantities of liquid (10–100 μL) and thorough rinsing of the pipette with the diluent contained in the receiving flask empties the liquid. Because of the small volume, improper use might lead to a large error. Hence, after the pipette is filled to the mark, wipe the outside to remove the excess fluid and then empty out the contents into the diluting fluid, which is also used in rinsing the inside of the pipette 3-4 times. There are a few other miscellaneous types of pipettes, which have limited use. In haemoglobin determination by photometric method, **Sahli pipette** is used as a blood-diluting pipette for counting blood cells. This is a TC pipette that holds 20 μL of blood; it is always washed with the diluent. The markings on the pipette do not indicate volume, they only indicate proportionate dilutions. **Oswald pipette** looks like a volumetric pipette where the delivery tip is considerably closer to the bulb (Figure 4.6). This pipette should be blown out, as indicated by the etched ring at the mouth piece. The large bulb near the tip makes the pipette well suited for the delivery of viscous fluid like blood. The fluid must be allowed to drain very slowly when these pipettes are used. No residual fluid should be left in the pipette or in its inside walls. The usual volumes of the Oswald pipette are between 1 and 2 mL.

FIGURE 4.6 *Oswald pipette*

Graduated pipettes have the advantage of delivering variable quantities of fluids into several containers after filling the pipette once, which can expedite the dispensing job.

They are, however, not as accurate as the volumetric pipettes. Graduated pipettes are most commonly used in clinical laboratories. **Mohr pipette** is a special graduated pipette where the last calibration mark is far from the tip. This provides better accuracy as the space between the tip of the pipette and the last calibration mark cannot be accurately determined. Unlike others, the serological pipette requires blowing out of the residual fluid after dispensing. It is usually graduated and the graduation markings continue up to the tip. The etched double band at the suction end identifies the **serological pipette**. The primary use of the serological pipette is to obtain serial dilutions of serum in determining titres. The 'blow out' character of the serological pipette helps in mixing and the results are semi-quantitative. Because of their rapid rate of delivery with a larger opening at the tip, they have poor precision, and hence should be avoided in biochemical determinations.

Automated pipettes are becoming increasingly popular (Figures 4.5 and 4.20). They are often 'blown out' pipettes with mechanical devices. They can use disposable tips or the latter can be recovered thorough washing followed by drying. These pipettes can dispense multiple volumes or a single volume, as desired. The use of automated pipettes has greatly helped in reducing biological hazards and increasing the speed of work. One word of caution—the automated pipettes must be calibrated more frequently or else the false sense of security results in a high degree of error. Avoid mouth pipetting by using various devices available in the market (Figures 3.4, 4.7, 4.19 and 4.20). The procedure to use the capillary micropipette is shown in Figure 4.20.

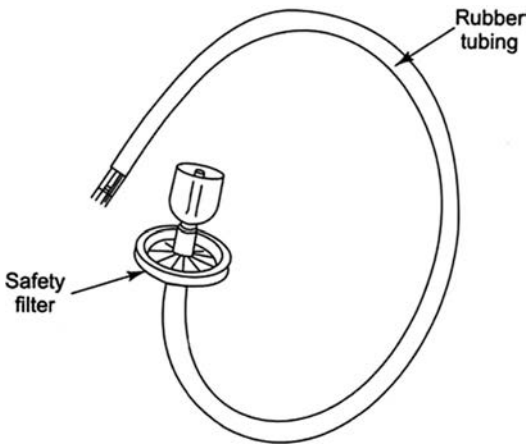

FIGURE 4.7 *Safety device in mouth pipetting*

The **burette** (Figure 4.3) is similar to a graduated pipette but differs in that a stopcock attached to the lower end controls the outflow of liquid. The graduation markings are etched on the cylindrical glass tubing, with the zero marking at the top, like the graduated pipette. Pouring through the opening at the top accomplishes filling. Before dispensing the liquid, bring the level to the zero mark, then dispense through the stopcock and finally read the volume dispensed by the difference of the liquid levels. Burettes are almost exclusively used for titration which is described later in this chapter. Volumetric flasks are flat-bottomed pear-shaped vessels with a long narrow neck that has a single calibration mark. They are fitted with a ground glass or plastic stopper. The volumetric flask contains only the specified volume of fluid as marked on the flask. The commonly used sizes of the volumetric flask in the laboratory are between 25 and 1000 mL capacities. Volumetric flasks are used in preparing solutions.

Micropipette

A single channel micropipette is the most used pipette in laboratories. Various kinds of micropipettes are available in the market. These micropipettes are used to transfer small amounts of liquid, usually down to 0.1 μL from one vessel to another.

General procedure

1. Check the pipette assembly before using the micropipette.
2. Never drop a micro pipette as this may alter the calibration.
3. Initially before use, depress the plunger knob several times. This acts to redistribute the lubricant within the pipette. It should feel smooth when the plunger knob is depressed and released.
4. The plastic pipette tip must be properly fitted onto the pipette; it should be straight and held firmly in place.
5. When using a plastic tip, pre-rinsing is always required (unless otherwise indicated in the procedure).
6. Pipette solutions that are at room temperature to avoid errors caused by contraction and/or expansion of fluids.
7. Hold the pipette in a vertical position to increase accuracy and precision.
8. Depress and release the plunger with a controlled and smooth action. Try to maintain the same speed of intake and delivery for all samples (consistency is the key).
9 Never allow the plunger knob to snap back as this will cause aspiration of the liquid past the pipette tip into the pipette contaminating its interior assembly. If this happens, alert your instructor as cleaning of the pipette is required.
10. Depress the plunger knob BEFORE inserting the pipette tip into the fluid. Depression of the plunger knob while it is in the solution will cause bubbles to form in the tip, resulting in an inaccurate measurement.
11. Insert the tip of the pipette into the sample at approximately the same depth each time. Try not to 'drown' the tip into the fluid (never deeper than ¼").
12. Any air bubble, regardless of its size, will invalidate the volume of liquid you are trying to pipette. If an air bubble appears in the pipette, re-sampling is required.
13. When delivering a sample to the receiving vessel, place plastic pipette tip inside the receiving vessel without touching the interior walls of the vessel. Touching the walls with the tip of the pipette alters the 'flow' of sample delivery and also the volume being delivered.
14. Position the sample tip so that the sample does not 'dribble' down the entire length of the interior walls of the receiving vessel. This may cause a loss of sample and invalid test results.
15. The plastic pipette tip SHOULD NEVER come in contact with the fluid in the receiving vessel while delivering the sample (unless otherwise instructed in the procedure).

Oxford Micropipette

Oxford micropipette is a TD (to-deliver) pipette and has 2 plunger stop positions: primary and secondary; as the plunger knob is depressed the first stop is encountered. This first depression of the plunger knob is the primary or calibrated movement that 'aspirates' the specified volume. The plunger knob may then be depressed to its lowest position. This is the secondary or blow out movement (similar to the 'blow-out' step when using a serologic pipette).

Procedure

1. Apply a clean plastic tip firmly to the pipette; verify that the tip is straight.
2. Depress the plunger knob to the FIRST (upper-most) stop position.

3. Immerse the tip approximately 1/8" into the solution.
4. Return the plunger knob to the release position, aspirating the sample into the plastic tip.
5. Slightly remove the plastic tip from the solution and return the sample to the original container: depress the plunger knob to the first (upper-most) stop, pause slightly, and then depress the plunger knob to the second (lowest) position.
6. Return the plunger knob to its release position.
7. Depress the plunger knob to the FIRST stop (upper-most stop).
8. Immerse the tip approximately 1/8" into the solution.
9. Return the plunger knob to the release position, aspirating the sample into the plastic tip.
10. Remove the plastic tip from the solution, and while doing this, gently 'swipe' the tip against the side of the tube to avoid delivering any fluid adhering to the outside of the plastic tip into the receiving vessel.
11. Place plastic tip inside the receiving vessel without touching the interior walls of the receiving vessel. Position the sample tip so that delivery of sample does not result in the sample dribbling down the entire length of the interior walls. Do not allow the pipette tip to touch any fluid in the receiving vessel.
12. Deliver the sample to the receiving vessel: depress the plunger knob to the first/upper stop, pause slightly, and then depress the plunger knob to the second/lowest position (this secondary movement is used to BLOW OUT the contents of the pipette tip and is similar to the blow out step used for serologic pipettes).
13. With the plunger knob still held in its lowest position, withdraw the pipette from vessel (this is done to eliminate aspirating some of the sample from the receiving vessel back into the plastic tip resulting in delivering a falsely low volume).
14. Return the plunger knob to the release position; remove and properly discard the plastic tip.
15. This is the pre-wetting step that is required when using plastic pipette tips.

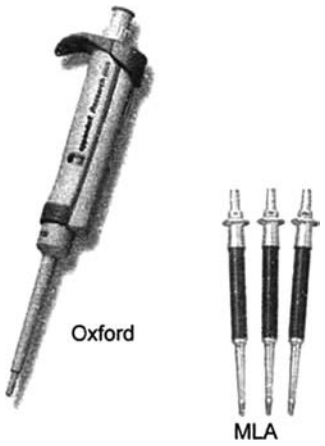

FIGURE 4.8 *Types of micropipettes*

MLA Pipette

This is a TD (to-deliver) pipette which has only 1 plunger stop position. The same plunger stroke is used for aspiration and delivery of sample 2. This type of pipette is most often used in clinical laboratories due to its ease of use. It is available in over 30 fixed volumes, from 5μL to l000μL. The slim, tapered design and location of the de-tipping mechanism enables easy access to narrow diameter tubes and is comfortable – especially for smaller hands. Smooth one-stroke action enhances repeatable results and requires less physical effort. In-lab calibration is performed with minimal efforts.

Procedure

1. Apply a clean plastic tip firmly to the pipette.
2. Depress the plunger knob completely.
3. Immerse the tip approximately 1/8" into the solution.
4. Return the plunger knob to the release position, aspirating the sample into the pipette tip.
5. Slightly remove the plastic tip from solution and return the sample to the original container: fully depress the plunger knob.
6. Return the plunger knob to its release position.

7. Again depress the plunger knob completely.
8. Immerse the tip approximately 1/8" into the solution.
9. Return the plunger knob to the release position, aspirating the sample into the pipette tip.
10. Remove the plastic tip from solution and while doing this, gently 'swipe' the tip against the side of the tube to avoid delivering any fluid adhering to the outside of the plastic tip into the receiving vessel.
11. Place the plastic tip inside the receiving vessel without touching the interior walls of the receiving vessel. Position sample tip so that delivery of sample does not result in the sample dribbling down the entire length of the interior walls; try to deliver the sample near the bottom of the vessel while not touching the fluid that is already in the receiving vessel. Do not allow the pipette tip to touch any fluid in the receiving vessel.
12. Deliver the sample to the receiving vessel and then fully depress the plunger knob.
13. With the plunger knob is fully depressed, withdraw the pipette from the vessel (this is done to eliminate aspirating some of the sample from the receiving vessel back into the plastic tip resulting in delivering a falsely low volume).
14. Return the plunger knob to the release position; remove and properly discard the plastic tip.

MLA Digital

The introduction of digital adjustable MLA pipettes has made the use pipettes all the more fast and enables easy volume selection. This is ideal in clinical laboratory conditions. A streamlined shape and smooth single-stroke operation make these pipettes remarkably easy to use.

Calibration

All micropipettes must be calibrated regularly, at least once a year. Depending on what type of work you do, you might have to do it more often.

Heating

The **Bunsen burner** is the most common device used in laboratories for heating (Figure 4.9). It is used not only for heating liquids but also for flame sterilization and in other laboratory operations. Kerosene oil stoves (Figure 4.1) are ideal for fast heating; however, one should be careful as it works under pressure. Electric heaters and hot plates are modern devices of heating if electricity is available. They have a temperature regulator and some of them may have a magnetic stirrer to mix fluids while heating. A plastic-coated magnetic stirring bar is dropped inside the beaker containing the solution to be mixed. The bar spins while the solution is heated, facilitating the dissolving process. In laboratories where electric supply is not regular, alternative heating arrangements like the use of a Bunsen burner should be available.

Use and care of Bunsen burner

The Bunsen burner provides an open flame for heating. It may be connected to the central gas line since it can bum bottled gas coming out of a cylinder. A well-adjusted Bunsen burner should ideally provide a hot, smokeless, clean and silent flame. In order to attain this, an understanding of the Bunsen burner components is necessary.

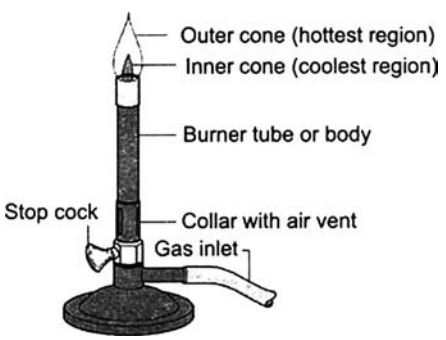

FIGURE 4.9 *Components of a Bunsen burner and heat regions of flame*

The Bunsen burner has three components—the main tube or body, the metal collar over the main tube and the nozzle that connects the burner with the gas supply. The metal collar has a hole that can turn around the main tube of the burner. At one position, the hole in the collar lines up with the hole in the main tube. The holes allow air to enter into the main tube of the burner to mix with the gas coming through the nozzle. The air and the gas mix in the main tube of the burner and the mixture then comes out of the top and starts burning. Proper blending of the burning gas and air is needed in order to obtain a clean and quiet flame. The control valve attached to the cylinder controls the flow of the burning gas and the amount of air entering the main body of the burner is controlled by the holes provided in the collar.

Procedure for using Bunsen burner

1. Close the air entry hole by misaligning it from the hole on the main tube.
2. Put a burning matchstick at the top of the burner and tum on the gas. The gas without air bums with a tall, smoky, yellow, silent, cool flame at the top of the burner (Figure 4.9).
3. Adjust the air entry hole (half open) so that the burner will be silent, hot and smokeless. If the air hole is fully open, there will be too much air and the flame will be short, smokeless, clear and noisy.
4. When the work is over, rum off the flame by turning off the gas supply.

Caution The Bunsen burner gives an open flame; therefore do not keep any flammables near the burner. As the flame is not always clearly visible, accidents might occur due to careless handling. The gas cylinder should be securely chained to the wall. Check all joints for any possible leak of gas, especially when a new cylinder is attached. Use soap water to check for leaks. Replace old washers promptly before dangerous leakage of gas occurs. Always close the cylinder valve before leaving the laboratory.

Maintenance of Glassware

Laboratory glassware is expensive. Proper care and handling reduce the risk of personal injury, add life to these items and keep the cost of laboratory operations down. Some common tips for the care and maintenance of glassware are provided below:

1. Never leave the glassware unattended when it is heated; it cracks or explodes when dry condition approaches.
2. Avoid thermal shock by putting the hot glassware on an asbestos pad or cardboard pad. Do not put it on a damp surface.
3. Avoid scratching of glass in its daily use. During the mixing of a solution in a beaker by means of a glass stirring rod, do not rub the bottom of the beaker, it scratches the glass and makes it weak. Whenever possible, use a rubber policeman (rubber-tipped glass rod) or use a plastic rod if the solution is not too hot. For cleaning, use plastic brushes instead of metal brushes; the latter scratches the glass. Scratches diminish thermal shock resistance of the glassware and might lead to breakage.
4. Use anti-bumping devices like glass beads at the time of boiling and use metal gauze to diffuse heat while heating liquids in a beaker or Erlenmeyer flask. Heat slowly and avoid overheating.
5. Use heat-resistant glass while preparing solutions of acids and alkalis; a considerable amount of heat may be generated during this process.
6. Fire polish glass tubing before attempting to insert it into rubber tubing.

General cleaning of glassware

Glassware used in clinical laboratories should be scrupulously clean in order to reduce errors. Residual detergents and acids might ruin specimens and valuable findings.

New Glassware

New glassware should be appropriately treated and cleaned before use. Newly purchased soft glassware (soda lime) may be slightly alkaline and should be treated overnight with 5% HCl (concentrated technical grade hydrochloric acid, 30%, diluted six times). This neutralizes free alkali found on the surface. This treatment is not necessary for borosilicate glass (hard glass). Acid-treated glassware must be first rinsed with tap water followed by thorough rinsing with distilled water or deionized water. Newly purchased borosilicate glassware is cleaned with detergent, followed by washing with tap water and then rinsing with distilled water or deionized water.

Old Glassware

1. Rinse all glassware immediately after use. Remember, dry glassware, such as the dry dishes after a meal, is difficult to clean; stains, markings, proteins, and other materials may get stubborn due to drying. Rinsing in hot tap water is desirable.
2. Place in a low-suds-detergent solution (2%). Do not use very concentrated detergent solution, which may not be completely removed, and this affects the test results. Dissolve the detergent completely before putting in the glassware. Preliminary soaking in the detergent can save time, reduce contamination problems and make the final washing simple. Soak for one hour.
3. Scrub thoroughly with a good quality brush (choose an appropriate brush for the type of glassware being cleaned). A mild abrasive might help cleaning but the abrasive should not scratch the glass. Make sure that the brush reaches all parts of the glassware, inside and outside. When possible, keep the glassware grouped together—beakers, flasks, test tubes etc. This makes the cleaning and washing easier. Almost all glassware will be thoroughly clean with hot soap solution and a good brush.
4. The next step is to wash the glassware under running tap water. Wash each item five times or more. All traces of detergent must be removed from both inside and outside during the rinsing process.
5. Finally rinse with distilled water, or deionized water, at least three times.
6. Dry glassware completely by keeping it in an oven (140°C). If an oven is not available, dry the glassware on the drying rack at room temperature overnight. Dry the burette in the inverted position on the burette stand.

Special Cleaning

If the glassware becomes unduly dirty due to coagulated organic matter or other substances, it must be cleaned with chromic acid cleaning solution.

Reagent

Caution Potassium dichromate (or sodium dichromate) and sulphuric acid are both powerful corrosive solutions and the mixture makes it even more so. While preparing or handling this solution, use an eye shield, heavy rubber gloves and a rubber apron. Avoid using this solution when other cleaning methods are available. Do not drop the cleaning solution on the floor; if it falls, wash it away thoroughly; shoes corrode if they come in contact with the cleaning solution.

Dissolve 100 g of potassium dichromate ($K_2Cr_20_7$) in 1000 mL of water in a heat-resistant beaker. Use powdered commercial or technical grade sodium or potassium dichromate. If the chemical is in crystal form, grind it to a fine powder in a mortar. Add 100 mL of concentrated sulphuric acid to the dichromate solution. Add the acid drop by drop and very carefully, stirring constantly with a long glass rod or plastic rod. Avoid scratching the bottom of the beaker with the glass rod; it weakens the beaker.

Caution Always add acid to water.

Store the fluid in a wide-mouthed porcelain jar with a lid. The storage jar should be big enough to accommodate glassware for overnight soaking. Keep some of the cleaning solution in an ungraduated plastic wide-mouthed cylinder for dipping pipettes. Always pre-wash the glassware before putting in the cleaning solution because some chemicals (like alcohol) can ruin the cleaning solution when they come in contact with it.

Discard the solution when it begins to turn greenish. Dilute with large volumes of water before discarding or carefully neutralize the diluted solution with sodium hydroxide. Do not let the solution drip on to the floor; it corrodes shoes. If clothes or skin are splashed with the cleaning fluid, they should immediately be washed in water and any residual acid neutralized with a weak alkali (sodium carbonate).

Procedure

1. Rinse the glassware in tap water and then dip in the cleaning solution for an hour (Figure 4.10). Rinsing with tap water is especially needed when the glassware contains alcohol (this spoils the cleaning solution). Longer treatment (overnight or 48 h) may be needed in case of more dirty glassware.

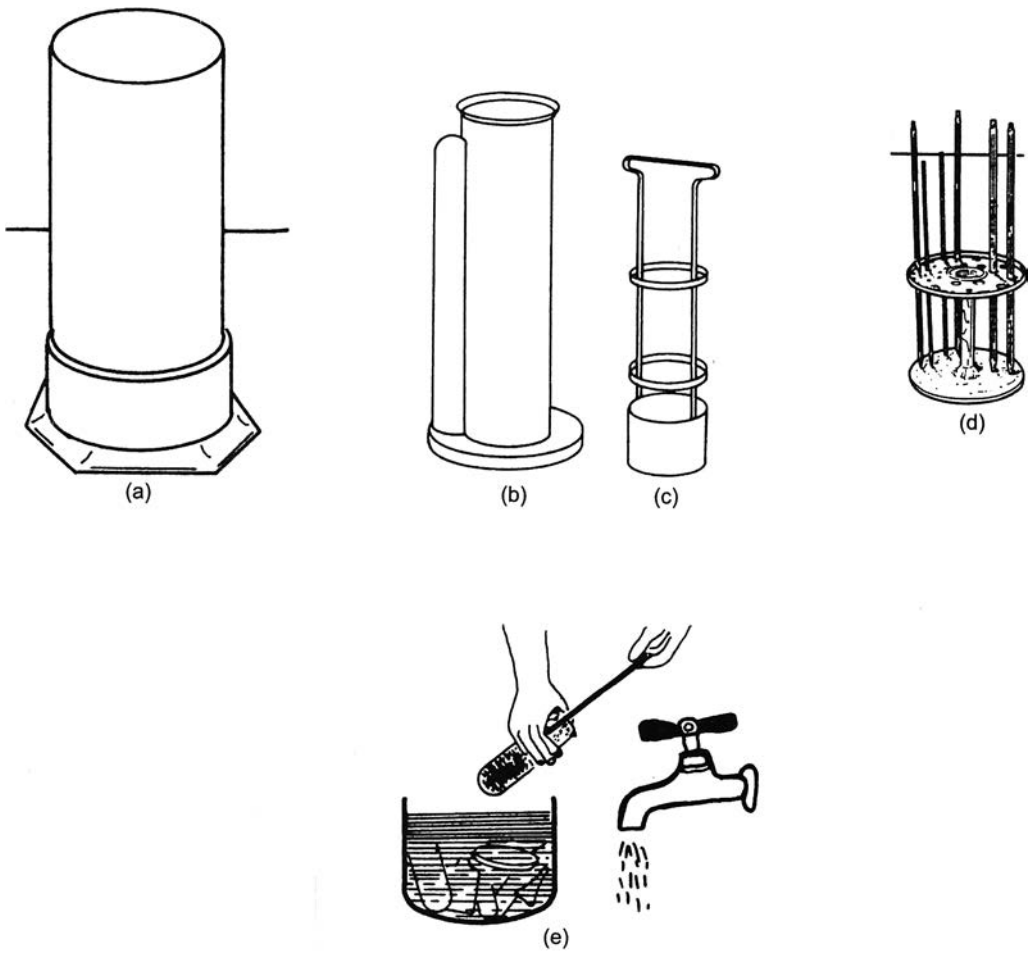

Figure 4.10 *Washing of glassware: (a) Plastic (or metal) container for holding pipette after use, (b) Automatic pipette washer with siphon arrangement, (c) Pipette holder for automatic washer, (d) Pipette stand for drying, (e) Other glass utensils are washed with hot soap and tap water. All lab ware used in analytical chemistry must be finally rinsed with deionized water.*

2. Wash under running tap water.
3. Rinse with distilled water or deionized water.

Other cleaners

The following cleaning solutions may be used in special cases:

1. Diluted hydrochloric acid—50% concentrated HCl, commercial grade (30% strength), in water removes iron stains.
2. Use nitric acid for stains due to Nessler's reagent (iodine).
3. Remove grease by boiling with weak alkali solution (sodium carbonate). Never use strong alkalis (NaOH, KOH). Ordinary grease can also be removed with acetone and ether (flammable). To remove silicone grease use sulphuric acid.

Note All the cleaning reagents must be washed away and the glassware should be rinsed finally with distilled water or deionized water.

Cleaning and storage of pipettes

With the advent of automatic pipette with disposable tips, use of classic pipettes has become rather rare. Yet some old-fashioned laboratories still use the classical pipettes occasionally. Hence this section is included.

Discard pipettes (tip up) in a metal or plastic cylinder, half-full with water, immediately after use. See that the water level is high enough to immerse the greater portion or the entire pipette. While dropping the pipette, take care not to chip the tip which makes the pipettes useless. Placing a pad of glass wool at the bottom of the jar prevents breakage. After the day's work, the pipettes are drained and then transferred to a cylinder or jar with detergent solution. If exceptionally dirty, put the pipettes in chromic acid solution. After soaking for several hours, or overnight, drain the pipettes and run tap water over and through them until all traces of dirt and acid (if used) are removed. The use of an automatic pipette washer is recommended for rinsing and cleaning (Figure 4.10). For the latter, an abundant supply of water will be needed. If the water supply is limited; leave the pipettes overnight in a large volume of fresh tap water. Finally wash with fresh tap water and rinse with distilled or deionized water 3 to 5 times. Dry the pipettes in the hot air oven. For quicker drying, rinse with acetone and then blow off with air.

Store the pipettes in a drawer divided into compartments for different sizes and types of pipettes (Figure 4.11).

Cleaning of micropipettes

In haematology and chemistry, micropipettes and blood diluting pipettes are washed with the help of suction pump or filter pump (Figure 4.12a). **Blood pipettes** (Sahli pipettes) are cleaned differently. Immediately after use, put the pipette in low-suds-detergent solution (2%). The pipette is then cleaned by suction with the help of a filter pump (Figure 4.12a). The detergent is first drawn in, followed by rinsing with tap water (with continued suction), and it is finally rinsed with deionized water. The pipettes can be dried in the oven. Alternatively, after the final rinse with deionized water, draw in acetone-alcohol mixture (1:1) and dry the pipette by air suction. In case the pipette is clogged, use suction bulb to suck and blow out. A horsehair or fine steel wire can be used to clear the clog (Figure 4.12c).

Cleaning of microscope slides and cover slips (Figure 4.13)

Microscope slides and cover slips are not disposable in developing countries. All attempts should be made to recover them after disinfection. Highly contaminating slides and cover slips are, however, destroyed. Commonly used microscope slides and cover slips must be cleaned thoroughly before use. This avoids biohazard and also facilitates the making of better smear and avoids false reports. The slides must be grease-free for their use in haematology.

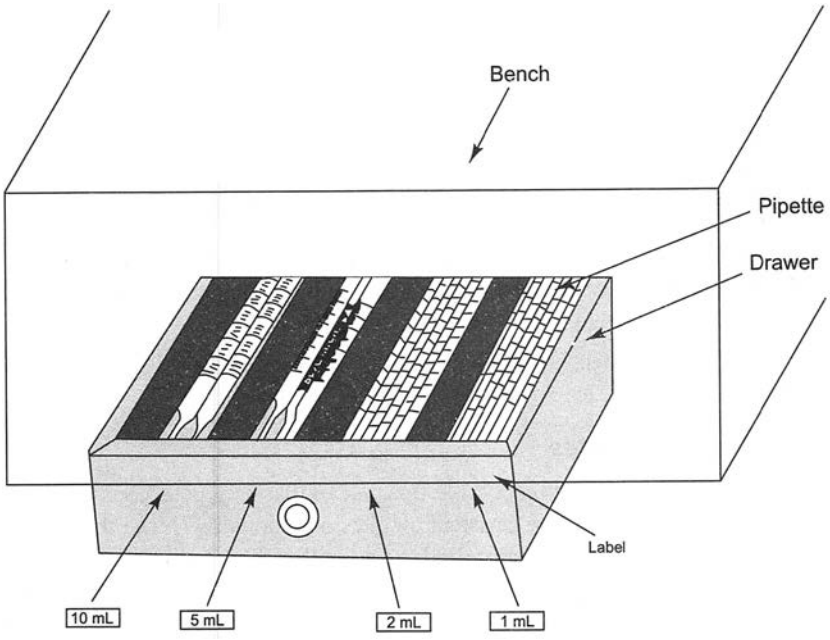

FIGURE 4.11 *Storing the pipettes in an organized manner*

Microscope slides must be free of scratches. The scratches might create artefacts. If the slides (and cover slips) are used in bacteriology or faecal examination, soak in 5% phenol or in Lysol solution (200 mL Lysol in 3 L of water). Alternatively, place the slides in glacial acetic acid for 10 min, rinse with distilled water and wipe dry with clean paper towels or cloth. Before use, wash the slide with alcohol and wipe dry. Use xylene or detergent to remove immersion oil and other material on the slide or cover slip.

New slides should also be cleaned after treating with a detergent solution. Often the new slides have undesirable residues left behind after they leave the factory. Keep the clean slides in paper wrap after dividing them into piles of 10 or 20 slides. Many laboratories number the slides before putting them in circulation. Etched numbers are permanent and are not destroyed by stain or other reagents. The numbers are etched, using diamond pencil and the slides are kept in small individual packs of 20 or more.

Dirty slides are often covered with immersion oil. Take the oily slides one by one and rub them with newspaper to remove as much of the oil as possible. Dipping in xylene helps in clearing the oil. After removing the oil, soak the slides in lukewarm water with mild detergent. Detergent containing enzymes are excellent for removing blood films. If the slides were used for infected specimens (e.g., urine, stools), treat them with detergent before cleaning. Weak detergent solution is recommended for cleaning slides. Heavy detergent requires long wash or leaves residues. Remove the slides one by one using forceps. If you must use your fingers, pick the slides up by their edges. Rinse each slide separately under the tap, and then soak for 30 min in a bowl of water. For a quick wash, empty the bowl of weak detergent and fill the bowl with clean water. Change the water three times and shaking the bowls vigorously each time for a thorough wash.

Pick each slide individually, wipe them and dry them thoroughly and then wrap them up in small packages like the way you did in case of new slides. If the slides cannot be rearranged sequentially, they will have a number to follow which is helpful to the technicians.

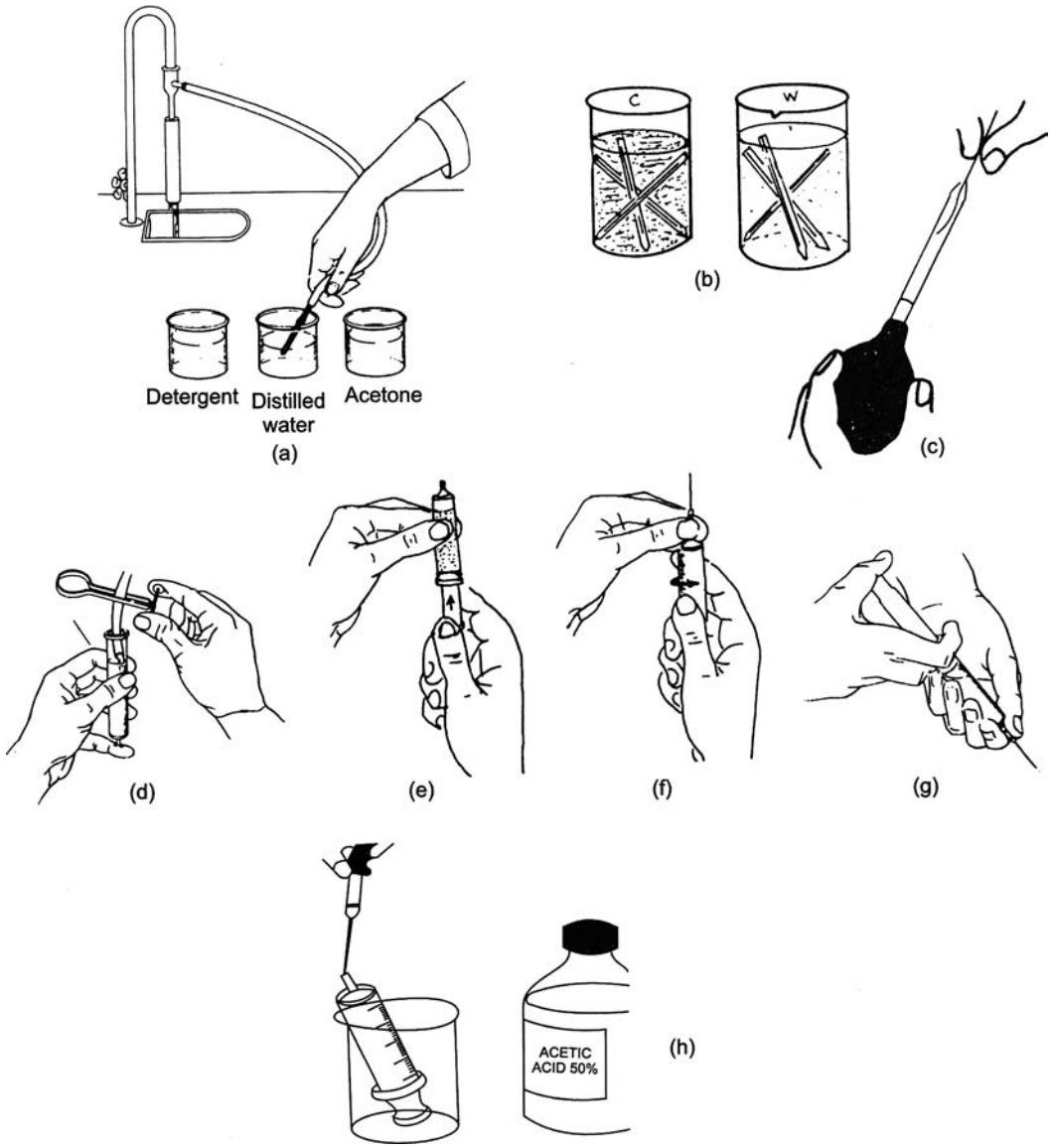

FIGURE 4.12 *Cleaning of a micropipette, (a) With the help of a suction pump suck the pipette through the following solutions in sequence – detergent, water and acetone/alcohol mixture (1:1, v/v), (b) In case of dirty pipettes, leave them in chromic acid solution overnight or longer, then soak in detergent solution and wash as described, (c) Clogged pipettes are opened up with horse's hair and then blow out; use fine stainless steel wires, if available, in place of horse's hair, (d) Syringes are cleaned first by filling with distilled water, (e) then put the piston, and (f) fit the needle, (g) Finally push out the distilled water through the needle, (h) Cleaning a blocked (reusable) syringe using acetic acid*

Special care is needed to recover the **cover slips**. The following steps are taken for the recovery of cover slips:

1. If the cover slips are stuck to slides they are first separated with the help of a needle and dropped in a mild detergent combined with disinfectant—one by one.

2. Make up the following solution in a large beaker and drop the cover slip into this solution: 200 mL of water, 3 mL of liquid detergent, 15 mL of household bleach (calcium or sodium hypochlorite) or 5 mL of quaternary ammonium disinfectant (further described in the following pages, under disinfectants).
3. Leave the cover slips to soak for 2–3 h, shaking gently from time to time.
4. Then rinse out the beaker containing the cover slips with tap water four times, shaking gently each time.
5. Give a final rinse with demineralized water.
6. Drain the cover slips by tipping them out carefully on to a pad of gauze.
7. Dry in a hot air oven at 60°C, if possible.
8. Keep clean, dry cover slips in a small Petri dish. If possible, use special cover slip forceps for taking them out.

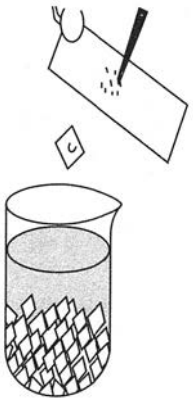

FIGURE 4.13 *Cleaning of cover slips*

Alternatively Dip the cover slips, used for oil immersion, in xylene and the leave in Lysol (or in any disinfectant) until ready to clean. When the container is half full with the cover slips, wash them in running tap water and finally rinse with deionized water. Separate them out, lay them flat on a piece of paper and dry them in an oven or polish them carefully with a piece of dry cloth.

Caution The cover slips are very thin and liable to break. Be careful in handling them. It is good practice to use forceps in handling cover slips while cleaning. Broken cover slips are discarded.

Cleaning of miscellaneous glassware

Haemocytometers are cleaned with mild detergent (2% sodium bicarbonate), followed by rinsing with cold water and dried with soft cloth or a piece of absorbent paper (sanitary towel). Do not rub the ruled portion of the chamber and do not touch this with the hand.

Wintrobe tubes pose a special problem in cleaning because of their narrow opening (Figure 4.14). After use, immerse the Wintrobe tubes in the upright position in a beaker and with the help of a Pasteur pipette fill the tubes with a mild detergent. Red blood cells will be immediately haemolysed. While handling the tubes, wear gloves in order to avoid biohazard. After immersing in the detergent for a while, hold the beaker under running tap water. The tubes will be fairly clean. Use the Pasteur pipette to empty the contents. If necessary, re-use the detergent to clean the inside with the help of the Pasteur pipette. Washing the narrow Wintrobe tubes requires a fine jet of water coming from the tap water through a Pasteur pipette or a special device (Figure 4.14). Finally, wash the tubes individually with deionized water and then dry in a hot air oven.

Glassware **contaminated** with an infectious agent must first be disinfected with 5% phenol before it is cleaned. If possible, boil the glassware, along with the disinfectant, on the stove for 30 min. Chemical cleaning of the glassware may not be necessary in case of their use in the microbiology laboratory; however, the glassware must be sterilized in an autoclave before it is re-used. Containers holding sputum specimens should be autoclaved for 30 min. Disposal of other specimens has been discussed separately.

Reusable **glass syringes** and needles should be kept clean and ready for use all the time. It is good practice to remove the plunger from the used syringe as soon as a sample has been

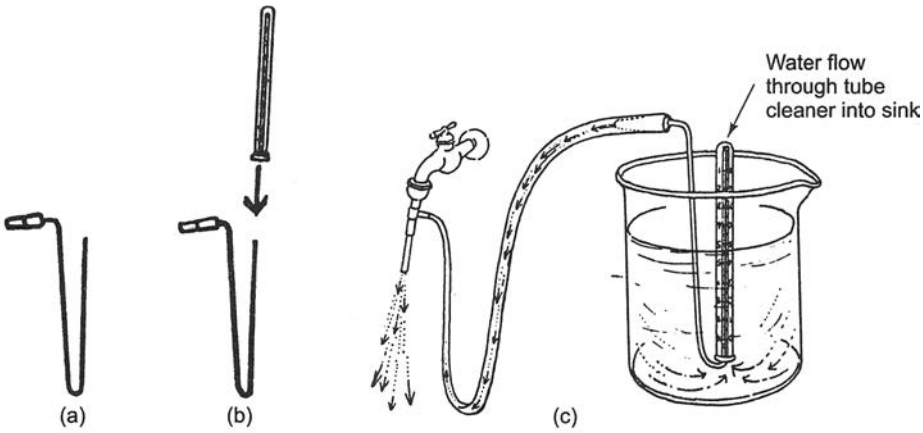

FIGURE 4.14 *Cleaning of Wintrobe tube: (a) Use special device with bent narrow metal tube*

Note: Bent glass tubing can be used but it breaks easily, (b) Place the Wintrobe tube on the metal tube, and (c) Clean the Wintrobe tube by suction of the water kept in a beaker

collected. Fill the barrel with water (Figure 4.12) or mild detergent, insert the plunger and force the water through the needle. Finally remove the needle and rinse the hub cavity. Do not let the specimen dry on the glass surface. Keep the set ready for use after sterilization while wrapped in paper.

Tips to release blocked piston: To loosen the piston, choose one of the following methods: (a) soak for 2 h in hot water (about 70°C). Stand the syringe on its end, piston down, (b) Pipette 50% acetic acid solution into the nozzle of the syringe with a fine Pasteur pipette. Leave for 10 min. After loosening the piston, soak the syringe for several hours in a bowl of 1 mmol/L hydrogen peroxide.

Tips to release blocked needle: Always rinse the needle after it is used while it is still attached to the syringe, then remove it and leave it to soak in hot water. To remove the blockage, use a nylon thread dipped in 50% acetic acid solution. Alternatively, you can use a stylet.

Use of disinfectants

The role of disinfectants is to destroy infectious agents. There are a number of disinfectants used in the laboratory. They include cresols, Lysol, iodine, household bleach (sodium and calcium hypochlorite), chloramines, calcium hydroxide, quaternary ammonium compounds and alcohols. As sodium or calcium hypochlorite is most commonly used, we will discuss their uses here. Others will be discussed in their respective places.

Sodium and calcium hypochlorite solutions (household bleaches) are very strong disinfectants. They are used in a number of laboratory and household applications. Hypochlorites are rapidly inactivated by particles of dust and organic materials and must be freshly prepared from stock solutions every day. Hypochlorites cause irritation of the skin, eyes and lungs. Strong undiluted solutions, as sold commercially, should contain 10% available chlorine. For preparing working dilutions, the following dilutions are recommended: Calcium hypochlorite is available in its solid form as powder or granules. It decomposes at a slower rate than sodium hypochlorite. A solution of 1% available chlorine is obtained by dissolving 14 g of calcium hypochlorite in 1 litre of water.

1. *Cleaning of glassware, slides and for swabbing bench surfaces:* 10 mL concentrated hypochlorite solution in 990 mL of water. This is equivalent to 0.1% available chlorine. Leave

the glassware in this solution for at least 12 h. Do not overfill the container. Change the container daily.

2. *For decontamination of blood spills and other specimens with high protein content:* 40 mL of concentrated hypochlorite solution in 360 mL of water (1% available chlorine).

Caution Calcium hypochlorite solutions are corrosive and can cause burns. Handle solutions of bleach carefully: wear rubber gloves to protect the hands and eye shields to prevent splashing in the eyes.

Filtration

Filtration is the process of separating solid particles from the liquid in which they are suspended. In this process the suspension is poured on a porous material called filter. The liquid passes through (called filtrate) and the filter retains the solid particles (called residue). Filter paper is most commonly used for filtering. It is folded (Figure 4.15) and placed on a funnel. The filter paper size should be large enough to fit into the funnel; it should never extend over the top.

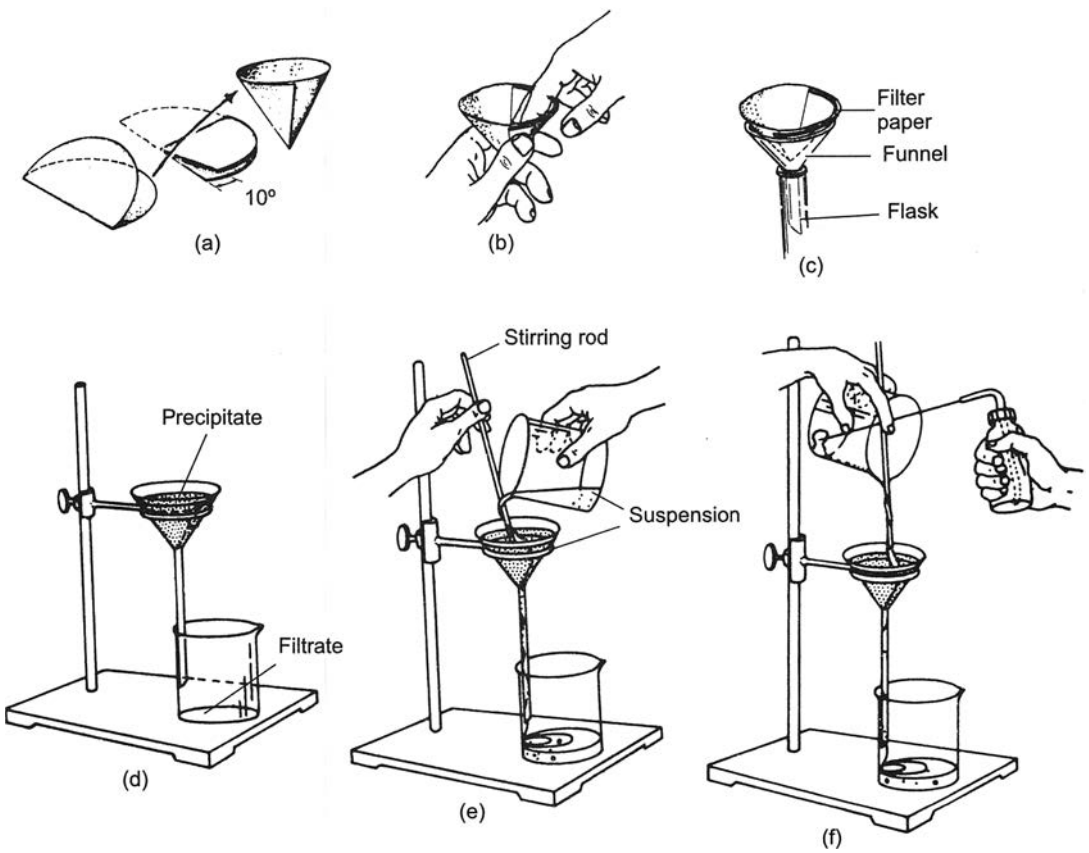

Figure 4.15 *Filtration technique: (a and b) Fold the filter paper in four quadrants, (c) Place it in the funnel. The funnel can be placed directly on a volumetric flask to catch the filtrate, (d) or on a funnel stand for filtration, (e) Use a glass rod to transfer the liquid and keep the stem touching the wall of the beaker, (f) Squirt distilled water with the help of a wash bottle in order to transfer precipitate from the beaker to the funnel. In case of quantitative transfer, take special care not to splatter the solution while transferring.*

Folding of the filter paper

Folding of the filter paper is done in two steps—first, fold the circular filter paper into a semicircle of two leaves and then fold this into a quadrant of four leaves. The folded filter paper is half-opened which gives it a cone shape with one side having three leaves and the other side one leaf. This is then fit snugly into the funnel.

Filtration Procedure

1. Hold the funnel on a funnel stand.
2. Insert the folded filter paper into the funnel and place a beaker under the funnel to catch the filtrate.
3. Moisten the filter paper with a little of the material to be filtered to make it adhere firmly. Use of water changes the concentration of the filtrate.
4. Slowly pour the liquid onto the portion of the filter paper consisting of three leaves. Do not pour directly on the apex of the cone but on the side. Fill the funnel as much as possible without overflowing.
5. Catch the filtrate in the receiving beaker while the stem of the funnel should rest against the side of the beaker (or other container) so that the filtrate runs down the wall without splattering.

Note

- If the funnel rests on the neck of the receiving vessel (e.g., volumetric flask), some provision must be made for the air to escape or the liquid will not pass through the neck of the funnel. Occasionally, an air bubble may try to push through the flowing filtrate if there is not enough space left for its escape. To avoid this, use a funnel with a narrow stem and do not allow the funnel to sit snugly on the neck of the receiving vessel.
- Filtration may be accelerated by the use of plaited filter paper. This is prepared as follows: Fold the filter paper into quarter as before and then continue folding to eighths and sixteenths of a circle, making each fold in the same direction. Now double each division upon itself in the opposite direction but do not crease all the way to the tip, because this weakens the paper.

Pipetting

Before using the pipette, familiarize yourself with the type of pipette you are using (TC, TD or serological) and also the graduation (Figure 4.16). If you want a single volume, use a volumetric pipette (Figure 4.17). If repeated amounts of the same volume are to be taken (e.g., 1 mL for 10 times), it will be faster with a graduated pipette (10 mL). A

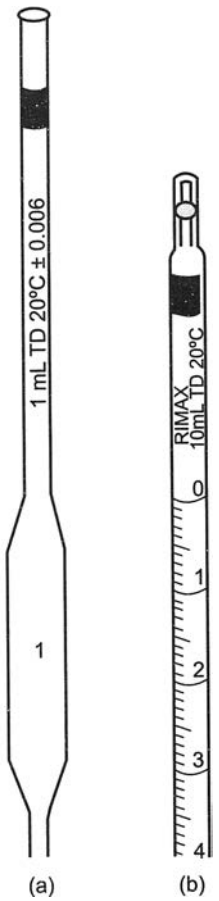

FIGURE 4.16 *Calibration markings of (a) volumetric pipette, and (b) graduated pipette. The divisions of the graduated pipette are equivalent to 0.1 mL.*

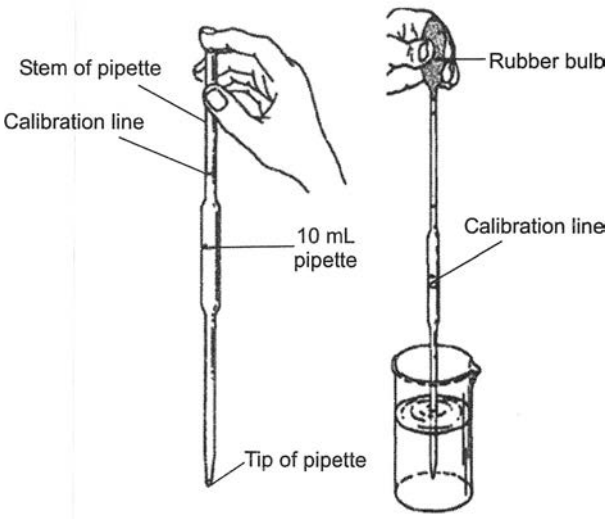

FIGURE 4.17 *Components of a volumetric pipette, (a) Finger control for dispensing fluid is done with mouth pipetting. This is discouraged in clinical laboratories, (b) Instead, one should use various devices available for automatic pipetting.*

graduated serological pipette can be used but when it reaches the end, it should be blown out. This might cause some error for high precision work. On the other hand, the serological pipettes are ideal for titre dilutions. Also check that the pipette is perfectly clean and no droplets of the dispensing fluid should remain on the sides of the pipette.

Use of volumetric pipette

The structural components of a volumetric pipette are shown in Figure 4.17. Following steps are involved in the pipetting techniques (Figure 4.18):

1. Hold the pipette lightly between the thumb and the last three fingers, leaving the index finger free.
2. Using a mechanical suction device or an aspirator bulb, draw the liquid up into the pipette until the liquid is well above the calibration mark.
3. Remove the bulb end quickly and cover the suction opening at the top of the pipette with the index finger.
4. Wipe the outside of the pipette with a piece of gauze or tissue paper to remove excess fluid.
5. Hold the pipette in a vertical position with the delivery tip against the inside of the original vessel. Carefully allow the liquid in the pipette to drain by gravity until the bottom of the meniscus is exactly at the calibration mark. The meniscus is the concave surface of the clear liquid. To get the meniscus to the graduation mark, do not entirely remove the index finger from the suction-hole end of the pipette, instead rolling the finger slightly over the opening; allow slow drainage to take place. If there is a hanging drop of fluid, touch the tip of the pipette to the side of the vessel in order to dispense the excess fluid.
6. Transfer the pipette into the receiving flask while holding the pipette in a vertical position or slightly inclined, and touch the tip of the pipette to the inside wall of the receiving vessel. Remove the index finger from the top of the pipette to permit free drainage. Keep the pipette close to the vertical position for correct drainage. The dispensing fluid trickles down the wall of the receiving vessel. To be certain that the drainage is as complete as possible, touch the delivery tip of the pipette to another area on the inside

wall of the receiving vessel. A to-deliver (TD) pipette retains a drop of the fluid at the tip; do not blow it out.

7. Remove the pipette from the receiving vessel and lay it down on a pipette holder if it is to be re-used or discard it into the container with detergent solution.

Sources of error

- Always wipe off the tip of the pipette to remove the liquid adhering to the outside of the pipette in order to avoid the 'carry over' volume.
- Never allow the tip of the pipette to touch the liquid of the original flask or receiving flask while dispensing; only touch the dry side wall of the vessel.
- With clear liquid, read the meniscus at the bottom, with the eye at the level of the meniscus (Figure 4.18). For coloured and viscous solutions, top of the meniscus must be read. Keep a white paper at the back if the meniscus is not clearly seen.

Calibration of pipette

After receiving a new batch of pipettes they must be calibrated (at least a few at random). This is particularly true for micropipettes, automated or manual. This is because the volume dispensed by the micropipette (e.g., specimen volume) multiplies the error when the final value is expressed in mg/100 mL or per litre. For calibration purposes, 20°C is considered to be the standard (marked on the glassware); however, the laboratory temperature is often above 20°C in developing countries. Hence, adjustments have to be made during calibration.

There are two methods of calibration: Gravimetric or weight method and Photometric method. Only the gravimetric method will be described here because of its simplicity. Photometric method, however, is recommended for TC pipettes. If the following method is adopted for the calibration of TC pipette, do not use any weighing vial (Step 1). Instead, pre-weigh the pipette (W_1), fill it with water and re-weigh (W_2). The difference of weight ($W_2 - W_1$) will be the weight of the water held by the pipette.

Gravimetric method

One gram of water occupies 1 mL volume at 20°C (Table 4.1). The weight of water varies with the temperature and for the same volume of 1 mL, it weighs less at temperature >20°C and more at lower temperature. The weight of water dispensed by the volumetric glassware (TD) should correspond to its volume following temperature correction.

Procedure

1. Take the weight of a weighing vial (W_1).
   Note If you are using a modern balance, take the weight of the vial.
2. Take the pipette to be calibrated and draw distilled water up to the calibration mark. Wipe the outside surface and dispense the distilled water held by the pipette into the pre-weighed vial.
3. Weigh the weighing vial with distilled water dispensed by the pipette (W_2).
4. The difference of weights ($W_2 - W_1$) is the weight of water dispensed the pipette.
5. Find out the actual volume of water (V_a) held by the pipette at the calibration temperature of 20°C, which may be different from the volume claimed by the manufacturer (V or stated volume).

Note Measure the volume of water dispensed by the pipette at least three times and take the average volume for the following calculations. Each time the water is dispensed note the increase of weight.

$$V_a = \frac{W_t}{F}$$

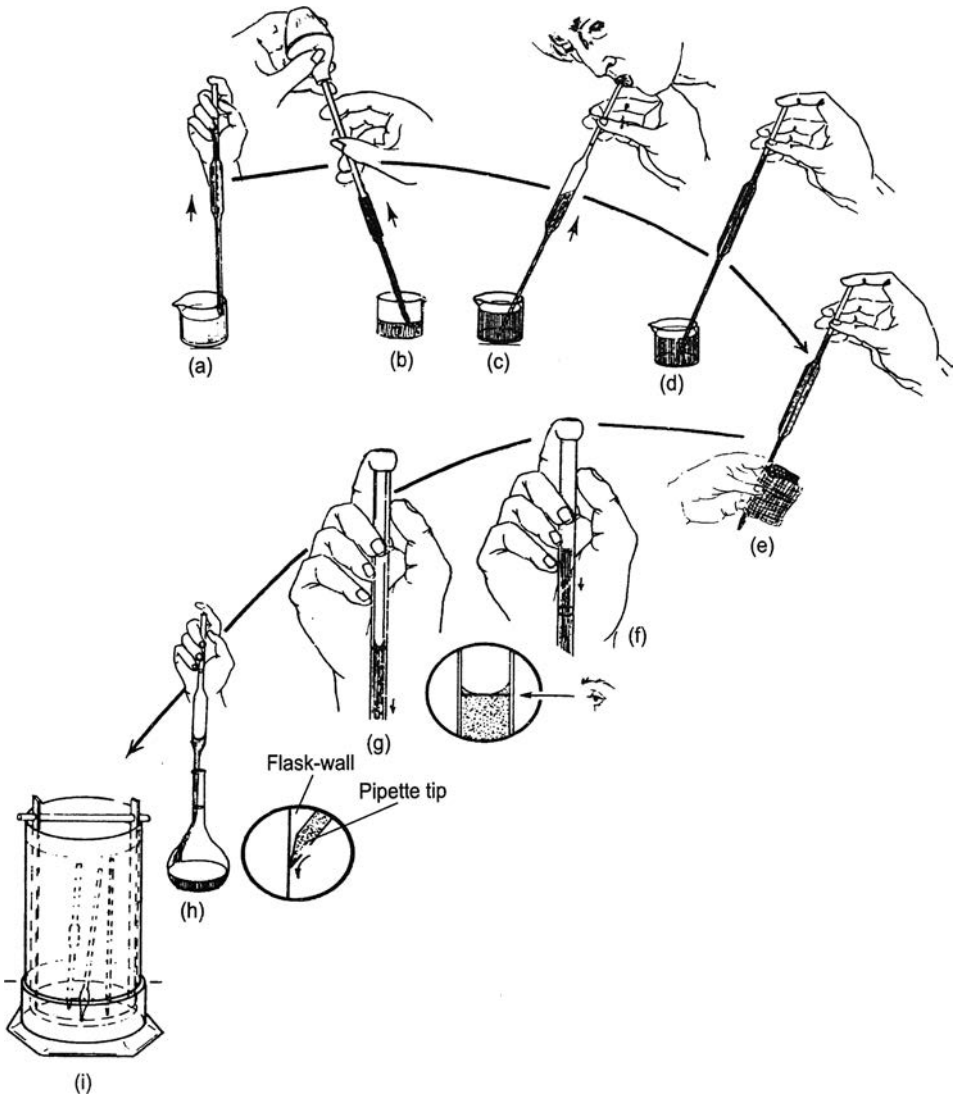

FIGURE 4.18 *Pipetting techniques: Suck the fluid with bulb or mouth (a and b). Note: Mouth pipetting is not recommended (c). Allow the fluid to pass the calibration mark (d). Hold the liquid by closing the sucking end with the fingertip and wipe the tip with a piece of cloth (surgical gauze) or absorbent paper (e). Then bring down the level of fluid gently, allowing air to leak through the fingertip (f) until the meniscus touches the calibration mark (g). Finally, take off the finger and allow the liquid to flow down the wall of the flask (h). After use, drop the pipette in the container with detergent. If the container is made of plastic, keep the tips of the pipettes facing down (i). If the container is made of metal, the tip should face up in order to avoid breakage of tip. The container should have sufficient detergent to keep the pipettes submerged.*

Where, V_a = Actual volume of water held by the pipette at 20°C

W_t = Weight of water dispensed by the TD pipette at the existing room temperature $(W_2 - W_1)$

F = Weight of 1 mL of water at the existing room temperature (consult Table 4.1)

TABLE 4.1 *Weight of 1 mL of water (F) at different temperatures*

Temperature (°C)	F(g)	Temperature (°C)	F(g)
15	1.0008	23	0.9994
16	1.0006	24	0.9992
17	1.0005	25	0.9989
18	1.0002	26	0.9987
19	1.0001	27	0.9984
20	1.0000	28	0.9982
21	0.9998	29	0.9979
22	0.9996	30	0.9976

Calculation of error

The error inherent in the volume stated by the manufacturer (V_a) is calculated as follows:

$$\text{Error (\%)} = \frac{D}{V_s} \times 100$$

Where, D = Average difference between the corrected volume (V_a) and stated volume (V_s)
V_s = Stated volume of the pipette

If the percentage deviation (error) is greater than ±5.0%, the pipette should not be used.

Note If the room temperature is under 25°C, ignore the temperature correction.

Precision check of pipette

The pipette can also be checked for precision by pipetting water into a weighing vial and weighing the amount delivered. The procedure is repeated 10 times. The 10 data points are used to compute the coefficient of variation (CV) for the pipette. Acceptable precision should be at least 5% with lower precision levels (i.e., 1–2%) being preferred. Method of calculating CV, mean and SD is presented in Chapter 7.

$$\text{CV (\%)} = \text{SD}\sqrt{\overline{X}} \times 100$$

Where CV = Coefficeient of variation
$\overline{X}$ = Mean of 10 data points
SD = Standard deviation of 10 data points

Example Weight of water (W_t) dispensed by 5 mL pipette (V_s, as claimed by the manufacturer) is 4.981 g. The temperature of the laboratory is 26°C. What is the actual volume (V_a) of the pipette? Determine the error.

$$\text{Actual volume } (V_a) = \frac{W_t}{F} = \frac{4.981}{0.9987} = 4.987$$

$$\text{Difference } (D) = V_s - V_a = 5.0 - 4.987 = 0.013$$

$$\text{Error (\%)} = \frac{D}{V_s} \times 100 = \frac{0.013}{5.0} \times 100 = 0.2$$

Use of capillary pipette

Use of capillary pipettes (to-contain, TC) for dispensing small volumes of liquid is becoming popular (Figure 4.19). The pipette is filled by capillary action without any other aid. If mouth

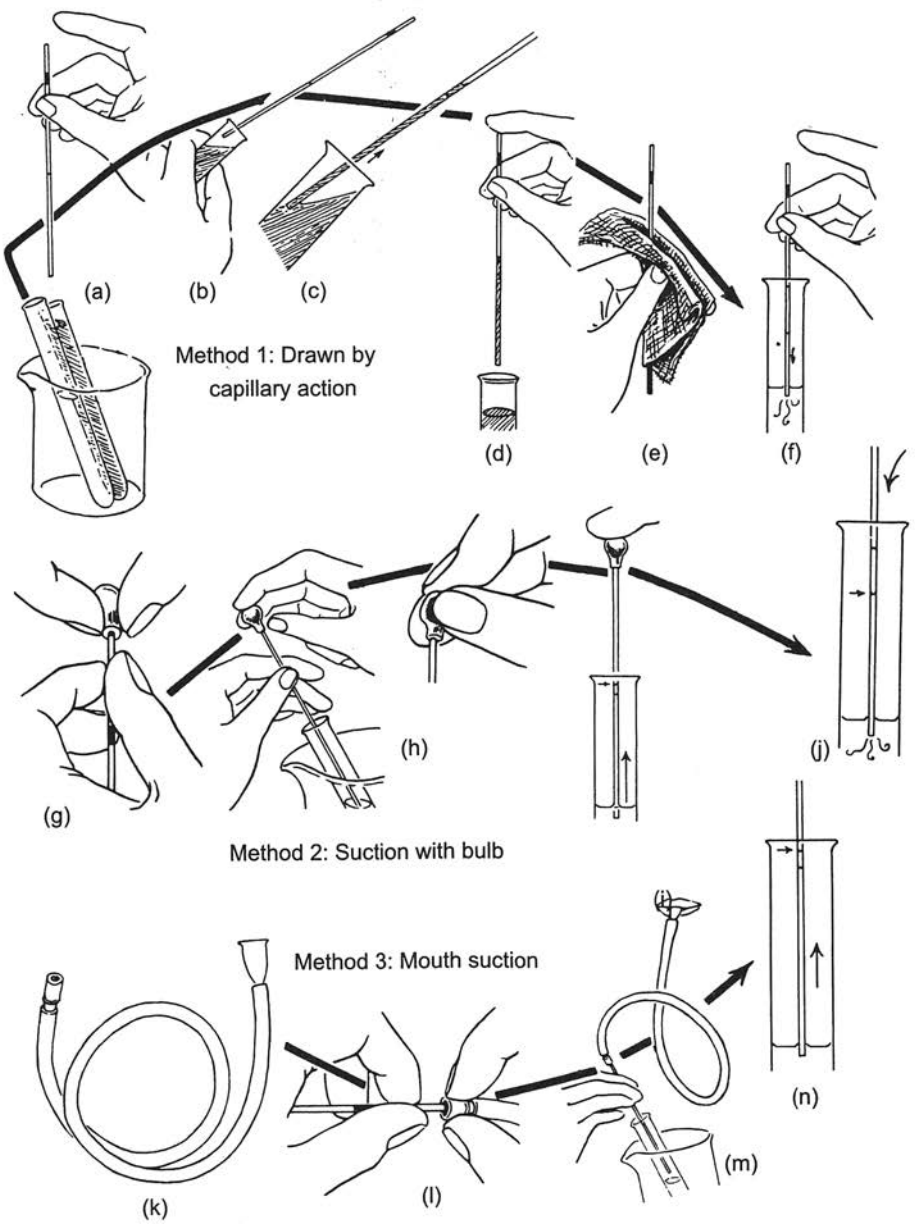

Figure 4.19 *Use of capillary micropipette, (a-d) Draw the fluid by capillary action up to the calibration mark, (e) Wipe the tip, and (f) Let the fluid flow out by the action of gravity.* ***Method 1*** *Wash the micropipette with the diluent by repeatedly dipping in and out. While dipping, make sure that the fluid level reaches the calibration mark in order to ensure complete washing.* ***Method 2*** *A special bulb with a small hole on top (other than the hole to hold the capillary micropipette) is used to create suction or for blowing out the air (g-i). For suction: squeeze the bulb (g), close the hole (h) and then release the squeezed bulb (i). For blowing out: close the hole and squeeze the bulb which is the opposite of suction (j).* ***Method 3*** *Suction tube is also used for mouth pipetting (k-n), which should be done with caution.*

suction is desired (not recommended) use suction tubing. Special suction bulb can also be used. In either case, wash out the capillary pipette with the diluent in which the aliquot is dispensed.

Calibrating droppers

The **droppers** are most commonly used for transferring fluids but it can also be used for measuring volumes of fluid. Use of dropper for measuring volume is not very common and is applicable only if the amount is not that critical. For example, for making increasing dilutions in ceramic cups during the estimate of titre, drop dilutions are convenient. One drop of serum mixed with one drop of saline is 1:2 dilutions. The approximate volume of each drop, delivered by the dropper, can also be determined by the following way:

Ordinary calibrated dropping pipettes (Figure 4.20) deliver 20 drops per mL of distilled water which is equivalent to 0.05 mL for each drop. This is variable and is determined by several factors like size of the bulb, diameter of the opening, etc. However, you can calibrate your own dropper (Figure 4.20):

1. Draw in distilled water to the maximum amount the pipette can hold.
2. Hold the dropping pipettor absolutely vertical to expel the drops.
3. Dispense the water in drops in a pre-weighed container which is weighed again after collecting the water.
4. If the number of drops is known and the weight of the water dispensed is known ($W_2 - W_1$, difference between final weight or W_2 and initial weight or W_1), the volume per drop can be determined by the following formula.

$$\frac{\text{Increase in weight } (W_2 - W_1)}{\text{Number of drops}} = \text{Volume of each drop of water}$$

We are assuming here that the density of water is 1 (1 mL if water weighs 1 g) at room temperature.

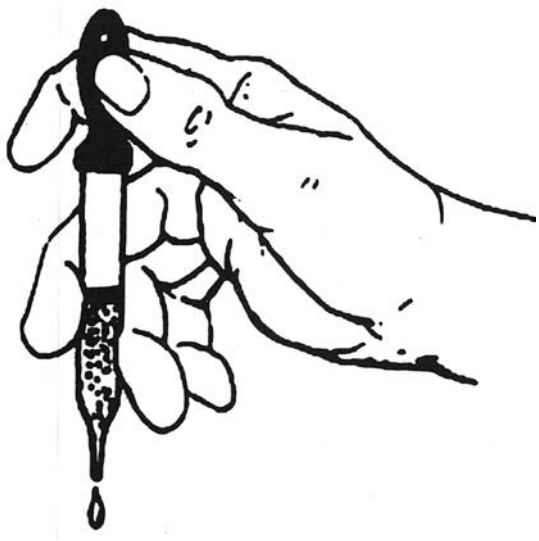

FIGURE 4.20 *Measuring the volume of fluid by drops*

Use of Vernier Scale

Taking readings from an instrument without Vernier scale involves some amount of guesswork (Figure 4.21). First check the graduation markings and find out the value of the subdivisions. If the pointer lies between the graduation marks, first read the marking provided on the scale and then guess the subsequent value after the decimal, considering 10 imaginary subdivisions. If a Vernier scale is provided, read the highest graduation marking on the main scale which is below the '0' mark of the Vernier scale. Then switch to the Vernier scale and tally the markings on the Vernier scale in increasing order until the first graduation mark of the Vernier scale coincides with any of the graduation marking on the main scale.

Note The above description of Vernier scale is applicable to old instruments (which are still in use in many developing countries). All modern instruments, however, have digital display of the reading.

Titration

Titration is a volumetric technique by which concentration of an unknown solution (usually an acid or base) is determined by reacting with a corresponding neutralizing solution of known strength (called standard solution). The end point of neutralization is marked by the change of colour of an indicator added to the titrating system.

Procedure

1. Prepare the burette for the titration. The burette should be clean, free from chips or cracks and the stopcock should turn easily and smoothly. If necessary, lubricate the stopcock lightly. To grease a clean stopcock, apply a bit of grease with the fingertip down the two sides of the stopcock away from the capillary bore. Then insert the stopcock in the burette and rotate it until a smooth covering of the whole stopcock is obtained. If the burette is equipped with a Teflon plug (plastic), the stopcock need not be lubricated. Rinse the burette with a little amount of the titrant which will later be used to fill the burette. Rinsing is done by rolling the titrant over the inside wall and discarding the rinsed solution through the stopcock. During rinsing, watch if the burette is clean.

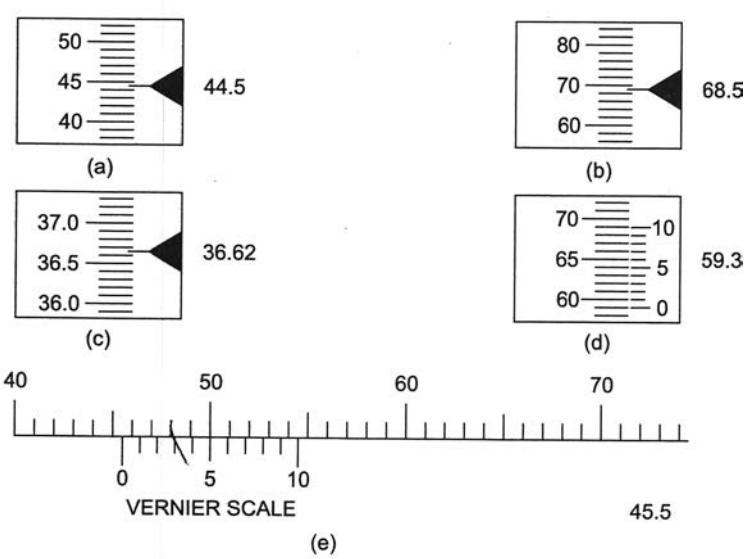

FIGURE 4.21 *Taking the reading (a-c) without Vernier scale, and (d and e) with Vernier scale*

A clean burette drains without any solution clinging to its sides; if the burette is dirty, there will be droplets of liquid clinging to the sides.

2. Fasten the burette clamp to the burette stand.
3. Fill the burette slowly and carefully with the titrant (unknown). Do not allow air bubbles to form inside the burette. A small beaker may be used during pouring and allowing the titrant to flow down the inside wall of the burette. Fill the titrant past the zero mark of the burette and then bring the meniscus exactly to the zero mark by draining. Use the stopcock to control the flow of the titrant (Figure 4.22). Collect the drained titrant in the beaker used for filling the burette.

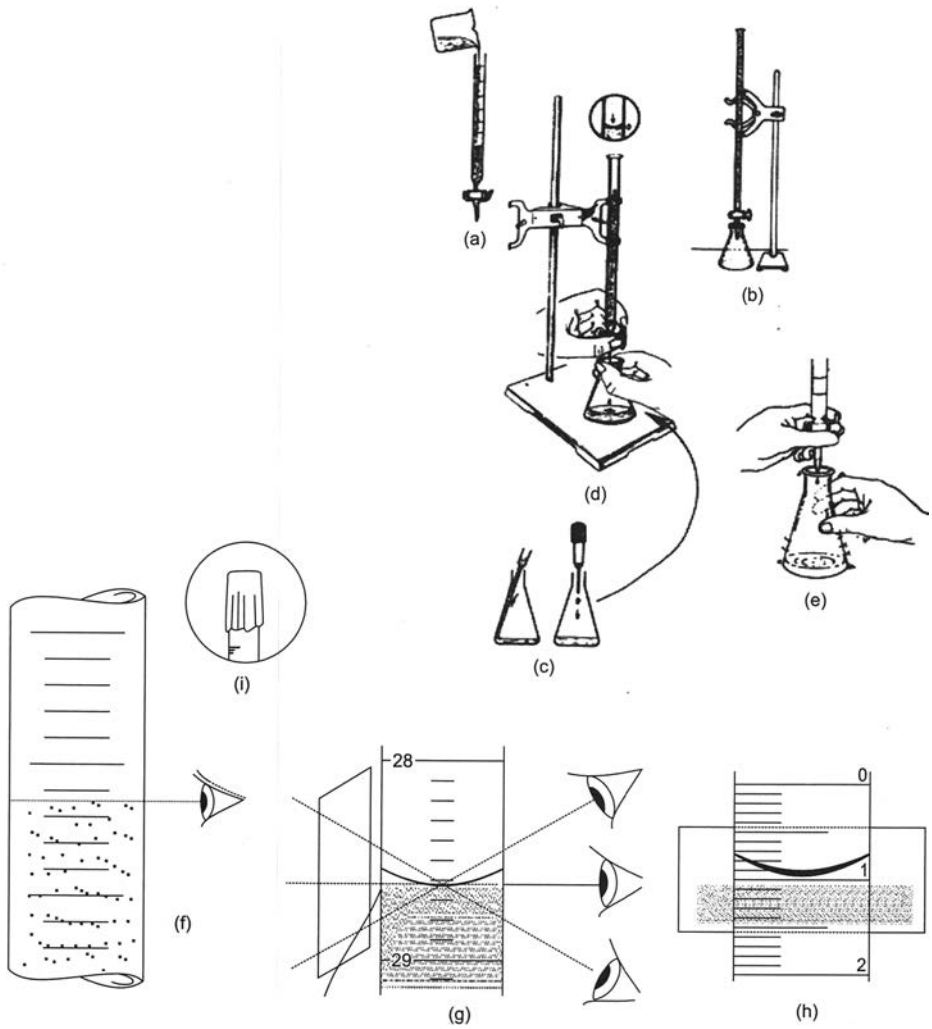

Figure 4.22 *Titration technique: (a) Fill the burette with the titrant or solution of known strength, (b) Fix the burette to the stand and remove the air bubble and bring the liquid level in the burette to the '0' mark, (c) Transfer a known volume of titrating fluid and a few drops of indicator in an Erlenmeyer flask, (d) and then proceed with titration by letting the titrant to flow out of burette into the flask, (e) Swirl the flask during titration, (f) Stop adding the titrant into the flask when the colour of the indicator changes. Read the level of the fluid in the burette at the meniscus, (g) Avoid parallax error, (h) A white paper on the back is helpful in taking the burette reading, (i) Cover the burette after use*

4. Measure the second solution (standard) into an Erlenmeyer flask (or beaker) with a volumetric pipette. Take extreme care to measure the volume accurately. Add the required amount of the indicator solution into the flask. If the volume is low, you can add 5–10 mL of distilled water to dilute the volume of fluid in the flask. The volume of the diluent is not critical, since it does not enter into the reaction or affect the volumes of the solutions that are being titrated. You can arrange three flasks for getting three titration results consecutively.
5. Before starting the titration, inspect the burette carefully for any air bubble trapped within the burette column or inside the stopcock or on the tip. Presence of an air bubble adds the air-volume to the volume of the titrant as an error. If air bubbles are present at the narrow end of the burette, drain the burette until the bubbles are all out and then refill the burette with the titrant. Finally, bring the meniscus to the zero mark. Keep the tip of the burette well within the Erlenmeyer flask, which contains the fluid to be titrated (standard) and clamp the burette at this position. Put one hand on the stopcock and use the other to swirl the flask. This will be awkward at first, but when practised it becomes natural.
6. Titration is done by adding the solution in the burette to the Erlenmeyer flask by rotating the stopcock carefully. For accurate reading, titration is done at least three times. Take three Erlenmeyer flasks in step (4) for three replicates. The titration readings of three replicates must be close; the second and the third readings should be almost identical,
7. Clean up the burette following titration by thoroughly rinsing the burette several times with tap water followed by rinsing with deionized or distilled water. Dry the burette by holding it on the burette stand in the inverted position with the stopcock open. Alternatively, the burette can be clamped to the burette stand in the upright position filled with deionized or distilled water. Never leave the titrant in the burette for an extended period after completing the titration. If the titrant is an alkali, it might 'freeze' the stopcock. In addition, the concentration of the titrant changes due to the evaporation or absorption of atmospheric carbon dioxide (in case of alkali). Cover the burette between titrations.
8. Calculate the strength of the unknown by the following formula:

$$\text{Conc. of unknown } (N) = \frac{\text{Volume of titrant} \times \text{Conc. of titrant } (N)}{\text{Volume of unknown}}$$

Common sources of error in titration

Common sources of error in titration include use of a dirty burette, presence of air bubbles in the burette, improper measurement of volume (in the burette or the pipette), improper way of taking readings, inaccurate determination of the end point and mistakes in calculation.

Helpful hints in handling stopcocks and joints

As stopcocks frequently pose problems for the user, the following notes may be helpful. Lubricate the stopcock before use. Hydrocarbon grease is recommended which is easily removed by an organic solvent (e.g., acetone or xylene). When storing burettes and other apparatus with glass stopcocks and joints (e.g., separating funnels), insert a thin strip of paper between the ground joint surfaces to prevent sticking. It is advisable to remove the lubricant or grease from the ground surfaces before storage. The modern plastic stoppers are more convenient to use and pose fewer problems.

Occasionally a ground-glass stopper or stopcock becomes firmly fixed in its socket and cannot be turned or removed. There are many ways of dealing with this problem (as discussed later). The quickest method is to tap the stopper or stopcock socket flange on a wooden surface or gently heat the socket (not the cone) in a localized flame. If the stopcock becomes loose

in its socket, put an appropriate rubber ring at the end that sticks out of the stopcock socket. Most stopcock plugs are made with a depression or channel cut in the small end of the plug for this purpose.

Storage, Handling and Preparation of Laboratory Reagents

Proper handling of laboratory reagents during storage and laboratory use is essential in order to maintain their purity and to prevent their deterioration. Reagents are of different grades with increasing purity—commercial grade, laboratory grade and analytical grade. The chemically pure (CP) or analytical reagent (AR) is marked on the bottle. Cost of the reagent increases with purity. You must know which kind of chemical is to be used. In preparing cleaning solution, the commercial grade is good enough, while in preparing a standard solution, the purest grade of the chemical is desirable. Always read the label provided by the manufacturer before using the reagent. The label clearly gives the nature of the reagent (flammable, poison), its storage condition (room temperature or refrigerator), formula weight, per cent impurity, concentration, etc. Chemicals must be stored according to the manufacturer's directions.

Storage of Reagents

Proper storage of laboratory reagents is necessary for maintaining the quality of the reagents. Reagents are usually stored on the shelf at room temperature. Arrange them alphabetically so that they can be located easily. Always replace the bottle promptly when the bottle is empty. Regularly check the inventory (Chapter 3). Orders for new bottles of reagent must be placed in advance so that the work does not suffer due to lack of planning. In general, put the hygroscopic chemicals in the desiccator; dry chemicals in a cool dry place; chemicals that deteriorate at room temperature in the refrigerator; flammables away from the naked flame; and volatile substances, flammables and ohemicals with irritating fumes should be handled under a fume hood. Storage conditions of some reagents are given below:

1. Store acids in glass-stoppered bottles preferably in a drip tray. Big-size Winchester bottles are stored at the floor level.
2. Store alcohols under lock and key and all details of their use should be recorded. Customs and excise officials periodically inspect the duty-free alcohol.
3. Label all poisonous chemicals **(POISON)** with red ink and keep them locked. Maintain a record of the amount issued and to whom it is issued.
4. Store flammables securely in metal containers if they are in larger quantities. Smaller quantities for daily laboratory use are kept in glass bottles with wax-lined or cork-lined bakelite screw caps. Never store flammable liquids and solvents on the bench tops or shelves over benches. They should be placed away from the flame, heat, sunlight and electrical switches. The cupboard under the bench is quite safe. Most organic solvents attack rubber and rubber bungs, and these should not be used. All containers with flammables must be clearly marked as **'FLAMMABLE'** with red ink. Never store flammables in an ordinary refrigerator. A specially made store room with a sunken floor is recommended so that in the event of breakage no liquid flows out from the room.
5. Some chemicals like picric acid and perchloric acid can become serious explosion hazards if they are allowed to dehydrate. The quantity of these chemicals kept in the laboratory should be limited in order to use the chemicals within six months.
6. Hygroscopic chemicals, such as phenol, trichloroacetic acid, ferric chloride, sodium carbonate, sodium hydroxide, potassium hydroxide and others must be stored in air tight containers or in a desiccator (Figure 4.1). A desiccator helps to keep the reagent dry. It contains desiccant in the lower section (e.g., calcium chloride, anhydrous calcium sulphate). The lid must be greased and tightly fitting. Some desiccators are provided

with suction arrangement. The desiccant must be in active state and, in some cases (e.g., calcium sulphate), the desiccant can be revived by heating at 100°C for several hours. Presence of an indicator, such as copper sulphate crystals, in the desiccant helps to determine the hydrated (pink) and dehydrated (blue) states. Replace the desiccant when it is too old. Storing in an incubator (37°C) may help in the rainy season but some chemicals might deteriorate. Reagents, such as trichloroacetic acid and ferric chloride, which are extremely hygroscopic, might have already absorbed water when they arrive in the laboratory. In such cases prepare a concentrated solution and determine the weight of the solute in the solution from the specific gravity of the solution.

7. **Photosensitive** chemicals (iodine, silver nitrate, potassium permanganate and sodium nitroprusside) must be stored in a dark, glass-stoppered bottle, as they decompose with exposure to light. Iodine has an additional problem; it attacks rubber, hence do not use rubber stoppers in case of bottles containing iodine.
8. Solutions of sodium hydroxide and potassium hydroxide should be stored in plastic bottles. The alkali attacks glass and long storage in a glass bottle will corrode the glass and the chemical composition of the solution will change. In addition, glass stoppers may get **'fixed'** due to the formation of salt when the alkali present around the stopper reacts with the carbon dioxide present in the air. Alkali solution in daily use can be stored in an aspirator which is connected to a soda lime guard tube that will absorb and prevent any carbon dioxide from entering the aspirator.
9. Reagent solutions after their preparation should be stored properly according to the direction of the test procedure. They should be properly **labelled with date.** If the solution is stored in the refrigerator, it should be brought to room temperature before use. It is good practice to take out only the amount needed for the day and leave the stock solution in the refrigerator.

Handling of Reagents

The following are some of the important facts to bear in mind while handling laboratory chemicals (Figure 4.23):

- Replace the covers of all reagent bottles promptly and securely.
- Diluted solutions have a shorter shelf life than concentrated stock solutions. Hence, do not dilute the stock solution until needed.
- Contamination of reagents is fatal to laboratory work.
  - During the transfer of a reagent from the bottle do not place the cap on the work bench (Figure 4.23). When a reagent is removed from a bottle, never put any of it back into the bottle.
  - Insert only a clean spatula or a clean pipette into the reagent bottle.
  - If in a series of tests, repeated transfer of the same reagent is required, leave the pipette in the bottle. Do not leave the reagent bottle open (without the cap) for prolonged periods.
- When water is mixed with strong acid (e.g., sulphuric acid) or alkali (sodium or potassium hydroxide) it gets heated. Never add water to acid. Add acid into water slowly and cool the solution under running tap water (Figure 4.23).
- Procedures that involve the transfer or heating of flammable liquids should be carried out in a fume hood in order to avoid fire hazard. Using electric mantles or steam baths for heating flammable liquids reduces the chance of ignition of the vapour. Open flames, electric hot plates or other devices that have exposed hot wires or surfaces should not be used.
- Chemicals should be dated on receipt, on opening and when made up into solution or mixtures. Containers used for the storage of reagent solutions need labels that clearly

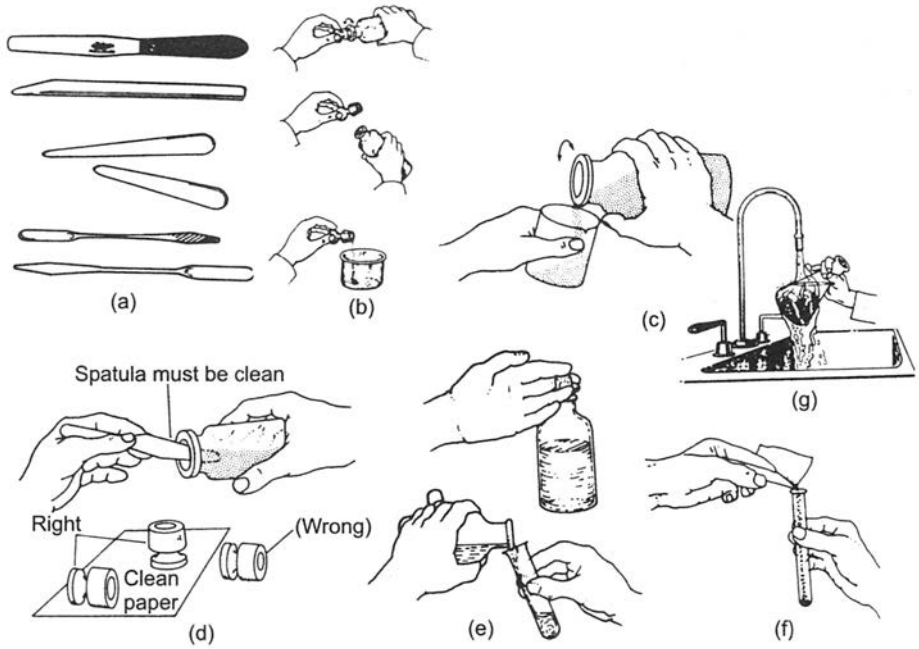

FIGURE 4.23 *Reagent handling procedure: (a) Reagent should not be touched by hand; Use an appropriate spatula, (b) the cap of the reagent bottle can be used to transfer solid reagent, or (c) the reagent bottle can be rolled on side, (d) never keep the cap directly on the table, (e) while transferring liquid reagents hold the cap between fingers, (f) Use a piece of weighing paper to transfer a solid, When mixing acid (especially sulphuric acid) and water, always add acid to water and not the other way; (g) cool the hot solution under running tap water*

indicate the contents, any specific hazard (flammable, poison, etc.) associated with the contents and the proper procedures for safe use, transfer, and disposal of the contents. Placing chemicals in bottles or cans in which other chemicals were purchased should be discouraged.

- Empty chemical containers should be thrown out into the trash baskets with their caps or stoppers off and only after being thoroughly rinsed with water.

Preparation of Laboratory Reagents

Commercially prepared kits with pre-weighed packages of solutions are becoming increasingly popular in clinical laboratories. The kits are ideal for smaller laboratories as they provide reliable reagents, save time and might prove to be cost effective. However, for bigger laboratories, except 'difficult to prepare chemicals', in-house preparation of reagents might prove to be economical. In addition, under the conditions existing in developing countries, the manufacturer may not be able to supply materials in time or reagents might deteriorate during transportation due to unexpected delays. Hence, it is essential that you learn the procedure of reagent preparation which calls for the technique of quantitative transfer.

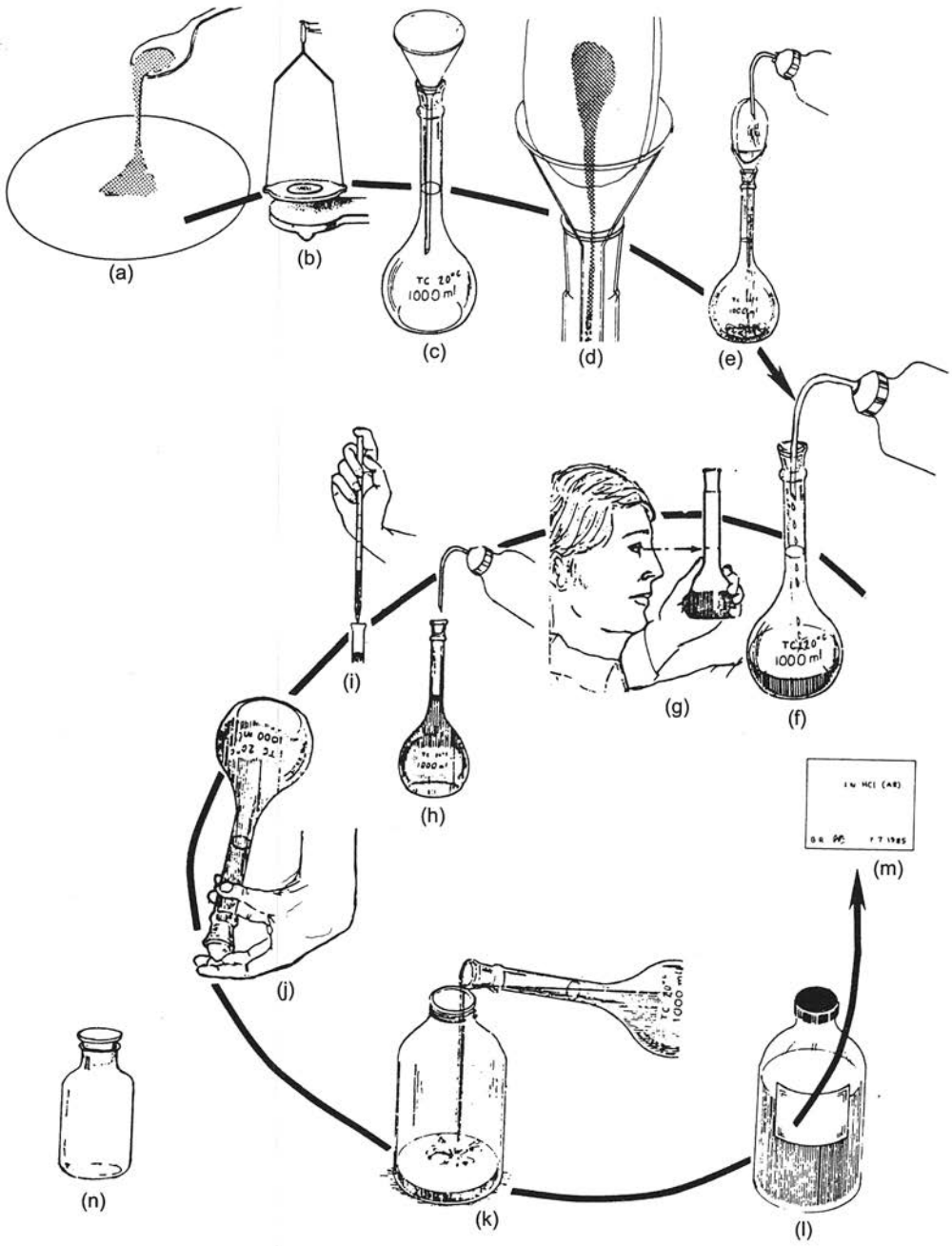

Figure 4.24 *Quantitative transfer and preparation of reagent solution, (a and b) Weigh the requisite quantity of reagent on a piece of weighing paper, (c and d) Transfer the weighed reagent into a volumetric flask; gently wash in the reagent without splattering, (e-h) Add more solvents and bring the level close to the volumetric mark, (i) Raise the meniscus and bring it in line with the volumetric mark by adding the solvent in drops, (j-l) Finally, mix the solution by inversion and transfer it to a reagent bottle, (m) Put the label, (n) Glass caps are preferred over Bakelite caps with strongly reactive reagents*

Quantitative transfer technique

Preparation of reagent solutions involves the technique of quantitative transfer which ensures the transfer of the entire quantity of solute weighed in preparing its solution (Figure 4.24).

1. Place a volumetric flask of suitable size next to the balance with a dry funnel over the neck. Make sure that the stem of the funnel hangs in the centre of the neck of the volumetric flask or long enough to extend into the centre of the flask. This leaves enough room for the displaced air to move out of the volumetric flask.
2. Weigh the empty weighing vial, watch glass or waxed-paper (W_1). You can take the weight (which is zeroing the balance with the weighing vial) on a modern balance. Now calculate the final weight (W_3) needed by adding the weight of the vial (W_1) and the desired weight of the chemical (W_2).
3. Set the balance to the final weight (W_3) and transfer the chemical into the vial or on the waxed-paper in instalments until the desired weight is attained.
4. Remove the watch glass with the weighed chemical from the balance and hold the watch glass (or waxed weighing paper) over the funnel and wash down the chemical into the flask by means of a gentle water jet squirted out of a wash bottle. Alternatively, dissolve the weighed chemical in a beaker and transfer the solution quantitatively (without any loss) into the volumetric flask. A glass stirring rod is used to facilitate the transfer process without splashing and the rod should remain with the weighing vessel until the transfer is complete (Figure 4.25). Rinse the watch glass and the stirring rod 3–5 times with small portions of the diluent, using a wash bottle. Each time, following rinsing, hold the beaker over the funnel and guide the fluid into the flask by touching the glass stirring rod to the lip of the beaker and hanging it down in the centre of the funnel. During rinsing, the solution sticking to the rod should also be washed down.

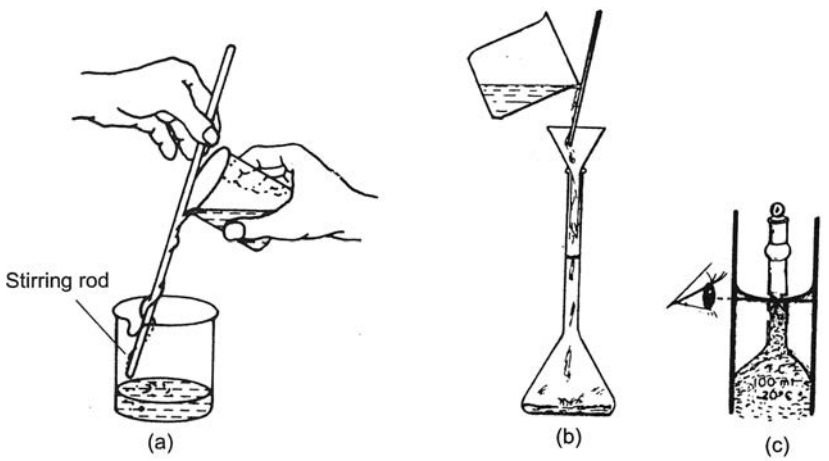

FIGURE 4.25 *(a) Quantitative transfer of solutions from one beaker to another beaker, or (b) From beaker to volumetric flask; use a glass rod to avoid splashing, (c) Finally bring the solution to the volumetric mark with the solvent and then mix*

5. Rinse the funnel with the diluent and remove it from the flask. Add sufficient amount of diluent to the flask to bring about dissolution of the solid. In many cases the volumetric flask is half-filled with the solvent before transferring the weighed solute. Be sure that the entire solid is dissolved. Allow for the cooling of the solution to room temperature if heat is evolved or applied during the process. Add the diluent close to the calibration mark.

6. Finally, with a Pasteur pipette or a dropper, bring the level of the fluid to the calibration mark. When the volume is made, the bottom of the meniscus must be exactly even with the calibration mark.
7. Stopper the flask and invert it several times to mix the contents.
8. Pour the reagent solution into the stock bottle and label. The label must indicate the name of the reagent, its concentration, any special warning (e.g., **POISON**), the date prepared and probable expiry date and the technician's name who prepared it.

Procedure to Prepare Solutions of Hygroscopic Substances

1. Weigh a weighing vial (W1).
2. Prepare a concentrated solution of the hygroscopic reagent which appears wet during storage. Make sure that the entire reagent is dissolved before you proceed to the next step.
3. Dispense a known volume (V mL) of the concentrated hygroscopic reagent solution into the pre-weighed vial and re-weigh the vial (W2) with the concentrated solution.
4. Determine the weight of the solution W_s from the difference of weights (W2 – W1).
5. Calculate the specific gravity (*S*) of the solution, which is the weight of concentrated solution per mL. As the specific gravity of water is 1 at 20°C (consider this at the existing room temperature), the added specific gravity, over 1, is due to the solute present.

$$S = \frac{W_s}{V} \text{ mL}$$

Example

$$\text{Weight of vial } (W_1) = 6.40 \text{ g}$$
$$\text{Weight of vial with solution } (W_2) = 15.65 \text{ g}$$
$$\text{Weight of solution } (W_s) = 15.65 - 6.40 = 9.25 \text{ g}$$

$$\text{Volume of solution} = 5 \text{ mL}$$

$$\text{Specific gravity of the solution} = \frac{9.25}{5} = 1.85$$

Or, weight of hygroscopic solute per mL of solution is 0.85 g.
This concentrated solution can now be diluted to the desired strength.

Example

For preparing a 3% TCA solution, take 3.5 mL of the concentrated solution which contains approximately 3 g of TCA and make this up to 100 mL volume in a volumetric flask. This is referred to as **quantum sufficit (q.s.).**

Techniques for Heating a Liquid in a Test Tube

While heating liquid in a test tube, hold the test tube with a test tube holder and keep it at an angle (Figure 4.26). Always keep the mouth of the test tube away from you. Put the tube over the flame of the Bunsen burner, starting to heat from the top. Slowly move the test tube upwards so that the bottom can be heated with full heat. Gently shake the test tube during heating in order to avoid bumping.

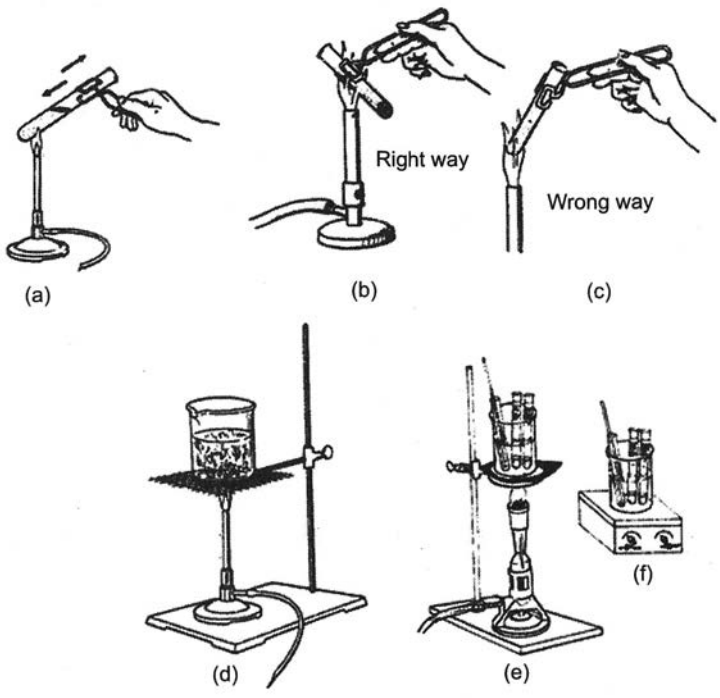

FIGURE 4.26 *Heating of liquid: (a) Liquid heated in a test tube needs constant agitation during heating in order to avoid lumping, (b and c) Hold the test tube opening away from you, keep the test tube in a slanted position and start heating from the top of the fluid, (d and e) Heating of liquid in a beaker requires special arrangement; use of wire gauze, with or without asbestos, helps to spread the heat; boiling water bath can be made with a beaker on a Bunsen burner, or (f) on a hot plate*

GRAPHICAL PRESENTATION OF DATA

Most analytic procedures require preparation of a calibration curve which requires knowledge of graphic presentation. In this graphic presentation, observations made from the instrument are presented in graphical form to visually describe their relationship with the quantities of standards taken for analysis. The calibration curve can then be used to determine the concentration of the unknown. If the curve is linear or a straight line, one can use the following formula based on the mathematical relationship of ratio and proportion indicating the direct correlation between the instrument response (e.g., absorbances, A_s and A_u) and the concentration of the standard (C_s).

$$C_u = \frac{A_u}{A_s} \times C_s$$

where, C_u = concentration of unknown; C_s = concentration of the standard; A_u = absorbance of the unknown; A_s = absorbance of the standard.

If the calibration curve is not linear, do not use the formula; rather determine the concentration of the unknown solution directly from the calibration curve.

Procedure

1. Take a linear graph paper.
2. Use the horizontal line at the bottom (called abscissa or x-axis) for constructing the scale of the concentration of standards, which is the independent variable. The scale should be made in a way that the maximal horizontal length of the graph is used. The divisions chosen cover the entire range of standards with convenient subdivisions for the plotting of the data (Table 4.2 and Figure 4.27a).

TABLE 4.2 *Absorbance readings at 550 nm for different concentrations of haemoglobin standards*

Concentration of standard (Hb g/dL)	Absorbance (550 nm)
0.0 (blank)	0.000
5.0	0.150
10.0	0.300
15.0	0.451
20.0	0.597

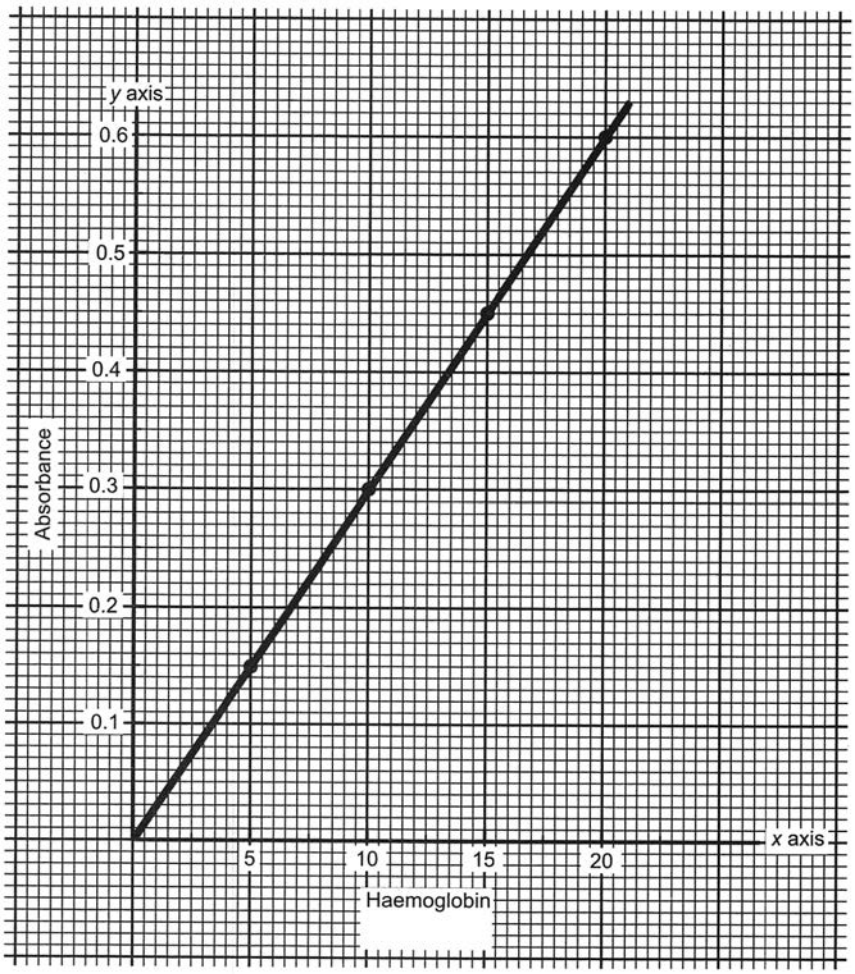

FIGURE 4.27(a) *Graphic presentation of data: Plotting of absorbance values*

3. Use the vertical line of the left margin (called ordinate or y-axis) for constructing the scale of the absorbance values to be plotted (Table 4.2). Here also, spread out the scale over the entire graph; choose the divisions that facilitates plotting of the data given in Table 4.2. The absorbance reading is the dependent variable that changes according to the concentration of the standard. Ordinarily the highest reading of the absorbance should not be more than '1' because the absorbance reading above 1 is not accurate and the error increases in a logarithmic scale. The scale (Figure 4.27a) is divided into ten major divisions and each major division represents 0.1. Each major division is further divided into 10 subdivisions leading to each subdivision being equivalent to 0.01.
4. The intersection of the abscissa and the ordinate (x- and y-axis, respectively) is called the origin. It represents the absorbance reading of zero and the standard concentration of zero. This is the reading of the blank (zero standard) against which the instrument was set to zero absorbance.
5. Begin to plot the absorbance readings against each standard.
6. When plotting is done, a smooth line is drawn, connecting the points. If the points do not fall on a smooth curve or straight line, the curve or line is drawn so that as many points miss the line on one side as on the other, that is, you should attempt to average out the misses. An ideal standard curve (Figure 4.27b) should show linearity and the slope of the curve should be close to 45° angle.

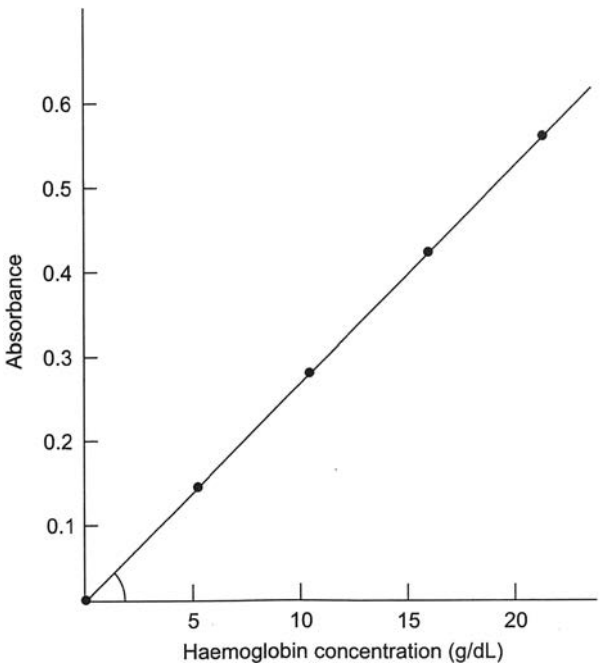

FIGURE 4.27(b) *Graphic presentation of data: Typical calibration curve of haemoglobin showing linearity with a slope close to 45° angle*

USE AND CARE OF COMMON LABORATORY INSTRUMENTS

A laboratory technician must learn the use and care of laboratory instruments. The efficient use of most instruments calls for some measure of training and skill. Improper use can ruin the equipment leading to loss of time and escalating expenses for the laboratory. In developing

countries, owing to the lack of readily available spare parts and trained repair-personnel, the instrument may never function as the factory-built one.

Before buying an instrument for the laboratory, first answer the following questions:

1. Is the instrument profitable for the laboratory?
2. Can the present laboratory personnel handle it?
3. Who will fix the instrument when it is out of order?
4. How long does it take to fix it?
5. Are the supportive services (reagents, replacement parts, etc.) readily available?

These questions are especially pertinent before importing a foreign instrument. Foreign instruments are excellent as long as they work. But when they go out of order, it is difficult to fix them locally. This is due to the non-availability of parts. The general manufacturing policy of foreign countries like the USA is to make their products fairly disposable and not to make them last forever because the technology is changing very fast. Developing nations with very limited buying potential cannot afford to purchase 'short-life' instruments. Manufacturers of analytical instruments are now coming up with items to suit the needs of developing countries, and India has taken the lead in this (*Analytical Instrumentation in India—A Review*, Raju, D.V.S, and Krishnamurthy, T.G., Elco News, India, 1982).

The laboratory supervisors frequently comment 'It is better to have a working second class locally made instrument which can be easily serviced than a first class instrument made in a foreign country without supportive services available'.

General Comments for the Use of Instruments

After the purchase of the instrument it has to be set up in the laboratory. Unpacking of the instrument should be done very carefully; follow the manufacturer's directions in unpacking. After unpacking the instrument and placing it at the proper place, look for the instruction manual. Read the instruction manual carefully. It provides information regarding operation, maintenance, trouble shooting and essential circuitry for repair jobs. No textbook can ever replace the description provided by the manufacturer for a specific machine. Never lose the instruction manual. The manufacturer does not replace it. Make copies for daily use.

Instruments are delicate and should be handled carefully. Do not be over enthusiastic in fixing things around the laboratory as an 'all round repair man'. At the other extreme, do not get panic-struck for every bit of trouble, read the instruction manual for trouble shooting. Make sure that the address for repair services is available from the dealer. Each instrument has an internal world of its own and any interference with unknown circuitry, without appropriate training and knowledge, can be an expensive experience for the laboratory. Hence a repair shop once advertised: *'It will cost you Rs. 100 to fix the machine if you have not tried to fix it, but the cost will be Rs. 200 if you have given a try (in fact, it may never get fixed)'.*

Microscope

A microscope magnifies the image of an object. The modern compound microscope (light microscope) is the most important apparatus in a medical laboratory and is also one of the most expensive ones (Figure 4.28). It is a precision instrument and needs careful handling. Improper use of the microscope leads to the loss of image clarity, consequently resulting in the loss of definition.

The light microscope uses white light, either external sunlight or the internal tungsten filament lamp, as the source of illumination. When viewed under the microscope, objects look dark or coloured, contrasted against a lighted background. In case of the dark-field microscope, a special dark-field condenser is used that lights up the object, like stars against a dark sky (Figure 4.29). This has limited use in the microbiology laboratory for observing spirochetes. The fluorescent microscope uses a special ultraviolet lamp as the source of

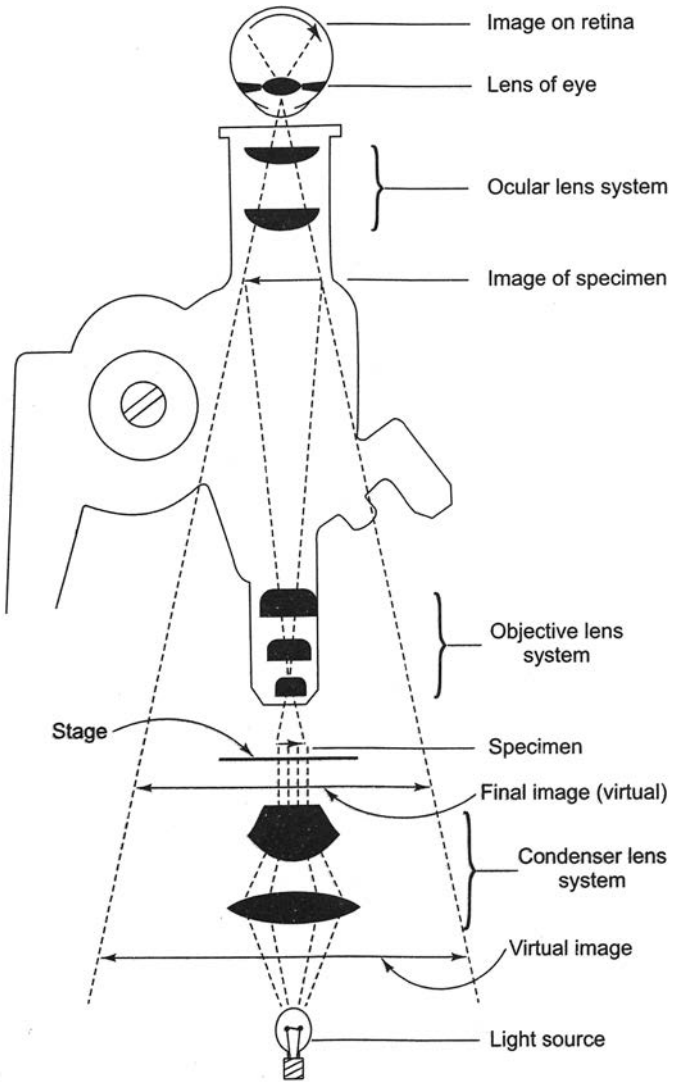

FIGURE 4.28 *The principle of bright-field (light) microscopy*

illumination (Figure 4.30). A fluorescent dye is attached to the object through laboratory procedures which glows when exposed to ultraviolet radiation. Fluorescent antibody testing requires this kind of microscope. In the following pages, only the commonest type, the light microscope, will be discussed.

Components of a light microscope

Components of two commonly used light microscopes—monocular and binocular—are shown in Figure 4.31. Basically they are the same except that the binocular microscope has two eye pieces (ocular), which allow the user to keep both eyes open while viewing through the microscope. Both the microscopes have three major systems—support system, illumination system and magnification system.

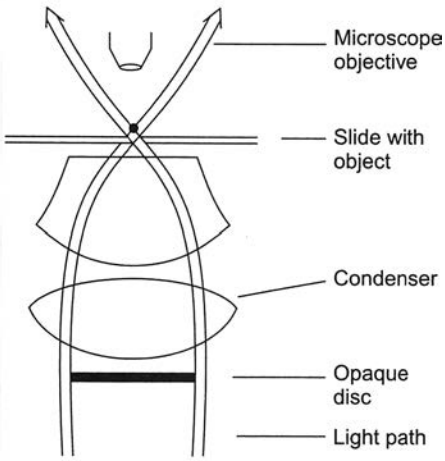

FIGURE 4.29 *Principles of dark-field microscopy*

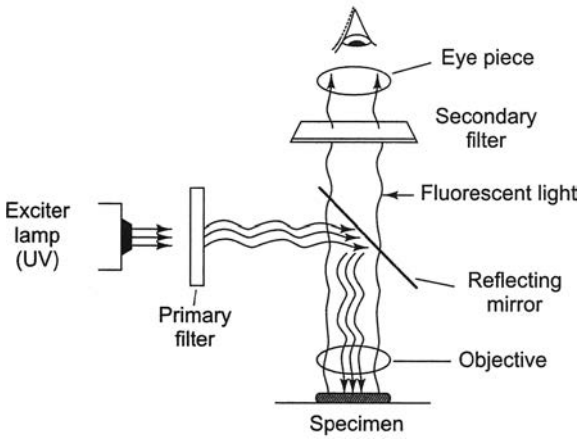

FIGURE 4.30 *Principle of fluorescent microscopy. High energy of short waves (ultraviolet) excites the specimen which emits visible light. The latter is passed through the barrier filter and the eye can see the fluorescent object.*

Support system

The support system holds the microscope components—the foot rest, the tube and limb, the revolving nose piece (objective changer), components of the illumination system and the stage. The foot rest is used to hold the microscope while transporting. The tube holds the optical system for magnification. The tube can be straight and fixed (older models) or bent and movable (modern models) for convenient viewing. The tube is hollow and holds the objectives at the lower end (near object) and the eye piece at the upper end (near eye). In case of the bent tube a special prism is put in the middle of the tube where the tube bends. The prism directs the light beam coming through the objective lenses towards the eye piece. In case of the binocular microscope, the light beam shifts further and reaches both the eye pieces. The revolving nose piece brings in line the objective required for viewing while holding others out of the way. Components of the illumination system attached to the support system or the main body of the microscope are discussed separately. The stage is used to hold the object slide in place for viewing. The stage has a central hole for the passage of light in

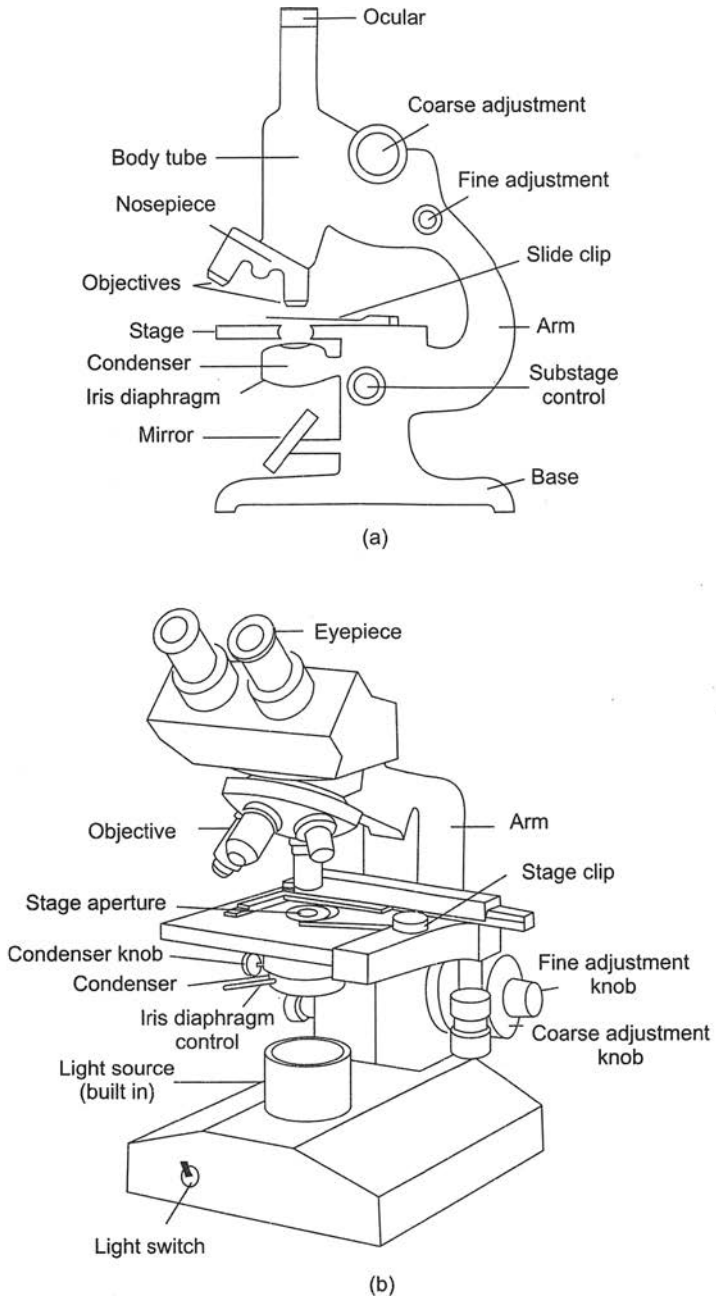

FIGURE 4.31 *Components of a microscope: (a) Monocular microscope, (b) Binocular microscope*

order to illuminate the object. The stage can be fixed, or a mechanical stage can be screwed (Figure 4.32) onto the fixed stage which has the capability of moving the object slide across the stage or along the stage, horizontally or vertically. The mechanical stage is useful for the controlled movement of the stage and complete viewing of the slide. This is indispensable for the differential count of leukocytes.

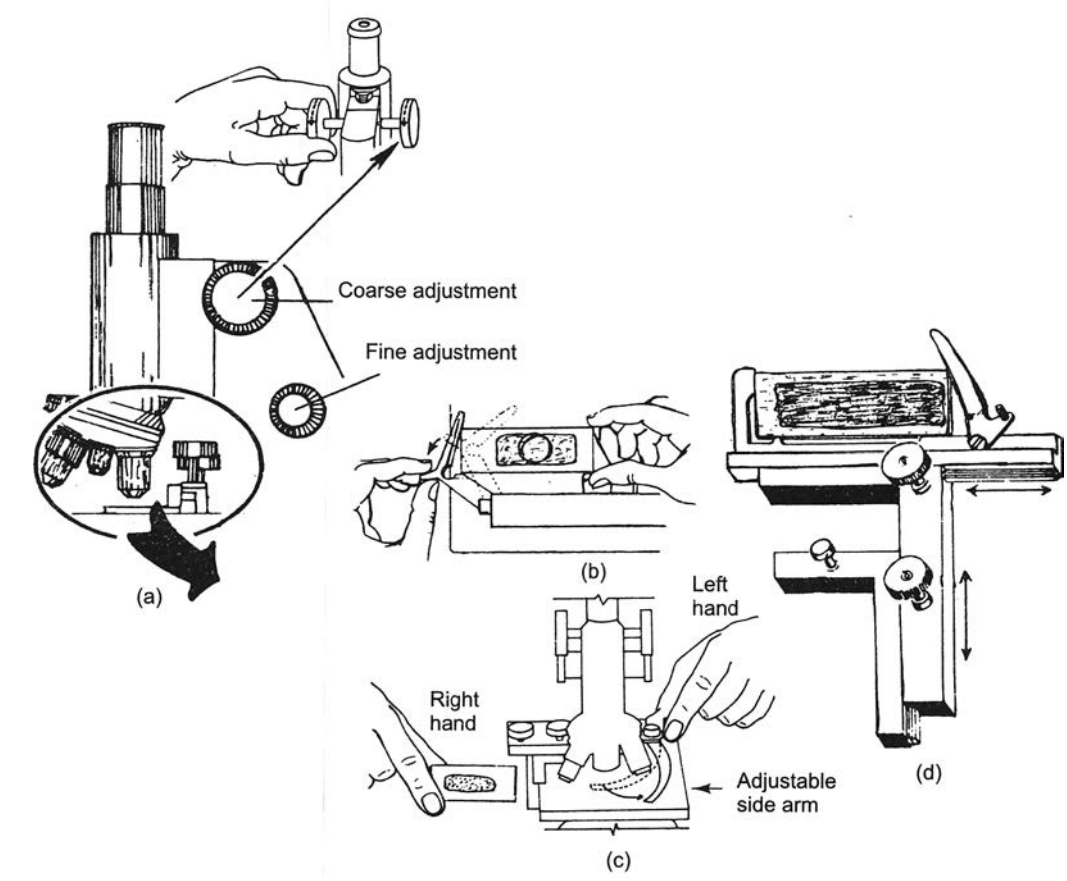

FIGURE 4.32 *(a) Focussing devices of a monocular microscope, (b and c) Use of mechanical stage, (d) The slide can be moved vertically and horizontally by using appropriate screws*

Focussing of the optical system can be done either by moving the stage up and down (older models) or by the movement of the limb (modern models). The ultimate goal is to place the object at different distances from the objective lens.

The support system helps to carry the microscope. Always carry the microscope in an upright position with one hand holding the base (Figure 4.33).

FIGURE 4.33 *Position for carrying the microscope*

Illuminating system

The illumination system (Figure 4.34) provides proper illumination to the object. The ultimate goal of the illumination system is to provide a uniform and soft-bright illumination of the entire field viewed under the microscope. A dazzling light takes away the contrast and the object may not be clear due to lack of definition.

Most modern microscopes have a built-in internal light source with an electric lamp. It provides better control of illumination. The lamp housing has a frosted tungsten

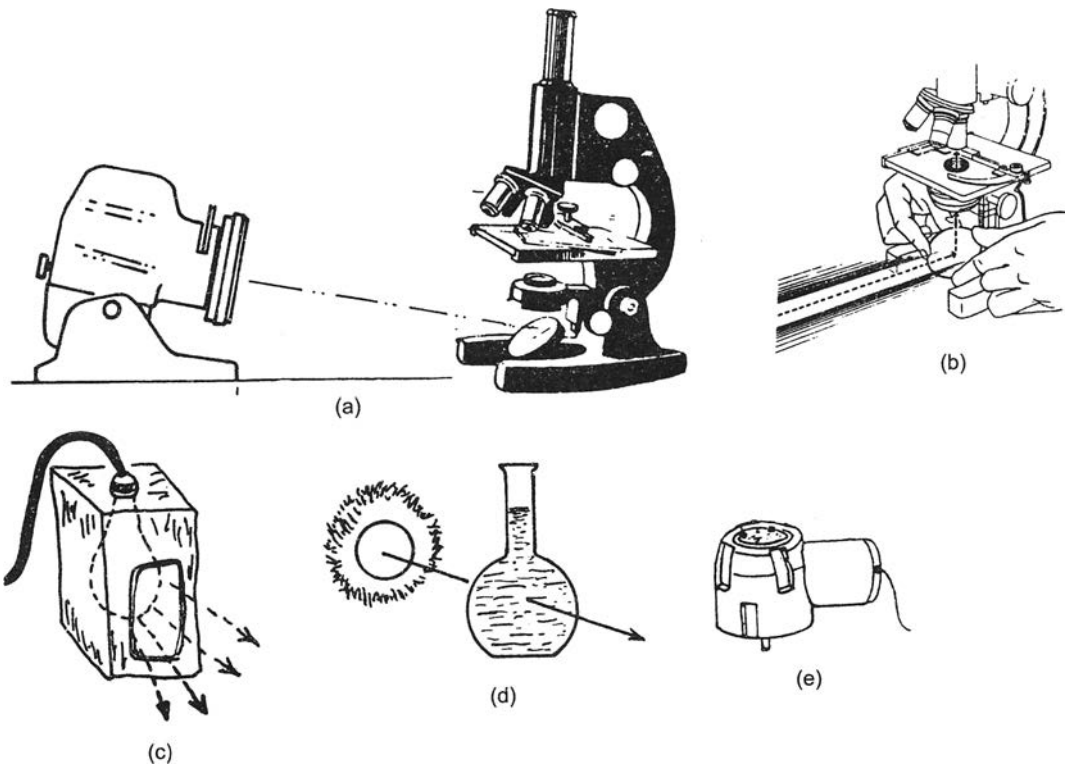

FIGURE 4.34 *Illumination of object: (a and b) Light from external source needs to be directed through the slide (object) placed on the stage of the microscope with the help of the mirror, (c) Open bulb should not be used, a tin-can cut on the side with the bulb socket on top can serve the purpose of routine microscopic examination, (d) Sunlight should not fall directly on the microscope; allow the light to pass through water before striking the mirror, (e) In-built lamp is always convenient and does not require any mirror adjustment*

lamp which is directly under the stage. A soothing sky-blue background can be obtained by using a blue filter over the light source. Always keep a spare bulb ready, in case the one in use is fused; and also read the instruction manual for changing the bulb.

The external light source can be from an electric lamp housed in a lamp box with a window, or from the sun. In case of the external light source, rays of light must be reflected by a mirror towards the object. The mirror is located under the condenser and has two surfaces—plane and concave. The plane surface is needed for the microscope with a condenser which concentrates the light that can pass through the narrow hole provided on the stage in order to illuminate the object placed over the opening. The concave side is used if the condenser is not provided in the microscope. Most of the modern microscopes have a condenser.

If the external light source is an electric lamp, a filter is placed in between the mirror and the lamp, which takes away the yellow colour of the incandescent lamp and provides a sky-blue background. If daylight is the alternative source of illumination, the microscope must be placed near a window. It should be illuminated by subdued light and should never be kept under direct sunlight. If bright sunlight is falling on the microscope, intercept it by a clear bottle or round flask containing water. This diffuses the light.

There are two other components that form a part of the illumination system of the microscope. These are the iris diaphragm and condenser (Figure 4.35). The iris diaphragm is located between the mirror and the condenser (nearest to the stage on the under surface). Its

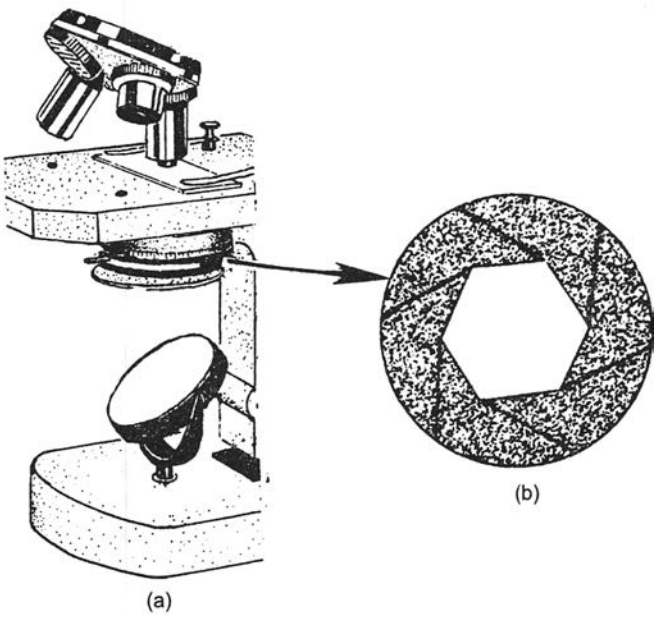

FIGURE 4.35 *Regulation of intensity of light with the use of diaphragm*

function is to regulate the amount of light that illuminates the object. The diaphragm can be closed for less light or opened for more light, according to the need by a control-hand provided with the shutter. The condenser concentrates light, which falls as parallel beams, in such a way that it passes through the opening on the stage. It is located immediately under the stage and can be raised for increased illumination. A knob is provided for changing the position of the condenser. The condenser should be centred and adjusted according to the manufacturer's directions. Most modern microscopes do not need any adjustment and for all practical purposes, the condenser is kept at the top position (raised) in order to provide maximum illumination, which is then regulated by the iris diaphragm.

Magnification system

The magnification system is the optical component of the microscope that magnifies the object placed on the stage. It is done by the combination of two series of lenses—the objective and the eye piece.

The **objective** stays near the object and it is located at the bottom of the tube. A group of three objectives are used—low-power (l0×), high-power (40×) and oil-immersion with highest magnifying power (l00×). These objectives are attached to a revolving nose piece that helps in the selection of the objective for viewing (Figure 4.36). When aligned with the tube, the objective performs the first magnification, producing a real image at the level of the eye piece. You can see the image if you place a piece of paper on the eye piece.

The **low-power objective** (l0×) is smallest in size, usually with a green ring on the objective for easy identification and when the image is in focus, the low-power objective has the largest working distance (5–6 mm). The working distance is the distance between the front lens (objective) and the slide on the stage. The working distance decreases with increasing magnification, and is 0.5–1.5 mm in case of the high-power and 0.15–0.20 mm in case of oil-immersion objective.

The **numerical aperture** (NA) indicates the ability to reveal closely adjacent details as separate and distinct. The numerical aperture is engraved on the sleeve next to the magnification,

for example the NA for low-power, high-power and oil-immersion objectives are respectively 0.30, 0.65 and 1.30. As you can see, the low-power objective has the smallest numerical aperture. Greater the NA, the greater is the resolving power. Resolving power of an objective is its ability to reveal closely adjacent details as separate and distinct. The greater the **resolving power** of the objective, the clearer is the image. The maximum resolving power of a good medical laboratory microscope is about 0.25 μm (the resolving power of the normal human eye is about 0.25 mm). Immersion oil increases the resolving power by conserving many light rays that would be lost by refraction if a dry objective was used (Figure 4.36). The illumination has also to be increased in the same order when the objectives are changed from low-power to higher powers. In addition, the field of view or the area under focus (both surface and depth) narrows down with increasing power of the objective.

The **high-power objective** (40×) is popularly known as the high-dry objective as different from the oil-immersion objective, which is also a high-power objective. The high-power objective bears a yellow ring and the oil-immersion objective bears a red ring at the collars of the objectives. The **oil-immersion objective** (l00×) has a very small lens which always looks

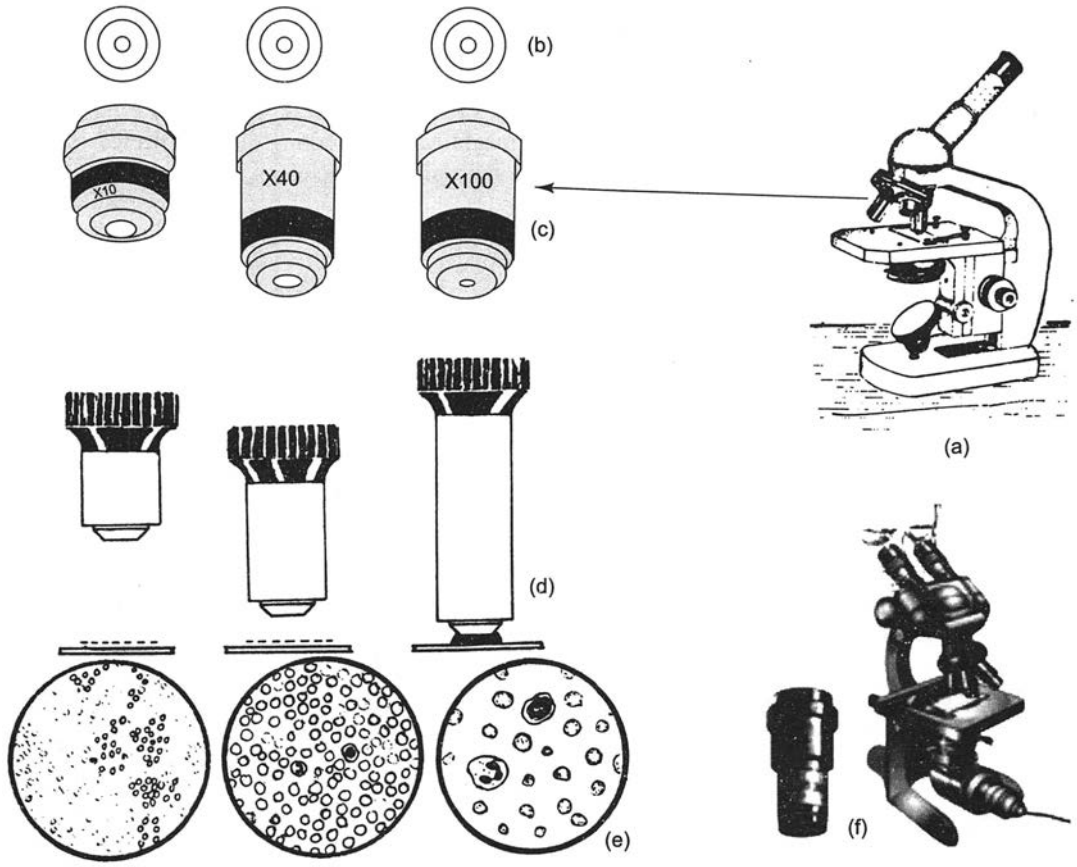

Figure 4.36 *(a) Use of microscope objectives: Location of objectives on a microscope, View of low-power, high-power and oil-immersion objectives from (b) bottom and (c) from side, (d) The working distance between the objective and the slide decreases as the objective is switched from lower power to higher power, (e) Comparative magnification of three objectives, (f) Binocular microscope with electric illumination is shown for comparison*

at the object through a drop of oil. The immersion oil establishes contact of the lens with the object slide. This increases the resolving power by conserving the light rays that would be lost by refraction if a dry objective was used (Figure 4.37). While working with the microscope one must comprehend that with increasing magnification, working distance decreases (Figure 4.37), numerical aperture increases, lens size decreases, field of view decreases and more illumination is required to see the object clearly.

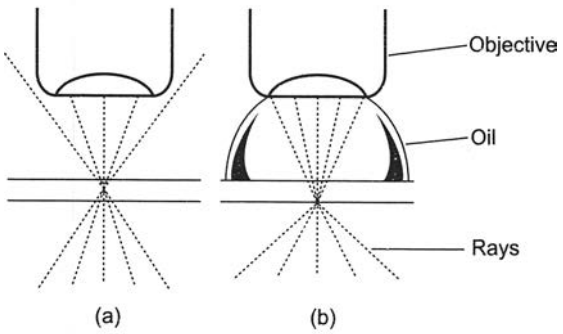

FIGURE 4.37 *Conservation of light by oil. (a) Rays of light are lost in the use of dry objective, (b) They are conserved by the immersion oil which improves the resolving power and provides a clearer image*

The **eye piece** is another system of lenses that is attached to the top of the microscope tube and is located close to the eye. The eye piece magnifies the real image made by the objective lens into a virtual image that cannot be put on a screen. The magnifying power of the eye piece is marked on the collar or on the top of the eye piece lens. A 5× eye piece magnifies the image produced by the objective five times and a 10× eye piece magnifies the image ten times. The commonly used eye piece has 10× magnifications.

The **total magnification** of the object is the multiplied value of the magnifying powers of the objective and the eye piece. Thus with 10× magnification of the eye piece, the low-power objective gives 10 × 10× = 100× total magnification; the high-power 10 × 40× = 400× magnification and for the oil-immersion lens, 10 × 100 = 1000× magnification. The cost of the lens increases with higher magnification (objective or eye piece).

Focussing of the objective is done by the adjustment system, which consists of a larger coarse adjustment screw and a smaller fine adjustment screw. The process of focussing consists of changing the working distance between the objective lens and the object.

The **number of eye pieces** in use differentiates the basic models of the microscope—monocular and binocular. The monocular microscope uses a single eye piece while binocular microscope uses two eye pieces. The former is cheaper and is recommended when daylight is used as the source of illumination. The binocular microscope requires greater intensity of light, which is only provided by electric bulbs. While using the monocular microscope both the right and the left eye should be alternately used. While using, both the eyes must be kept open and the eye not viewing through the microscope must either be covered or 'blinded out' by practice. This decreases the fatigue of the eyes. For long hours of work a binocular microscope is recommended. It is easy to use and less strenuous to the eyes. The binocular microscope may require some adjustment with the two eye pieces in order to fit the individual's interpupillary distance (distance between the two eyes), and also to focus the two eye pieces separately so that both eyes see the image equally. The manufacturer provides the procedure for the adjustment.

Using a microscope

1. Place the microscope on a stable place on a laboratory bench. The stool should have a convenient height to let you see through the eye piece without bending. Always sit straight while working with the microscope. Place the microscope near the window if daylight is used for illumination.
2. Provide illumination from any of the three sources—built-in lamp, external lamp or sunlight. The built-in lamp is most convenient and requires little adjustment. If the lamp is housed outside, place the lamp at 20 cm distance from the microscope, turn on the lamp and allow the light coming out of the lamp-housing window to fall on the mirror. Use the concave side of the mirror if there is no condenser and use the plane side if there is a condenser; keep the condenser at the top position. If the external lamp is fitted with lenses to concentrate the light, keep a piece of paper on the mirror, focus the filament on the paper and then direct the light through the object slide. If the microscope uses daylight, place the microscope near the window and direct the subdued light by means of the mirror to get maximum illumination.
3. Direct the path of light to pass through the hole of the stage with maximum intensity while setting the mirror (look from the side). This step will not be necessary in case of a built-in lamp.
4. Put the slide between the clips provided on the stage.
5. Revolve the nose piece and align the low-power objective (10×) to examine the object on the slide. The objective must click into place.
6. Look through the eye piece and not into it. Adjust the illumination to improve contrast. In case of low-power, the illumination is cut down to the minimum by reducing the aperture size.
7. If you are using a binocular microscope, adjust the interpupillary distance to suit your need and also focus the movable eye piece so that both the eye pieces are matched.
8. Put one hand on the focussing knob (coarse or fine, one at a time) and the other on the screw to move the stage. Use the coarse adjustment knob for bringing the object close to focus and get a sharp focus with the fine adjustment knob. The low-power objective is commonly used to screen the field of view. Bring the object of interest in the centre.
9. Switch to high-power (40×) and increase the illumination as needed. While changing the objective by rotating the nose piece make sure that the objective clicks into place. Repeat the process of focussing as described earlier by using the coarse adjustment knob and fine adjustment knob in sequence. Modern microscopes are par focal, that is, once the object is focussed under any objective, it stays in focus for all objectives and no further adjustments will be necessary. This character is often lost as the microscope gets older with constant use. As the high-power does not normally touch the slide (check carefully the distance between the slide and the high-power objective at its lowest position), the use of the coarse and fine adjustment knobs may not be so critical and they can be switched freely. However, if the high-power lens tends to touch a thick slide or a slide with a cover slip, always focus by the anti-clockwise movement of the coarse adjustment knob so as to increase the working distance by getting away from the slide.
10. After screening under the low-power and examining under the high-power, oil-immersion objective is used for obtaining greater details of the object. Swing away the high-power objective and put a small drop of immersion oil over the path of light. This will be the place where the oil-immersion objective comes and rests. Increase illumination to the maximum. Turn the nose piece and set the oil-immersion objective in position; make sure that the objective has clicked into place. Use the fine adjustment knob to get the object in focus. If this fails, look from the side, keep the eye level with

the slide and lower the objective carefully with the coarse adjustment knob until the oil-immersion objective touches the oil (Figure 4.38a). Lower the objective further down (caution!) and stop when the oil-immersion objective touches the slide; avoid pressing hard on the preparation. First focus the object with the coarse adjustment knob while increasing the gap between the slide and the objective (anti-clock movement of knob). Finally, finish focussing with the fine adjustment knob, which can move in any direction—clockwise or anticlockwise.

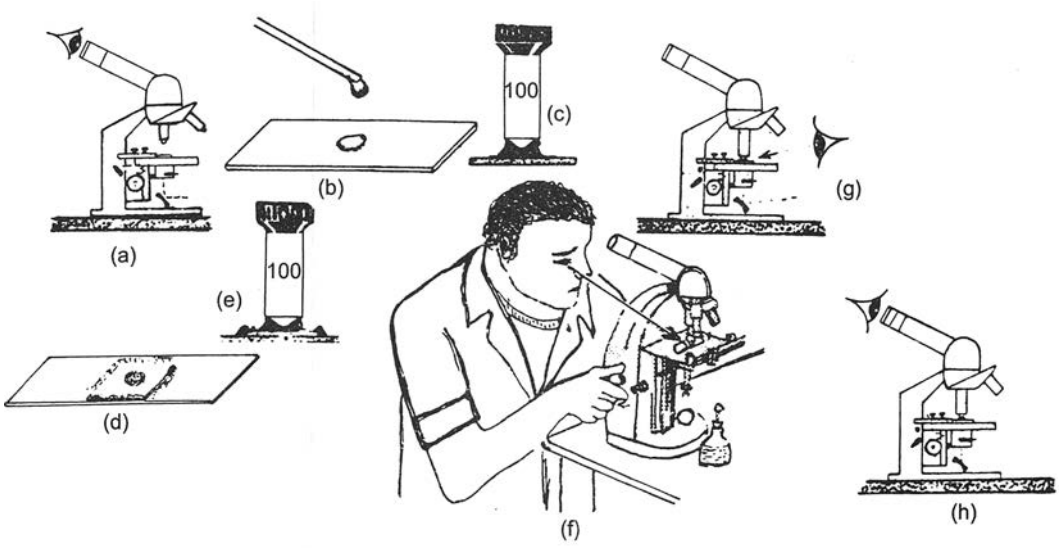

FIGURE 4.38(a) *Use of oil-immersion objective: (a) First screen with low-power and high-power; centre the object under the high-power objective, (b) Swing the objective to one side and put a drop of immersion oil on the object, (c) Bring the oil-immersion objective in line, (d and e) The objective will probably touch the immersion oil and will come under focus. In case of wet mount, the coverslip must be fixed to the slide with petroleum jelly and wax mixture. If the oil-immersion objective does not come in focus, refocus with the following procedure: (f and g) Look from the side and slowly lower the objective with the coarse adjustment until the objective touches the slide, (h) Then look through the eye piece and focus with the fine adjustment*

Trouble shooting tips

- If the field of view is not clear, check illumination, check the mirror position (when applicable), open the diaphragm if necessary, check the condenser position (it must be at the top level), and check the objective position. If the problem continues, take out the objective and clean it (Figure 4.39).
- Dark shadows in the field of view suggest a dirty eye piece. If the shadow moves when the eye piece moves, take out the eye piece and clean it.
- An unclear image under the oil-immersion objective suggests trouble with the slide or with the objective. First check the slide by taking it out (do not forget to swing out the objective); check the position (it should not be viewed from reverse position, smear side up); change the cover slip if too thick; check for air bubbles by holding the slide against the light; check whether the immersion oil has become too thick and the slide has stuck to the objective. Use xylene to thin the immersion oil.
- Occasionally, the object will not come into focus even when the objective is in the lowermost position, and the fine adjustment screw is not able to bring the objective any

closer to the slide. This happens when the fine adjustment screw reaches the end of the thread before the object is brought to focus. To correct this, turn back the fine adjustment screw in the reverse direction for several turns. Then focus the object carefully with the coarse adjustment knob and finally sharpen the focus by turning the fine adjustment knob. It is a good practice to keep the fine adjustment knob in the middle position. To check the position, turn the fine adjustment knob to either extreme end, then turn back, counting each turn until the knob reaches the midpoint.

- If the field of view looks oval, check the objective for the correct position. Whenever the objective is changed, it must click into place.

Measuring the size of a microscopic object

The size of the object seen under the microscope could be good criteria for its identification. The following procedure may be helpful to report the size of the microscopic object (Figure 4.38b).

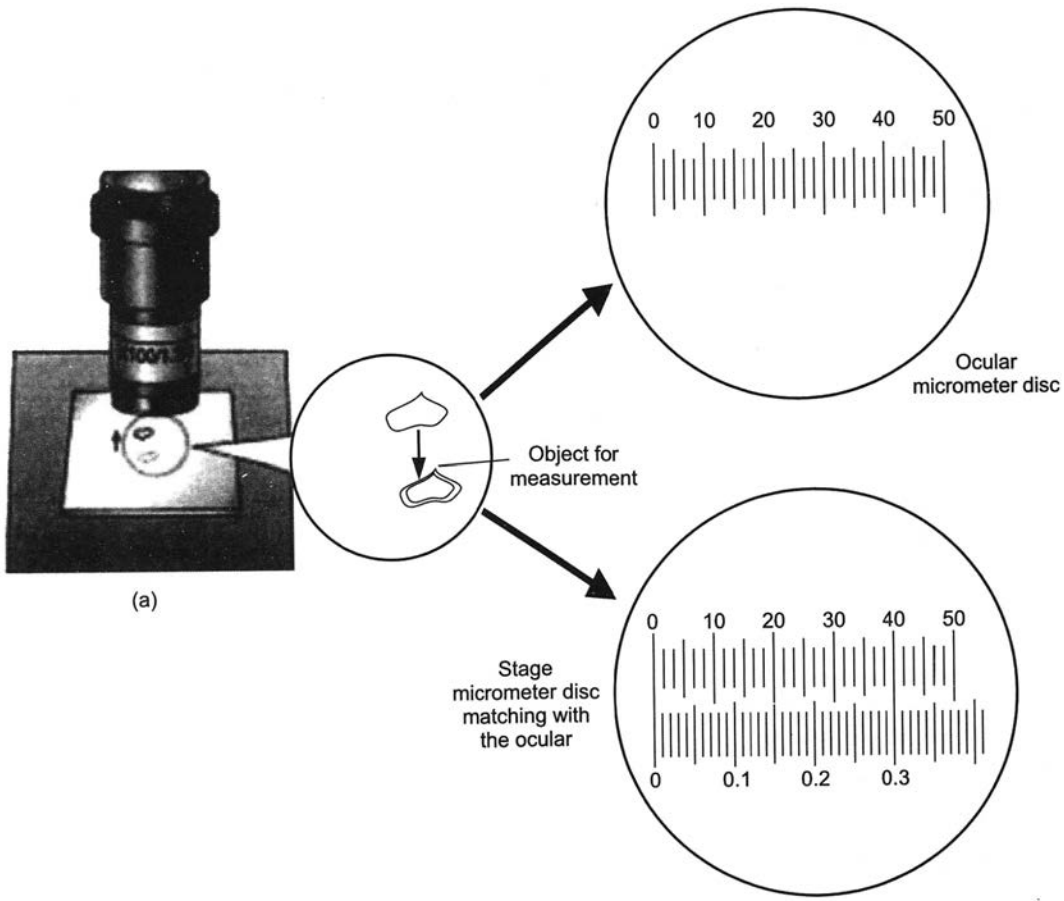

FIGURE 4.38(B) *Measuring the size of a microbe under the microscope*

Materials required

- Microscope with higher power objectives (40×, 100×)
- Slide with the object for measurement
- 10× objective

- Ocular micrometer disc: This is placed inside the objective (10×). This has calibration without any unit. It is matched with the stage micrometer to provide the measure of each division.
- Stage micrometer: The stage micrometer is a calibrated micrometer slide that is used to calibrate the ocular.
- Lens paper
- Immersion oil

Procedure

1. Put the high-power objective (100×) in place. If your microscope is not par focal (i.e., when the objective remains more or loss in focus when changed from a low-power objective to a higher power objective), raise the nose piece before changing to the higher power objective and re focus. Before changing the objective make sure that the object examined is in the middle of the field, so that it is not lost after changing the objective.
2. Take out the eye piece. Unscrew the eye lens of the ocular.
3. Place the ocular micrometer with the engraved scale face-down in the ocular. Use lens paper to handle the micrometer disc. Do not touch the lens.
4. Replace the lens carefully.
5. Place the ocular with the micrometer in the ocular tube of the microscope.
6. Put the calibrated stage micrometer on the stage of the microscope and focus on the scale. You should be able to clearly distinguish the 0.1- and 0.01-mm subdivisions. Adjust the stage micrometer so that the 0-mm line coincides with the 0-mm line of the ocular micrometer.
7. Look for another set of lines where the scale of the stage micrometer coincides with that of the ocular micrometer. This set of lines should be as far away from the 0-mm line as possible (Figure 4.38b). The distance between the two coinciding sets of lines varies, depending on the magnification of the objective of the microscope. Count the number of 0.1-mm subdivisions of the stage micrometer scale between the 0-line and the second set of coinciding lines.
8. Count the number of subdivisions of the ocular micrometer scale between the 0-line and the second set of coinciding lines.
9. Calculate the proportion of a millimeter that is measured by a single ocular unit using the following formula:

$$\frac{\text{Stage reading (mm)} \times 1000\ \mu\text{m}}{\text{Ocular reading} \times 1\ \text{mm}} = \text{Ocular units } (\mu\text{m})$$

Example

With a high-power objective (40×), the calculation is as follows

$$\frac{0.1\ \text{mm} \times 1000\ \mu\text{m}}{50\ \text{units} \times 1\ \text{mm}} = 2\ \mu\text{m}$$

Note Corresponding objectives should not be exchanged for a calibrated objective, but must be separately calibrated. The ocular containing the micrometer disc should be stored until required. Each microscope that is to be used for measuring the size of organisms must be individually calibrated.

Routine care and maintenance of a microscope

A microscope is an expensive and delicate piece of equipment which, if properly maintained, can render long years of service.

Cleaning of Lenses

Lenses are cleaned in a special way in order to avoid dust-scratches. If the lens is taken out for cleaning (eye piece or objective) choose a place which is free from air draft and is not dusty. The eye piece is pulled out while the objective is unscrewed from the nose piece. During cleaning, first blow off the dust particles from the surface with the help of a rubber bulb or paintbrush. Rubbing with a lens paper or toilet paper or with a soft old non-fluffy cloth follows this. Use chamois leather, if it is available. While cleaning lenses breathing on it by mouth is a good practice but do not clean it by spitting or blowing on it by mouth and never touch a lens or mirror with the fingers.

The **oil-immersion objective** may require more frequent cleaning. Make sure that there is no immersion oil left on the oil-immersion objective after use. Use clean soft tissue paper for removing the oil by repeated gentle rubbing on the surface. Move the cloth across and not circularly. The optical surface may be finally cleaned with a special solution, consisting of the following:

- 90% petroleum ether (boiling point 60–80°C)
- 20% 2-propanol

Caution Do not use 95% ethanol, xylene or toluene for cleaning the lenses, since these substances dissolve the cement. The dripping solvent may seep in and dissolve the cement holding the lens in the socket. If at all, soak a piece of soft tissue in the solution and rub the paper lightly and gently on the surface of the lens. They can, however, be used for cleaning mirrors. A piece of chamois leather or a non-fluffy rag is a preferred choice over toilet paper or tissue paper.

- By viewing from the side, a complete cleaning of the lens surface can be checked (Figure 4.39).

Precautions

The following precautions must be observed at all times while handling the microscope:

- Carry the microscope by holding its limb with one hand with the other hand under the foot rest (Figure 4.33). Never swing the microscope while carrying it.
- Never dip the objectives in organic solvents, as this might cause the lenses to become detached.
- Never use ordinary paper to clean the lenses. It will scratch.
- Never touch the lenses with your fingers. It will leave finger prints.
- Never clean the support or the stage with xylene or acetone.
- Never clean the lenses of the eye pieces and objectives with cloth or paper. This might remove the anti-reflecting coating. Use soft camel hairbrush, a fine paint brush or a blower instead.
- Before storing the microscope after the day's work, clean the lenses.
- Never leave the microscope without the eye pieces unless the openings are plugged.
- Never press the objective on to the slide, since both the slide and the objective might break. Take care when focussing the microscope.
- Keep the mechanical stage clean.
- Do not dismantle the optical components, as this might cause misalignment. The optical surfaces should be cleaned with lens cleaning tissue or soft tissue paper.
- Never put the microscope away with immersion oil on the objective. Remove any oil on the lenses. This must be done every day after work. Mild soap solution is suitable for most cleaning.
- Use organic solvents only in accordance with the manufacturer's recommendations.
- When changing the bulb, avoid touching the glass with your fingers, as fingerprints reduce the intensity of illumination.

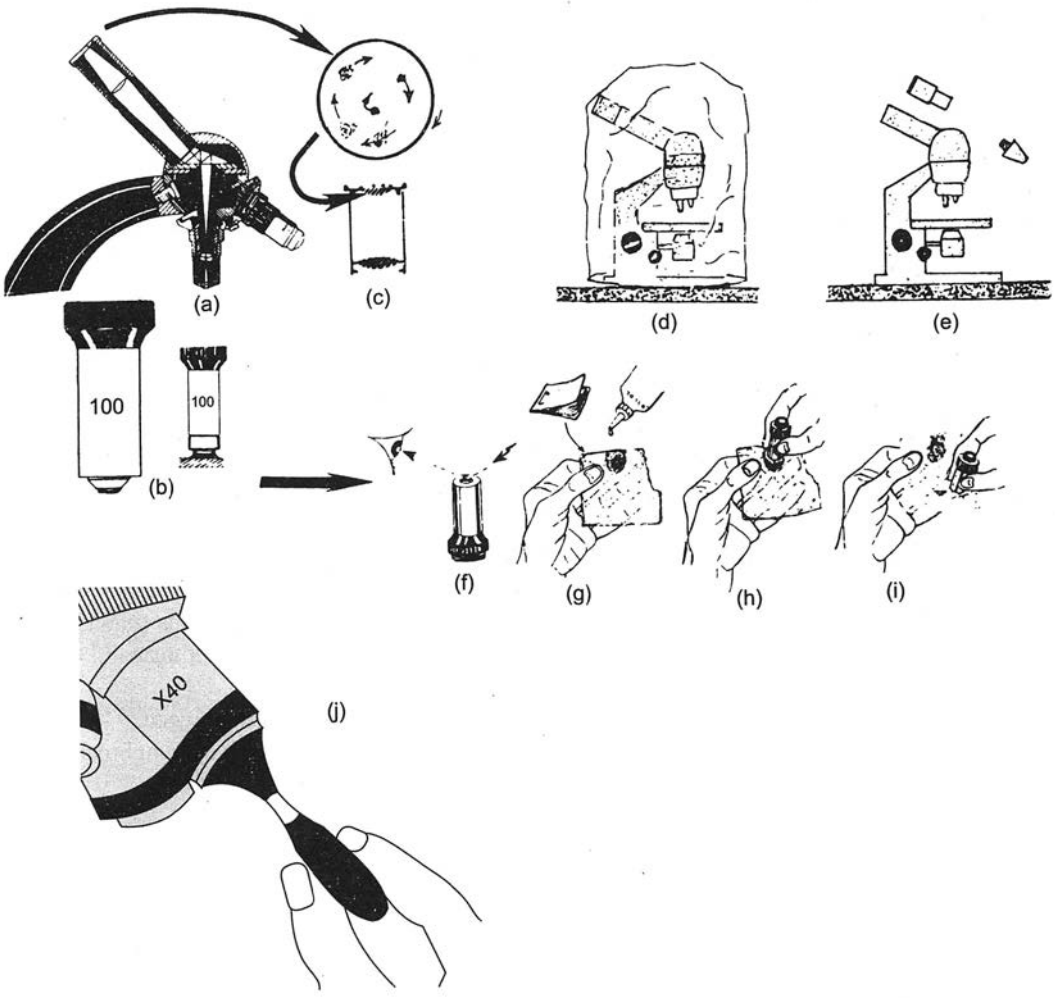

FIGURE 4.39 *Maintenance of microscope: (a) Eye piece and the objective must be kept clean and protected from dirt, (b) Never leave the oil on the oil-immersion objective when the microscope is stored, (c) While viewing through the microscope if you see a dirty field, turn the eye piece and see if the dirt is moving, (d) If it moves, take out the eye piece and clean. Always keep the microscope covered, (e) Never take out the objective without covering the tube, (f-i) If the objective looks smudged or dirty against reflected light, clean it with lens paper soaked with xylol and finally with dry lens paper; do not use alcohol or spirit for cleaning lenses, (j) Soft camel hair brush is recommended for cleaning the objective*

- To maximize the lifespan of bulbs, adjust the voltage with a dimmer switch to give the lowest required light intensity.
- If the mains voltage fluctuates excessively, use a voltage stabilizer.
- In hot dry climates, the main problem is dust. Avoid all dust accumulation by keeping the microscope in an airtight plastic cover when not in use.
- At the end of the day's work, clean the microscope thoroughly by blowing air over it with a rubber bulb. Also wipe off dust from the lens surface with a soft camel hairbrush or a fine paintbrush or a blower. If dust particles remain on the surface of the objective, clean it with special lens tissue paper. The microscope must be cleaned daily to get rid of the dust, its worst enemy.

- Never allow direct sunlight to fall on the microscope; put a plastic cover on it when not in use during the day. A common way of storing it is under a bell jar resting on a glass plate with a sealed edge (use grease), and with a desiccant inside the bell jar. Alternatively keep it inside a heated cupboard (heat the cupboard with a 40 W lamp). Never store the microscope in its wooden box.
- Areas with high humidity might grow fungi on the microscope, lens surfaces, grooves of the screws and under the paint. This might render the instrument useless. To prevent this from happening, always keep the microscope in airtight plastic cover when not in use. Inside the cover keep a dish filled with blue silica to dry the air under the cover. (The silica turns red when it has lost its capacity to absorb moisture from the air. It can be simply regenerated by heating in a hot air oven or over a fire.)
- While working with the microscope, do not pull out the object-slide from the stage without swinging out the oil-immersion objective; the slide might scratch the objective. Do not use the oil-immersion objective on a wet mount. If this is essential, use a cover slip. Also remember not to push the oil-immersion objective through the slide; it might crack both the slide as well as the objective. It is easy to prepare another slide but difficult to replace an expensive oil-immersion objective.
- Heavy contamination can be removed with mild soap solutions. Grease and oil can be removed with the special cleaning solution of distilled water and 95% ethanol (50:50).
  Caution This is not suitable for cleaning the optical surface. The microscope must be cleaned daily.
- The mechanical parts (coarse adjustments screw, fine adjustment screw, condenser focussing and mechanical stage) should be periodically cleaned and lubricated with machine oil to make them run freely.

Centrifuges

Centrifuges are devices by which a suspension of solid material in liquid phase is spun at high speed in order to separate the liquid phase from the solid phase. The principle of centrifugation is that when a body is rotated in a circular movement at a high speed, it creates a force that drives the body away from the centre of the circular movement (Figure 4.40). This is called centrifugal force. At the time of centrifugation, the centrifugal force of spinning pushes the solid particles of higher density 'outwards', which pack in the narrow bottom of the centrifuge tube and form a pellet. The packing of the solid particles facilitates the separation of the supernatant which is then decanted out.

Types of centrifuges

The common laboratory centrifuge is used for the separation of serum, precipitates and sediments of various body fluids. The haematology laboratory, however, uses a different kind of centrifuge for determining haematocrit, while the blood bank uses the high-speed angle-head centrifuge. All centrifuges must be properly used in order to ensure longer years of service.

Most modern centrifuges are electrically operated. Hand-driven (manual) centrifuges are seen in areas where either electricity is not available or the supply of electricity is not dependable. Hand centrifuges (Figure 4.40) can hold only two to four centrifuge tubes and run at a slow speed. The most common uses of hand-driven centrifuges are to obtain urinary sediment for microscopic examination and for the concentration of parasites in faecal material. They are not as safe as the electrically operated ones.

The two most common types of centrifuges are table-top model and floor model. The floor models are large, often with the arrangements for refrigeration and are seen in the blood bank. The table-top laboratory centrifuges can be broadly classified as: (a) free-floating type or horizontal type [Figure 4.40 (b–d)], and (b) angle-head type (Figure 4.41b). In the free-floating

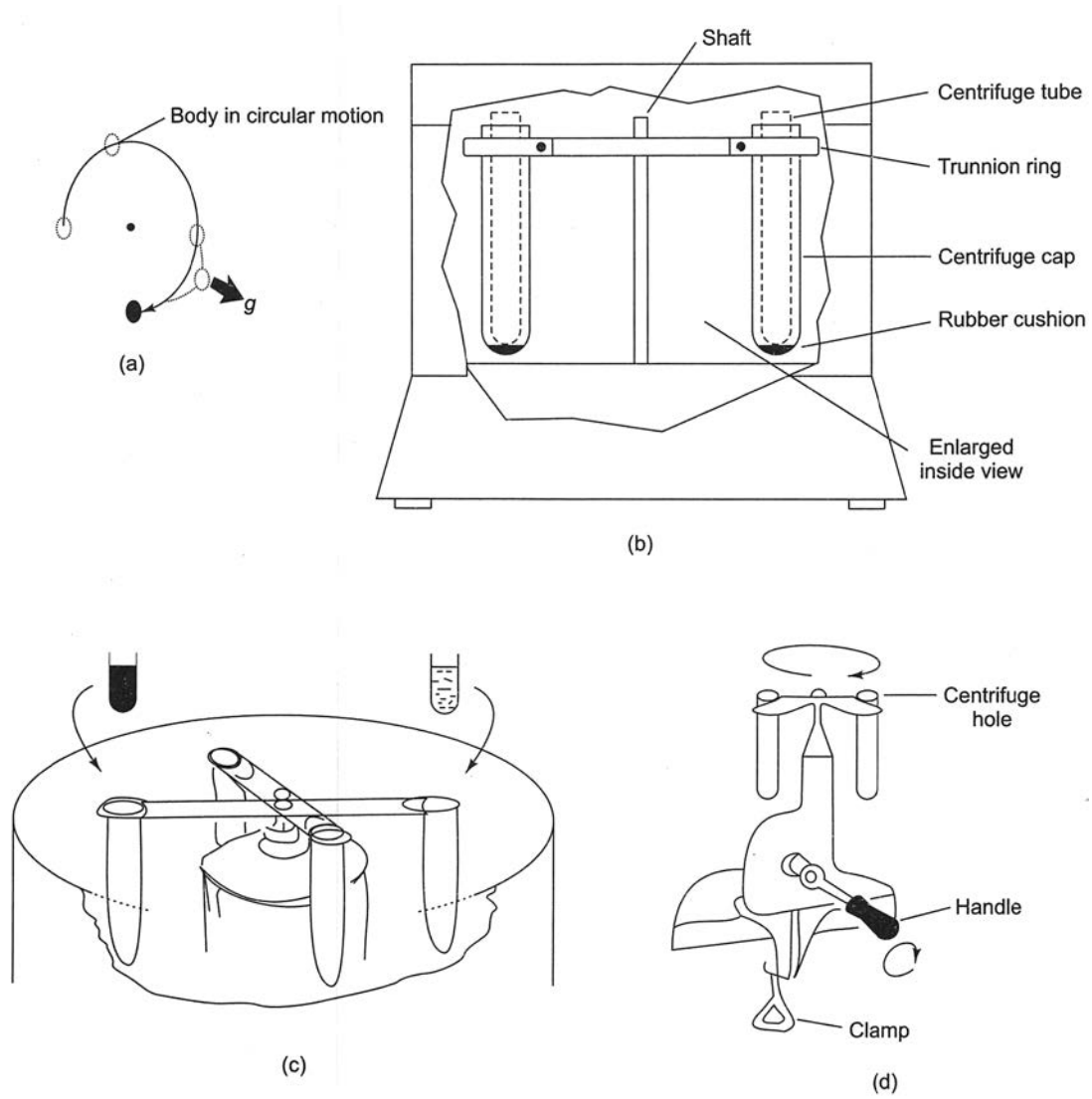

FIGURE 4.40 *(a) Principle of centrifugation, (b and c) Free-floating centrifuges – electrically operated, and (d) Hand operated*

type, the centrifuge tubes holding the material to be centrifuged stay in a vertical position when the centrifuge is at rest (Figures 4.42); but assume the horizontal position when the centrifuge revolves. As a result, the sediment surface stays in a straight line at right angles to the centrifuge tube wall. The free-floating type centrifuge does not hold more than four to eight centrifuge tubes (15 mL) and the maximum speed that it can attain is about 1800 rpm (or 2000 G). In the case of the angle-head centrifuge, the tubes are held in a rigid position at a fixed angle of 45°. Either having the trunnions fixed at an angle or using a cone-shaped solid head does this. The angle-head centrifuge holds more centrifuge tubes, can run at a higher speed, the sediment is laid at an angle and there is also less chance that the sediment will be disturbed when the centrifuge stops (Figures 4.42 and 4.43). The angle-head centrifuge, which can attain high speed in a short time, is preferred in the blood bank in order to observe haemagglutination.

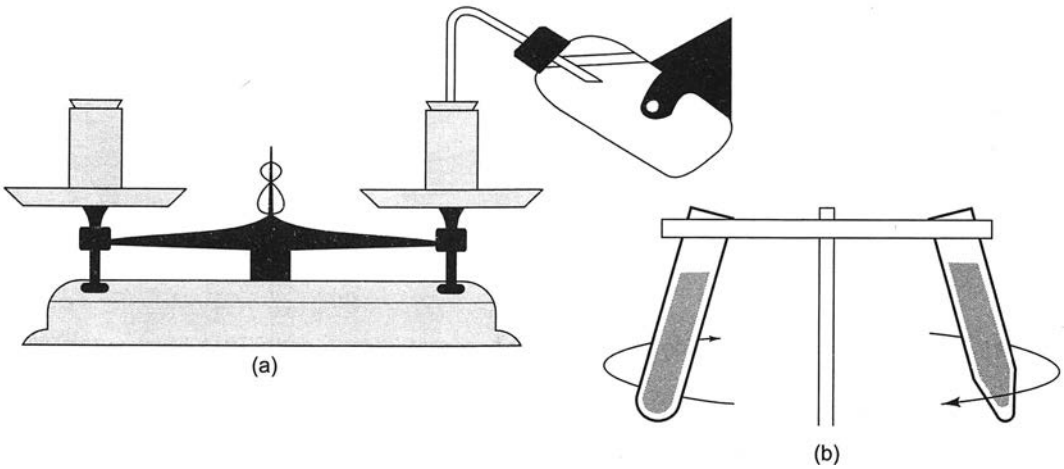

FIGURE 4.41 (a) *(a) Balance the opposite-side-sockets before starting the centrifuge, (b) The tubes need not be identical. An unbalanced centrifuge (by weight) will vibrate and may cause breakage of the centrifuge tubes and damage to the centrifuge head.*

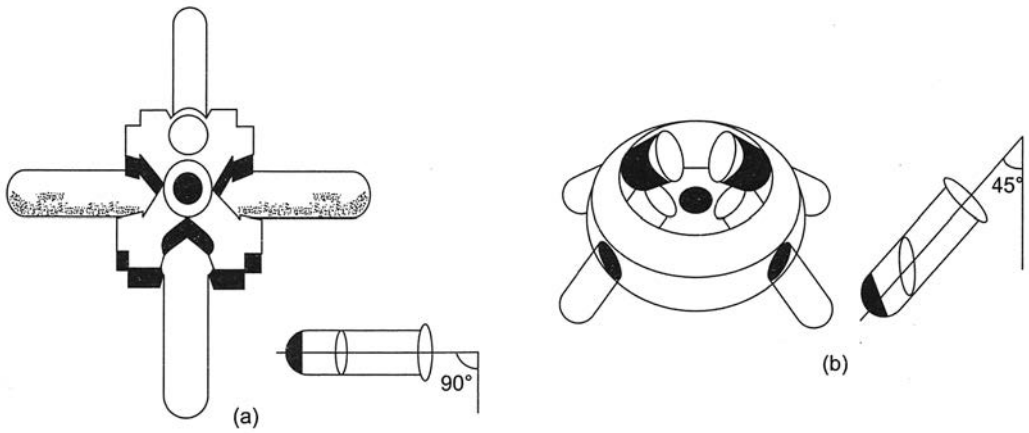

FIGURE 4.41 (b) *Swing out packing as compared to angle head packing*

Components of a clinical centrifuge

The clinical centrifuge (Figure 4.42) has three basic components—the central shaft which rotates at high speed and is driven by a hidden motor; the head which is fixed to the shaft and carries the centrifuge tubes in a bucket or cup or hole and the chamber (bowl) in which the shaft with the centrifuge spins. Electrical centrifuges have a rheostat to regulate the speed and some of them are provided with a timer or a tachometer which measures the rate of spinning. The centrifuge has a lid, which must be closed during the running of the centrifuge. It is a good practice to keep the lid closed all the time, even when the centrifuge is not working. The centrifuge tubes are either made of glass or of plastic; they should be able to withstand the stress during centrifugation. Make sure that the soft rubber cushions are present at the base of the sockets. If they are missing, replace them promptly. Their absence might cause the centrifuge tubes to break.

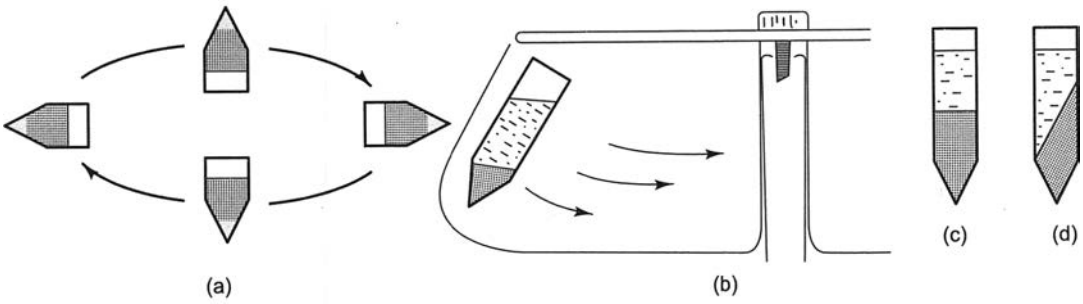

FIGURE 4.42 *Swing motion of (a) free-floating and (b) angular-head centrifuges, (c and d) The tubes show their respective packing of sediment*

Procedure of centrifugation

1. Place the centrifuge on a skid-free padding or rubber cushion so that it does not slide away. Make sure that the centrifuge is away from the edge of the table. Also check the soft rubber cushions placed at the bottom of the sockets before running the machine. While working with infectious material, keep the centrifuge inside the hood and keep the sockets closed with a lid.
2. Label the centrifuge tubes before filling in the fluid to be centrifuged. Use a wax pencil and make sure that the number does not rub off.
3. The centrifuge must be balanced before running (Figure 4.41a). In other words, the centrifuge tubes put in opposite sockets must be of equal weight. Failure to balance the load in a centrifuge can lead to severe vibration of the centrifuge with possible loss of samples and hence shortens the life of the machine. Ordinarily, balancing is done by taking equal volumes of fluids in a pair of centrifuge tubes, and they are put in opposite sockets. If the number of centrifuge tubes with specimens is odd, take an empty tube and fill it with water to match. If the fluids are of different densities, matching by volume is not recommended; weigh the tubes on a physical balance before putting them in opposite sockets.
4. Close the lid and start the motor. Never run the centrifuge without closing the lid.
5. Gradually increase the speed until the desired speed is reached. Put on the timer if it is provided with the centrifuge or use an alarm clock.
6. Stop the centrifuge after the desired period. Let the centrifuge stop gradually. If brakes are provided, use them only sparingly, or else the sediment will be disturbed. Never try to stop the centrifuge by holding the shaft.
7. Remove the tubes slowly and carefully without disturbing the pellet.

Maintenance of centrifuge

Maintenance of the centrifuge should be carried out according to the manufacturer's directions. If lubrication has to be done on a regular basis, include this in the maintenance schedule. The centrifuge and the work area around it should be kept clean at all times. Check that the soft cushions at the base of the sockets are in place before running the centrifuge. Never take the cushions out unless for cleaning. Glass tubes break due to the pressure on the metal. Cracked or damaged centrifuge tubes should not be used. They may not be able to withstand the stresses of centrifugal force and valuable samples can be lost. Whenever a centrifuge tube breaks inside the centrifuge cup, it is most important that both the cup and the rubber cushion in the cup be cleaned well to prevent further breakage by glass particles left behind. After cleaning the bucket (or socket), make sure that the cushions

are back. Clean the bowl regularly with phenol-soaked absorbent paper. In case of breakage of centrifuge tubes containing microbiological specimens, the bowl of the centrifuge must be treated with a disinfectant (5% phenol or Lysol). While cleaning, wear a pair of gloves and disinfect the gloves after the clean-up. Soak the cup in phenol water for 1-2 h before it is washed.

Standardization of centrifuge

Centrifugal force is the principal factor that determines the separation of the solid from the liquid phase in a suspension. The centrifugal force depends on speed as well as on the size of the centrifuge head. The usual expression of revolutions per minute (rpm) gives only the centrifugation speed and does not actually express the centrifugal force. The centrifugation speed and the centrifugal force are, however, directly proportional.

Formula to convert RPM of centrifuge to RCF or g-force (or G-force)

The force exerted on a particle in a centrifuge is a simple function of the *rotation speed of the centrifuge* and *the radius of rotation (R).*

The actual equation is:

$$\text{RCF or G-force} = 1.12 \times R \times (\text{rpm}/1000)^2$$

Here, RCF is the relative centrifugal force or g (G); R = radius of rotation measured from center of the pivot to the bottom of the centrifuge tube (It is 240 mm in Figure 4.43a).

Where, *R* = radius in millimeters (mm), rpm = revolutions per minute (shown on the tachometer provided with the centrifuge)

The use of a **nomogram** (Figure 4.43b) is more convenient for determining the RCF if the radius of the centrifuge head and the rate of spinning (rpm) are known.

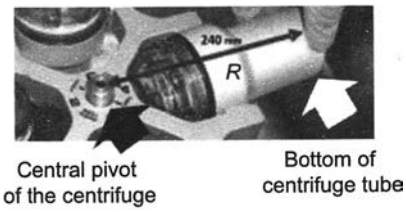

FIGURE 4.43(a)

Example *r* = 9 cm; RCF recommended for the test = 1500 G; the rpm to be set is equivalent to about 3700, which is found out by laying a straight edge that touches 9 cm and 1500 G and passes over 3700 rpm.

The **time of centrifugation** is an important consideration in applying centrifugal force and is the period of time required to move the heavier particles to the bottom of the tube before the lighter particles, at the requisite speed. Hence, the tubes are spun for a specified period to obtain the desired effect. If the centrifuge is unable to accomplish the set goal, the time of centrifugation is changed. For example, if the haematocrit value of normal blood (male) is found to be 52% for the centrifuge in use, increase the time until the standard value of 47% is reached. Keep this time as constant for comparing with the test specimens.

Balances

A balance is an important instrument in the laboratory which measures the weight of a substance. Balances are of different sensitivities depending on their use. The physical balance is less sensitive than the analytical balance. For routine laboratory purposes, the **sensitivity of a balance** can be considered to be the smallest mass that can be weighed accurately. For example, a physical balance may require more weight to move the pointer than the analytical balance.

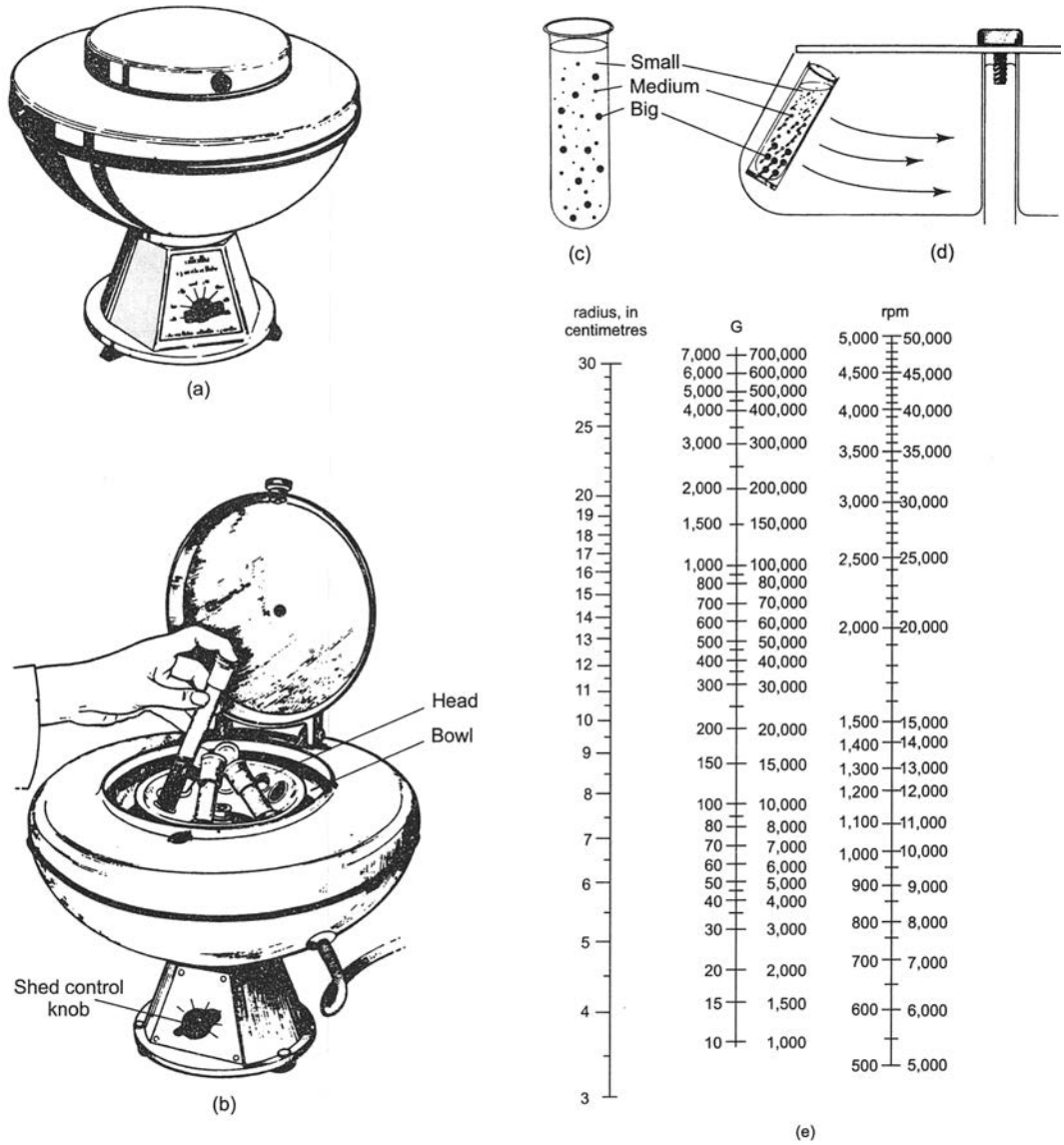

FIGURE 4.43(b) *(a and b) Common laboratory centrifuge with angular head, (c) Distribution of particles in suspension, (d) Effect of centrifugation on the sedimentation of the particles, (e) Use of nomogram for G value; connect the value of the radius (cm) and the revolution per minute; alternatively use the formula*

Most of the balances in current use in developing countries are the old-fashioned double-pan balances (Figure 4.44). The substance is put on a pan which is counter-balanced by known weights on the other side of the pivot. Rider is used to add smaller weights. In recent years, single-pan balances have been introduced which are replacing the older double-pan balances (Figure 4.44). These have internal counterweights, which are added or removed by turning a knob on the outside of the balance case. More improved balances have a digital read out and electronic operation of the weighing process.

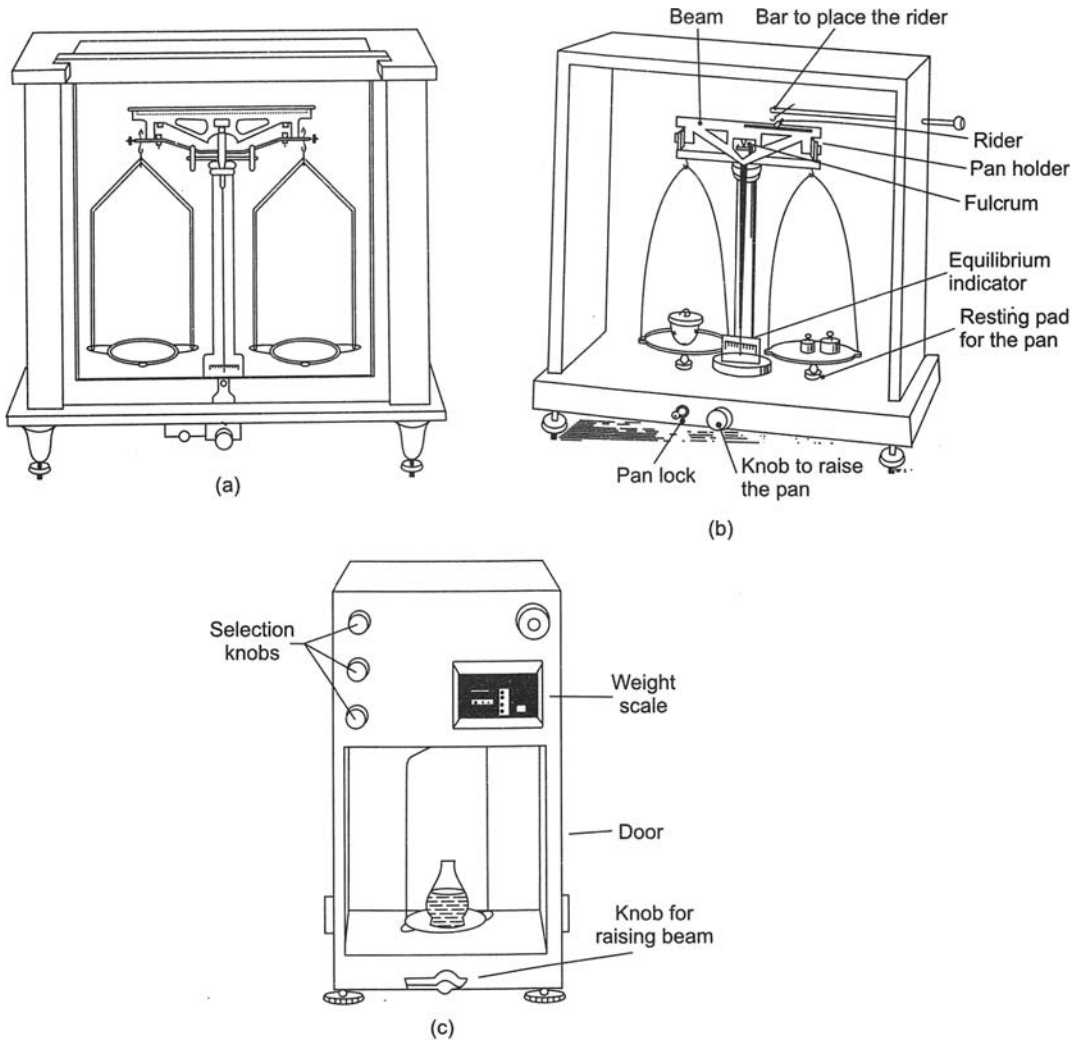

FIGURE 4.44 *Different types of analytical balances: (a and b) Double-pan balances, (c) Single-pan automatic electric balance*

Physical balance

Physical balances are used for relatively crude weight measurements with accuracy up to 10–100 mg. They are faster and easier to weigh on and are cheaper than analytical balances. The triple beam balance is more common in clinical laboratories (Figure 4.45). It has the advantage of not requiring a large set of weights like the older type of double-pan balance.

Components of a triple beam balance

The base of the balance holds the pan (Figure 4.45), the beams and the pillar. The pillar is located on the side of the balance which has a '0' mark for tarring and balancing. The container with the weight of the substance on the pan balances the weights on the beams. The pointer swings on the pillar of the balance and when rested to '0', indicates that the weight

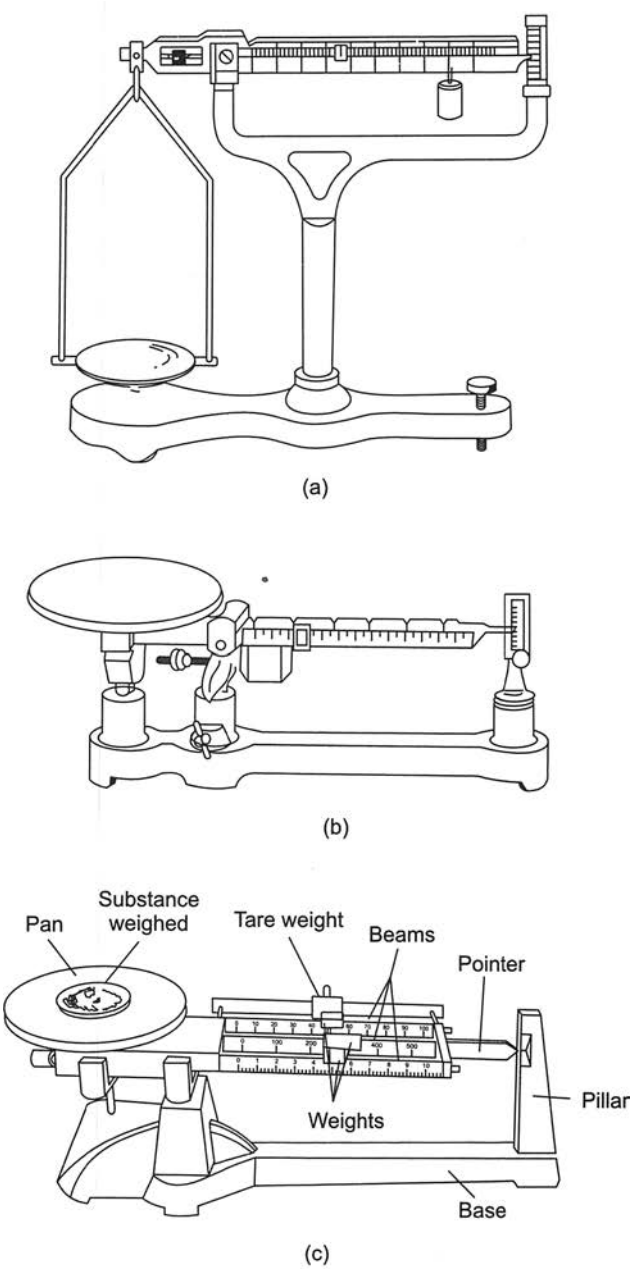

FIGURE 4.45 *(a-c) Single-pan physical balances of different types. The triple beam balance (c) is of superior quality and most commonly used. Each beam bears different range of weights.*

is balanced. The poising nut adjusts the balance before weighing the substance; at the end of the adjustment, all the slide weights are at '0' and the pointer is at '0'. A tare beam is supplied with some of the improved balances; this is used to 'zero' substance during weighing. The three beams are illustrated in Figure 4.46.

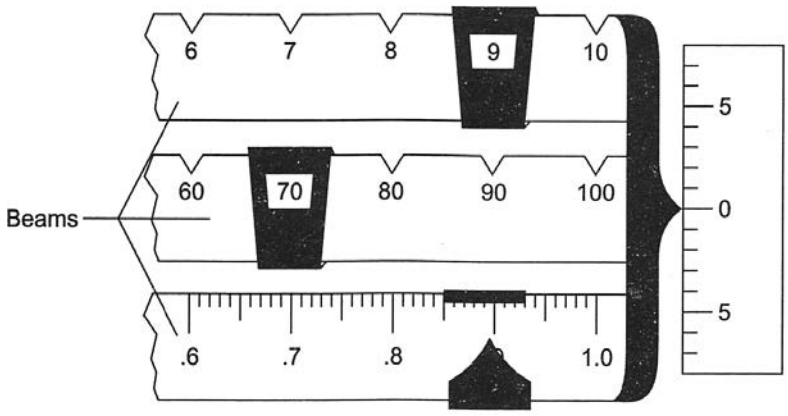

FIGURE 4.46 *Use of triple beam balance (single pan). The reading is 79.89.*

Use of double-pan physical balance

The use of a double-pan physical balance with two beams of sliding weight is described here (Figure 4.47). This type of physical balance is more commonly seen in the clinical laboratories of developing countries.

1. Zero setting:
   - Move the poising nut to the middle of its screw and push all the weights to their '0' position, the extreme left notch.
   - Check that the pointer is swinging freely. If it is touching the side of the pillar, move the pointer a little forward. The pointer should move equally to both sides of the '0' mark in the centre. If not, move the poising nut for 'zeroing' of the balance.
2. Determining the container weight:
   - Model with tare weight: Put the container on the pan and move the tare weight until the pointer swings equally to both sides of '0'. The balance is again poised and the weight of the container is nullified.
   - Model without tare weight: Find out the weight of the container by moving the weights on the arms at different ranges until the pointer shows equal swing on both sides of '0'. For example, if after the weight-setting for the container, the middle beam weight (range 0–500 g) is at '0', tare beam weight (range 0-100 g) at 10 and the front beam weight (range 0–10) at 5.1, the weight of the container is 15.1 g.
3. Calculation of final weight:
   Make a note of the weight of the container (15.1 g, in the above example) and add this weight to the required weight of the substance (e.g., 384.2), which comes to 384.2 + 15.1 = 399.3 g.
4. Setting the weight:
   - Set the middle beam weight (range 0–500 g) to the 300 g position.
   - Then set the tare beam weight (range 0–100 g) to 90 g position.
   - Finally, set the front beam weight (range 0–10 g) to 9.3 g position.

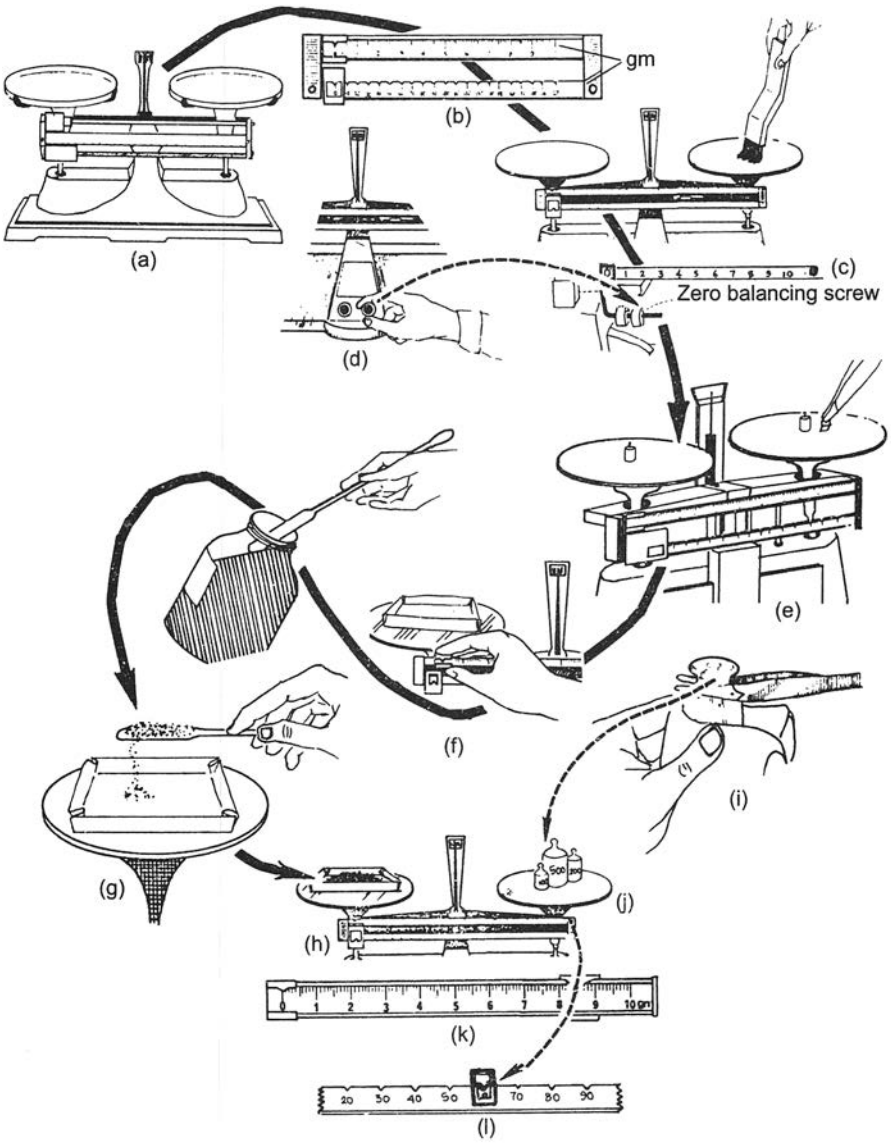

FIGURE 4.47 *Use of double-pan physical balance: (a, b, c) Examine the balance and its scale, (d) Locate the zero adjustment knob, (e) Clean the pans and balance them with the zero adjustment screw; check the sensitivity by putting equal weights on both pans and gradually increase the small weight (mg) until the pointer begins to move, (f) Put the weighing paper on the left pan and balance the pan by moving the weight on the scale, (g-l) Record the weight; add the reagent on the weighing paper and weigh again. Add the weights placed on the pan and weights indicated on the scales (1–10 and 1–200 gm). Actual weight of the reagent is the difference of the final weight and the weight of the weighing paper.*

Note Always check the scale of the graduation on each beam. If there are ten divisions between 0 and 1 g on the front beam (0–10 g range), each division is equivalent to 0.1 g or 100 mg. If there are five divisions, it will be equivalent to 0.2 g or 200 mg.

Analytical balance

Analytical balances are more accurate, with a sensitivity of 0.1 mg or lower. Most analytical balances have a maximum weight limit which means that the balance should not be used for weighing substances beyond the tolerance point. The balance is enclosed in a glass case to avoid air draft. The balance should be placed on a firm table, preferably made of concrete, to minimize disturbance during weighing.

There are two basic types of analytical balances, the double-pan type and the single-pan type. In the former case, two pans are suspended from a cross-beam, material to be weighed is put on the left pan and counterweights are put on the right pan (Figure 4.44). Counterweights of less than 100 mg are manipulated by the rider which is placed on the cross-beam. This type of balance is more common, although the sophisticated single-pan balances are seen in advanced private laboratories. Single-pan automatic balances have the weights built inside and are added by manipulating dials which indicate the weights added (Figure 4.44). Analytical balances are expensive and must be handled carefully.

- Select a balance that suits the requirement.
- Never put the substance directly on the pan. Use the watch glass or weighing paper; beakers and other containers are also used provided they are not too heavy.
- The balance must be in an area which is least disturbed.
- All substances must be weighed at room temperature.
- Load and unload the balance only when the pan is arrested.
- If the standard weights are to be placed manually, always use forceps to pick up the weights.
- Always balance the empty pans before using the balance. In case of a suspended double-pan balance, a screw is attached to each end of the cross-beam (Figure 4.44), which is screwed out (increases weight on that side) or screwed in (decreases weight on that side). Initial adjustment of the unloaded balance to a reading of zero is necessary.

Colorimeters and Spectrophotometers

Colorimeters and spectrophotometers are mostly used in the clinical biochemistry laboratory. The instrument measures absorbance (A) or optical density (OD) of coloured solutions in the visible range. The use of spectrophotometers in the ultraviolet range is not yet popular in the laboratories of developing countries. If the measurement is done visually with filters, the instrument is called comparator (Figure 4.48). Modern spectrophotometers are capable of providing specific quality of light (expressed in 'nm' wavelength) with the help of prisms or diffraction grating. Colorimeters use filters that provide ranges close to the selected wavelengths. There are several types of colorimeters and spectrophotometers in the market; a few are illustrated in Figure 4.48.

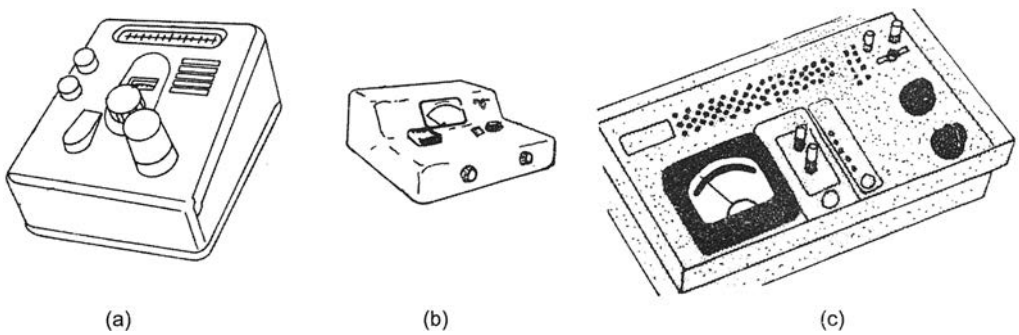

FIGURE 4.48 *Commonly used photometers in laboratories*

Refrigerators

A refrigerator is necessary in any clinical laboratory. It prolongs the life of perishable materials by cooling. Low temperature slows down biochemical activities and thereby arrests deterioration. In the clinical laboratory, the refrigerator stores media, reagents antisera, antibiotic disks and other material. Special refrigerators are used in the blood bank, which maintain temperatures close to 4°C (± 1°C). With the blood bank refrigerator, temperature must be constantly recorded on a chart.

For smaller laboratories performing routine diagnostic tests, household refrigerators are satisfactory, whereas in large laboratories commercial refrigerators or walk-in cold rooms are provided. Choose two smaller refrigerators rather than one large one with double capacity. This is because in case of mechanical breakdown of one of the refrigerators there will be less interruption and stored materials can be saved.

The refrigerator is divided into two compartments—the freezing compartment and the cooling compartment. The freezing compartment is used for keeping substances in frozen state (sera, certain antibiotic discs and others). The temperature of the freezing compartment is usually between –15 and –20°C. The cooling compartment outside the freezing compartment should be 4–6°C. Check the temperature of the cooling compartment daily—place a thermometer, dipped in a bottle of water at a corner of the cooling compartment.

Laboratory Water

Water supply to the laboratory is a basic requirement. The water supplied to the laboratory can be classified as tap water, deionized water, distilled water and deionized distilled water. The clinical laboratory needs an abundant supply of clean water, which may not always be available. Because the composition of tap water varies widely it cannot be used for preparing reagent solutions. The primary use of tap water is washing, but it is the source of other forms of purified water.

Storage of water is important for areas where water can be scarce in certain parts of the year or the water comes from a tank or well which may go dry. Hence, the laboratory must have its own reserve supply probably in plastic containers. Decant the water that has been stored before filtering.

Obtaining Clean Water

Tap water can be filtered to reduce crude contaminations and if minerals (electrolytes) are removed by chemical treatment, tap water becomes deionized water. The deionized water, however, is not free from organic matter. Distillation is another process to get purified water, it may be free from organic and inorganic materials dissolved in water but it may contain volatile gases. Deionized distilled water or double-distilled water is the purest form and may be used in preparing standard solutions. If tap water shows a deposit after storing in a bottle for about 3 h, it requires filtration.

Filtration

A porcelain or sintered glass filter can be attached to the tap. Alternatively, water is first kept in a bucket, a filter with a siphon arrangement is immersed in the bucket and the clean water is drawn out into a bottle (Figure 4.49). It is important that the filter is cleaned at least once a month by dismantling and washing in boiling filtered water. In dry seasons, water may be scarce; it should then be stored in plastic containers or glass containers (Figure 4.49). Water used for washing can be stored in metal drums. Stored water must be decanted for filtering so that the sediments can be avoided.

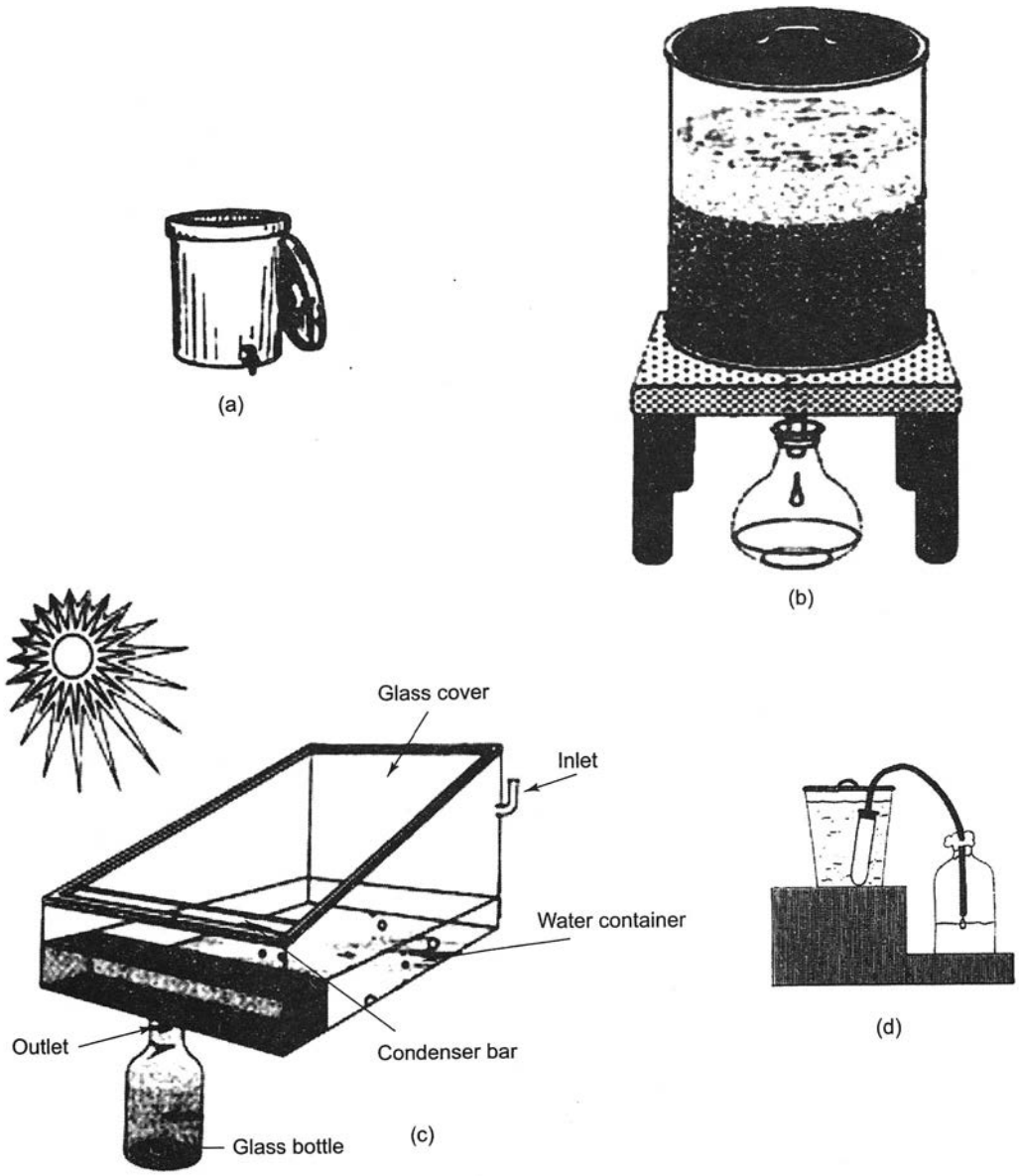

FIGURE 4.49 *(a) Plastic water storage container, (b) Water filtration through sand filter and gravel, (c) Solar still and its components to get distilled water, (d) Water filtration through porous unglazed porcelain*

Using a sand filter with gravel at the bottom (Figure 4.49b) provides reasonable amounts of clean water but the water is not free from minerals and volatile organic compounds.

Tap water and filtered water contain undesirable minerals and organic matter. Hence, they are not always suitable for the preparation of many reagents and solutions in the laboratory.

Solar still

Distilled water is used for the preparation of reagents and as a final rinse for some glassware before drying. For laboratories in remote areas and with limited resources, a simple

solar-powered water still (Figure 4.49) can provide water for the preparation of reagents. Commercially available distilled water may have absorbed volatile gases. Freshly collected distilled water from solar still or distilled water prepared in the laboratory is best suited for preparing laboratory reagents. Always store the distilled water in glass or plastic containers and use the distilled water, prepared the same week, for preparing laboratory reagents.

Solar water stills can be easily constructed using a clean plastic container with two compartments (one large and one small) and a large surface area, over which is placed a glass cover in a sloping position. Water is poured into the large compartment from which it is evaporated by the sun. It condenses on the glass cover and drops into the small compartment. The small compartment has an outlet at the bottom through which the distilled water can pass into a glass bottle placed underneath the container. In tropical climates, 2–7 L of distilled water can be produced daily from a solar still with a surface area of 1 m^2 .

Important Collect the distilled water in a glass or plastic container, not in a metal container. Replace or replenish the water when the level reaches a height corresponding to the last quarter of the compartment. It will contain residue.

Distillation equipment

Distilled water prepared in most laboratories is by boiling ordinary water. The steam generated from the boiling water is then cooled down while passing through a condenser. The condenser carries cold running tap water which forms a jacket around the central tube in which steam condenses into water. This distilled water is then collected in a flask or bottle (Figure 4.50).

The distillation apparatus (Figure 4.50) can be made of glass or metal (alembics). Heating of the distillation apparatus can either be accomplished with gas or kerosene or electricity. If properly done, single-distilled water is sufficiently pure for most laboratory work. Double-distilled water is used only for special purposes. Discard the first 10% of the distillate which contains the volatile gases. Similarly, distillation should be discontinued when the last 10% of the water is remaining. Hence, if 2 L of water are distilled, the first 200 mL of distillate and the last 200 mL of residual water should be discarded. The water may be freed of organic or nitrogenous compounds by adding a little potassium permanganate to the water (1 g/L) before distilling. Glass stills are expensive, require electric heating and are more fragile. The glass-distilled water is more pure and the still yields higher quantities of the distillate. While the glass still is in operation, it should be attended occasionally in order to check the flow of water as well as the water level of the distillation flask. Use only the round bottom flask for boiling the water during distillation. If the glass distillation flask is dry, it will crack.

If the heating is done with a gas burner, gas stills, made of copper or stainless steel, can be used which provide single-distilled water. It should be of a capacity that will yield 1–2 L of distillate per hour. The size of the distillation set must be adequate to meet the needs of the laboratory. The choice of the distillation equipment depends on the facilities available. Distilled water should not be made in large quantities and stored over a long period of time. Prepare only enough distilled water to last a few days. Store the distilled water in large plastic or glass bottles which should always be kept stoppered. Distilled water is only free from electrolytes and other substances but it is not sterile.

Testing the distilled water

It is a good practice to test distilled water occasionally for the presence of chloride and sulphate ions (Figure 4.50).

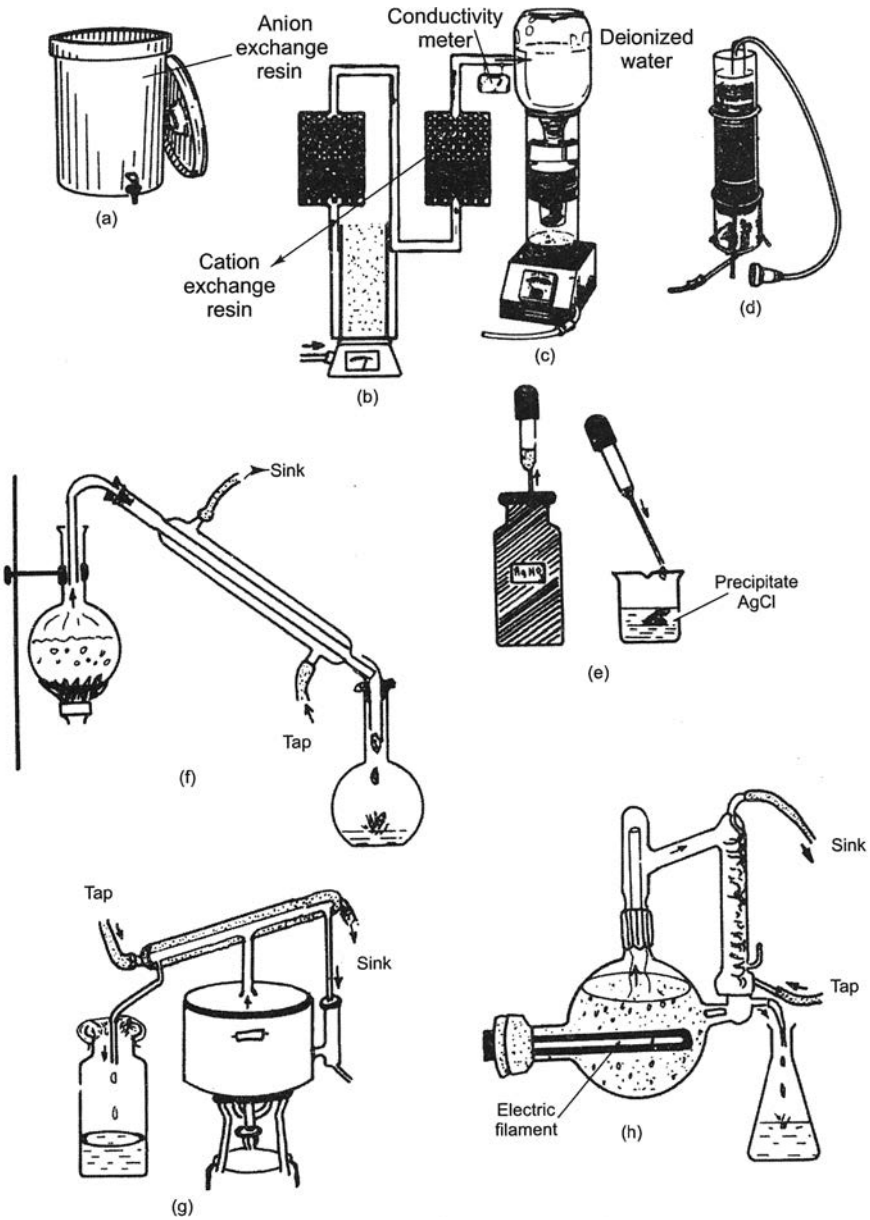

FIGURE 4.50 *Laboratory water–demineralized and distilled, (a) Ion exchange columns in the water line of the laboratory, (b-d) Various types ofdeionizers are used in order to get mineral-free water; the purity of deionized water is tested by the resistance metre or by chemical procedure, (e) When the deionized water produces turbidity with silver nitrate, presence of chloride (and mineral) is suspected, (f) Distilled water is prepared on a small scale with the help of a laboratory distillation set, (g) Metal stills and (h) Glass stills are used to obtain distilled water in a large scale; the latter is more pure*

Reagents

- Nitric acid, concentrated. Keep in a dropping bottle.
- Glacial acetic acid. Keep in a dropping bottle.
- Silver nitrate solution (2.5% aqueous). Dissolve 2.5 g of silver nitrate in distilled water and dilute to 100 mL with distilled water. Keep the solution in a dark-coloured bottle.
- Barium chloride solution (10% aqueous). Keep in a dropping bottle.

Procedure

1. Place in a small size beaker, 10 mL of the distilled water to be tested.
2. Add to it two drops of nitric acid and 1 mL of silver nitrate solution. The presence of a white cloud or white precipitate indicates the presence of chloride ions. Pure distilled water remains clear.
3. In another beaker, place 10 mL of the distilled water to be tested.
4. Add five drops of acetic acid and mix.
5. Then add five drops of 10% barium chloride solution and mix.
6. The formation of a white granular cloud or precipitate indicates the presence of sulphate ions. Pure distilled water remains clear.

Demineralizer

Mineral salts are electrolytes and are in an ionized state in solution. When they are removed from water, the latter is called demineralized water or deionized water. The electrolytes are chemically removed from the tap water by passing it through a column of ion-exchange resins which retains all the mineral ions or dissolved mineral salts. The deionized water is not free from organic matter and other non-ionizable substances. Preparation of deionized water is cheaper than preparing distilled water and the former can be substituted for distilled water in some laboratory operations. It is most useful in rinsing glassware and is pure enough for preparing most laboratory reagents, including stains.

The commercially available deionizers are made of three components—the receiving end of the deionizer, the resin column and the eluting end of the deionizer (Figure 4.50). The receiving end is connected to the tap or water reservoir. When the demineralizer has to be run, open the tap and allow the water to ran slowly (read the recommendation of the manufacturer for the rate of running the water). The resin column receives the water from the receiving end and purifies it by removing the electrolytes as the water slowly passes through the column. The eluting end of the deionizer is the outlet for the deionized water. It is connected to a closed container to collect the purified water. Many deionizers are provided with a meter to measure the resistivity or conductivity as an indication of the ionic purity of the demineralized water. Resistivity and conductivity are reciprocally related. The demineralized water should be of poor conductivity and high resistivity. The conductivity-testing meter is attached to the eluting end. If the testing meter is not available, test the purity of water for chloride and sulphate in the same way as described earlier. The resin column should be changed when the column is discoloured or when the demineralized water shows the presence of chloride and sulphate ions.

After using for a period of time, the resin column should be revived according to the manufacturer's directions. Reviving is done by treating the resin with dilute acid or dilute alkali. Always check the conductivity before re-using the resin column.

Quality control of demineralized water

One should keep in mind that demineralized water is free from ions but not necessarily free from organic compounds. Use of a conductivity meter in the deionizer line is recommended in order to obtain a dependable supply of deionized water (Figure 4.50). The conductivity

meter registers the resistivity of the water resulting from the presence of ions. The more complete the demineralization, the higher the electrical resistivity of the water. If the needle stops at a point below 2 mega-ohm/cm (a measure of resistivity), the cartridge of ion-exchange resin granules has been used for too long and must be replaced or reactivated.

If the ion-exchange column does not have a conductivity meter, determine the pH of the water supply flowing into the apparatus and the pH of the water coming out from the other end. If the pH remains the same, the resin column is no longer active. Demineralized water should have a pH between 6.6 and 7.0.

Another way to test the demineralized water is to pass a weak solution of sodium chloride (cooking salt) through the resin, then carry out the test of chloride with silver nitrate (1.7%). The method is described with the testing of distilled water. Silver nitrate solution gives white precipitate in the presence of chloride.

Some manufacturers advise to keep an eye on the colour of the resin column. It rums black when inactive. It then needs to be reactivated.

Use of Demineralized Water

Demineralized water is lot cheaper to make than distilled water. It also replaces the use of distilled water in many cases:

- Demineralized water can be used for rinsing glassware before drying.
- All the reagents used in medical laboratories, including stains, can be prepared from demineralized water.

Water for Human Consumption

Occasionally the laboratory may be involved in testing the water which is meant for human consumption. If the water is contaminated, government officials must be immediately informed so that appropriate measures can be taken, such as cleaning and disinfection ('shock chlorination') with high doses of chlorine or bleaching powder. In some parts of developing countries, wells may be the only source of water for consumption, which should be well-protected.

Water should be considered as unfit for human consumption if it contains:

- an undue amount of solid particles causing turbidity
- nitrites (indicating decomposition of organic matter)
- toxic substances (lead, arsenic, mercury, fluoride)
- pathogenic organisms (e.g., *Escherichia coli*, indicating faecal pollution)

Sampling for Laboratory Testing

Water sample for laboratory testing should be collected carefully so that it is representative of the water mass. For example, in case of well, tie the bottle with a stone and lower the bottle (narrow neck) below the surface to collect the water sample. Do not touch the wall of the well. For river stream or tank, collect from the middle. In case of tap water, cleanse the outlet of the tap thoroughly after removing all attachments (particularly rubber hoses) and allow the tap to run for a few minutes. If the specimen is meant for laboratory culture in search of pathogenic organisms, always use sterilized bottles for sample collection. Also, flame the tip of the tap with burning alcohol swab before the collection of the sample. Take a large amount of the sample so that results are reliable. All water samples must be quickly processed in order to avoid contamination. Chlorinated water, meant for laboratory culture, must be treated with sodium thiosulphate solution. Sodium thiosulphate dechlorinates the water.

Procedure
In a 250-mL sampling bottle, add 0.2 mL of 10% (w/v) sodium thiosulphate solution, place the cover and sterilize the bottle. The sterilized bottle with thiosulphate will be used for collecting specimens for laboratory culture.

Procedure of Laboratory Testing

The water for human consumption should be tested both physically as well as chemically.

Physical examination

Report colour, appearance (turbidity), taste and odour.

Chemical examination

The precipitation method described earlier only indicates the presence of chloride. In case of water for human consumption, some amount of chlorine (0.2 ppm) is added to prevent bacterial growth. Excess amount of chlorine (1.0 ppm) is, however, harmful. The level of chlorine is easily determined by a colorimetric procedure using orthotolidine solution and a visually operated comparator.

Principle
Orthotolidine forms a coloured complex with chlorine and the intensity of the colour is proportional to the amount of the chlorine present in the sample. The colour varies from light yellow (low) to deep orange (high) depending on the concentration of chlorine. The colour is compared visually against distilled water (blank) using a comparator or a colorimeter.

Equipment
Chloroscope (comparator) or colorimeter.
Test tubes (10 mL) with 5-mL markings and Pasteur pipette or dropper
Test tube rack

Reagents

1. **Orthotolidine solution**
   Orthotolidine dihydrochloride 1.35 g
   Distilled water 500 mL
   Dissolve orthotolidine in water.
2. **Dilute hydrochloric acid solution**
   Concentrated hydrochloric acid 150 mL
   Distilled water 350 mL
   Pour hydrochloric acid into water.
3. **Working solution** Mix solutions (1) and (2) with constant stirring. Store in amber-coloured dropping bottle.
4. **Standard solutions**
   - *Stock standard solution*
     Dissolve 1.6 g of sodium chloride in distilled water and make the volume to 1000 mL. This is equivalent to 1000 ppm.
   - *Working standards*
     Mix 0.1 mL of the stock with 1000, 500,200 and 100 mL of distilled water, respectively. This gives the following concentrations of chloride solutions: 0.1, 0.2, 0.5 and 1.0 ppm.

Procedure
Follow the instructions provided by the manufacturer while using the chloroscope.

Alternatively

1. Take six test tubes (10 mL) with markings at 5 mL and put them on a test tube rack.
2. Fill each tube to the mark (5 mL) with distilled water, water sample and four standards (0.1, 0.2, 0.5 and 1.0 ppm).
3. Add four drops of OT reagent to each and mix.
4. Compare the colour visually or use a comparator.
5. If the concentration exceeds 0.5 ppm, the water is not suitable for human consumption.

Note If the water sample gives too intense a colour, dilute accordingly and multiply the result with the dilution factor. If a colorimeter is available, use blue filter and prepare a standard curve. Running of standards may not be necessary if a standard curve is available. Prepare fresh standard curve intermittently.

Common Laboratory Equipment

The laboratory has an array of various other equipment, which are commonly used for day to day work. Only a few important ones are mentioned here.

Mixer

When preparing solutions, the solute has to be dissolved in the solvent by the process of mixing. This is usually done in a beaker or Erlenmeyer flask with the help of a glass stirring rod or plastic rod. Care should be taken during mixing so as to avoid hitting the glass bottom of the container with the stirrer. This weakens the glass container and it might crack easily with slight mechanical or thermal shock.

Mixing of fluid in a test tube is done manually by flipping at the bottom of the test tube or by rolling between the palms (Figure 4.51). This prevents the fluid from spilling. If the tube is full to the top, use plastic strips to cover the tube and then gently turn over. Plastic strips are difficult to get in India, and hence, choose a bigger size test tube and then mix. Do not use your thumb to close the test tube during mixing.

Modern laboratories use various time-saving mixers. They are divided into three basic types: vibrators, rotators and shakers. The vortex is a vibrator type of mixer, which is used for mixing the contents of test tubes. Shakers are not common in clinical laboratories. Rotators rotate the fluid within its containers. A common type of rotator is the magnetic stirrer, which has a rotating drive magnet below the flat surface that holds the beaker or flask (Figure 4.51). A plastic-coated bar magnet dropped into the solution rotates because of the attraction to the drive magnet. A magnetic stirrer may also be built inside the electric hot plate, which is useful in preparing a solution while the latter is heated. There are several other types of mixers such as the blood mixer, which slowly mixes blood for haematological examination; the micro-plate mixer, which is used in serology for micro-titration and the platelet mixer used in the blood bank for the gentle rotation of platelet-rich plasma held in transfer packs.

Liquid Dispenser

Liquid dispensers have become common in most laboratories (Figure 4.52). Their function is to dispense a pre-fixed volume of fluid from a reservoir of reagent solution. Most of the dispensers are hand-operated and deliver variable volumes of fluids. These are broadly divided into micro-volume dispensers, which deliver 0.5–5.0 mL and macro-volume dispensers which deliver 1–20 mL. These dispensers are ideal for monotonous pipetting operations. Most of the dispensers are attached to the top of the reagent bottle and operate on a plunger-type action.

Procedure

1. Prepare the reagent and place it in the bottle of the dispenser (some dispensers fits on various bottles with the same size of screw neck).
2. Screw the dispenser on the top; securely tighten the screw.

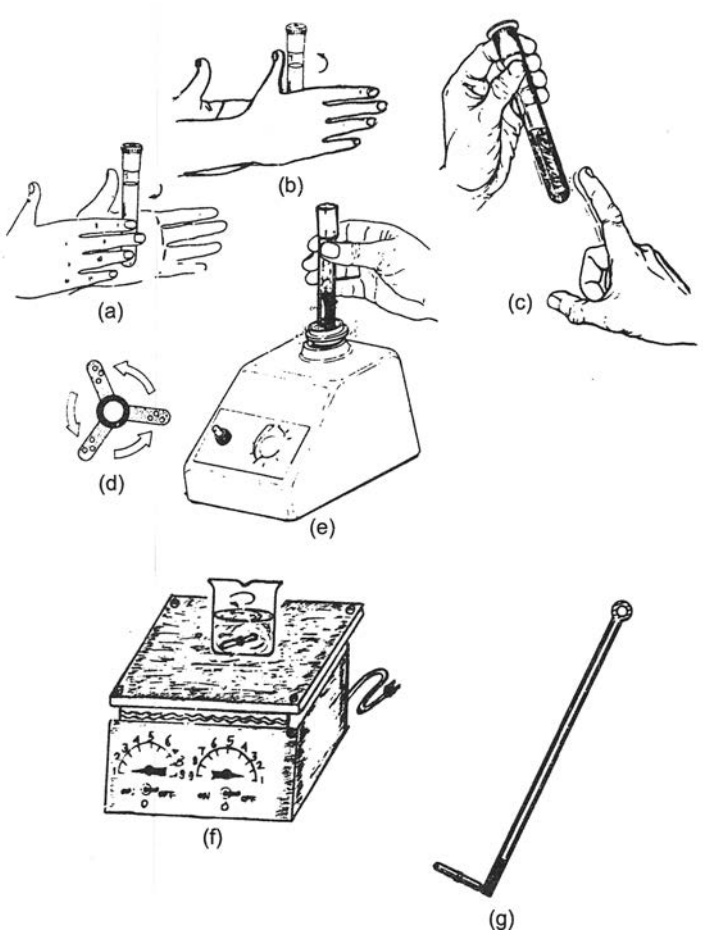

Figure 4.51 *Mixing of liquid in a test tube and beaker: (a and b) Manual mixing of liquid in a test tube can be accomplished by rolling the test tubes between palms, or (c) Tapping the bottom of the tube, (d) Mechanical devices for mixing liquid in test tubes are the rotator, and (e) Vortex mixer, (f) When the liquid is in a beaker mixing can done by using magnetic stirrer with or without a heating arrangement, (g) The magnet is retrieved after mixing by the bar retriever*

3. Adjust the knob to the desired volume.
4. Drive off the air by repeated dispensing of the liquid reagent and collect the reagent in a clean beaker to be returned to the reagent bottle.
5. Calibrate the volume of fluid dispensed by measuring the dispensed fluid with the help of a small-size graduated cylinder or by the weight method described earlier under calibration of pipettes. The use of dispensers has made work easier for the technician. Unlike the technician, the dispenser does not get fatigued, which improves the accuracy of the test. Automated dispensers are capable of dispensing equal volumes of fluid in a series of test tubes or any other containers. They can be driven by a motor or can be operated manually.

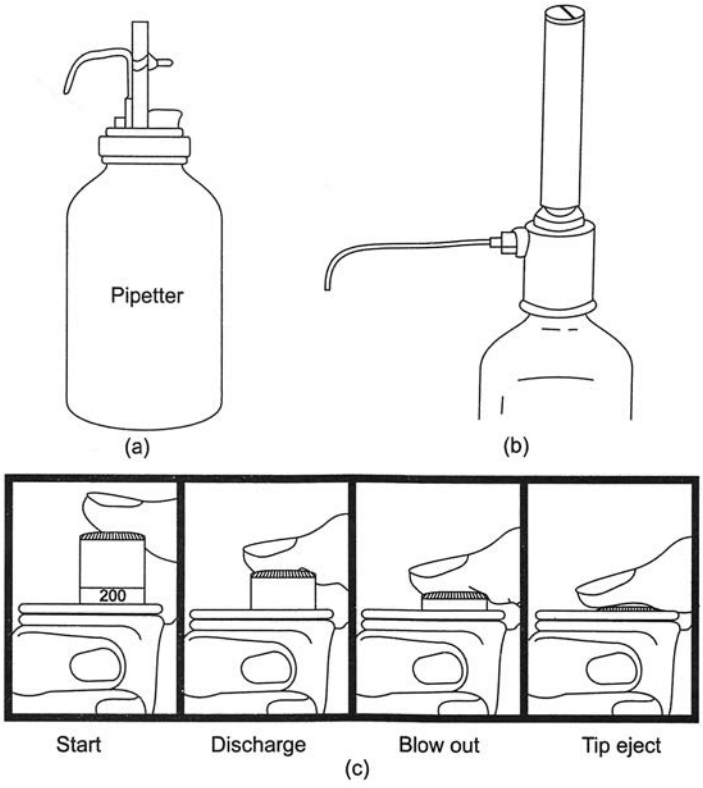

FIGURE 4.52 *(a and b) Various types of popular liquid dispensers, (c) Use of automatic pipettor*

Diluter

A diluter dilutes a solution with a solvent or another solution according to the need of the technician. The diluter works on the principle of alternate valve working system. In one stroke it picks up the pre-fixed volume of the fluid to be diluted and in the next stroke it picks up the pre-fixed volume of the diluent. This mechanism is applied in some of the automated systems used in chemistry (Clinicon Corona, Boehringer Mannheim, Petra Laboratory Glassware Industries, Haryana, UP, India).

The manual diluter consists of a diluent reservoir, a self-cleaning syringe and a dispensing tip. It has two volume-adjustment settings—one for the specimen and the other for the diluent. It works by three-stroke actions. In the first stroke, the specimen is picked up. Wipe the outside of the tip before going on to the next step. This avoids contamination and error in volume measurement. In the second stroke the diluent is picked up and in the third stroke, the specimen is dispensed, followed by the diluent. The diluent rinses the syringe while flowing out.

Automatic electrically operated diluters are becoming increasingly popular. They have various uses both as a pipettor and as a diluter. The blood diluters for cell counts are with fixed sample/diluent ratios for the enumeration of blood cells. In chemistry and serology, the dilutions are changed according to the need. Two mixing fluids are drawn into separate syringes or tubes which can be individually set. When the start button is pushed, the fluids (sample and diluent) are withdrawn into their respective syringes. After this operation is complete, the dispense button is pushed which discharge the fluids together into the receiving container. The syringes are interchangeable to different sizes.

Filter Pump

The function of a filter pump creates moderate suction for micropipettes. Greater suction can only be created by an electrically operated suction pump which is used for the culture of microbes. For the operation of the filter pump, water supply under reasonable pressure is needed. The filter pump is attached to the water tap and water is allowed to run freely. The water under pressure comes out of a narrow nozzle, which is caught in a small funnel. As the water rashes from the nozzle to the funnel it catches and traps air (Figure 4.53). This sucks air from around the nozzle and so from the suction tube outlet.

The components of the filter pump consist of the inlet for water, the suction outlet, a funnel-shaped bottom tube to receive the flowing water coming out of the narrow neck of the water inlet and the outlet for water. Places with limited supply of water will not be able to use suction pump sun by water flow.

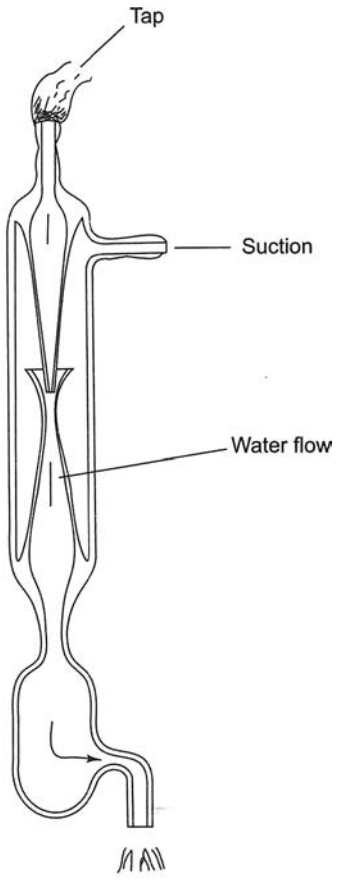

Figure 4.53 *Suction pump*

Special Laboratory Equipment

Incubator

Incubators are temperature-controlled chambers, which are well insulated. If the hot air oven (Figure 4.54) is set at lower temperature (37°C), it can be used as an incubator. Incubators are commonly used for growing bacterial cultures in the microbiology laboratory.

Water Baths

There are two types of water baths–incubator water bath and boiling water bath.

Incubator water bath

The incubator water bath has a constant temperature device which is electrically operated, and is controlled by a thermostat. The thermostat holds the temperature within the desired limits, temperature is usually kept at 37°C but occasionally it is kept at 56°C (e.g., inactivation of complement) or other temperatures. The water bath maintains better control of the temperature than the incubator.

Procedure to use incubator water bath

1. Fill the water bath with deionized water (avoid deposition of minerals present in tap water). See that the thermometer attached to the bath is immersed in the water. The level of water should be such that the test tubes to be immersed in the bath do not get drowned or float up. Put on the lid.

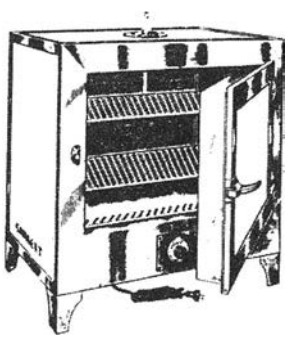

Figure 4.54 *Hot air oven used for sterilization of glassware and metallic utensils*

2. Turn on the switch and raise the thermostat (the knob moves clockwise); the indicator lamp starts to glow.
   - Watch the thermometer until the water reaches the desired temperature.
   - Turn down the thermostat until the indicator lamp turns off.
   - Allow the temperature to be maintained for a few hours before the water bath is used. The thermostat may have to be adjusted during this period.
   - Once the temperature is set, do not turn off the water bath even after use.

As a part of maintenance, keep the water bath clean; replace the water when it looks dirty. Check the water level and temperature at regular intervals. Never allow the water bath to dry up.

Boiling water bath

Boiling water baths are needed in some chemical reactions and for preparing solutions. This does not require any temperature setting and as long as the water is boiling, the temperature stays around 100°C (Figure 4.26).

Procedure of making a boiling water bath

1. Take a large size heat-resistant beaker and add water till half full.
2. Put the beaker on a Bunsen burner or electric hot plate. If a Bunsen burner is used, a wire gauze should be placed under the beaker.
3. Place the test tubes with the solution inside the boiling water bath. If the water stops boiling with the insertion of the test tubes, due to cooling effect, set the time only when the water re-starts boiling.

Equipment for Sterilization

Sterilization is applied in the destruction of pathogenic microbes in a contaminated sample and for the active exclusion of unwanted organisms from culture media and surgical equipment. Various methods of sterilization are discussed separately under microbiological techniques (Chapter 19). Here we will concentrate on two items of equipment, which are essential parts of a clinical laboratory—the hot air oven and the autoclave. In both cases, heat is used in the process of sterilization, but in the hot air oven dry heat is used, while in the autoclave, it is moist heat.

Hot air oven sterilization

The hot air oven uses a dry heat sterilization process and is applied for the sterilization of articles made of glass or metal (e.g., syringes, needles, scalpels, pipettes, etc.). It cannot be used for liquid media. This method is more efficient than the boiling water method (Figure 4.54). The inner chamber of the oven has adjustable shelves. The thermostat is used for adjusting the temperature. When the temperature is on the rise, it is indicated by the pilot lamp. The actual temperature of the oven is shown by a thermometer which sticks out for reading. The temperature setting is done in the same way as described under the water bath.

Sterilization procedure

1. Wrap the glassware (Petri dishes, pipettes, specimen tubes, syringes, containers for urine collection, etc.) in white paper sheets and tie with string. Separate the piston and barrel. The sucking end of the pipette can be plugged with non-absorbent cotton wool (pipettes used for handling specimens) and put in a metal container with the metal lid kept loose (or off) during sterilization (Figure 4.55). Pasteur pipettes are put in large size test tubes (or other glass containers) and then plugged with cotton. Needles must be

well protected keeping the pointed end facing down and resting on a cotton pad. They can be kept in tubes or put in a paper or cloth wrap and placed in a metal tray, which is then sterilized. Keep the lid of the metal tray separate during sterilization and replace it promptly following sterilization. This allows the steam to come in contact with the material sterilized.

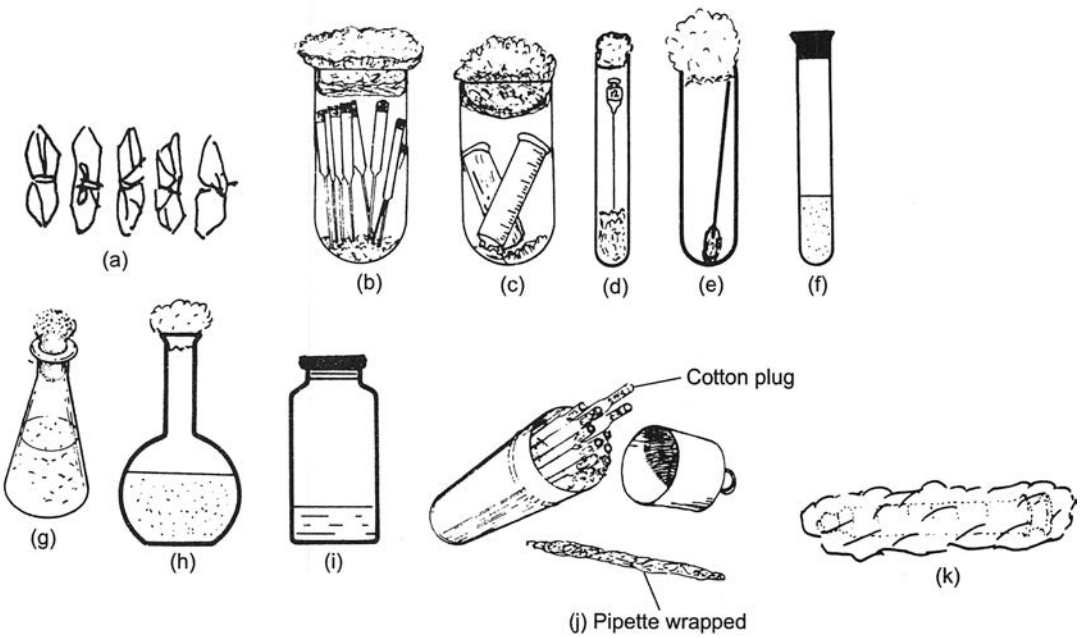

FIGURE 4.55 *(a) Preparation of material for sterilization by autoclaving surgical materials are wrapped in cloth, (b-e) Pasteur pipettes, needles and cotton swabs are placed in test tubes, (f-i) media and other fluids are placed in test tubes, flasks and bottles, (j-k) pipettes are either put in metal containers or wrapped in paper for sterilization by hot air oven or by the autoclave*

2. Switch on the oven; the indicator light, if provided, will be on. Set the thermostat at 175°C.
   Note Do not totally rely on the temperature setting shown by the equipment. It is advisable that the oven is checked regularly with a thermometer for the temperature setting.
3. Leave the materials for dry heat sterilization inside the oven for one hour (two hours if the material is heavy or bulky).
4. Switch off the oven and wait until the temperature falls to 40°C.
5. Remove the sterilized materials and store.
   Note The white wrapping paper used should turn brown; when pale yellow, the oven is not hot enough; if it is blackened or charred, the oven is too hot.

Autoclave (steam sterilization)

Autoclaving is the most effective and most commonly used method of sterilization (Figure 4.56). Properly carried out, autoclaving kills all micro-organisms, thus creating sterile conditions. It works on the principle that steam put under pressure reaches temperatures above the temperature of boiling water. The temperature of boiling water or steam is 100°C,

while steam under 15 lb (or psi) pressure will be 121 °C. Steam is efficient, in that it penetrates wrappings more rapidly than hot air to reach the surface of items to be sterilized. Hence, during steam sterilization or autoclaving, all covers are removed.

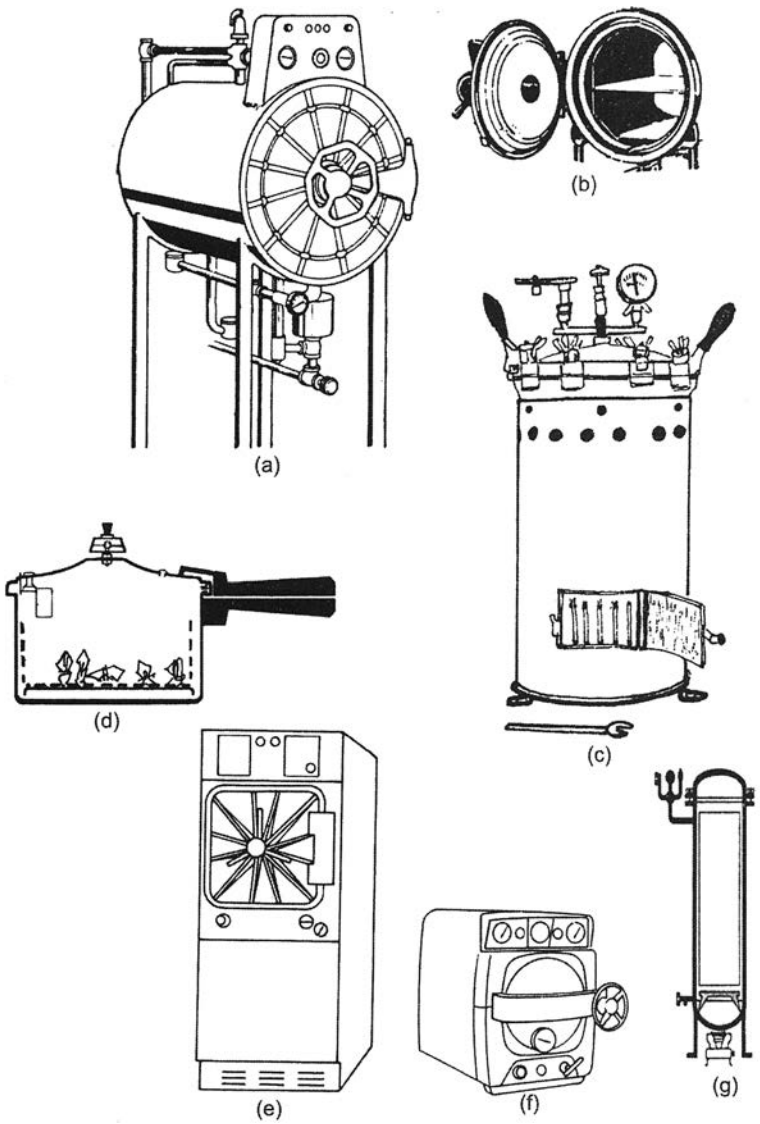

Figure 4.56 *Equipment for steam sterilization: (a and b) Horizontal type autoclave, (c) Vertical type autoclave and (d) Pressure cooker, (e-g) Modern autoclaves - floor model and (f) Table model*

Most items (syringes, needles, media, glassware and others) that require sterilization can be autoclaved satisfactorily. The first step in preparation for autoclaving is to clean items thoroughly. The next step is to wrap the items in individual packs (Figure 4.55).

While autoclaving, the technician should bear in mind that the steam pressure has no effect on sterilization in the ranges used with the autoclave. Pressure is needed to maintain the steam at temperatures above 100°C. Hence, timing for sterilization should be noted from the point when the temperature reaches 121°C and not when the pressure reaches 15 lb (15 psi).

Most microbiology laboratories have an autoclave to sterilize media and other materials; however, domestic pressure cookers are equally efficient and can be used in small size laboratories.

Preparation of material for autoclaving

Materials must be properly wrapped before putting in the pressure cooker, autoclave or hot air oven for sterilization (Figure 4.55). Solid materials are prepared in the same way as described earlier. Liquid media are kept in conical flasks, round bottom flasks or in test tubes held in a beaker. Test tubes without a lip are preferred. Cotton plugs, metal caps, plastic caps or aluminium foil are used to cover the vessel. Do not fill the liquid to the top or leave one-quarter space for the liquid to expand or boil without touching the lid. If it is necessary to use rubber stoppers, screw caps, or plastic caps; they should be loosely set in place in order to allow air to escape, to prevent the containers from bursting or blowing off the caps as steam is generated and to allow steam to penetrate easily.

Pressure cooker (steam sterilization)

Domestic pressure cookers are large saucepans designed to cook food very quickly, using steam under pressure (Figure 4.56). They are cheap, take less space than the autoclave but have limited space in which to keep the materials for sterilization. Small size autoclaves are ideal for smaller laboratories.

The pressure cooker has a lid with a clamp that sits on a rubber ring. The pressure valve on the lid indicates the presence of steam pressure within the sterilizing chamber. The lid should not be opened following sterilization, until the pressure valve drops; the drop in pressure valve indicates lack of steam pressure. The safety valve regulates the desired steam pressure by releasing the excess pressure of the steam. The valves are available with different weights in order to attain 10 or 15 lb (psi) steam pressures. For sterilization, only the weight of 15 lb (psi) is used.

Procedure for using a pressure cooker

1. Fill the bottom of the cooker with water. The water level should be such as not to touch the basket in which materials are kept.
2. Place the wrapped materials to be sterilized in the basket. Never lay articles flat, rather keep them in an upright position so that steam may circulate between them.
3. Fit the lid securely; check that the safety valve is functioning properly.
4. Start heating the stove and keep on high heat until steam starts to escape continuously. Allow the steam to escape for a while (5 min). This replaces the air with steam.
5. Put on the 15 lb (psi) weight and continue heating.
6. Wait until the valve begins to whistle. When it does, reduce the heat and note the time (0 min). Leave on moderate heat for 20 min.
7. Turn off the heat. Leave to cool slowly if the sterilizing material is liquid; otherwise, the pressure cooker can be cooled under the tap for fast cooling.
8. Open the lid after the pressure valve has dropped.
9. Remove the sterilized materials, and if necessary, put them in the oven for drying.

Laboratory autoclave

There are two types of laboratory autoclaves—vertical and horizontal (Figure 4.56). The vertical autoclaves are in a standing position and materials are inserted into the sterilizing chamber from the top. These are heated either by a gas burner or a kerosene stove. The horizontal types of autoclaves have the door on the side and materials are placed at eye level. These autoclaves are heated by electric elements.

Sterilization procedure with vertical autoclave

1. Fill the bottom of the boiler with water (up to the basket support). Do not let the water touch the basket; if necessary, drain out the excess water.
2. Put the basket with materials to be sterilized in the chamber and close the lid.

   Caution Make sure that the rubber washer is in its groove.
3. Screw down the lid clamps evenly and firmly but not too tightly.
4. Open the air outlet valve and turn on the burner to heat the water inside the boiler.
5. Continue heating until a jet of steam appears through the air outlet valve. Wait for 5 min to drive out the air and for the boiler to contain saturated steam.
6. Close the air outlet valve.
7. Tighten the lid clamps and reduce the heat slightly.
8. Watch the temperature gauge until 121°C is reached (approximately 120°C). Provide medium heat that maintains the temperature without producing excess steam. See that the needle on the dial remains close to the desired temperature. Start timing when the temperature reaches 121°C.
9. Continue sterilizing the material for 20 min (specimen containers are sterilized for 30 min).
10. Turn off the heat as soon as the required time is up.
11. Open the air outlet valve when the temperature falls below 100°C. This equalizes the pressure inside and outside the chamber.
12. Unscrew the clamps when the hissing sound stops, indicating the drop of steam pressure to zero.
13. Take off the lid and leave the materials to cool.
14. Remove the basket when it is cool. The condensed water on the surface of the sterilized materials can be dried in an incubator. Do not open the packages until they are ready to be used.

Sterilization procedure with horizontal autoclave

1. Load the sterilizer with the material to be sterilized.
2. Close and lock the door.
3. Open the air outlet valve to allow the air in the chamber to be displaced by the incoming steam.
4. Open the steam supply valve. This admits steam to the sterilizing chamber from the steam line.
5. Watch the temperature gauge as it approaches 100°C. The air is discharged from the chamber by this time. Close the operating valve.
6. Continue to watch the temperature gauge, which keeps rising beyond 100°C because the steam is under pressure. Autoclaves for routine laboratory sterilization are usually set for 15 lb (psi) pressure, giving a temperature of 121°C.
7. Begin to time the sterilization when the temperature reaches 121°C (and not the pressure of 15 lb). This is because the pressure may reach 15 lb, while the temperature may be lower if the air is not completely removed. The temperature is a better index than the pressure.
8. After 20 min or the prescribed time, shut off the autoclave by closing the steam supply valve. Wait until the pressure falls to zero on the gauge. If materials that were sterilized did not contain liquids you can open the operating valve to release the pressure more rapidly.

   Caution This should be done only when there is no liquid. If liquids are in the container, such sudden release of pressure causes them to boil up, wetting the plugs and blowing them out. It is therefore always necessary to let the pressure fall when liquids are being sterilized.

9. Open the door of the autoclave only when the pressure gauge shows 0 indicating no more steam pressure.

Precautions for using the autoclave

- Do not touch the valves (drainage valve or safety valve) when the autoclave is under pressure.
- Do not open the autoclave until the pressure has gone down to 0.
- Stay away from the steam when the door (or lid) is opened. It might cause serious burns. Wear heat-resistant gloves while removing the hot basket and flasks.
- Always watch the pressure which might cause accidents if the safety valve is not properly functioning.
- Do not leave the autoclave unattended when the steam pressure is on the rise.
- It is not good practice to leave the autoclave to cool for a long period (overnight) following sterilization. If it is left for several hours without the outflow valve being opened, a vacuum forms and the sterilized material may break.

Testing of autoclave

It is important to check at intervals whether the autoclave is working properly. This is an important part of the quality control programme. There are two ways of testing the efficiency and maintaining qualify control of the materials sterilized.

Indicator fluids or papers with a dye are commercially available. These change colours if the correct temperature-time combination has been employed. Efficiency of the autoclave can also be checked by culture method using heat-resistant live spores of *Bacillus stearothermophilus*. The sterilized spores are cultured in trypticase soya broth for 7 days at 55°C. If the autoclave is working properly, the organism fails to grow.

Laboratory incinerator

Disposal of laboratory materials requires special arrangements. All discarded materials from the laboratory are considered to be contaminated and infectious. This problem has also been discussed in Chapter 5. Much of the urine and faecal materials are flushed into the sewer but solid materials, such as cardboard boxes, papers, soiled clothes and such flammable materials need to be incinerated. Many laboratories use 'home- made' incinerators (Figure 4.57) which is made of a big strong metal drum. Two-thirds of the way down the drum, a metal grating with holes is placed on a metal bracket. The bottom is cut out for ventilation and for taking out ash. The vent at the bottom must have a door to keep the incinerator closed when not in use. The vent is made below the level of grating. The incinerator stands on 3–4 legs. A removal lid is made at the top which is kept closed when the incinerator is not in use.

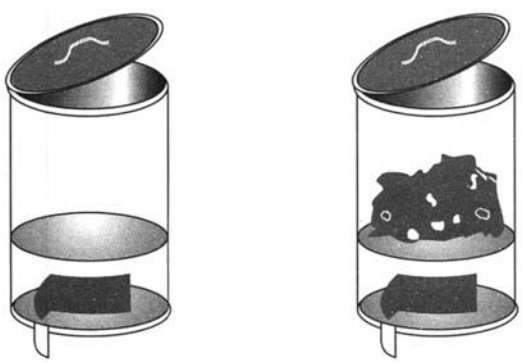

FIGURE 4.57 *Incineration*

Review Questions

1. Identify the following and state their uses: cuvette, funnel, graduated cylinder, Pasteur pipette and desiccator.
2. What is the difference between borosilicate and soda lime glass? Which of these is heat resistant?
3. What are the advantages and disadvantages of plastic ware?
4. What is volumetric glassware? Give examples.
5. How would you clean the following: pipette, Wintrobe tube, Sahli pipette, graduated cylinder, beaker and burette?
6. If a contaminated centrifuge tube is broken inside a centrifuge, how would you clean the centrifuge?
7. How does the serological pipette differ from volumetric pipette?
8. How would you calibrate a 100-µL pipette?
9. What is a Vernier scale? How is it used?
10. How would you store the following chemicals: ether, acetone, sodium hydroxide, sodium chloride and ammonia?
11. Prepare a standard curve from the following data:

Protein concentrations of standards (g/dL)	0	2	4	6	8
Absorbance readings	0.00	0.11	0.22	0.32	0.45

12. What are the principal components of a microscope? State their functions.
13. What is the magnification of a high-dry objective if the eye piece is 10×?
14. How do you clean an oil-immersion objective after use?
15. Describe the steps involved in using the oil-immersion lens.
16. How do you clean the cover slips?
17. What are the common disinfectants used in the laboratory? How would you disinfect the slides?
18. What will happen if you add water to the acid, instead of acid into water?
19. Why are the cover slips so thin compared with the slides?
20. How would you measure the size of a microbe under the microscope?
21. Why do you balance a centrifuge?
22. Describe the principle of operation and use of the following equipment: deionizer, autoclave and colorimeter.
23. What is the difference between sterilization and disinfection?
24. What is the advantage of steam sterilization over boiling water?
25. What is the effect of steam pressure during steam sterilization?
26. How would you check the purity of water? Would you consider demineralized water as sterile water?
27. What will happen if you mix alcohol with dichromate cleaning solution?
28. Why should you wear gloves while handling household bleach?
29. Describe the difference between 'weight loss' and 'weight gain' methods in weighing a reagent.
30. A technician was asked to prepare a 5% salt solution. He measured 5 g of the salt and transferred it into a beaker. Then he added 100 mL of water into the beaker with salt. Will the final solution be 5% or will it be less than 5% or more than 5%?
31. How would you measure the volume of a drop while using a Pasteur pipette?
32. What substances are incinerated in the laboratory?

Chapter 5

Specimen Handling and Laboratory Records

Chhotelaal Pande and Anant Kumar Pandey

Chapter Outline

Overview

In order to understand the role of laboratories in testing and reporting, one has to overview the process of information transfer. It is important to realize that there are three components, known in laboratory jargon as phases, associated with laboratory testing:

- Pre-analytical phase
- Analytical phase
- Post-analytical phase

Pre-analytical phase

The pre-analytical phase of laboratory testing refers to the situations and actions that take place prior to the specimen collection, during the collection, and during the processing-storage-transportation of the specimen. Phlebotomists and medical assistants often participate in this phase of laboratory testing. The importance of this phase cannot be emphasized enough. Generally speaking, the majority of problems associated with laboratory tests result from inadequate or inappropriate specimen collection, processing, storage, and transportation.

Analytical phase

The analytical phase of laboratory testing refers to the performance of the test been ordered. This phase also includes maintenance and calibration of laboratory equipment and instruments associated with the testing and performing quality control measures, which are in place to validate the test reagents and kits, the testing process, and training of the laboratory personnel performing the tests.

Post-analytical phase

The post-analytical phase of laboratory testing includes the processes associated with the recording and reporting of laboratory results, storage and/or disposal of specimens after testing, and provision and patient notification of test results. Even if the other two phases of testing occur without any exception, if this phase is not handled appropriately, then the overall experience will not be positive and may negatively affect patient's treatment.

Collection and Pre-Analytical Handling of Specimens

In order to obtain a specimen, either the patient can be transported to a central medical facility or the specimen already drawn from the patient can be sent to a laboratory for analysis. The latter is usually more convenient and cost-effective. One should keep in mind that specimen(s) start deteriorating as soon as they are removed from the body. However, such deteriorations can be slowed down by refrigeration, though it cannot be stopped altogether. Prompt fixation of tissues and smears will further allow the retention of morphology 'close' to normal conditions and stop further changes; however, prompt fixations of such specimens are not practical for all studies. The proverbial statement often emphasizes the importance of a specimen, '*The results are only as good as the specimen'*. A mishandled specimen may not only give erroneous results but also misguide the clinician responsible for initiating the treatment for the patient.

The most frequent source of variation affecting laboratory analysis in a well-functioning laboratory is not the laboratory investigation itself but specimen preparation and errors in the identification or labelling of a specimen. This chapter describes the recommended procedures for collection and transportation of the following specimens:

- Blood for biochemical, microscopic and microbiological investigations
- Urine
- Stool
- Throat swab, oral swab, nasopharyngeal swab, conjunctival swab, ear swab, urethral swab, and vaginal swab
- Sputum
- Body fluids—pleural, cerebrospinal, peritoneal (ascetic), pericardial and synovial
- Specimens from patients with sexually transmitted diseases

Collection of Specimens

Different persons collect specimens. **Attendants** in a hospital ward often collect stool and urine while most often it is the **phlebotomist** who draws the blood specimen. Collections of spinal fluids, serous fluid, bone marrow, tissue biopsy and many others involve greater risks for the patients and therefore they require greater technical skills to collect such specimens. Hence, a **senior nurse or an attending physician** may draw these specimens. The person collecting the specimen should be competent, calm and cheerful and must be skilled in explaining the contemplated procedure(s) in simple words. This may set the patient's mind at rest; otherwise the patient's anxiety might affect both the quality of the drawn specimen and results obtained.

Every specimen must be accompanied by a **request slip** (Chapter Appendix 5.1A). The request slip, among many other personal identification data, must mention clearly the patient's full name and date of birth. Person drawing the specimen must ask the name and date of birth for verification. Many hospitals use an armband for each patient, so that misidentification of patients at the time of collecting specimens, which is often a common source of error, can be avoided.

Many laboratories provide sticker ID **labels to be attached to specimen** collecting containers in order to minimize the error due to labelling. Smaller laboratories, however, may use adhesive tapes and indelible pencil or pens to identify specimens corresponding to numbers provided by the registration desk.

Pre-analytical Handling of Specimens

Some of the specimens such as blood, faeces, sputum, ulcer or pustule fluid and urine, should be considered as **high-risk specimens.** Specimens thought to contain dangerous pathogens must be handled with special care and transported with great caution. Special care is needed in **mailing specimens** in order to avoid biohazard. The technicians must know how to pack infectious materials, avoid breakage of and leakage from containers, as well as how to open packages containing infectious specimens. Loss and spoilage of specimens not only lead to loss of time in rendering patient care but also irritate patients owing to inconvenience and distress inflicted on them by repetitive drawing of specimens. Such actions also spoil the image of both the laboratory personnel and laboratories themselves.

Specimens must be processed as early as possible. **Temporary storage** of specimens in refrigerator is permissible only in some cases but not for all. For example, CSF specimens must be processed immediately, while other specimens may be stored for 2–8 h, or for several days, depending on the type of specimen. The technician should be aware of the waiting period and storage conditions of the specimen as delineated below in Table 5.1.

TABLE 5.1 *Specimen transport and storage*

Specimen	Purpose	Container/Preservative	Specimen amount	Holding temperature RT (room temperature)	Storage time
Blood	Culture	Tubes with sodium polyanethol sulphonate	8.3 mL	RT	24 h
Serum	Biochemistry	Blood collected without anticoagulant in sterile tubes.	5–7 mL	4°C to RT	2 days
	Serology	Blood collected in sterile tubes. For preservation add merthiolate (1:5000) or sodium azide (lg/L)	5–7 mL	4°C to RT	2–3 days
	Field collection	Collected on filter paper, dried.	1–2 drops	RT to 37°C	3 weeks
Vaginal/ urethral secretions	Culture gonococcus	Bottle/Transgrow medium	Swab	RT to 37°C	4 days
CSF	Culture, bacteria	Bottle/Transgrow	1–2 mL	RT to 37°C	4 days
		In tube, Cary-Blair	1–2 mL	RT	2 days
	Serology	In sterile tube	1 mL	4°C to RT	1 week
Stool	Culture bacteria	Tubes/Cary–Blair medium or Ames medium	Swab	RT	2 weeks
	Occult blood Microscopy (protozoa, worm, cyst, eggs)	Tube/MIF (merthiolate, iodine, formaldehyde) preservative 5 m L	Swab 1–2 g	RT RT	2 days Indefinite
Hair, nail, skin scraping	Microscopy, fungus	Envelope or screw-cap tube/none	Several pieces	4°C to RT	1 week
Pus	Culture, bacteria	Tube/sterile	1 mL	RT	3 days
Sputum	Culture, Tuberculosis, bromide (25 mL)	Tubes/silica gel	Swab	RT	10 days
Throat swab	Culture, bacteria	Tube/silica gel Loeffler's medium	Swab	RT	24 h
Urine	Biochemistry culture	Container, Bottle or tube/ sterile Tube/boric acid 1%	10 mL	4°C to RT	1 h
	Microscopy (Schistosoma eggs)	4 drops commercial bleach, and 2 drops HCl	10 mL	RT	Indefinite

Biopsy materials submitted for histological and cytological studies should be **fixed as early as possible**. Fixed specimens can be examined even after a long period. The most commonly tested specimens in different laboratories are listed in Table 5.2 and the various containers used in collecting specimens are also shown in Figure 5.1.

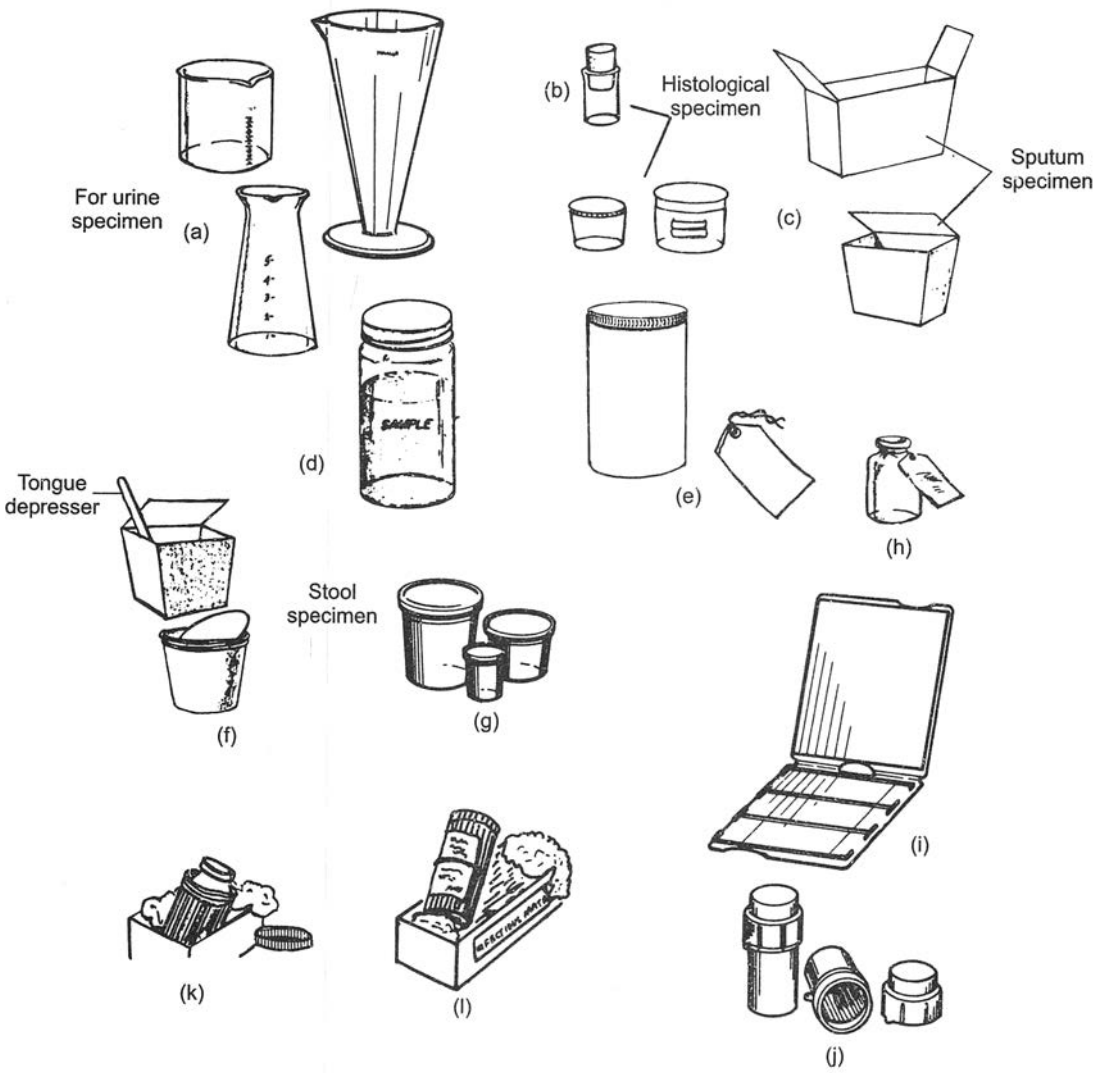

FIGURE 5.1 *Containers for collecting specimens: (a) Urine specimen, (b) Histological specimen, (c, g) Sputum specimen, (d) Liquid specimen, (e) Container for mailing specimen with tag, (f, g) Stool specimen, (h) Blood specimen, (i,j) Slide-holders for mailing, and (k, 1) Other materials prepared for mailing.*

Some of the specimens are more precious than others. Thus, Cerebrospinal fluid (CSF), biopsy and blood specimens of new-borns are more valuable than stool or urine specimens. These precious specimens must get priority over others. They get the **Stat** (an abbreviation of the Latin word **statim**, which means **immediately**) status for immediate analysis.

Pathogenic microbes pose a special problem in a specimen. Contaminating organisms (commensal), which may be harmless, might overwhelm the pathogenic microbes. Hence, various transportation systems are adopted and preservatives used to maintain the viability of micro-organisms while suppressing the growth of contaminating organisms. Many special media (e.g., Cary–Blair, Amies, etc.) help in the transportation of specimens with suspected pathogens. As a rule of thumb, **fresher the specimen, greater is the likelihood of a successful**

laboratory analysis. Use of such strong preservatives, like commercial household bleach (2 mL per 100 mL urine) or hydrochloric acid (0.1 molar), will not destroy eggs of parasites and specimens can be preserved for an indefinite period.

Procedures for Common Laboratory Specimens

Collection, processing and storage are important **pre-analytical steps** that determine meaningful diagnostic test results. Any error in these steps might affect results of an investigation to the extent that results are without clinical relevance in spite of correct analytical performance. This section describes recommended procedures for collection and processing of commonly arriving specimens in the laboratory. Table 5.2 gives the commonly submitted specimens in different sections of clinical laboratories.

Table 5.2 *Specimens handled by various sections of a clinical laboratory*

Laboratory section	Specimen
Haematology	Whole blood (EDTA-anticoagulated blood)
Coagulation	Plasma (citrated-blood)
Blood bank	Serum (clotted blood) or citrated whole blood
Bacteriology	Throat swab, blood, urine, stool, wound exudate, CSF and others
Mycology	Skin scrapings, wound exudate, urine, CSF and other body fluids
Parasitology	Stool, urine and blood
Serology	Serum (clotted blood)
Clinical pathology	Urine and other body fluids (CSF, pleural fluid, etc.)
Histology and cytology	Blood smear, Tissue biopsy and PAP Smears
Biochemistry	Serum, whole blood for blood gases

Blood

Blood is one of the most common specimens studied in various sections of clinical laboratory to detect blood–related disorders, metabolic disorders and infections and also to assess the general conditions of health.

The vascular system consists of the following three parts, all of which may be used for blood collection:

- The arterial system
- The venous system
- The capillary system

While the concentration of some analytes do not change from one body compartment to another, for others they might vary as a consequence of changes in metabolism or in the distribution of analytes between different body compartments. For example, concentrations of blood gases in arterial blood vary from those in venous blood. Oxygen concentrations are higher in arterial blood, while carbon dioxide concentrations are higher in venous blood. Arterial glucose concentrations are higher than venous glucose concentrations. Protein concentrations in capillary blood are higher than in venous blood (*Basis of Quality Assurance for Intermediate and Peripheral Laboratories*, 2nd Ed. by Mohamed M. El-Nageh, Walter Appel, Kraesten Engback, Claus C. Heuck, Jozef Vandepitte and Anders Kallner, WHO Publication, Cairo, 2002).

Types of blood specimens

Technique of arterial blood sampling is more complicated than any other sampling techniques because of the higher blood pressure of the arterial blood vessel system, which increases the risk of haemorrhage. A physician should usually perform it. Therefore, it will not be further described in this manual. **Venous blood** sampling is the method of choice for adults. For venepuncture, open (with a needle and open tube) and closed (with a needle and syringe or vacuum tube) techniques are in use. Closed systems reduce the risk of infection.

Blood will clot within a few minutes after it is removed from the body unless an anticoagulant (a chemical) is used to stop the process of clotting. Anticoagulated blood is also known as **whole blood**. For haematological studies, unclotted whole blood is needed. **Plasma** (fluid portion of unclotted blood) is obtained from the anticoagulated blood. Citrated plasma is needed for coagulation studies. **Serum** (fluid portion of clotted blood) is obtained from clotted blood, which is collected without any added anticoagulant to the blood sample. The blood banks usually require clotted blood for blood grouping and cross-matching. However, for blood grouping, EDTA-anticoagulated blood can also be used.

Blood is commonly collected by **venepuncture**. In case of venepuncture, blood is taken from the vein with the help of a sterilized dry syringe. This will be dealt in greater details in the following section. The alternate method is to draw blood through a skin **puncture**, which has a limited use because of the small quantity of the specimen obtained through this procedure. It is, however, ideal for paediatric patients and is acceptable for micro-methods.

During epidemiological surveys in the field, rapid **blood films from individuals** can be drawn and applied directly onto microscopic slides. The procedure is discussed in following pages. Specimen from skin puncture is used for basic haematological studies. For maximizing the yield of successful test results, in certain type of parasitic infections such as filariasis, malaria, etc., the drawing of blood specimens has to coincide with the presence of high density of parasites in the blood stream. If filariasis is suspected, blood drawn at midnight while a person is asleep and in the case of malaria, blood drawn while the person is experiencing fever and chills are more likely to result in the successful yield of tests.

Transportation of **dry blood specimen** is ideal for field surveys of some serological tests. Blood can be collected by skin puncture and preserved on filter paper for subsequent serological tests in the laboratory. The method is further elaborated in the following pages.

Haematological tests are used to study the cellular components of blood. Hence the blood specimens should be kept as close to their natural conditions as circumstances permit. One should be cognizant of the fact that blood will clot within few minutes if left without any anticoagulant. The clot destroys the cellular components and makes the specimen unfit for further studies. Usage of anticoagulant in the blood-collecting container prevents clot formation. Most anticoagulants remove calcium, an essential ingredient to form a blood clot. EDTA (ethylenediaminetetraacetate) and citrate belong to this group. Both these chemicals, however, change the pH of the whole blood and make it unfit for certain chemical measurements (serum calcium, blood gases and pH). Heparin, on the other hand, destroys some of the intermediates of the clot-forming mechanism. It is not an anticoagulant of choice in a haematology laboratory as it interferes in the staining reactions. Use of heparin and fluoride (oxalated) is limited for the determination of blood gases (and pH) and plasma glucose, respectively. These do not affect the pH.

Collection of blood specimen

The two most common methods of blood specimen collection are venepuncture and skin puncture.

Venepuncture

The volume of blood obtained by venepuncture is sufficient to carry out multiple tests. Venepuncture can be performed either by the syringe method or by the vacuum tube method. The latter is disposable and is not very practical for developing countries because of its high cost.

Preparation

Before proceeding to collect the blood from a patient, assemble all the necessary materials (Figure 5.2), viz., the containers for the blood collection, disposable needle and syringe, tourniquet, disinfectant (methylated spirit or 70% alcohol swab). Do not forget to include in the materials needed, personal protective items such as gloves for the technician and a mask (if indicated), also for use by the technician. In highly infectious cases, additional protective equipment may be necessary which has to be discussed with the patient's physician.

Blood collecting containers

Preparation of Blood Collection Containers

Containers for blood collection must be prepared before collecting the blood by venepuncture. These containers must be thoroughly cleaned with no trace of detergent and thoroughly disinfected by locally approved laboratory procedures. Specific amount of anticoagulant is to be added and dried inside the container. This minimizes the error due to dilution. The only exception to this is the citrate, which is added as a solution. All previously prepared containers can be stored at room temperature except the ones with citrate. The latter is stored in a refrigerator. For easy identification, containers must be labelled differently with stoppers of different colours.

EDTA (Ethylenediaminetetraacetate, Sequestrene, Vercene)

1. ***Preparing the anticoagulant solution:*** A 10% solution of dipotassium salt of EDTA (do not use disodium salt as it is less soluble) is needed. To make this, dissolve 10 g of the salt in about 80 mL of water in a 100-mL volumetric flask and then bring the volume of the solution up to 100-mL mark (q.s.: This procedure is referred as *quantum sufficit*).
2. ***Preparing containers for blood collection:*** There are two kinds of blood collecting containers used in developing countries—penicillin bottles and 15-mL test tubes with lid. The penicillin bottles are more widely used because they are cheaper. Clean the blood collecting containers thoroughly with detergent followed by washing with tap water and deionized water. As the amount of anticoagulant added to the container is meant for a specific amount of blood specimen, the container must be previously marked for the amount of blood specimen to be collected. For this, mark ahead of time with a diamond pen, the levels for 2.5 and 5.0 mL of fluid on different penicillin bottles. Use a burette or pipette with 0.01-mL graduation to make the volume markings. Then dispense 0.05 mL (equivalent to 5 mg of EDTA) in the 2.5-mL marked bottles and 0.1 mL (equivalent to 10 mg EDTA) in 5-mL marked bottles. (***Note:*** The EDTA concentration will be finally 2 mg/mL of whole blood). Dry the anticoagulant within the bottle by keeping it overnight in an incubator (37°C). Do not keep the rubber cap on during drying; replace it after drying. Use a colour code (the universal colour is violet or purple for EDTA) by putting a paint mark on the bottle for identification.

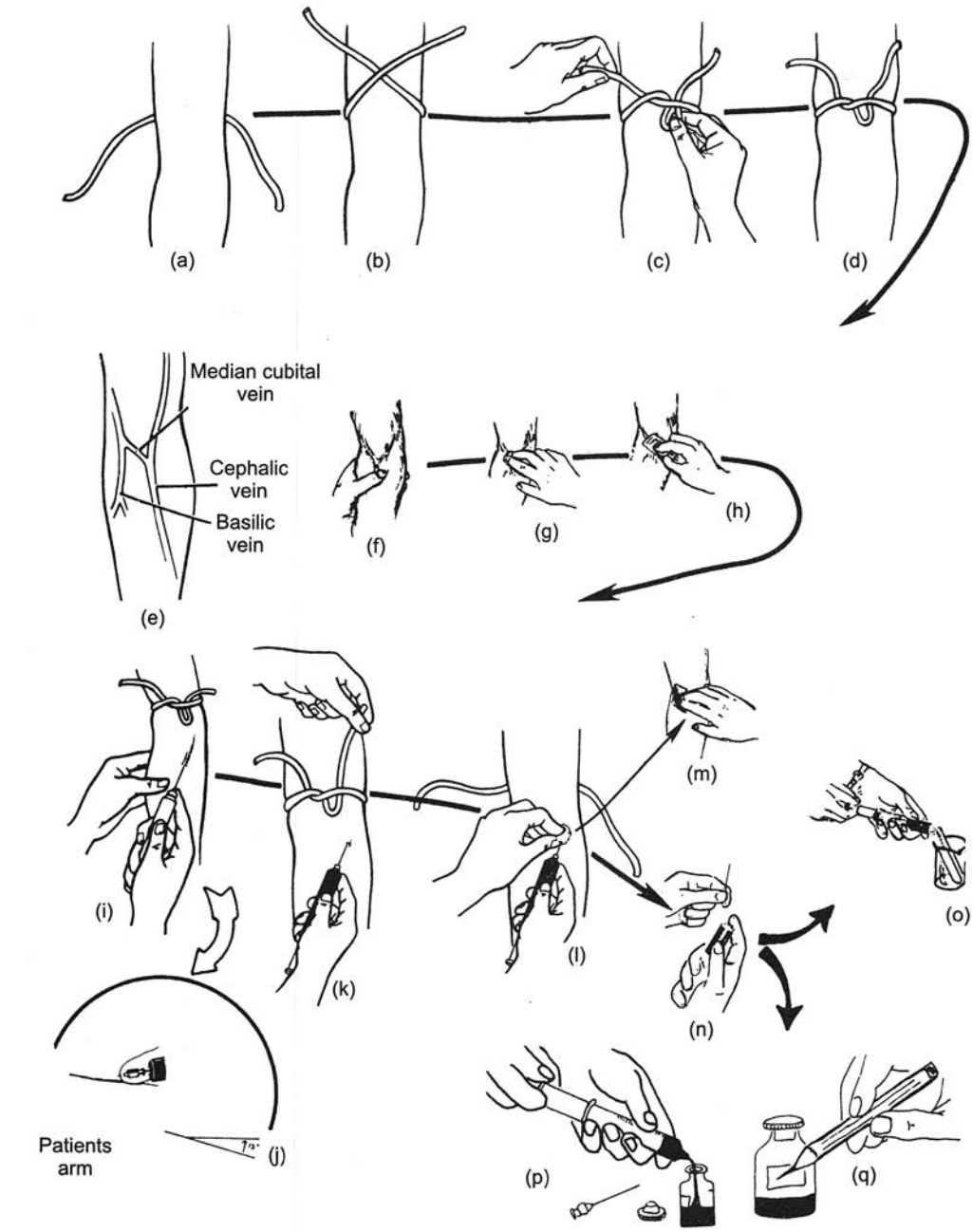

FIGURE 5.2 *Collection of blood by venepuncture: (α–d) Tie the tourniquet, Prepare the site for blood collection — (e–g) selection of commonly used veins, (h) Application of antiseptic; (i,j) Penetrate the needle, (k) Draw blood after releasing the tourniquet, (l, m) Withdraw the needle and apply pressure with sterile cotton in order to stop bleeding from the punctured spot, (n) Remove the needle from syringe, (o, p) Collect blood in an appropriate container*

Use of blood collection bottles

At the time of blood collection, collect the requisite amount of blood in the marked-bottle and mix the blood gently and thoroughly by swirling. Blood must be drawn to the appropriate mark (2.5 or 5.0 mL). If the blood is less in volume, the anticoagulant concentration will be more, resulting in distortion of cells. On the other hand, if the blood is more in quantity, the anticoagulant may not be fully effective and clotting might occur which will affect the results. The final concentration of anticoagulant should be 1.5 ± 0.3 mg/mL.

Double oxalate

Fluoride combined with oxalate is recommended for blood glucose determination, which is described later. Many laboratories use oxalated blood for coagulation studies but citrated blood is recommended.

The anticoagulant solution contains 1.2% ammonium oxalate and 0.8% potassium oxalate. Deliver 0.25 mL of the double oxalate solution into penicillin bottles with a 2.5-mL mark and 0.50 mL in those with a 5.0 mL-mark.

Evaporate the anticoagulant solution in the blood collection bottles in an oven (60°C) or in an incubator (37°C). Put the rubber cap after drying the fluid and then put the colour code on the bottle (grey is the colour of choice).

Sodium citrate

Prepare a 0.106 M solution of trisodium citrate in distilled water: This is equivalent to 2.73 g/100 mL solution of the trisodium salt. Sterilize it. This anticoagulant is used in solution form and is not dried inside the container. The penicillin bottles are specially marked for 2 and 10-mL marks. Give the colour code (blue is recommended). Add 0.4 and 1 mL of the sterilized anticoagulant solution in bottles marked 2 and 10-mL, respectively. After the mixing of blood specimen with the anticoagulant, the ratio of anticoagulant solution and whole blood comes to 1:4 and 1:9, respectively. The former is used for ESR (erythrocyte sedimentation rate) and the latter for coagulation studies. The Westergren method now modifies the ESR method where EDTA-anticoagulated blood is used. By this modification, a special blood collection procedure for haematological studies is avoided. The results are not significantly different so as to affect the clinical diagnosis. This will be further discussed in the haematology section.

Sodium fluoride–potassium oxalate

This anticoagulant is used for preparing blood specimens for the determination of glucose and urea in plasma by non-enzymatic methods. Fluoride inhibits glycolic enzymes and thereby prevents loss of glucose during transportation or delay in specimen handling. As fluoride is not a strong anticoagulant, it is mixed with oxalate. With the advent of newer enzymatic methods, the use of this anticoagulant is now limited.

The anticoagulant is made of 1.2% sodium fluoride (poison!) and 6.0% of potassium oxalate. Grind 1.2 and 6 g of the respective salts into a fine powder. Quantitatively, transfer the powder into a 100-mL volumetric flask, dissolve in 80 mL of distilled water and then make it to a 100-mL volume. Add 0.25 mL of the above solution into the blood collection vials with 5-mL mark and evaporate to dryness in an oven or incubator.

Heparin

This is rarely used in tropical countries and is limited to the determination of blood gases and electrolytes. This chemical is expensive and is not very stable in hot climates. Use commercially available heparinized blood collection tubes, which show the level of blood to be collected. In addition, heparinized blood is unsuitable for microscopic studies since cells are distorted more rapidly than in EDTA-anticoagulated blood.

Special note

1. All blood collecting tubes or vials must have airspace to facilitate mixing of reagents. As a rule of thumb this should be a volume corresponding to about one-fifth of the tube.
2. All tubes and vials must have rubber caps (stoppers) to facilitate transportation.

Blood collection by venepuncture

Supplies

- ***Needle:*** Sterilized sharp needles of bore size 18–20 gauge (medium, 1.2–0.9 mm) for adults and 23 gauge (0.5 mm) for children are needed. The bevel length should be medium (20 mm) for adults and short (15 mm) for children. The use of disposable needles is recommended. In case of reusable ones, the needlepoint should be kept sharp and assurance of their sterility ensured. Keep a stock of sterile needles in small glass tubes and the point should rest on a pad of non–absorbent (sterile) cotton wool and the tube should be plugged with the same material. A sterilized needle should be attached to the syringe under aseptic condition (Figure 5.2).
- ***Syringe:*** Syringes of different capacities, 2-, 5-, 10- and 20-mL, should be available. The size of the syringe to be used depends on the amount of blood needed. There are different types of connections between the needle base and the syringe tip; always read the manufacturer's instructions before using them. Check the end of each syringe for its right fit for the needle. At any stage of assembly of the needle and syringe, do not touch the needle tip and keep the assembled syringe and needle inside a sterilized tube or covered with sterile gauze. With reusable glass syringes, the fit of the plunger and barrel and the integrity of the syringe tip should be checked. Disposable plastic syringes come in a sterilized wrap and they have sharper needles for single use. Now-a-days, sterilized disposable plastic syringe with needle is recommended due to life threatening infections such as HIV.
- Following the use of a reusable syringe, it should be rinsed in cold water to remove the blood. All glass syringes should be properly sterilized and perfectly dry before their use. A wet syringe, the presence of detergent (not properly washed), the use of too fine a needle and emptying the syringe without removing the needle are some of the causes that lead to haemolysis. Haemolysed blood is unacceptable of a number of analyses done in haematology, chemistry and serology.
- ***Tourniquet:*** This is a soft rubber tubing of 2–5 mm bore and 30–40 cm length. A flat elastic rubber strip can also be used. The tourniquet is applied to the patient's arm to slow the blood flow and make the veins more prominent. This helps to select the puncture site for blood drawing.

Procedure

The procedure of venepuncture comprises of three steps—preparation, drawing blood and clean up.

A. Preparation

1. Introduce yourself to the patient and be pleasant. Read the patient's request form carefully. Identify the patient by name, date of birth and accession number. If the patient should be fasting before giving blood, inquire if the patient has done so.
2. Decide how much of blood is needed and arrange the correct tube(s) to be used for each test. For example, if haemogram is requested, 2 mL of blood in EDTA-tube will be sufficient, whereas, if the blood is needed in the biochemistry laboratory, 10 mL of blood in a plain container without any anticoagulant would be needed. This will yield the required amount of seram.

3. Plan the sequence in which blood samples must be taken. For example, the first 1 mL of blood must be discarded when blood is taken for a coagulation assay. So, collect this in the second tube, which will be used for other tests.
4. Before taking the blood, wash your hands with soap and water. If necessary, wear gloves if the patient is suspected of harbouring an infection or contagion. The phlebotomist, to minimize patient's apprehension, should explain the procedure of blood collection.
5. Fix the needle on to the syringe, touching only the top of the needle. Test the needle and syringe to make sure that the needle is not blocked and the syringe is airtight. Place the end of the needle in the sterile tube until ready for use.
6. Ask the patient to sit alongside the table used for taking blood. The patient should sit on a chair so that he/she can comfortably stretch horizontally the left or right arm. It also applies to a patient in the horizontal position (in bed). The elbow may be supported by an armrest.
7. The working arm (often the right arm) should be selected for blood collection, since on this side veins are easily accessible. Lay the patient's arm on the table, palm upwards and support it by placing a small cushion under the elbow. If the patient is in bed, lay his/her arm in an outstretched position. The correct site to take the blood is from the vein at the bend of the elbow, at the point where the vein is thickest and most easily visible. If possible, choose one of the branches forming a Y (median cubital or elbow vein) just above their junction. This is the most common site for blood collection. Alternatively, you can choose basilic or cephalic veins (Figure 5.4).
   Caution Never draw blood from a standing patient or patient sitting on a high stool. The phlebotomist should be prepared for the occasional patient who may faint and should be trained to administer first-aid techniques should this occur.
8. Fix the needle on to the syringe, touching only the top (syringe end) of the needle. Test the needle and syringe to make sure that the needle is not blocked and the syringe is airtight. Place the end of the needle in the sterile tube until ready for use.

B. Drawing Blood

1. Apply the tourniquet as described below.

a. Place the tourniquet under the patient's arm just above the bend in the elbow. With the right hand, wrap the tourniquet firmly around the arm and hold the ends.
b. With the left hand, pull one of the tourniquet's ends across.
c. Loop the end under the main part of the tourniquet half way through in a slipknot. The tourniquet should be just tight enough to slow down the blood flow and distend the veins; however, it must not be so tight that the blood flow in the arteries is reduced. The slipknot can be easily released when the tourniquet is to be removed.

2. Ask the patient to open and close his or her hand several times to swell the veins. Using the index finger of your left hand, feel for the vein where you will introduce the needle. Palpate the filled vein about 5–7 cm distal from the tourniquet and identify the site for vein puncture (Figure 5.3).
3. Disinfect the site on the skin with a sterile gauze or cotton swab soaked with 70% alcohol.
4. Take the syringe in your right hand, holding your index finger against the top of the needle.
5. Position the needle with the bevel uppermost and hold it at a low angle (about 150–250) to the skin surface with the tip directed towards the tourniquet. Make the venepuncture entering the centre of the vein without hesitation in the direction of the anatomical course of the vein to avoid injury to the interior wall of the vessel, which would cause pain to the patient.
   Important Never approach a vein from the side.

6. You will feel the needle going through—the layer of skin, which is resistant, than the wall of the vein, which is less resistant (more flexible). So, there will be a sudden loss of resistance as you penetrate the needle (Figure 5.4). Push the needle along the line of the vein to a depth of 1.0–1.5 cm.
7. With your left hand pull back the piston of the syringe slowly. Blood should appear in the syringe. Maintain a continuous blood flow, but avoid development of a strong negative pressure in the syringe chamber, which may cause collapse of the vein, foaming and haemolysis of red blood cells.
   Important The person collecting the blood sample must ensure that clotting does not occur during collection (e.g., due to a lengthy procedure and/or to multiple attempts with the same needle) and that there is adequate blood flow without turbulence.
8. Continue to withdraw the piston to fill the syringe with the required amount of blood.
9. After the requisite amount of blood is withdrawn, place a sterile dry gauze pad under the needle still sticking in the vein.
10. Open the tourniquet, by pulling the looped end and allow blood to flow freely in the vein. Do not pull the syringe and needle out prior to opening the tourniquet.
11. Apply a dry swab over the hidden point of the needle. Withdraw the needle in one rapid movement from under the swab (Figure 5.3).
12. Ask the patient to press firmly on the cotton wool swab for 3 min, keeping the arm outstretched. Bending the arm back over the swab is not recommended because of the risk of a haematoma.
13. Remove the needle from the syringe.
14. Expel the blood slowly out of the syringe gently down the side of the container tube or vial. Fill specimen tubes or vials with the blood up to the mark. Immediately invert tubes or vials that contain the anticoagulant several times to ensure complete mixing of anticoagulants. The mixing should be thorough but gentle in order to prevent clotting without causing haemolysis.
    Note If several tubes are to be filled from the amount of blood drawn, the following sequence is recommended:
    - Blood culture tube (Bacteriology)
    - Plain tube for serum (Biochemical analysis)
    - Tube with anticoagulant (whole blood—haematology and coagulation)
    - Other additives
15. Label tubes or bottles clearly with the patient ID, date of specimen collection and the accession number.
16. Before the patient leaves, re-inspect the venepuncture site to ascertain that the bleeding has stopped. If the bleeding has stopped, apply an adhesive tape over the cotton wool swab on the wound; otherwise, continue to apply pressure until the bleeding stops. Do not leave the patient until the bleeding stops.

C. Cleanup

1. Rinse the needle and syringe at once with cold water, then rinse in a disinfectant (see disinfection procedure) and give a final rinse. Prompt rinsing prevents clotting of blood in the syringe or lumen of the needle. Many laboratories prefer to leave the syringe and the needle in water with a mild detergent. Then they are washed later thoroughly prior to sterilization.
2. Place the rinsed needles and syringes in small glass tubes plugged with non-absorbent cotton wool and sterilize in an autoclave (see Sterilization). If dry-heat sterilization is done in an oven, do not use cotton wool.
3. Never use a needle or syringe on another person before it has been re-sterilized. Disposable needles must only be used once, as they cannot be re-sterilized.

Special Notes

Patient preparation

For the measurement of certain analytes, it should be ascertained that the patient has fasted for 12 h. Inquire from the patient if he/she followed the physician's instructions.

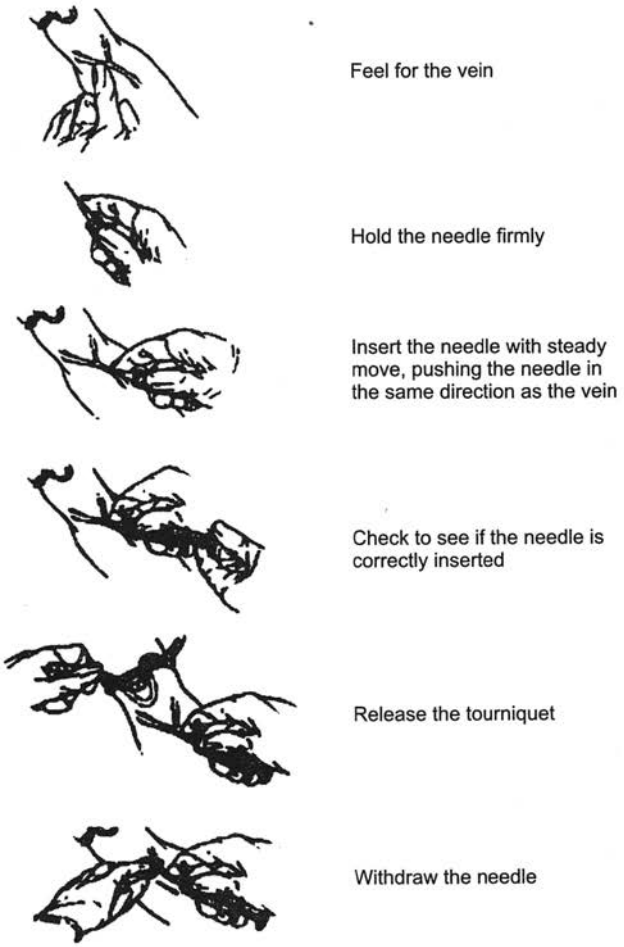

FIGURE 5.3 *Basic steps in venepuncture*

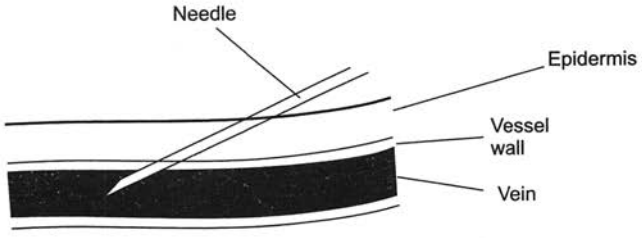

FIGURE 5.4 *Cross-section of puncture site during blood collection by venepuncture method*

Preventing haematoma during venepuncture

- Preferably use veins in the elbow area and only major veins.
- Be careful that the bevel of the needle is fully inside the vein.
- Be careful not to transverse the vein.
- Loosen the tourniquet and ensure haemostasis with a dry sterile cotton ball before pulling out the needle.

Special Circumstances

- When patients are receiving a transfusion or infusion, blood should never be collected near the infusion site or from the catheter but from the opposite arm. If neither arm is free, an ankle vein is the site of choice for the venepuncture. The rationale for this is to avoid collecting any substance that has been infused (e.g., glucose, potassium gentamicin and heparin) and which could give rise to misleading interpretation of results.
- In the weak or elderly patients, the venous pressure may be so low that the pressure of the needle may collapse the vein. Take extra care to draw the blood slowly.
- If the patient's clothing is too tight above the venepuncture site, it slows down the flow of blood and it may also cause haematoma. Loosen the cloth before drawing blood.

Capillary puncture

A capillary is a small blood vessel connecting the small arteries (arterioles) to the small veins (venules). The **capillary blood is obtained by skin puncture.** It provides only small quantities of blood specimens for making a blood smear or haematocrit determination. Skin puncture specimen is preferred over venepuncture specimen for the microscopic study of a blood smear avoiding the patient's traumatic experience during venepuncture. However the small amount of specimen collected by skin puncture is inadequate for most routine tests.

Capillary blood samples are more difficult to handle than venous blood samples and should only be used in special circumstances, e.g., neonates and infants, control check for diabetes, severe anaemic patients, blood gas analysis and in certain patients in whom venous blood sampling is difficult. Squeezing of the finger and subsequent dilution with interstitial fluid is the major reason for errors in capillary sampling, e.g., a decrease of concentrations of components and particulate matter (e.g., blood cells and parasites) and haemolysis. To reduce this, remove the first drop of blood before collecting the sample with a free flow of blood. Capillary blood is also used for preparing thin and thick blood films for the diagnosis of malaria and other blood parasites (Borrelia, trypanosomes, microfilariae).

Site for capillary blood collection (Figure 5.5)

- Ear-lobes in adults and children
- Fingertips in adults and children

Note Finger punctures should not be performed in infants, as there is a risk of injuring the bone. Puncturing the plantar surfaces of the heel of small infants can also damage the heel bone. Use big toe and medial and lateral positions of the planter surface of the foot of a neonate.

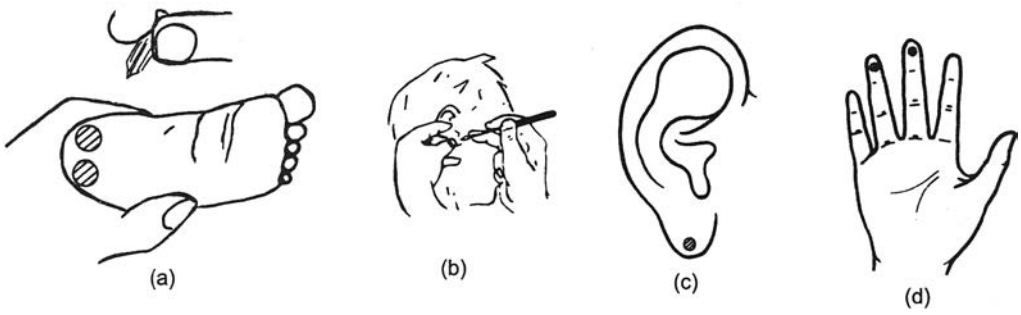

FIGURE 5.5 *Sites for skin puncture: (a) Toe puncture for infants, (b and c) Ear puncture, (d) Finger puncture*

Supplies

- Cotton ball and sterile gauze pad.
- Capillary tubes (may be coated with heparin to avoid blood clotting prior to the laboratory investigation).
- Disinfectant (ethanol or isopropanol, 70%).
- Lancet or needle, sterile.

Procedure (Figure 5.6)

1. Check the things you need. Allow the patient to sit comfortably.
2. Find a spot on the middle finger or ring finger of the left hand. The spot is located on the side of the finger, which is less sensitive than the tip. Toe puncture and ear lobe puncture are done in case of infants. Avoid previously punctured sites.
3. Increase the blood flow by rubbing or carefully warming the skin in order to enhance the accumulation of capillary blood similar in composition to that of arterial blood. Use of warm water may be advisable for old patients. Cold skin might prevent a free flow of blood which is essential.
4. Cleanse the puncture site with a sterile gauze pad soaked with 70% alcohol.
5. Dry the puncture site with a sterile gauze pad (or air dry), so that residual alcohol will not mix with out-flowing blood; otherwise haemolysis may occur. Wet puncture site also does not allow the oozing out blood to form into a well-rounded drop, which is necessary for good results especially when the drop is used for smearing on the slide.
6. Grab the finger firmly and quickly puncture the site with a sterile lancet. The puncture should be 2–3 mm deep. A deep puncture hurts no more than a superficial one and it gives considerably more satisfactory flow of blood. If a good puncture has been made, the blood flows freely. If it does not, use gentle pressure to make the blood form a round drop. Excessive squeezing causes dilution of the blood with tissue fluid. Discard the lancet in the appropriate disposable container. Used lancets should never be left lying on the work area.
7. Wipe away the first drop of blood with dry sterile cotton wool or gauze. The first drop of blood is contaminated with tissue fluid and will interfere with the laboratory results if used. The succeeding drops are used for laboratory tests.
8. Collect the out flowing blood in a capillary tube by holding the tube to the blood drop. This will be used for the haematocrit determination or sucking into the Sahli pipette for haemoglobin determination or for blood count. By touching the blood drop on a clean slide, smear can be made on the site. About 20 μL, of blood will probably be needed in completing laboratory requirements.
9. After blood collection, press the puncture site with a dry sterile gauze pad to stop further bleeding.

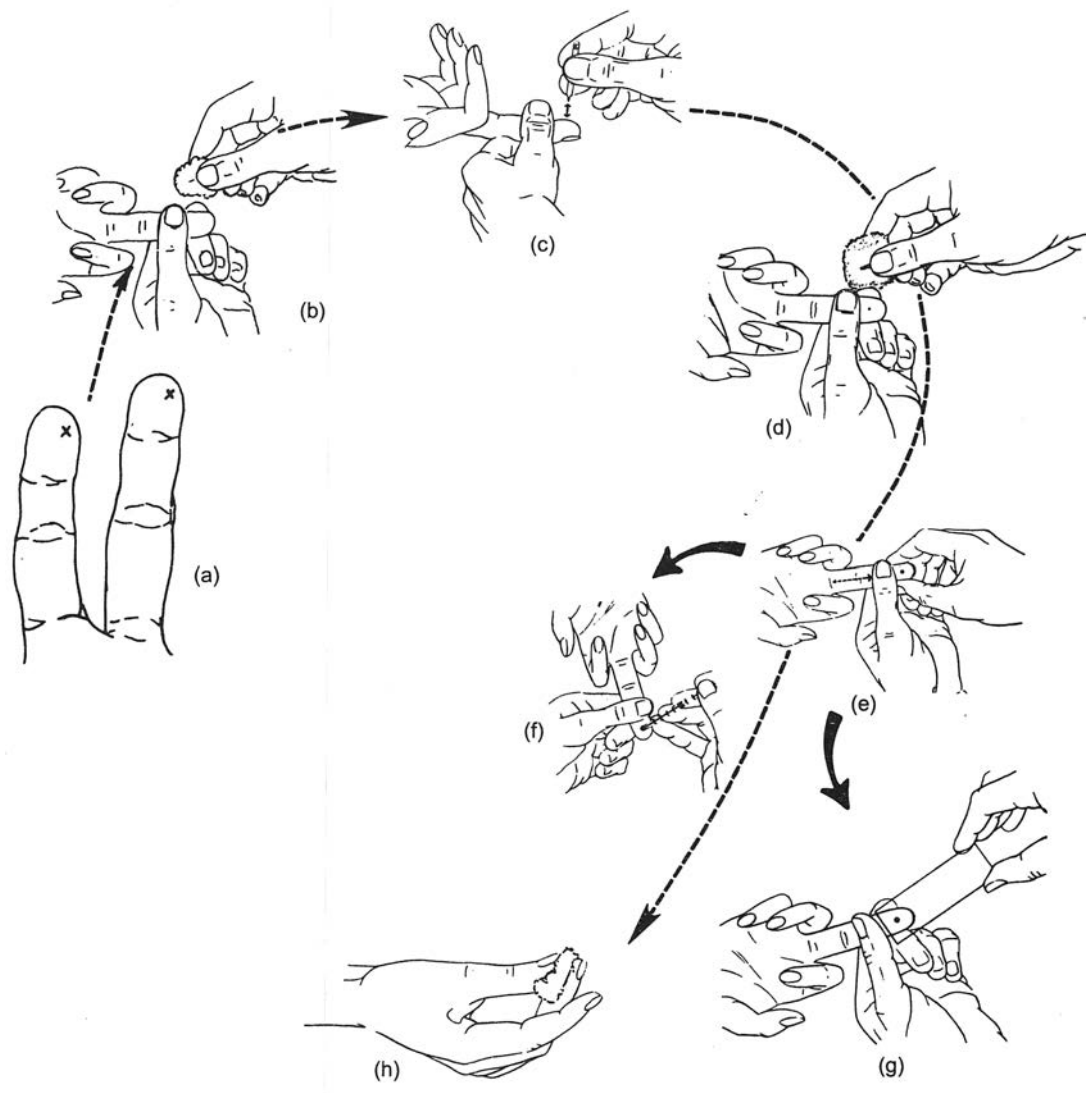

FIGURE 5.6 *Finger stick technique: (a) Site for finger stick, (b) Disinfect the site, (c) Puncture, (d) Wipe off the first drop of blood, (e) Gently squeeze the finger and produce a large rounded drop of blood, (f) Drawing up the blood for cell count, (g) Place a drop on the microscope slide for preparing smear, and finally, (h) Press a wad of cotton on the puncture site in order to stop the bleeding. Fix the cotton with an adhesive tape if necessary.*

Things to remember

- To avoid infection do not puncture the same site twice.
- Never touch the tip of a sterile blade or keep it on the table. If a disposable lancet is not available, you can use broken glass chips or needle sterilized in alcohol.
- Do not touch the blood; use rubber gloves, if available. All specimens are potentially hazardous. Hepatitis B viras and AIDS virus (acquired immune deficiency syndrome) are transmitted through the blood and other body fluids.

Processing of blood specimens

Blood specimens collected by venepuncture are occasionally processed in the collection area or they are sent directly to the testing areas for further processing and testing. Early processing or separation of blood components is recommended for more reliable results. Plasma is the liquid portion of anticoagulated blood, while serum is the liquid portion of clotted blood. Plasma contains fibrinogen along with the soluble ingredients of the fluid component of blood. Serum, on the other hand, has all chemicals of plasma fluid, except the fibrinogen that is used up in the clot formation.

Preparation of plasma

1. Collect blood in a tube with dry anticoagulant. Make sure the tube is not wet. This will cause haemolysis.
2. Mix instantly by gentle inversion. This yields whole blood.
3. Whole blood is now centrifuged at 2500 rpm for 10 min. This separates the cellular component of the blood, which goes to the bottom as red-coloured, from its liquid component of the blood, which stays at the top.
4. Take out the liquid portion (plasma) into a separate tube for further use. Plasma is commonly used in coagulation laboratory and in blood grouping.

Preparation of serum with centrifugation

1. Collect blood in a plain tube without any anticoagulant.
2. Allow the blood to clot for 30–60 min at room temperature (Figure 5.7).
3. Release the clot from the wall of the tube by means of an applicator stick.
4. Centrifuge at 2500 rpm for 10 min.
5. The liquid portion at the top is the serum. Transfer the serum into another test tube for further testing.
6. If electric centrifuge is not available, one can obtain serum with the help of a hand centrifuge.

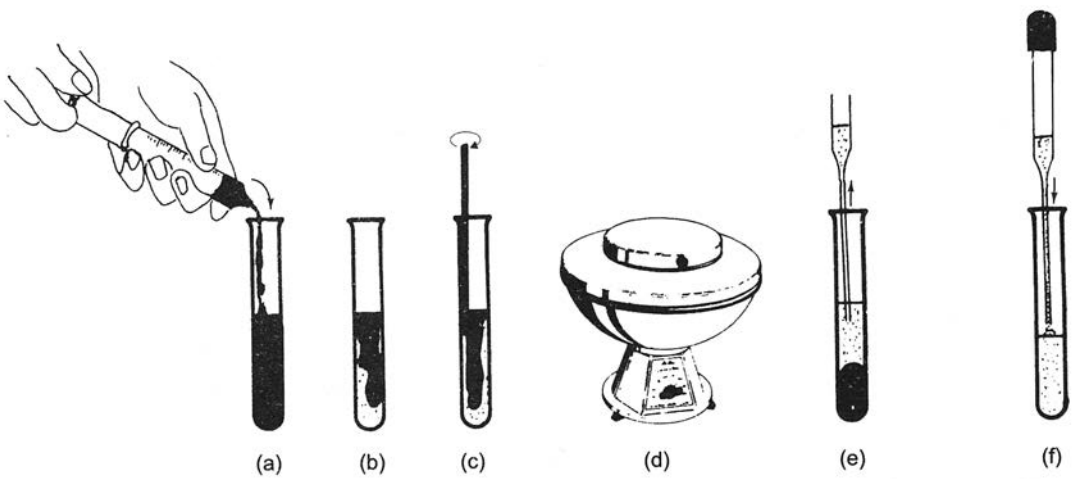

FIGURE 5.7 *Preparation of serum following centrifugation of clotted blood: (a and b) Allow the blood to clot for 30–60 min at room temperature, (c) Release the clot from the wall of the tube by means of an applicator stick, (d) Centrifuge, and (e and f) Transfer the serum to another test tube*

Preparation of serum without centrifugation

Under some circumstances, when the blood is collected in penicillin bottles, the following method may be applicable to obtain the serum (Figure 5.8):

1. Collect the blood in a clean penicillin bottle without anticoagulant.
2. Allow the blood to clot at room temperature for an hour.
3. Separate the serum after an hour with the help of a Pasteur pipette.

Hand centrifuge can be used to separate serum from clotted blood in laboratories where electricity is not available. Serum should be removed from the clotted blood as soon as possible after centrifugation. This is now been done conveniently with the use of special gel (serum separator vacuum tube) but that may not be available in developing countries.

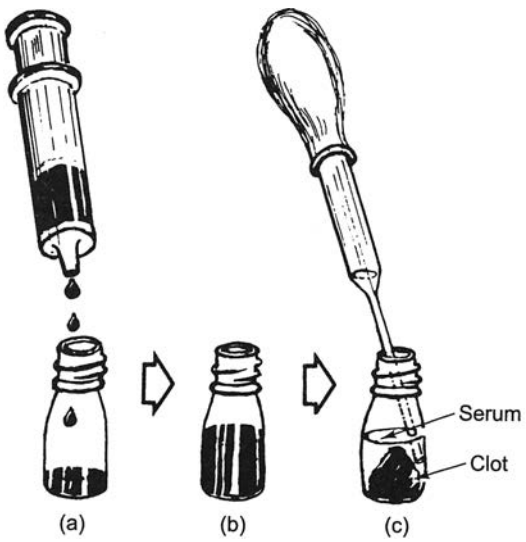

FIGURE 5.8 *Preparation of serum without centrifugation: (a) Collect blood in a clean penicillin bottle without anticoagulant, (b) Allow the blood to clot, and (c) Separate the serum after 1 h with the help of a Pasteur pipette*

Storage of blood specimens

Blood is a living tissue that retains considerable activity even after sampling. Thus, glucose is metabolized by erythrocytes, being their only source of energy. The metabolism can, however be suppressed or inhibited by the addition of fluoride ion, monoiodoacetate and to some extent by EDTA. Other substances may be degraded, such as enzymes and coagulation factors, light sensitive bilirubin and some hormones, e.g., insulin. Storage and transportation of whole blood, whether clotted or not, might cause haemolysis or leakage of intracellular components, e.g., potassium. Even in tubes containing a separation gel or separation device, blood cannot be safely stored or transported without separating the cells. The method of separating plasma and serum has been described. Serum and plasma can be stored for prolonged times at lowered temperatures or frozen. There are some analytes, however, that do not withstand freezing and are better stored at 4°C, e.g., S-lactate dehydrogenase and some coagulation factors; others are better kept at room temperature, e.g., the prothrombin time will be shortened by activation of coagulation factor VII at lower temperatures. Table 5.1 indicates the common changes found in stored blood specimens.

Repeated freezing and thawing might introduce uncontrollable errors. Frozen serum or plasma remains non-homogenous and needs prolonged, careful mixing without foam formation before being investigated. All stored samples should be properly capped to prevent

changes in concentration as a result of evaporation. In blood, plasma or serum, the concentration or activity of an analyte changes with time (Table 5.1) depending upon storage conditions. The activity of some analytes changes within a short time. Serum stored for a long time is not recommended for electrophoresis. The results of determination of triglycerides and cholesterol may be erroneous when using cooled, old-stored serum because of non-homogeneity of specimens. Routine investigations should therefore be made using fresh specimens. When samples are to be preserved for more than a week, they should be rapidly frozen to –20°C.

Clotting of blood proceeds faster in glass tubes than in plastic tubes, where it may continue for hours if the reaction is not artificially accelerated. Plasma or serum should be separated from blood cells. The exposure of fluids to direct sunlight must be avoided, as it causes degradation of bilirubin.

Analytes affected by prolonged storage of blood specimens

Potassium, chloride, phosphate, creatinine (determined by Jaffe reaction), glucose, serum iron, lactate dehydrogenase (LDH), alkaline phosphatase, AST, coagulation factors—V, VI and VII, blood gases, blood count, PT and APTT and ESR.

Table 5.1 shows the stability of the common analytes measured in clinical chemistry after storage in refrigerator at 4°C in a stoppered tube (data taken from Basics of Quality Assurance for Intermediate and Peripheral Laboratories, 2nd ed., El-Nageh, Mohamed, WHO Regional Office for the Eastern Mediterranean, Cairo, Egypt, 2002).

Things to remember during storage of blood specimens and their subsequent analysis

- Do not refrigerate blood specimens submitted for bacterial culture. Most other tests can be performed with refrigerated blood specimens.
- For serological investigation, the serum can be frozen for later study. A refrigerated serum specimen can be used for most biochemical analysis for certain durations of time (Table 5.1).
- For red cell investigation (haematocrit, haemoglobin concentration, red cell count), refrigerated blood specimen is acceptable for 8 h. For white cell investigation (leukocyte count, differential count), results are unreliable after 4 h. Specimen for platelet count should not be refrigerated and the test must be completed within 2 h.
- A blood film fixed in methanol can be stored for an unlimited period.
- For requisite volume needed for an assay (blood or serum), the refrigerated blood specimen must first be brought to room temperature. A cold specimen of the same volume yields false high value.

Transportation of blood specimens

Transportation of blood specimens to reference laboratory poses a special problem. The specimen must be properly secured as to avoid any breakage or leakage. All specimens are potential biohazard and must be handled carefully. In order to avoid haemolysis, serum may be separated before transportation. Whole blood transport should be in well-padded containers in order to avoid physical injury to cells. Equal care should be taken at the time of opening the packages.

Filter paper technique for collection and transport of body fluid specimens

The transport of liquid specimens soaked in filter paper is a simple and safe procedure. The dried blood can be used for such things as serological tests for syphilis, yaws and protozoan diseases. Blood is collected with the help of a disposable lancet soaked in a filter paper mounted on a card, dried thoroughly and stored until tested. The dried blood sample can

be used for such things as serological tests for syphilis, yaws and certain protozoan diseases. The filter paper can be used in any format but is commonly incorporated into a form or card specifically constructed for sample collection. This facilitates transportation in a pre-designed plastic bag with a zip lock and can be held in a transportation envelope without folding. The technique can be adopted for other body fluids as well. Whatman No. 1 filter paper (4 cm × 3 cm strips), about the size of a microscope slide, or Schleicher and Schuell filter papers are commonly used.

1. Always use gloves before blood sampling. It is, however, important that the filter paper surface does not come into contact with gloved hands or any other material, solutions and lotions, prior to and during the entire process of sampling. Hold the filter paper by the edge.
2. Complete the patient data (name, accession number and date of birth) on the card with a ballpoint pen or a pencil (never use ink). Many commercially available filter papers may have a circle in the centre, otherwise make the outline with a pencil (about 2 cm diameter) in advance. You can make a standard dice to make the circle with constant size. Make sure that you do not touch the circled area. Use of commercially available standardized filter paper gives better results and is recommended for maintaining quality.
3. Complete the patient's identification data on the narrow side of the filter paper without touching the circled area of the filter paper.
4. Clean the puncture site on the patient's fingertip, or heel of an infant, with 70% alcohol. Wipe the site dry with sterile gauze. Use a sterile disposable lancet for puncture. If disposable lancets are not available, a heat-sterile needle can be used.
5. Dab the lancet and let the first drop of blood ooze out; wipe away the first drop of blood with dry sterile gauze.
6. Then allow a large drop of blood to form on the puncture site. Soak the blood on the circle of the filter paper until the circle is completely filled.
   Caution
   - Avoid squeezing the puncture site as this might cause haemolysis of the specimen (a source of error) and dilution of blood with tissue fluids.
   - Do not layer successive drops of blood in the printed circle because this may cause caking.
   - Do not apply blood more than once.
   - Apply blood to one side of the filter paper only.
   - Incompletely soaked circles are not acceptable.
7. Allow the blood specimen to dry for 3 h in the air in a horizontal and preferably elevated position (to avoid contamination and to allow better air circulation around the card for drying). Do not let the specimen come into contact with any surface, direct heat or sunlight. Do not refrigerate samples.
8. Place each dry specimen card into an envelope or a small plastic bag, add a few granules of desiccant and close the envelope hermetically for mailing. Store the filter paper in a small plastic bag with zip lock in a cool dark dry place. Specimens that have been stored up to three months, under appropriate conditions, may give acceptable results.

Blood for bacterial culture

Blood specimens for **bacteriological culture** can be preserved using liquid (sodium polyanethol sulphonate) in normal saline solution. The tube and preservative must be sterile. Liquoid is an anticoagulant and also neutralizes bactericidal substance in fresh blood. It can be autoclaved in the collection tube for sterilization. For approximately 8.3 mL of blood sample, 1.7 mL of 0.35% liquid is used. In order to avoid repetition, this will be further elaborated in the Microbiology section.

Urine

Urine is collected for chemical, cytological and microbiological investigations. Various metabolic disorders, renal dysfunction and urinary tract infection are diagnosed from urinalysis. Most often the specimen is referred to the clinical pathology laboratory, which deals with various body fluids.

Arandom urine specimen collected in a clean, but non-sterile container is used for qualitative and/or semi-quantitative examination of contents, such as glucose, protein, pH, specific gravity, bile pigments and the possible presence of blood, pus or crystals. Often the first morning-voided specimens are requested because it gives the urine concentration most accurately. Otherwise the time of collection or volume of the voiding is not important. Quantitative urine analysis is helpful for assessment of kidney function. For chemical and microbiological examination, the urine must be collected 'clean', as any discharge or pus from the vagina or external genitals added to the urine invalidates the examination.

For the proof of eggs of *Schistosomes*, a random urine sample should preferably be collected between 10 am and 2 pm as the concentration of eggs are greater during this period, particularly in the last drops of the passed urine. Exercise prior to the collection results in the excretion of more eggs.

Collection of urine specimen

Specimens are submitted either as single discharge, timed or random (200–300 mL) or as 24-h discharge (1000–1500 mL). The former is collected in a wide-mouthed disposable wax-lined container or other containers (Figure 5.1). The procedure is described here.

Patients not requiring assistance (outpatient and those visiting doctor's office)

1. Give the patient a clean, preferably sterile container of appropriate size (50 mL or more). The container must be free of detergents, which may cause false determinations. The container should be pre-labelled with identification data (ID) of the patient.
   Note For quantitative chemical investigation the whole volume of urine excreted during 24–h period must be carefully collected in a 2-L container.
2. Instruct the patient before the collection, preferably with illustrations. Tell him or her not to touch the inside or rim of the container. Ensure that his/her hands are clean.

   Male
   - If not circumcised, withdraw the foreskin.
   - Let the patient begin to urinate, but passing the first portion into the toilet.
   - Collect the midstream sample of urine in the container and pass the remainder into the toilet.

   Female
   - Instruct the patient to squat over the toilet and separate the labia minora with one hand.
   - The patient should void the first portion of urine into the toilet, while with her hand, keeping labia separated.
   - The midstream sample of urine should be collected in the clean sterile container, without contaminating the lip or inside of the container with the hand, or inguinal or perineal area. The remainder should be passed into the toilet.
3. The container should be capped without holding its top.

Bedridden patient requiring assistance (inpatient and those in hospital ward)

1. Prepare a clean, sterile container of appropriate size, cotton balls, gauze pads, soapy water and bedpan.

2. Explain to the patient how urine will be collected. Before collecting the urine, label the container with the patient's ID. Use appropriate containers for male, female and infant patients.
3. Collection procedure will be different for male, female or infant.

Male

- Withdraw the foreskin and clean the glans with cotton balls soaked with soapy water, while working away from the urethra.
- Ask the patient to urinate into the bedpan.
- Collect the midstream sample of urine in the specimen container.

Female

- Place the patient in lithotomic position.
- Wearing sterile gloves separate the *labia minora* with one hand and carefully clean around the urethra with cotton balls soaked with soapy water while wiping from front to back.
  Note The labia minora (singular: *labium minus*), also known as the inner labia, inner lips, vaginal lips, or nymphaea, are two flaps of skin on either side of the human vaginal opening, situated between the labia majora (outer labia, or outer lips).
- Rinse the cleansed area with two successive sterile, water-soaked cotton balls, wiping from front to back.
- Ask the patient to urinate into the bedpan.
- Collect the midstream sample of urine in the appropriate container without contaminating the container ('clean catch').
- Any excess urine is passed into the bedpan.

Infants

- Prepare a clean sterile plastic bag of appropriate size, cotton balls, gauze pads and soapy water.
- Let the child drink as much water as possible just prior to the urine collection.
- Clean the external genitalia as described before.
- Clean the external genitalia as described before.
- Seat the child on the lap of the mother, nurse or ward attendant.
- Collect as much urine as possible in the container from the urinating child.
  Note For quantitative chemical investigation, the child is not allowed to drink prior to urination and special attention is required for the collection of the total volume excreted over 12 or 24 h.

Processing of urine specimen

A single discharge urine specimen goes to the urine analysis laboratory for examination within one hour for investigations. The reason is the fast growth of contaminating bacteria. A specimen containing 10^3 bacteria per millilitre, after collection may be 10^5 bacterial/mL two hours later when kept at room temperature. If transport cannot be immediately assured, the specimen should be refrigerated and processed within 24 h. Additives are not required for routine analysis. The urine specimen for laboratory culture is discussed separately in the microbiology chapter. Urine should be collected in a sterile container and inoculated within 2 h and within 4 h, if refrigerated.

If delay is expected, many laboratories spin the urine specimen (12 mL), discard the supernatant, which is used for chemical screening and save the sediment in the centrifuge tube with a drop of 10% formaldehyde solution. This is good for 2 days. The timings of the single discharge might vary (early morning or after a meal) according to the examination sought.

An early morning specimen ('first catch') is desirable for most tests, except urobilinogen for which an afternoon (2 pm) specimen is required.

The 24–h discharge is used for the quantitative assay of various chemical discharged into the urine during that period. Some preservative (e.g., toluene, chloroform, thymol, formalin) must be added to avoid the growth of micro-organisms and change in constituents of urine. Most preservatives, however, interfere with one or more chemical tests except toluene. Add sufficient toluene to the urine to form a very thin layer on the top of the container. No chemical preservative should be added to urine specimen submitted for microbiological and pregnancy tests.

Do not allow the urine to freeze. If the urine is relatively concentrated due to high specific gravity, cooling might result in the formation of heavy precipitate of crystals or amorphous material. These may obscure important cellular elements in the urine.

Stool

Stool specimens are submitted to the laboratory for the diagnosis of intestinal problems—intestinal bleeding (occult blood), parasitic infection, bacterial dysentery (shigellosis), enteric fever (salmonellosis), diarrhoea, digestive problems or abnormalities in the function of the pancreas (fatty stool). The preliminary diagnosis should be mentioned in the request slip in order to facilitate stool collection and stool examination. This is because the same specimen can be used for a number of tests and procedures of stool collection are different. In addition, appropriate clinical information may justify the inclusion of special culture media, e.g., suspicion of cholera, post-antibiotic diarrhoea (*Clostridium difficile*) and haemorrhagic colitis (*Escherichia coli*).

Collection of stool specimens

Various containers are used in collecting stool. A waxed cardboard box, an empty tin can with a lid, a plastic pot with a lid (polypot), wax-lined cups with lids or a special container with a spoon attached to the lid are used by the laboratory or by the attending nurse. Penicillin bottles, matchboxes and banana leaves should not be used as containers as they expose the nursing and laboratory staff to the risk of infection. For liquid stool, only plastic pots are recommended. A plastic bag (no leaks) may be used, but put this in a can in order to avoid any spillage; close the bag tightly. These specimens are good for parasitological examinations. In order to study bacterial infection, stool must be collected on clean paper and a swab is dipped into the stool and taken to the laboratory in a test tube. A clean bedpan can also be used instead of paper but avoid contamination with urine. An anal swab can also be taken.

Procedure

1. Faecal material should be collected directly in the container (as described earlier) or clean bedpan, or on a paper towel or piece of toilet tissue.
2. The material then should be transferred to a suitable container provided with a lid. The specimen collected should be at least 5 g of faeces for parasitological and bacteriological examination and 50 g for chemical analysis. Parts that contain blood and/or mucus, when present, should be selected for parasitological and bacteriological analysis. The specimen should not be contaminated with urine.
3. Rectal swab should only be taken if the patient is unable to produce a stool specimen. A cotton-tipped swab, which need not necessarily be sterile, is introduced past the anal sphincter, rotated and withdrawn and inserted into a tightly closed tube. If the swab is not immediately processed it must be placed in a suitable transport medium (see in the next section), the stick broken off and the tube or bottle tightly closed before transportation.

4. Specimens for bacteriological examination should be transported to the laboratory and processed within a few hours. In case of delay the specimen should be refrigerated. If longer delays cannot be avoided a special transport medium should be used. In such media, pathogens survive for up to 1 week even at room temperature, although refrigeration is preferable.

Never allow the stool to dry; always keep the lid on. Use a wooden tongue depressor or plastic spoon to transfer specimens inside the laboratory. The clinician, the nurse or the technician should inspect every stool specimen and record observations on the request form. Note the consistency (water, liquid, mushy, formed) and the presence of blood, pus or mucus. In rare instances, adult parasitic worms can be recognized with the naked eye: *Ascaris, Enterobius* and tapeworm segments.

Always examine the liquid specimens first for they might contain motile amoebae (amoebic dysentery) that die quickly. For the detection of motile forms of *Entamoeba histolytica* and other protozoa, suspected stools should be examined within one hour after defecation, without preliminary refrigeration. It is recommended to examine a wet mount in saline of freshly passed faeces immediately by microscope.

Never keep the stool on the request form; keep the form at a side under a weight.

Preservation and transportation of stool specimen

Transportation of stool to the reference laboratory may be necessary. Placing it in a transport medium should then preserve the stool. For overnight culture, put it in the GN broth (enrichment medium) and plate it on the following day. If it is put in Cary–Blair medium, it is good for 4 weeks and is good for 2 weeks in buffered glycerol saline. If the stool is kept in 107 formaldehyde (15 mL formaldehyde solution in a 30-mL bottle holding 5 mL of specimen), eggs, larvae and cysts of parasites could be preserved for an indefinite period. The liquid stool should be kept in thiomersal iodine and formaldehyde solution or in polyvinyl alcohol (PVA) fixative. The vegetative bodies (trophozoites) of the protozoa will stay indefinitely. Most of the preservatives are easy to prepare in the peripheral laboratories.

Cary–Blair Transport Medium

This medium can be used for all stool cultures with enteric pathogen, including *Vibrio cholerae* (cholera). The preservation time is 4 weeks.

Sodium thioglycollate	1.5 g
Anhydrous disodium hydrogen phosphate (Na_2HPO_4)	1.1 g
Sodium chloride	5.0 g
Agar	5.0 g
Distilled water	990 mL
pH	8.4

Mix the above chemicals in clean glassware of 2-L capacity. Heat until the solution becomes just clear. Cool to 50°C and add 9 mL of freshly prepared aqueous calcium chloride solution (1%), then adjust the pH to 8.4.

Pour 7 mL of the solution into previously rinsed and sterilized 9-mL screw-capped vials. Steam vials containing media for 15 min, cool and tighten caps.

Buffered glycerol saline solution

This medium is only a second choice in case Cary–Blair medium is not available. It has 2 weeks of preservation time and can be used in all stool cultures except *Vibrio cholerae*. It need not be sterilized. This medium prevents intestinal flora from overgrowing the enteric bacilli.

Sodium chloride	4.2 g
Anhydrous dipotassium phosphate (K_2HPO_4)	3.1 g
Phenol red	3.0 mg
Distilled water	700 mL
Glycerol	300 mL
Final pH	7.2

Dissolve salts in water and glycerol and fill the specimen bottles (about 10-mL capacity) with about 5 mL of the solution. Place the stool swab or rectal swab in the transport medium (break the stick, if necessary) and send it directly to the bacteriology laboratory for culture.

Note The pH of the medium can be tested with pH paper. If the indicator colour changes from pink to yellow, discard the medium and prepare a fresh one.

Merthiolate-Iodine-Formaldehyde (MIF) solution

1. ***Formaldehyde solution*** (10%) Mix 100 mL of commercial formaldehyde (37% formalin) with 300 mL of distilled water.
   Caution The reagent is poisonous and corrosive, handle carefully.
2. **Merthiolate (thiomersal)-iodine-formaidehyde (MIF) solution**:
   A. *Stock solution of thiomersal-formaldehyde (tincture of thiomersal)*

1:1000 (Merthiolate, Lilly)	200 mL
10% Formaldehyde solution	25 mL
Glycerol	5 mL
Distilled water	250 mL

   Store in a brown bottle.
   B. *Stock solution of iodine (aqueous 5% w/v)*
   Dissolve 10 g of potassium iodide (KI) in about 50 mL of water in a graduated beaker. Then add 5 g of iodine, mix until dissolved and then add water to 100 mL.
   C. *Working solution of MIF*
   Mix 9.4 mL of Solution A (thiomersal) with 0.6 mL of Solution B (iodine).

Throat Swab

A throat swab is submitted for the diagnosis of infection in the **upper respiratory tract** and the most common pathogen sought for is *Streptococcus pyogenes* and (in endemic countries) for *Corynebacterium diphtheriae*. Even other organisms are found; they not reported until mentioned in the request slip. Diagnosis of diphtheria in a patient should be made as soon as possible. Another pathogen of concern is *Bordetella pertussis* that causes an acute inflammatory reaction, called whooping cough. More on this will be discussed in the Microbiology section.

Supplies

Cotton swab, transport medium (Stuart, Amies or Cary–Blair), tongue depressor.

Procedure

1. Collect the specimen before the administration of antimicrobial therapy.
2. Focus the light so that the oral cavity is well illuminated and ask the patient to open the mouth widely and breathe deeply.
3. Depress the tongue gently with a tongue depressor. Ask the patient to 'ah' while a swab is carefully introduced over the tongue into the oropharynx. Care should be taken to avoid touching the lips, palate or the tongue with the swab.
4. Rub the swab firmly over the back of the throat, both tonsils and any areas of inflammation, exudation or ulceration. A separate swab should be taken for microscopy if diphtheria, candidiasis or Vincent angina is suspected.

5. If the swab is to be processed within 1–2 h, place it in a sterile test tube and close with stopper. If the processing will be delayed, place the swab in a transport medium (Loeffler medium). Remove the stopper without touching the sterile end, insert the swab into the transport medium, break off the non-sterile part of the swab, which has been in contact with fingers and replace stopper. This is specially recommended in case of diphtheria bacilli. Transportation of a swab in Transgrow medium or Stuart medium is rarely necessary except for epidemiological surveys in order to preserve *Neisseria meningitidis* found in the upper respiratory tract.

Sputum

A distinction must be made between saliva and sputum. Saliva is secreted by the salivary glands and is limited in the oral region. Sputum is the material coughed up from the throat and lungs. It is usually examined to determine the presence of lungs or upper respiratory tract disease. In some countries, sputum can also be used for the microscopic detection of bronchopulmonary parasites: ova of *Paragonimus* spp. and larvae of *Strongyloides stercoralis*. For the diagnosis of pulmonary tuberculosis, sputum is submitted to the laboratory. The first expectorate coughed out in the early morning is the most desirable specimen for the laboratory investigation.

With the exception of the diagnosis of tuberculosis, microbiological investigation of sputum specimen is often useless for the correct treatment of the patient. It may even give misleading results and lead to unnecessary treatment with broad-spectrum antibiotics. This is because all expectorated sputum is contaminated to some degree with secretions of the oropharyngeal cavity, which contain a wide variety of commensal bacteria. Some of these are potential pathogens for the lower respiratory tract (pneumococci, *Haemophilus influenzae*). Contamination with oropharyngeal secretions should be minimal, since the sputum must reflect the infectious process in the bronchi and the lungs. This requires good technique for expectoration and collection of the specimen.

Supplies

Sputum is collected in a wide-mouthed glass bottle of about 50-mL capacity with a screw top or preferably in a disposable plastic jar with tight-fitting lids. Place 25 mL cetylpyridinium bromide (0.6% in distilled water) in the container. This acts as a preservative. Preservation time is about 10 days.

Procedure

1. Collected prior to antimicrobial therapy.
2. Sputum should preferably be collected in the morning. The patient should be standing, if possible or sitting upright in bed.
3. The patient should take a very deep breath to fill lungs with air and expirate in one breath, coughing as hard and deeply as possible. The sputum coughed up should be spat directly into the container.
4. If the volume of the sputum is not sufficient, the procedure may be repeated. A well-collected specimen on a single occasion, however, is better than a specimen collected over several hours. A 24-h collection of sputum or repeated (three) specimens is useful only for the diagnosis of mycobacterium disease (tuberculosis).
5. Macroscopic evaluation of sputum should be made before sending the specimen to the laboratory. The technician should briefly inspect its appearance and record it on the request form as follows:
   - a watery, white-frothy or mucoid sputum generally represents pharyngeal secretions and should not be examined for non-tuberculous infections;

- a purulent, mucopurulent (mixture of pus and purulent flecks) yellow, green or brown blood-stained sputum is generally acceptable.

6. After the collection of sputum, the cap of the container must be tightened and the specimen must be sent immediately to the laboratory. In case of delay, the specimen should be kept in the refrigerator.
7. Microscopic screening of the sputum, based on the evaluation of the proportion of leukocytes versus squamous cells, should be done immediately after collection. The leukocyte count should be at least 10 times the count of squamous cells. Attention should also be paid to the predominant type of bacteria in the purulent part of the sputum.

When the patient does not expectorate, as is often the case in children, tracheal secretion can be aspirated through a nasopharyngeal catheter. The culture results, however, are difficult to interpret in children because of the presence of potential pathogens (pneumococci and *H. influenzae*) in the normal pharyngeal flora.

For severely ill, hospitalized patients who are suspected to suffer from non-tuberculous bronchopulmonary infection, special techniques are available for collecting deep respiratory tract secretions with no or minimal contamination by the oropharyngeal flora.

Send the specimen, collected in bottle, to the reference laboratory after careful packing. Cardboard boxes, made in the laboratory (Figure 5.1) are also used if the specimen is collected inside the laboratory and from patient without suspected tuberculosis.

Miscellaneous Specimens

Cerebrospinal fluid

Cerebrospinal fluid (CSF) is a body fluid that surrounds the spinal cord and the brain. The CSF compartment is separated from the blood system by the blood-liquor barrier. This barrier prevents blood cells and the majority of plasma proteins from entering the CSF, while water-soluble small molecules, such as glucose, penetrate the barrier. Normal CSF is therefore almost void of cells and appears colourless and transparent.

Considerable risk is involved in collecting a CSF specimen and hence it is performed only by an attending physician in the hospital or by an experienced nurse. All CSF specimens should be considered as Stat (urgent). Laboratory study of CSF is for the diagnosis of problems related to the brain or central nervous system (CNS). Collect the specimen in three tubes. The first one (bloody) is used for bacterial culture and the other two for microscopy and chemical screening. *Neisseria meningitidis* is the most commonly sought bacterial organism in CSF. It is highly fragile and may die out during transportation. Place the CSF specimen (first collection) in Stuart transport medium ('Transgrow medium' is not always available in developing countries). The CSF specimen in Stuart medium will be preserved up to two days. **Do not chill the CSF specimen on medium.** The pathogen is cold sensitive and dies when chilled.

Pleural and peritoneal fluids

Exudate or transudate may accumulate in the pleural cavity or peritoneal space of patients who are suffering from certain diseases. These fluids can be aspirated for therapeutic and/or diagnostic reasons. Only physicians or trained nurses are allowed to conduct the procedure for obtaining the specimen. The aspiration of the accumulated fluid carries a high risk of injury to the associated vital organs of the body.

Physical examination of the aspirate must be done immediately. Normally, aspirates are yellowish and transparent. Under clinical conditions, it can be bloody, turbid, milky or greenish. The aspirate should be sent, without delay, to the laboratory for microscopic, chemical and microbiological investigations.

Hair, nails and skin scrapings

Hair, nails and cutaneous tissues are submitted to the mycology laboratory (Chapter 21 in Volume II) for the diagnosis of ringworm and other dermatophyte infections. For transportation to the laboratory, put the infected material in a screw-capped bottle or in an envelope. Do not use bottles with rubber stopper or a cotton plug. The specimen is preserved at room temperature for more than a week.

Tissue biopsy

Tissues are removed by surgery at the operation theatre and sent to the histopathology laboratory for microscopic examination to find out if the removed tissue is inflammatory or neoplastic (new growth or cancerous). If the tissue is neoplastic, the nature of the growth whether benign or malignant can be ascertained. A biopsy is a small portion of a tissue taken out by means of a special biopsy needle in order to diagnose certain diseases of organs such as the liver or the kidney. A biopsy may not require an elaborate surgical procedure. Both the physician always collect tissue and biopsy specimens and they need to be immediately fixed in order to avoid changes in tissue structure. The choice of the fixative largely depends on the tissue and the purpose of histological studies. Two of the most commonly used fixatives are formaldehyde solution for tissues and Zenker fixative for biopsy materials.

Needle biopsies are sent to the laboratory in a container carrying a fixative. Commonly used containers are usually made of glass (30-mL), with a plastic cap and wide mouth. These are often known as 'pill bottles' (Figure 5.1). Various other sizes are also available. The technician's job is limited to assisting the attending pathologist—arranging the request slip, marking the accession number, recording the gross observations dictated by the attending pathologist, labelling the specimen bottle which should tally with the record, adding fixative and recording in the register the number of tissue bits received along with the outline of the bits (shape). The last helps in the identification of the tissue in case of any mix-up.

Note The technician must be very careful to avoid any mix-up of specimens. Never separate the identification slip from the tissue during its processing in the laboratory. Never leave the tissue-bit exposed to the air for a prolonged period; it should always be under the fixative fluid. Transfer the biopsy materials gently with a scalpel blade or on a piece of stiff paper. Do not use forceps since this may damage the tissue.

Fixatives

1. ***Neutral formaldehyde solution (pH 7.2–7.3):*** This is used for all tissue biopsies.
   Commercial formaldehyde (37% formalin) 10 mL
   Saline (0.85% NaCl) 90 mL
   Commercial formaldehyde is slightly acidic. Hence, before diluting, it is first neutralized (pH 7.0) by adding a few drops of sodium carbonate solution (5%) before diluting with saline. Check the pH with pH testing paper. Phosphate buffer is also used in place of sodium carbonate.
   Caution The formaldehyde solution is corrosive and poisonous.
   For routine histology the above solution is frequently used for initial fixation and for tissue processing.
2. ***Zenker's fixative***
   Caution This is a poisonous solution and should be prepared carefully by an experienced technician. Handle reagents carefully especially mercuric chloride and acetic acid.

- Weigh the following ingredients and put them in a 1-L beaker.

Potassium dichromate	25 g
Mercuric chloride	50 g
Sodium sulphate	10 g

- Add about 800 mL of water and dissolve by stirring.
- When dissolved, make the solution up to 1000 mL in a graduated cylinder.
- Transfer the solution to a 1-L reagent bottle and label it.
- Just before use, add 5 mL of glacial acetic acid in 100 mL of the above solution.

This is a good fixative for bloody (congested) specimens and trichrome stains.

3. ***Dichromate fixatives***
   Time of fixation (24 h) is critical in dichromate fixative. Tissue should be washed after fixation and transferred to 70% ethanol. Failure to wash the tissue after fixation may cause pigments to be precipitated. Extensive shrinkage occurs when tissues are processed to paraffin blocks.
   Miller's or Moller's solution

Potassium dichromate	2.5 g
Sodium sulphate	1.0 g
Distilled water	100 mL

4. ***Picric acid fixatives***
   Many picric acid fixatives require a saturated aqueous solution of picric acid. Aqueous picric acid (2.1%) will produce a saturated solution and 5% picric acid will give a saturated solution in absolute ethanol.
5. ***Bouin's solution***

Saturated aqueous solution of picric acid	1500 mL
Formaldehyde (37%)	500 mL
Glacial acetic acid	100 mL

Bouin's solution is an excellent general fixative for connective tissue stains. The yellow colour can be removed with 70% ethanol, lithium carbonate, or another acid dye, separately or during the staining sequence. Bouin's solution destroys membranes; therefore cannot be recovered from Bouin's fixative tissue and there may be extensive shrinkage of larger specimens.

Specimens for sexually transmitted diseases

Sexually transmitted diseases (STDs) are caused by a large variety of viruses, bacterial, fungi and parasites. With a few exceptions all these micro-organisms are too **delicate and fastidious** to grow *in vitro*. Their identification depends therefore upon the collection of an appropriate specimen and upon its transportation under optimal conditions to the laboratory. As the laboratory diagnosis of *Chlamydia trachomatis* and herpes simplex virus is generally not possible at the primary or intermediate health-care level, specimen management for those organisms will not be considered here. All specimens should be collected **before the administration of antibiotics** or the application of topical drags.

Gonorrhoeae

Diagnosis of acute gonorrhoea in the male can often be made from a Gram-stained direct smear of exudates from the urethra. High vaginal swab can be collected by trained female nurse or gynaecologist for Gram stain. However, culture is usually necessary to diagnose gonorrhoea in the female and to diagnose chronic gonorrhoea. Transport of specimen is done on Transgrow medium at 35–37°C if possible. The specimen is taken and streaked on the surface of the medium with a swab or bacteriological loop. A non-growth transport medium such as Cary–Blair medium or Amies medium can also be used. The sampling swab is thrust

into the tube of medium and broken off at the tube lip before capping the tube. This method is a second choice for exudates specimen transport.

Mailing Laboratory Specimens

Laboratory facilities are not available in remote areas away from urban centres. Hence, specimens may have to be dispatched by mail (Figure 5.1). The prime consideration in the mailing of laboratory specimens is to have the specimen reach its destination rapidly, without loss, and without contamination. Mailing containers are of various types, made of metal (aluminum), wood, cardboard and plastics. If containers are used, package specimens carefully with a sufficient amount of absorbent material around the inner specimen container to absorb any leaking fluid. Newspaper and non-absorbent cotton wool are commonly used as packing materials. Pay careful attention to securing the cap of the container. Some cardboard containers are provided with metal screw caps and bottoms. These are recommended. It is suggested that the container be labelled as:

First Class Mail
GLASS: FRAGILE
(If the inner container is made of glass)
LABORATORY SPECIMENS
Infectious Material

If glass containers are used, use thick-walled bottles or test tubes with tight screw caps. If several tubes are packed in the same can, wrap them individually in soft paper or cloth to provide adequate insulation between the tubes. The following hints may be further helpful:

1. Send slides placed between pieces of cardboard and later tied tightly with a thread.
2. Send skin scrapings and hair in an envelope and seal the envelope. Write on the cover 'Infectious Material'.
3. Send stool and other specimens in plastic bottles (unbreakable). Hermetically seal the cap (preferably a screw cap with inner rubber lining) with adhesive sticker plaster or wax. The latter may melt in hot climates. Wedge the bottle with absorbent cotton wool.
4. Keep the request slip (examination requested) along with the patient's name, age, sex, address, type of specimen, referring physician, his clinical note of provisional diagnosis and other details attached to the specimen container. Use a rubber band to wrap the slip or staple it to the plastic bag in which it will be placed.
5. Place the bottle in a plastic bag with wrapping paper, then put in a can with a screw cap. Always double-pack the specimen container.

Receiving Specimens

1. Open the package carefully on several layers of newspaper. Use a pair of gloves and wear an apron. This precaution is necessary in case the specimen arrives in a broken container.
2. Keep the requisition slip with the specimen; do not separate it. It may get mixed up.
3. If the specimen arrives in a broken container, save the specimen bottle with the specimen. Wipe the outside of the specimen container with phenol (5%) and transfer the specimen to another container. Attach the requisition slip to the new container.
4. If the requisition slip is spoiled, re-write the details in another requisition form and discard the former with the packing materials.
5. Sterilize the packing material before discarding.

Stability of analytes

The technician should always keep in mind that the analysis is as good as the specimen. Hence, he should be alert about the stability of analytes or else his reports may be misleading. Table 5.3 lists the duration of stability of various analytes determined in the serum specimen.

TABLE 5.3 *Stability of analytes in sterile serum stored in a stoppered test tube at 4°C (d = days)*

Analytes	Stability
Bilirubin	In fresh serum only
Chloride	10 d
Creatinine	24 h
Iron	7 d
Potassium	14 d
Sodium	14 d
Phosphate	7 d
Triglyceride	2 d
Urea	3 d
Uric acid	5 d
Alkaline phosphatase	7 d
Cholinesterase	7 d
AST	3 d, 8% decrease
ALT	3 d, 10% decrease
LDH	7 d

REPORTING OF LABORATORY RESULTS

Once the specimen has been processed through the laboratory, the **laboratory results must be transmitted back to the source** who sought the information—the health care provider or the physician. In most cases, reporting to the physician is done directly on the request slip (Chapter Appendix 5.1 A, B); however, the laboratory may choose to provide more detailed report which is attached to the physician's request slip. An example of a laboratory report is shown in Table 5.4.

System of Reporting

The results are reported in three ways: qualitative, semi-quantitative and quantitative. In case of quantitative reports (e.g., clinical biochemistry), normal values of the laboratory are quoted and the technician may choose to mark out high (H) or low (L) values.

Qualitative

Qualitative reports give a general impression without any numerical value. Examples of such reports are:

- Positive or negative (+ or –)
- Present or absent (+ or –)

Semiquantitative

This kind of report is half way between qualitative and quantitative reports. The actual numerical value is considerably subjective and varies from technician to technician and yet the physician is able to assess the severity of the situation to some extent.

Negative	–	0
Doubtful	±	
Mild	+	(1+)
Moderate	++	(2+)
Severe	+++	(3+)
Gross	++++	(4+)

Above notations are used to communicate different things. In blood banking, it may indicate the degree of agglutination; in protein test for CSF by the Pandy method (*see Clinical Pathology section*) it shows the degree of precipitation; in parasitology, it is useful in giving an idea of the number of parasites (density) seen under the microscope. Such expressions as 'few', 'many', 'abundant', are also semi-quantitative measures. Some common codes used by technicians to communicate semi-quantitative results are given below:

ML = mild
FR = fair
MO = moderate
SR = severe
OC = occasional
AF = a few
AD = adequate
SI = slightly increased
IN = increased
LO = low
VL = very low

Quantitative

Results are reported in quantitative terms. The units are more often in mg/100 mL, but some are in g/100 mL(e.g., protein concentration) and electrolytes are in mmol/L or mEq/L. The clearance rate is expressed in mL/min. All quantitative results are compared against a standard. Quality control serum must be analysed at the beginning of the day.

Reference range in quantitative report

The quantitative laboratory report document lists the results for the tests performed, as well as the reference ranges (also known as normal ranges) that have been established by the laboratory for the tests. Reference ranges are the results that are expected 95% of the time for a particular laboratory test in the general healthy population. A range is necessary instead of a particular value because of differences in the population due to age, race, and gender. Geographical locations may also affect the reference range, as will the testing methods used by that laboratory. A notation will be present on the laboratory report for all results that are outside the expected reference range for that patient, based on demographics, testing methods, and the tests ordered. The gender and age of the patient may affect the reference range used for interpretation of the results, so it is critical that this information is provided whenever a sample is collected.

The laboratory report will also include the date and time of the specimen collection, the name and identification number for the patient, and the name and address of the laboratory where the test was performed. The specimen source is identified, as well as the date and time at which the report was generated.

TABLE 5.4 *Sample laboratory report*

Patient: Parameshwar Bhatia **DOB:** 08/07/1960 **Sex:** Female **ID#** 764900	**Date:** 7/20/2014 **Time** 11 a.m. **REPORT STATUS:** Final			
Procedure	**Result values (* Abnormal)**	**Out of range (Abnormal) High or Low**	**Reference range**	**Units**
Rapid strep screen	Positive			
Urinalysis				
Appearance	Clear			
Colour	Yellow			
Glucose	Negative			
Ketone	Negative			
Specific gravity	1.010		1.002–1.030	
Occult blood	Negative			
pH	6.0		5–7.9	
Protein	Negative			
Nitrate	Negative			
Leukocyte	Negative			
Electrolytes				
Sodium	141		136–145	mEq/L
Potassium	4.8		3.5–5.0	mEq/L
Chloride	100		96–106	mEq/L
CO_2	25		22–30	mEq/L
Haematology				
Haemoglobin	10.9*	Low	12–16	g/dL
Haematocrit	33*	Low	37–48	%

Reporting Abnormal Values

Each laboratory report sheet must have the following information: date, accession number, laboratory number (provided by the technician), name of patient with date of birth and name of referring physician. The record must indicate steps taken for quality assurance. All test results must correspond to the accession number provided by the receiving clerk. The reporting form should show the normal values and flag the 'abnormal' results in order to alert the physician (Book Appendix B-E given at the end of Volume III). If results indicate 'Panic' condition, the physician or ward must be immediately informed. The supervisor must approve all reporting of results.

It is recognized that critical laboratory values (low or high) indicate the need for prompt clinical intervention. The healthcare professional reviews records for any sudden change in values that may also signal alarm. Critical (panic) laboratory values represent serious medical

conditions that may be life threatening unless immediate actions are taken. Notification and collaboration with the clinician and other healthcare team members must take place when critical values are identified so that prompt treatment can begin.

One of the biggest responsibilities of the technician is to alert the physician and the nurse in the hospital ward if a patient's life is in danger. Thus the technician must be aware of the panic values (Book Appendix E given at the end of Volume III). Abnormal values are highlighted in the routine laboratory reporting but personal calls will be necessary if the laboratory test results hit the panic value.

There are several ways in which the report reaches the physician–hand-delivery, e-mail, fax, or by courier service. In many modern laboratories, the information is transmitted through "online service" by dedicated laboratory link. This helps the healthcare provider to view the results on site. The results must be reviewed as soon as possible so that appropriate action can be taken for those outside the normal reference range. Laboratory reports are legal documents and are a part of the patient's health record. A delayed action from the part of the physician may be questioned and reviewed. If found guilty of negligence, the physician may liable to be sued.

Discarding Specimens after Use

Keep all specimens until their examination is complete. All tests specimens must be discarded in such a manner as to render them entirely without danger to the laboratory worker. Specimens should never be emptied into ordinary sinks. It is equally important that the sewage leaving the laboratory not be contaminated. A specially constructed hopper connecting with the sewer system should be provided in every laboratory. The lid of the hopper should be kept closed when not in use. The following are some of the methods recommended for disposing of the most important specimens.

1. ***Blood and blood clot:*** Loosen the clot from the wall of the test tube or penicillin bottle (container commonly used in developing countries) with not known to be infectious (haematological), discard the collected specimens directly into the special waste hopper and flush thoroughly. If specimens are suspected of containing infectious organisms (microbiological), cover the container with water and autoclave. Then discard the blood and water into the hopper.
2. ***Urine, body fluids, CSF, saliva, stomach contents:*** Pour down the drain without preliminary treatment unless suspected to contain pathogenic organism. In this case, add two volumes of 5% phenol and let stand 2 h before discarding into the drain. Rinse the container well.
3. ***Bacterial cultures:*** Autoclave cultures in Petri dishes or test tubes and place solid materials in the incinerator. Discard media, which are liquid when warm by washing them down the drain with large quantities of hot water. Swabs with infectious material must be discarded in the disinfectant solution (5% phenol) after use. At the end of the day, swabs are collected and sterilized.
4. ***Stool:*** Discard in the special hopper. Treat with disinfectant before discarding, if known to contain infectious agent. Incinerate the container (if disposable) or cover with 5% phenol before cleaning.

Clinical Laboratory Records

The clinical laboratory has to communicate not only with the physician but also with various external agencies such as hospital management, health department of the government and suppliers of laboratory products. Hence the communication system established by the laboratory can be easily followed and meaningful. The importance of laboratory records cannot be

overemphasized. The records help in tracing the patient's history, keeping accurate statistics, tracing back errors and avoiding confusion in communication between the technician, the laboratory and the physician. Let us take a closer look as the patient arrives in the laboratory with the physician's laboratory test request form.

Requisition record

A physician following the physical examination of the patient makes a test request. The requisition slip bears the patient's ID and the name of the referring doctor. A list of diagnostic tests offered by the laboratory is listed in the request slip for the physician to check. A properly filled request slip may be sent to the ward for collection of the appropriate specimen or the specimen may be collected in the laboratory itself. The latter is often done for outdoor patients.

Samples of requisition slips are given in the Chapter Appendix 5.1. The same request form may be used for reporting of the result as well. The physician must communicate properly with the technician through the request slip by providing the provisional or presumptive diagnosis based on physical examination and clinical history of the patient. This will help the technician to be aware of the physician's expectations and choose a certain course of testing (e.g., media for the culture of a specific infectious agent).

Accession record

Guided by the physician, the patient arrives in the laboratory with his request slip (Chapter Appendix 5.1) for giving specimen that is needed for the laboratory test. Many tests require **patient's preparation** that may be noted in the request slip. Patient should understand his/her role in the preparation and cooperate in order to comply with the test requirement. This education to the patient is a crucial part of getting reliable finding. **The patient should keep in mind that a bad specimen will always yield bad and unreliable result.**

The laboratory may also receive specimens collected by external agencies. They all must have their identifications and request slip to process.

The **registration desk** records the date, the patient's name and the referring physician, and the test(s) requested. Only blood and urine are collected at the collection site of the laboratory while other specimens come from home (faecal material) or from the ward. Each specimen gets an accession number allotted by the registration desk if not assigned by the hospital. All laboratory records must bear the number assigned by the hospital even if the laboratory uses its own code. This has been greatly facilitated with the bar code that originates from the registration desk.

The **phlebotomist** reads tests to be performed and collects the specimen in appropriate containers. In many cases the registration desk prints out the sticker label that is attached to the collection tube.

If the laboratory receives specimen from an external source, the specimen should bear the identification number from the source laboratory with name of the patient along with the physician's request slip. The laboratory now makes a note of the specimen identification, provided by the external source, and then inscribes its own number for internal communication. The request slip bears the laboratory accession number for future references.

Many modern laboratories now use the bar code system to identify the specimen that provides all details as entered by the receiving clerk. In all communication, this bar-coded specimen provides all details, including results. The supervisor can check anytime the current status of the test as the information gets distributed through the computer connection. In peripheral laboratories, these are done manually, keeping in mind the care needed to avoid any transcription error. The clinical laboratory has to communicate not only with the physician but also with various external agencies such as hospital management, government

health department and suppliers of laboratory products. The record helps in tracking the patient's history, any error incurred, laboratory maintenance and inventory, profit and loss, history of people working for the laboratory and many more. The following are some of the important records that the laboratory maintains. This starts with the specimen's arrival.

The log book (Chapter Appendix 5.2) of the registration desk records the accession number, which is assigned to each specimen before it is collected. Patient's ID (name, sex and date of birth) goes with the accession number. Other records in the laboratory register includes—type of patient (outpatient or inpatient), referring doctor's name, date and time of receiving specimen, type of specimen received and the test requested.

The receiving clerk may print out several stickers to be attached to each specimen, which is then passed on to the phlebotomist. All specimens, which need immediate attention, called STAT, are recorded and given the top priority in analysis. Others are considered as routine.

The specimen, along with the request slip, is distributed to the various sections of the laboratory for the completion of tests. It may take a single day or several days (in microbiology) to complete a test. After the completion of tests, results are recorded in the technician's laboratory book with the date and time of reporting results to the supervisor for its further communication. If the receiving clerk received any additional information about the specimen or the patient, it is also recorded in the register under the column of 'comments'. Thus any discrepancy is easily caught on a day-to-day basis.

Laboratory record book

The central laboratory register provides an accession number to the requisition slip which is sequentially recorded in a register or log book (Chapter Appendix 5.2). The logbook may keep the record of **urgent** (technically referred as **Stat**) specimen separately. The logbook must show clearly the patient's ID, type of patient (indoor or outdoor), referring doctor, date and time, type of specimen received and tests requested. Specimens with the corresponding request slips are distributed to the various sections of the laboratory for the completion of tests. It may take a single day or several days (e.g., in microbiology) to complete a test. After the completion of tests which may take a single day or several days (e.g., in microbiology laboratory), results are recorded in the laboratory register with the date and time of reporting results to the physician through the request slip. The register also records any information sent by phone. Thus any discrepancy is easily caught on a day-to-day basis.

Diagnostic tests are carried out in the different sections and these sections keep their own record of the test done and its results (Chapter Appendix 5.3). They all use the same accession number as shown in the main register. Wide variation exists between the various sections of the laboratory (histology, microbiology, haematology and others) regarding keeping of records. For example, the histology laboratory maintains the accession register but no detailed observation report. The pathologist maintains the latter. The technician of the microbiology laboratory, on the other hand, must make sequential observations through direct examination, laboratory culture and biochemical testing. The physician should be posted with the preliminary information (direct examination) within 24 h and later after 24–48 h, unless the culture takes a longer time to grow (e.g., mycotic agents). Under emergency condition (e.g., positive findings of budding yeast in CSF), the physician must be informed immediately.

Date-wise test results, along with the corresponding accessional number of the specimen and quality confrol data, are recorded in a bound register or electronically in a hard drive. Normal values, as established by the laboratory, are on record for referral. The **technician flags all abnormal results** in order to alert the supervisor and the physician. Most automated instruments do this job automatically. The supervisor checks findings and transmits them to the central office or directly to the physician. Quality control results are not communicated to the physician. As the arrangements are, the physician can directly look into the result through

the computer screen or wait for the hard copy to arrive. As the patient has an ID number, all results and clinical history can be immediately matched. **Electronic record** keeping on computer disks, magnetic tapes, microfilm, microfiche or optical disks are acceptable. They require considerably less physical space and provide rapid retrieval of information. This has been elaborated in Chapter 2.

Technician's record book

This is the diary of the technician. It keeps all the raw data, calculations and results reported, along with the accession number provided by the laboratory. The technician's record book (Chapter Appendix 5.4) should also indicate the result of the quality control and any maintenance record.

When the technician communicates results to the central desk through the request/reporting slip, results are entered into the central registration book and the receiving clerk sends the requisition slip with the report to the pathologist for signature. This is then despatched to the physician who requested the test. If the patient needs a copy, he/she gets it from the physician. **The laboratory does not send the result directly to the patient**, unless it is asked to do so.

Note Urgent specimens (**STAT)**, like cerebrospinal fluid, are handled specially and are given priority over other specimens.

Record of laboratory procedures

The laboratory **maintains a hard copy of all the procedures** followed in the laboratory. Any new procedure introduced in the laboratory must get the approval of the supervisor and the pathologist. The laboratory record also provides working plan of the laboratory and responsibilities of each worker. This helps the new employees to learn the laboratory runs. The record also provides the direction for handling specimens, which fall out of line. For example, if specimens are not analysed within two hours, particular attention must be paid to the **preservation of the specimen**. A procedure that sets criteria for acceptable specimens and provides proper notification on receipt when a specimen is not properly labelled or has insufficient quantity is required.

As a policy, the laboratory must perform testing **only at the written or electronic request of an authorized person**, and the test requisition or medical chart containing the requisition must be retained for two years.

A copy of the **procedure**, to be followed by the technician at the work area **should be readily available in the bench drawer** or laboratory manual. This is for the benefit of less-experienced operator. A common cause of error is when different operators use a slightly different technique because of excessive concern for speed or lack of understanding about the proper technique. As part of the procedure, reagents must be dated and initialized when received, opened and prepared and routinely checked for outdating. Calibration procedures, linearity protocols and preventive maintenance procedures are usually specified by the manufacturer but, unless spelled out in the procedure, will frequently be forgotten or lost. Since procedures can have manufacturer-initiated analytical changes as well as changes that naturally evolve in office practice, each method should be reviewed and compared to recent package inserts on an annual basis.

Safety records

The clinical laboratory is a place that has **a potential risk of getting infection or laboratory accident.** Hence, the laboratory **should impose strict safety regulations** for the safety of its workers. These rules and regulations must be documented for reference and reminded to

the people associated with the laboratory from time to time. Fire drills, safety workshops and other methods may keep the laboratory personnel conscious of dangers involved. One should keep in mind that hospital-acquired infection is more dangerous than the infection occurred by natural means.

Quality assurance record

Quality assurance must be an ongoing process and should be on record for all laboratories. Laboratories with reputation must participate in an accredited proficiency program and be tested in every procedure for which the laboratory is certified. Government agencies should inspect the performance of laboratories from time-to-time and look into their laboratory records.

Laboratory inventory

The laboratory should store its chemicals and supplies in a safe place. Inflammable materials should be in a safety vault. Inventory check should be a regular feature and inventory book should be available to all inquiring authorities (Chapter Appendix 5.5). The supervisor or his assistant usually does the inventory check so that the laboratory does not run short of supplies. Outdated chemicals and reagents must be removed and chemicals no longer used with the updated methodologies must be rejected. Disposal of chemicals must be done carefully and government regulations must be honoured. Chapter Appendix 5.5 gives the inventory check of items of daily supply in four drawers in a bacteriology laboratory and on the bench top. This is just an example. The laboratory should develop similar inventory for all areas of the laboratory. The inventory tracks down loss and helps in future planning.

The accuracy of the work performed in a laboratory can usually be estimated from the orderliness of the laboratory. Laboratory procedures require a sequence of actions, which must be conducted correctly and methodically. The laboratory worker must be sure that all materials required to run his tests will be available when and where needed. An effective system of inventory control provides economical laboratory management and avoids unnecessary delays in obtaining test results.

The materials used in the laboratory are broadly classified as expendable items (e.g., cotton swabs, matches) and non-expendable items (e.g., instruments, inoculating needles, bottles). The expendable items need to be replenished. Thus a supply will be needed. The **storeroom of the** laboratory holds the bulk material and materials for daily use are checked out as needed. A smart technician always plans ahead of time. In the following pages, methods of maintaining inventory are described.

Workbench inventory

Workbench inventory (Chapter Appendix 5.5) is done daily. The inventory is taken in the evening hours so that all supplies are ready before new specimens arrive on the following morning.

The drawers under the bench should be well organized with proper compartments for pipettes, rubber tubing, glass rods and others. Number the drawers and list the materials as they are kept in these drawers. Take a daily inventory and replenish items used up during the day's work. A typical example of a bacteriology work bench inventory is shown in Chapter Appendix 5.5. Modify this according to your needs. Remember, an effective system of inventory control provides economic laboratory management and avoids unnecessary delays in obtaining test results. This inventory can include items such as glassware, reagent solutions, culture media (in the bacteriology laboratory), etc. The daily record of supplies serves as an

important aid in measuring the laboratory work load and is a source of information for requisitions and for more general inventories of stock rooms.

Store room inventory

At least one person of the laboratory staff should be assigned to take care of the store room, prepare orders or requisitions, check orders received, distribute supplies as needed and keep a running inventory. An easy way to maintain an inventory list is to prepare an index card for each item (10 cm × 15 cm). Each card keeps an account of the number of items in hand and orders are placed before it is exhausted. An example of the inventory card is shown in the Chapter Appendix 5.6.

When materials are received, the storekeeper notes on the card the date, the amount of material received and the total amount in hand. When materials are distributed, he adds to the card the date, the amount of material distributed and the balance in hand. Periodic review of these cards will indicate those regularly used items, which must be ordered.

It is considered to be a good practice to have a notebook close at hand in the storeroom in which to make notes concerning items to be ordered. Do not depend on your memory. When it is evident that an item needs to be re-ordered and you cannot take time to check the inventory card, make a note immediately in the notebook.

While ordering chemicals, care must be taken to order the **proper grade** and proper quantity. Some of the chemicals might not stay good forever. Similarly, proper storage conditions must be provided for the materials arriving. For example, some of the materials need to be refrigerated. The store must have a large size refrigerator and, if possible, a freezer, to store the perishable goods. Although large quantities are economical, an excessive amount might be wasteful as it may be spoiled or may get contaminated when taken out in small quantities. In addition, bulky containers are difficult to handle.

If materials are issued in small containers, make sure that the empty container is first labelled before it is filled. Furthermore, never place in a container any solution or material other than that stated on the label. Bottles of highly poisonous solutions or reagents should be labelled **POISON**. If an unmarked container is found and there is no assurance as to what the contents may be, discard the entire container and its contents. Large containers of chemicals that are opened from time-to-time to dispense a portion of contents should be dated when the container is first opened. It is most important that every container be correctly labelled as to its contents.

Record of laboratory personnel

Qualifications of all laboratory workers must be recorded and available to appropriate government agencies when called for. The laboratory deals with the public and hence, the government has the responsibility to follow up in case of any controversy. Unqualified and untrained workers in the laboratory might be subjected to legal suits. The pathologist is responsible for their performance. Patient's record, however, is not released until the court asks the pathologist to do so. The patient is protected by the privacy act.

Continuing education of all laboratory workers, including pathologists, is an important component for the efficient operation of the laboratory. The Internet has come very handy and can be accessed from anywhere in the world.

All laboratory workers should be encouraged to participate in professional workshops, whenever it is available and affordable. This keeps the laboratory workers updated, which is necessary to work in the medical field. The continuing program attended by workers should be recorded in their personal file. All trainings must be 'methodology-based' and not on academic achievement.

Retention of Records, Reports and Specimens

Retention time for records, reports and specimens are given in Table 5.4. The records may be referred for inquiries and follow-ups. Some of the valuable reports may be kept for 20 years while others may only be short-lived. With the use of computers, records are now kept almost permanently unless there is a computer crash. Hence, it is advised to have a hard copy whose retention time is indicated in Table 5.5. For safety reasons, all records should be kept in a hard disk separate from the computer memory.

TABLE 5.5 *Retention time for records, reports and specimen collected by the laboratory*

Item	Record type	Retention
Records	Request slip, test record, quality control, procedure manual, proficiency testing, instrument maintenance	2 years
	Personnel record	30 years
	Blood bank donor, blood bank employee who did compatibility testing	5 years
Reports	Laboratory reports	2 years
	Autopsy, pathology, bone marrow, cytopathology	20 years
Specimens	Serum and other body fluids	24 h
	Blood smears (routine)	7 days
	Blood smears (special)	1 year

REVIEW QUESTIONS

1. What type of specimen would you collect for patient suspected of anaemia, bleeding disorder, renal infection, hookworm infection, syphilis and meningitis?
2. What is a Stat specimen? Why is the CSF specimen considered to be Stat?
3. How is the blood specimen collected for the haematology laboratory? Why is ammonium oxalate not used as an anticoagulant for blood urea nitrogen (BUN) determination?
4. State the procedures involved in venepuncture.
5. What are the advantages and disadvantages of skin puncture?
6. How would you prepare serum for the determination of total protein?
7. How is the sputum specimen collected for the diagnosis of pulmonary tuberculosis?
8. How is the stool specimen, suspected of cholera infection, collected and transported to the reference laboratory?
9. What is the purpose of a tissue biopsy? Who collects the tissue biopsy specimen?
10. When would one collect skin scrapings? How are these mailed and processed in the laboratory?
11. You have received a clotted blood specimen. Following the laboratory work how would you dispose it off?
12. State the precautions to be taken in mailing a sputum specimen from a patient suspected of tuberculosis.
13. What is MIF? When is this used?
14. What is the basic composition of Cary–Blair transport medium? When is this used?
15. How would you collect the following specimens?
    Blood, Urine, Sputum and Throat Swab
16. How would you maintain a stock register?
17. How would you take the daily inventory so that you are not interrupted during the laboratory testing?

18. What is the difference between qualitative and quantitative reporting of data?
19. What is the difference between laboratory record book and technician's record book?
20. What do you understand by 'Logbook'?
21. What is the significance of keeping laboratory records?
22. Why is it necessary to have a daily inventory?
23. How does the technician report his/her results to the physician?
24. What do you understand by 'panic values'? What would you do if you find the result of the patient alarming?

Appendix

Sample Copies of Laboratory Forms and Records

The following annexure includes typical forms used in various laboratories in developing countries. The forms presented here are modified from the forms used in Vijaya Hospital, Madras, India. The forms might not be complete and do not include all the tests requested by a physician, but the general pattern of recording will be obvious.

Appendix 5.1 A

LABORATORY REQUISITION AND TEST REPORT FORM (Traditional)

Date: _____ Hospital address:_____ Telephone: _____

Patient's Name: _____ Sex: _____

Date of birth: _____ Address:_____

Telephone # _____ Emergency contact: _____

Inpatient No. (IP): _____ Outpatient No. (OP): _____

Ward: _____ Bed: _____ Date of admission: _____

Requested by Dr.: _____ Provisional diagnosis: _____

Type of specimen: _____

Specimen collected by: _____ On (date): _____Time: _____(A.M./P.M.)

Specimen accession No.: _____
(given by the receiving clerk and entered in the log-book)

Test requested: _____ Date of submitting report: _____
(Use attached form for reporting)

Technician/Supervisor reporting: _____ (initial)

Appendix 5.1B

EXAMPLE OF A MODERN LABORATORY REQUEST FORM

LABORATORY ORDER REQUEST — **Bar code**

PHYSICIAN — Date

Last Name — First name — Middle initial — Patient ID — Date of Birth

Physician — Date specimen taken — Special instruction — Technician

ICD-9 code(s) — Bill to: ☐ Insurance ☐ Office ☐ Patient

PROFILES	ICD-9 CODE #	INDIVIDUAL PROCEDURES	ICD-9 CODE #		INDIVIDUAL PROCEDURES	ICD-9 CODE #
		HEMATOLOGY/COAGULATION			**CHEMISTRY PROCEDURES**	
☐ **BASIC METABOLIC PROFILE** This includes calcium creatinine, sodium, carbon dioxide, glucose, urea, chloride, potassium		CBC				
		PT				
		PTT				
		Hgb				
☐ **ELECTROLYTE PANEL** This includes sodium, potassium, chloride, carbon dioxide		HCT				
		ESR (sed. rate)				
		Haemoglobin A1-C				
		SEROLOGY				
☐ **COMPREHENSIVE METABOLIC PANEL** This includes: Albumin, total bilirubin, BUN, calcium, chloride, creatinine, glucose, alkaline phosphatase, potassium, total protein, sodium, ALT/GPT, AST/SGOT		RPR				
		CRP (C-Reactive protein)				
		ASO (with titer)				
		RA (Rheumatoid factor)				
		ANA (anti-nuclear antibody)				
		SSA/SBB				
		HAA/Widal/Other (circle or mention)				
		URINE PROCEDURES				
☐ **HEPATIC-FUNCTION TEST** This includes: Albumin, total bilirubin, direct bilirubin, alkaline phophatase, total protein, ALT/SGPT, AST/SGOT		Urinalysis				
		Urine culture with sensitivity				
		24-hour Urine Protein				
		24-hour Urine Creatinine with clearance				
		24-hour Urine, Creatinine			**OTHER PROCEDURES**	
		Random urine, Sodium			1	
☐ **LIPID PROFILE** This includes: Cholesterol, Triglyceride, HDL, LDL.		Random urine, Potassium			2	
		Random urine, Chloride			3	
		Random urine, Myoglobin			4	
		Random urine, Osmolality			5	
MICROBIOLOGY AND CULTURES					6	
	Throat culture				7	
	Sputum culture				8	
	Strep A screen (swab)				9	
	Gram stain (Note source)				10	
Source		**Random urine Osmolality**				

Appendix 5.2

DAILY RECORD OF ACCESSION REGISTER

Day: _____ Date: _____

Time	Accession #*	Referring physician	Name of patient	Patient's ID	Sex	Date of birth	Specimen**	Test requested	Report	Date reported	Signature of tech.

*A11 Laboratory records refer to this number
** Put red asterisk on **Emergency** (Stat) specimens

Appendix 5.3

LABORATORY RECORD BOOK

IA. HAEMATOLOGY (Routine)

Date received	Specimen Accession #	Date received	Referring physician	Name of patient	Sex	Date of birth	Result (Normal values)	Date reported	Signature of tech.
Hb (g/dL)							Male 14–18 Female 12–16		
Hct (%)							Male 0–54 Female 37–47		
RBC count ($\times 10^6/\mu L$							Male 5.51 Female 5.01		
MCV (fL (cu mμ))							82–92		
MCHC (%)							32–36		
MCH (pg or picogram)							27–32		
WBC ($\times 10^3$ per μL)							(5–10) $\times 10^3$		
Differential count Seg. Neutrophil (%)							40–75		
Band Neutrophil (%)							3–6		
Lymphocytes (%)							20–45		
Monocytes (%)							1–10		
Eos (%)							1–6		
Baso (%)							0–0.5		
RBC/WBC morphology							Check the abnormalities noted		
Osmotic Fragility Haemolysis at g/L NaCl									
Blood parasite									
Others									

Check in red if it is a stat (emergency)

Atypical Cells (qualitative, check if found): Atypical lymphocytes _____ Plasma cells _____ Promyelocytes _____ Myelocytes _____ Metamyelocytes _____ Blast cells _____ Nucleated Red Blood Cells _____ Others _____

RBC Morphology (Qualitative, check if found): Hypochromasia, Polychromasia, Target cells, Basophilic stippling, Anisocytosls, Macrocytes, Spherocytes, Howell Jolly bodies, Poikilocytosis, Microcytes, Elliptocytes, Cabot rings, Others

IB. SPECIAL HAEMATOLOGY

Date received	Specimen Accession #	Date received	Referring physician	Name of patient	Sex	Date of birth/ Age	Result (Normal values)	Date reported	Signature of tech.
Reticulocyte count (%)							1–2		
Platelet (estimate) ($\times 10^5$ per μL)							1.5–4.0		
ESR (mm/hr) (Westergren method)							Male 0–5 Female 0–7		
Platelet count ($\times 10^5$ per μL							(1.5–4.0)		
Median corpuscular fragility g/LNaCl							Haemolysis between 4.6 and 5.9		
Test for sickling							(+/–)		
Foetal haemoglobin (Alkaline denaturation method)							<2% in children over 2 years and in adults		
Coagulation Laboratory Bleeding time (Ivy method) (min)							2–5		
Clotting time (Lee and White) (min)							5–11		
PT (sec)							10–14		
APTT (sec)							25–35		
Clot retraction (%) in 1 h							43–64		

2. BLOOD BANK

Request for blood

I.D of Patient (recipient) _____

Number of blood units needed _____

Type of blood needed: Whole blood _____ Packed cells _____ Leukocyte poor blood _____

Platelet rich blood _____ Others _____

Patient ID	Blood grouping	Rh typing	Cross-matching Saline Protein Coombs	Rh antibody titre	Coombs test

Requesting Doctor: _____ (signature)

Date: _____ Time: _____

At the time of delivery of blood

Date: _____ Time: _____

Donor No. (from the bottle): _____ Patient's ID: _____

Cross-matched by: _____ Signature: _____

Number of units supplied: _____

Any comment: _____ Checked by: _____

Signature of delivery clerk: _____

Blood Bank Laboratory Record

Donor Register

Date	Serial number	Donor ID	Blood group and Rh type				Laboratory Tests			Amount of blood collected*	Donor No. (same as on the Bottle #)
			Anti-A	Anti-B	Anti-AB	Anti-D	Hb	HAA	VDRL		

* 1 Unit = 500 mL

Laboratory Record of Cross-Matching

Date	Patient ID (recipient)	Donor ID (on the bottle)	Patient's (recipient) blood grouping							Donor's blood grouping								Cross-matching				Referring physician
			Forward				Reverse			Forward					Reverse							Tech. Sig.
			Anti-A	Anti-B	Anti-AB	Anti-D	A cell	B cell	0 cell	Du	Anti-A	Anti-B	Anti-AB	Anti-D	A cell	B cell	0 cell	SL	Alb IS	Alb Inc	Alb AC	

SA, saline; IS, instant spin; Inc, incubation at 37°C; AC, auto-control.
Donor ID is the number given on the blood collection bottle

3. BACTERIOLOGY AND MYCOLOGY

Date	Specimen #	Referring physician	Laboratory test result			Identification of infectious agent	Date of reporting
			Direct examination	Laboratory culture	Biochemical and other tests		

Antibiotic sensitivity test (antibiogram)		Report
*commonly requested drug sensitivity tests		
Achromycin	Gentamicin	Rovamycin
Amoxycillin	Kanamycin	Septran
Ampicillin	Ledermvcin	Streptomycin
Bactrim	Lincomycin	Sulfiosoxazole
Cephaloridine	Methenamine	Sulfisoxazole
Chloromycetin	Mandelate	Sulphadiazine
Cloxacillin	Novobiocin	Sulphamerazine
Colomycin	Penicillin	Sulphamethoxy-pyridazone
Erythromycin	Polymyxin	Sulphasomid
Furadantin	Pyopen	Tetracycline
Furazolidone	Rifamicin	Tetradox

4. SEROLOGY

Please note: Specify with the test if titre is to be determined.

Date	Specimen#	Patient ID	Referring physician	LABORATORY TESTS								Positive control	Negative control	Date reported
				VDRL	CRP	Widal	RA	ASO	C-RP	HAA	Others*			

* Others include: Brucells, Rose-Waaler, Frei's test, Paul-Bunnel test, cold agglutination, pregnancy test etc.

5. URINE ANALYSIS

Date	Specimen #	Patient ID #	Referring Physician	LABORATORY TESTS											
				Sp.Gr.	App.	Colour	Micros.*	Addis count	pH	Pro-tein	Sug-ar	Ke-tone	Bili	Uro.	Oth-ers

* Microscopic examination includes reporting from:
(a) Wet mount of: RBC/h.p., WBC/h.p., Epithelial cells/h.p., Casts, Crystal, Ova, Proto zoa (T. vaginallis), yeast, cellular bodies.
(b) Stained preparation: Gram stained.

6. FAECAL ANALYSIS

Date	Specimen #	Patient ID#	Presumptive diagnosis	Referring Physician	LABORATORY TESTS				Date of reporting
					Physical* Examination	Microscopic Examination**		Occult blood	Others	
						Direct	Cone.			

Note Indicate intensity of the parasite, when present.
* Physical examination: Colour, form (consistency), presence of mucus, blood, adult parasite, and gross parasite component.
** Microscopic examination: Mention method of concentration (flotation, sedimentation). Report: Presence of ova, protozoa (form), red cells, pus cells (WBC), others.

7. BODY FLUIDS

Date	Speci-men ID	Patient ID	Presumptive diagnosis	Referring physician	Laboratory Tests							Date of reporting
					Physical	Microscopic		Bio-chemical***	Microbiology			
						Total cell count*	Diff.**	Globulin (Pandy) Glucose Chlorides	Lab. Culture	Grams stain	AF stain	

* Total cell count reports: white cell count and red cell count.
** Differential count (WBC) reports: % PMN and % lymphocytes.
*** Normal values: Globulin (Pandy test) faint opalescence.
Glucose (40–80 mg/dL); Chlorides (113–127 mEq/L).

8A. CLINICAL BIOCHEMISTRY (Routine)

Laboratory Tests	Unit	Date	Specimen #	Patient I.D.	Referring physician	Laboratory report	Laboratory normal value	Date reporting	Tech.
Glucose	mg/dL								
Fasting	mg/dL						65–105		
Random	mg/dL								
Postprandial	mg/dL								
Urea (BUN)	mg/dL						15–45		
Creatinine	mg/dL						0.5–1.5		
Uric acid	mg/dL						2.1–7.4		
Total protein	g/dL						6.0–8.0		
Albumin	g/dL						3.3–5.3		
Globulin	g/dL						1.8–3.3		
A/G ratio							1.13–1.15		
Cholesterol	mg/dL						150–250		
ALP	U/L						Adult: 15–65 Children:70–150		
ACP (total)	U/L						2.5–11		
ACP (Titrate labile)	U/L						0.2–3.5		
LDH	U/L						95–200		
SGOT(AST)	U/L						12–30		
SGPT (ALT)	U/L						7–30		
CPK	U/L						10–120		
Amylase	U/L						110–330		
Na	mmol/L						135–145		
K	mmol/L						3.5–5.5		
Cl	mmol/L						98–109		
Bicarbonate (venous blood)	mmol/L						22–28		
Ca	mg/dL						Adult 8.5–10.5 Children 9.5–11.0		
Phosphate	mg/dL						Adult 2.5–4.8 Children 2.5–5.5		
TGL	mg/dL						50–150		
Bilirubin direct	mg/dL						0.1–0.4		
Bilirubin indirect	mg/dL						0.1–0.9		
Bilirubin total	mg/dL						0.2–1.3		
Creatinine clearance	mL/minute						Male:113–167 Female:117–135		
Others									

8B. CLINICAL BIOCHEMISTRY (Special)

Laboratory Test: 24 h urine specimen	Unit	Date	Specimen #	Patient .D.	Referring physician	Laboratory report	Laboratory normal value	Date reporting	Tech.
Volume/24-hr	mL						750–2000		
Chloride	mEq/L						110–250		
Calcium	mg/day						100–300		
Creatinine (varies with body weight)	mg/day						1.5–2.0		
VMA	mg/day						1.5–7.5		
17-Ketosteroid	?						8–15		
Protein	?						10–100		
Urobilinogen	?						0.4–1.0		

9. HISTOPATHOLOGY AND EXFOLIATE CYTOLOGY

Date	Specimen source	Specimen ID	Patient ID	Referring physician	Comments	Date reporting	Pathologist
	Histology						
	Biopsy						
	Surgery						
	Others						
	Exfoliate cytology						
	Cervicovaginal						
	Vaginal pool						
	Pleural fluid						
	Esophageal						
	Gastric						
	Sputum						
	Urine						
	Bronchial						

Appendix 5.4

Technician's Record Book

Only a few examples are quoted here for guidance. In general, the register must have columns for date of performing the test, laboratory serial number, specimen ID and series of tests performed by the specific technician. Enter results directly into the register. It is bad practice to take readings on loose sheets and then transfer the information into the register. This may incur error. All instrument readings must be recorded (see haemoglobin determination) into the register.

1. Haemoglobin Determination

* Number given daily by the technician for the convenience of writing on the glassware during the performance of the test.

Note A similar record is kept for all tests in other areas as well. The technician must write the actual reading so that the calculation can be checked.

Date	Laboratory serial#	Specimen Accession #	Instrument reading			Hb concentration (g/dL)	Signature of tech.
			Standard	Control	Test		

2. Bacteriology

Date	Specimen accession #	Referring physician	Preliminary report	Laboratory observations				Antibiotic sensitivity test	Signature of tech.
				Direct Exam, (unstained)	Direct Exam, (stained)	Pri-mary culture	Secondary* culture		

In all secondary cultures, follow the same specimen accession number in order to avoid transcription errors. If the number is too long, renumber by date and note the laboratory number on the second column, along with the accession number.

3. Body Fluid Examination

A. Sputum

Date	Specimen accession #	Referring physician	Physical				Microscopic						Protein	Others	Tech.
			Volume	Colour	Blood	Others	Wet mount			Stained smear					
							Organisms	Cells	Other findings	Wright stain	Gram stain	Acid fast			

B. Others

Date	Specimen accession #	Referring physician	LABORATORY TESTS						Signature of Tech.
			Physical	Microscopic examination				Protein	Others	
				Differential count		Stained smear				
				PMN	Lymph	Gram	AF			

4. Clinical Biochemistry

A. Routine Analysis

Date	Specimen accession #	Laboratory Test	Methodology	Instrument Reading				Calculated value	Date reported	Signature of Tech.
				Blank	Standard	Control	Test			

B. Special Analysis
Creatinine Clearance Study (Glomerular filtration rate)

Date	Ref. Phys.	Patient ID	Specimen	Instrument Rd. (A)			Creat, cone. (mg/dL)	Time (AM/PM)			Vol. of urine (mL)	Urine vol per min (mL)	Creat clearance (mL/hr)	Sig. of tech.
				Blank	Stand.	Test		Start	Finish	Total				
		#1	Urine											
			Plasma											
		#2	Urine											
			Plasma											
		#3	Urine											
			Plasma											

Appendix 5.5

Daily Inventory of Supplies

Work Station No. 3: Bacteriology (a sample)

Location	Item	No. in stock	No. used during the day	Balance
Drawer No. 1	Sterile pipettes* 10 mL	15	0	15
	5mL	5	3	2
	1 mL	10	g	2
Drawer No. 2	Petri dishes *	4 packages(12 in each)	8	3 packages
Drawer No. 3	Inoculating needle	2	NE	2
	Inoculating loop	2	NE	2
	Pasteur pipettes	3 dozens	12	2 dozens
	Coton swabs	2 dozens	18	6
	Rubber bulbs	2	NE	2
Drawer No. 4	Microscope slides	4 boxes (50 in each)	12	4 boxes +
	Cover slips	1 box (500 in each)	4	1 box (496)
	Test tube racks	2	NE	2
Top bench	Bunsen burner	1	NE	1
Continue for all articles in the laboratory	* If reusable, replace with new sterile articles		NE = Nonexpendable	

Appendix 5.6

INVENTORY CARD FOR LABORATORY SUPPLIES

Item:_____

Address of supplier: _____

_____

_____

Cost:_____ (unit price)

Date	Amount received	Amount issued	Name to whom issued	Balance in hand	Store clerk signature

Chapter

6

Units of Measurement and Preparation of Reagent Solutions

Tara Chattoraj

Chapter Outline

All quantitative measurements are expressed in some kind of unit. Units of measurements are used in our daily life and in scientific work. In clinical laboratory, measurements are used to indicate the number of cells or quantity of substances in patient's blood, serum or other fluids. In order to communicate properly, it is important that all the measurements be expressed by a universally accepted system. In this chapter, we will try to introduce the basic International System of Units (SI)—**the metric system**—and its application in the preparation of laboratory solutions.

International System of Measurement: The Metric System

The metric system is the system of measurements used in clinical laboratories. In many developing countries, the metric system is also used in everyday life although other local systems and the English systems also exist.

Laboratory units are used for the measurement of weight, length, time and concentration of solutions. As different units were used in different parts of the world, scientific communication became difficult. As a result, an International System of Units was introduced in 1971 and was called SIU (Le Système International d'unités). It accepts **the metric system of measurement**.

The SI system uses seven dimensionally independent units of measurement (Table 6.1) to provide logical and consistent measurements (Length, Mass, Time, Quantity of Substance, Temperature, Luminous Intensity and Electric Current). Dimensionally independent means that there is no way to meaningfully convert one unit into another. For example, there is no way to express time (seconds) into length (metres). It is also called the system of base units because all other units (density, concentration) are based off of these seven units. Of these seven, in the common clinical laboratory, only the first five are used (Table 6.2).

TABLE 6.1 *Common Laboratory Units*

Quantity	Unit	Symbol
Length	Metre	m
Weight	Gram	g
Volume	Litre	L or I
Temperature	Celsius	°C
Quantity of substances	Mole	mol
Concentration	Molarity	M
Time	Seconds, Minutes, Hours	s, min, h

TABLE 6.2 *SI base units as used in clinical laboratories*

Older ternis	New SI equivalent
Micron (μ)	micrometre (μm; 10^{-6} m)
Cubic micron (μ^3)	femtolitre (fL; 10^{-5} L)
Micro-micro-gram (μμg)	picogram (pg; 10^{-12} g)
Microgram (mcg)	microgram (μg; 10^{-6} g)
Millimicron (mμ)	nanometre (nm; 10^{-9} m)
Lambda (λ)	microlitre (μL; 10^{-6} L)
Cubic millimetre (mm^3)	microlitre (μL; 10^{-6} L)
Angstrom (Å)	nm × 10^{-1}

Often when converting into the SI system, the number that expresses the amount changes. For example, 1 inch is equivalent to 2.54 centimetres. Occasionally, the numbers stay the same. For example, chloride remains same in both the systems: 95–105 mEq/L (conventional) and 95–105 mmol/L (SI). We will discuss these units later. See Appendix D at the end of Volume III for more information.

This is called the metric system because it is based on the fundamental unit of distance, the metre. In the metric system, metre (m) is the basic unit used to measure distance, gram (g) is the basic unit to compare mass, and litre (L) is the basic unit used to measure volume (Table 6.1). For the measurement of temperature, Celsius (°C) is the recommended unit, while Fahrenheit (°F) is still in use but is growing obsolete in the scientific field. The conversion nomogram for Fahrenheit and Centigrade is given in Figure 6.1. Mole (molecular mass in grams) is the weight measurement in chemical language. The concentration of a solution is expressed in molarity, a concept which we will introduced later.

For the conversion of temperature unit, the following formula is used:

$$\frac{C}{5} = \frac{F-32}{9}$$

where, C = temperature in Celsius and F = temperature in Fahrenheit
Alternatively, the nomogram provided in Figure 6.1 can be used and is also recommended.

Examples

(a) Convert 56°C to °F

$$\frac{56}{5} = \frac{F-32}{9}$$

$$F = 132.8^{o}$$

(b) Convert 120°F to °C

$$\frac{C}{5} = \frac{120-32}{9}$$

$$C = 48.8^{o}$$

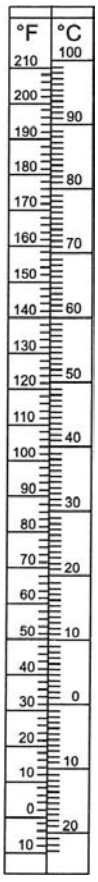

FIGURE 6.1 *Conversion of Fahrenheit scale to Centigrade scale*

UNITS OF MEASUREMENT

The SI system, like the metric system, uses decimal notations and the units are based on powers of 10. The SI system is convenient because by multiplying or dividing by increments of 10 (or by a power of 10) one can obtain units larger or smaller than the basic units (metre, gram and litre). Prefixes indicating the proper power are often added to the basic units to indicate larger or smaller units. For example, 'kilo' means 1000, which is used to express 1000 metres as 1 kilometre, 1000 grams as 1 kilogram and 1000 litres as 1 kilolitre. The prefixes and their definitions remain the same for all the basic units, i.e., metre, gram and litre.

Most laboratory measurements involve very small amounts of substances. So in laboratory analyses, it is more common to use units smaller than the basic units. Two common prefixes are 'milli', which means one-thousandth (0.001 or 10^{-3}), and 'centi' which means one hundredth (0.01 or 10^{-2}). For example, 1 millilitre is 0.001 L or 10^{-3} L. Thus the inter-conversion of millilitre and litre can be expressed as follows:

1000 mL or 10^{3} mL = 1 L
1 mL = 0.001 L or 10^{-3} L

To indicate even farther smaller weights, volumes and lengths, prefixes micro-, nano-, and pico- are used and each of these in sequence is one thousand times smaller than the previous one (Table 6.3). Thus, 1 g is 10^{3} mg, 10^{6} μg and so on.

TABLE 6.3 *Metric Weight Measurements*

Scale	Weight
Unit (1)	1 gram (g); older expression—gm
Miili (10^{-3})	1 milligram (mg) = 10^{-3} g (0.001 g)
Micro (10^{-6})	1 microgram (μg) = 10^{-6} (0.000001)
Nano (10^{-9})	1 nanogram (ng) = 10^{-9} g (0.000000001)
Pico (10^{-12})	1 picogram (pg) = 10^{-12} g (0.000000000001)

Following the above relationship, one can express weight, volume, length and concentration of solutions as follows:

Weight

$1\ g = 10^3\ mg = 10^6\ \mu g = 10^9\ ng = 10^{12}\ pg$

Volume

$1\ L = 10^3\ mL = 10^6\ \mu L, = 10^9\ nL = 10^{12}\ pL$

Length

$1\ m = 10^3\ mm = 10^6\ \mu m = 10^9\ nm = 10^{12}\ pm$

Concentration

(We will introduce these units in more detail later.)

$1\ mol = 10^3\ mmol = 10^6\ \mu mol = 10^9\ nmol$, etc.

$1\ Eq = 10^3\ mEq = 10^6\ \mu Eq$

To give an idea of the microscopic size of these measurements, the comparison of sizes of red blood cells (7 μm) with bacteria is given in Figure 6.2.

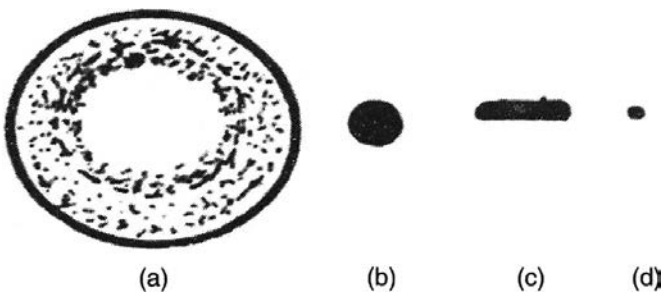

FIGURE 6.2 *Relative size of (a) red blood cells and bacteria, (b) coccus, (c) bacillus and (d) virus*

A few other commonly used prefixes of the metric system are:

1 decilitre (dL) = 100 mL
1 centimetre (cm) = 10 mm
1 cubic centimetre (cc) = 1 mL

In addition, the knowledge of the following conversion of English units to metric units may be necessary as they are still used occasionally.

1 inch (in) = 2.54 cm
1 pound (lb) = 454 g
1 quart (qt) = 0.95 L
1 fluid ounce (fl oz) = 30 mL

Metric System in Expressing Concentration of Solutions

Weight and mass are formally distinct concepts. However, for our purpose, we can use the terms interchangeably. When dealing with chemicals in the laboratory, it is critical to understand how to derive physical properties, such as weight, from their chemical formulas. Molecular weight (MW) and equivalent weight (Eq. Wt.) are the two ways of expressing the weight of substances. The latter is useful in expressing the strength of solutions.

Molecular weight is the weight of a molecule attained by totalling the weight of its constituent atoms. The weights of constituent atoms are listed in the periodic table. Conceptually,

this number corresponds to the weight of one mole of those atoms. Thus, one mole of sodium chloride (NaCl) weighs 58.5 g because one mole of Na (23.0) added to one mole of Cl (35.5) equals 58.5. A mole is the unit weight, like gram, and can be expressed in the same way as milli, micro, nano and other smaller weights.

Equivalent weight has limited use in clinical laboratories except in expressing the concentration of electrolytes. This is because alkalis and acids are electrolytes and classically they were expressed in normality, a unit of concentration. This will be further discussed later in this chapter. Equivalent weight is the molecular weight of a substance divided by its valence, where valence is a number that indicates how many atoms of hydrogen can unite with one atom of another element or the number of replaceable hydrogen or hydroxyl ions in a compound. For example, the valence of chloride is 1 as it forms HCl because it attaches to only one atom of hydrogen. On the other hand, the valence of barium in $Ba(OH)_2$ is 2 because 2 hydroxyl groups attach to one barium atom. Similarly, the valence of all these compounds is 1 :HC1 (hydrochloric acid), HNO_3 (nitric acid), KCl (potassium chloride), NaCl (sodium chloride), while it is 2 for H,SO_4 (sulphuric acid). Since equivalent weight is the molecular weight of a substance divided by its valence, it follows that the equivalent weight of HCl is 36.5 g because the molecular weight of HCl (1 + 35.5 = 36.5 g) divided by its valence (1) is 36.5. However, the molecular weight of H_2SO_4 is 49 g because the molecular weight of sulphuric acid (2 + 32 + 64 = 98 g) divided by its valence (2) is equal to 49.

Reagent Solutions

A solution typically refers to a soluble solid substance dissolved in a liquid. The former is called the solute and the latter is called the solvent. Reagent solutions are frequently prepared in the laboratory. These solutions are prepared according to specifications provided in the test procedures. One basic way of expressing the concentration of a solution is expressed by weight/volume ratio, or the weight of solute in the total volume of solvent (w/v). In case of solution in which one liquid is dissolved in another liquid, we use the volume/volume (v/v), for e.g., alcohol in water or acid in water. There are also other ways of expressing the concentration of a solution which will be discussed later.

Types of reagent solutions

There are three commonly used types of reagent solutions—stock solution, working solution and standard solution.

Stock reagent solution

This is a concentrated reagent solution, which is diluted to prepare a working solution. The stock solution has a longer shelf-life than the working solution and occupies less space in storage.

Working reagent solution

The working reagent solution can be diluted from the stock solution or prepared directly with the reagent chemical following the recommended procedures described before (Chapter 4).

Standard solution

The standard solution is also known as the reference solution that is used to find the concentration of the unknown. It should be made with extreme care as the accuracy of the results greatly depends on the value of this solution.

Expressions of Concentration of Solutions

The weight/volume (w/v) ratio is the most commonly used method of expressing the concentration of solutions. Let us try to understand this concept in greater detail.

If 1 g of table salt (NaCl) is dissolved in enough water to bring the solution to 10 mL volume, the concentration of the solution is 1/10 in weight/volume (w/v) ratio. This concentration of sodium chloride solution can be expressed in different ways that mean the same amount (Tables 6.4 and 6.5).

Table 6.4 *Expressions of concentration in various units*

Reagent preparation	Prepared solution	%	g/dL	mol/L	M
1 g of NaCl dissolved in water and made to 10 mL volume	1 g/10mL	10%	10 g/dL	1.7 mol/L	1.7 M

Note Molecular weight of NaCl (sodium chloride or common salt) is 23 + 35.5 = 58.5

Table 6.5 *Definitions of the strengths of solutions*

Concentration	Definition	Example
% (percent)	Whole weight in g (or volume mL) out of 100 mL	0.85% NaCl (0.85 g or 850 mg of NaCl in 100 mL solution). 95% Ethanol (95 mL of pure ethanol in 100 mL of ethanol solution)
M (mol/L)	Molecular weight in gram/L of solution	0.1 M NaCl/L (0.1 mole NaCl = 5.9 g NaCl/L. MW NaCl = 59)
N (Normality) Applied in the measurement of the strength of acid and base	Equivalent weight of an acid or base dissolved and made to a litre of solution	1 N HCl (36.5 g HCl/L)

Accuracy is critical in the clinical laboratory setting. Generally, for preparing 100 mL of 10% solution, the weight of .10 g should only be close to the first place decimal and the volume measurement may be made in a 100-mL graduated cylinder. In other words, 'percent' most often carries the meaning of 'not so accurate', while exact weight expression implies accuracy both in weighing and in volume measurement. Volume measurements are important for preparing many solutions and particularly, the standard solutions.

For example, the weight of 10.01 g or 9.99 g will not make much difference for preparing a 10% solution. In order to prepare a standard solution of sodium chloride (NaCl) with a concentration of 140 mmol Na/L, we must use a precise amount of NaCl.

If we want a standard solution with concentration 0.5 mol/L, we need to calculate how many grams of NaCl to add to 1 L of water. As we calculated earlier, 1 mole of NaCl weighs 58.5 g. Since we only need 0.5 moles for 1 L, we take exactly half that amount or 29.25 g and add it to 1 L of water. For solutions of considerably weaker concentration, far smaller

amounts of solute will be needed. For these, even greater care should be taken to ensure that amounts are exact because even a small miscalculation can have a relatively large impact on the desired concentration.

In the case of mixing two miscible liquids, for example alcohol in water, the concentration of the solution is in volume/volume (v/v) ratio. Thus if 80 mL of absolute alcohol mixed with 20 mL of water, then the solution is 80% alcohol. This will be applied for various acids and other reagents found in the liquid form.

You must be acquainted with the various expressions of reagent concentration so that you can understand the procedure and effectively communicate with the scientific world. We will discuss here the expressions that have been used in this book.

Percent solution (%)

Definition One gram of a solute in 100 mL of solution is 1 percent. It is expressed in different ways: percent, percent, %, g/dL or g/100 mL.

Example 5 g of solute in 100 mL solution will be 5%. The expression of mg% is technically wrong. It should be written as mg/100 mL or mg/dL. For the preparation of 0.85% sodium chloride, weigh 850 mg (0.85 g) of NaCl in about 80 mL (this volume is unimportant) of water in a 100-mL volumetric flask, dissolve the salt and then dilute the solution to 100-mL mark. The mixing of 850 mg of NaCl with 100 mL of water will be incorrect. The final solution will be more than 100 mL. If 1 L of the above saline solution is required, you have to weigh 8.5 g.

Molar solution (M)

Definition 1 mole (gram molecular weight) of solute in 1000 mL of solution is equivalent to 1 molar solution (1 M). It is expressed in different ways; 1 M, 1 mol/1, 1 mol/L (abbreviated) or 1 mole/L.

Example 58.5 g of NaCl in 1000 mL (1 L) solution is equivalent to 1 M (1 mol/L) and 58.5 mg of NaCl in 1000 mL (1 L) solution is equivalent to 1 mM.

Molar solution is a part of the metric system and hence such expressions as millimolar (mM), micromolar (μM) and others follow the same pattern as other units explained earlier. As a general rale of thumb, remember M = g MW/L; mM = mg MW/L; μM = μg MW/L, etc.

Avogadro constant

Before concluding our discussion on 'mole' we need to refer to 'Avogadro constant' (also called Avogadro's number expressed by symbols L, N_A). This is an important theoretical concept that defines mole. Although in the environment of diagnostic laboratories we will probably never come across this term.

Avogadro's number is equal to $6.02214179 \times 10^{23}$ and refers to the number of atoms in one mole of a substance. Avogadro's number is dimensionless; like saying 'a dozen' which means 12 entities of anything; let that be egg or marbles. Atomic weight on the periodic table refers to the mass of Avogadro's number of atoms of that element. For example, one mole, or 6.022×10^{23} atoms, of carbon is equal to 12.011 g. This number applies to both pure element as well as compounds. In case of pure element, it is the number of atoms while in case of compounds it will be the number of molecules. Thus 18 g water (H_2O) will have 6.022×10^{23} molecules of water in 1 mole of water.

Normal solution (N)

Definition A measurement of concentration based on equivalent weight. 1N equals 1 equivalent weight (in grams) of solute per litre of solution.

Example The equivalent weight of sodium hydroxide (NaOH) is 40 g because one mole of NaOH (23 g + 16 g + 1 g = 40 g) divided by the valence of Na (1) is equal to 40 g. When 1 equivalent weight (40 g) is dissolved in 1 L of water the concentration is equal to 1 N.

Example 1 equivalent weight of sulphuric acid (H_2SO_4) is equal to one mole of H_2SO_4 (2 g + 32 g + 64 g = 98 g) divided by its valence (2). Therefore when one equivalent weight of H_2SO_4, i.e. 49 g, is added to 1 L of water the concentration is 1 N.

Note 1N sulphuric acid is 0.5 M, since 1 mole of sulphuric acid is two times its equivalent weight because one molecule of the acid contains two reactive hydrogen atoms.

The expression of normality is only limited to acids and bases (alkalis) as equinormal solutions of acids and bases take equal volume to neutralize each other. Thus in **titration** the following formula is applicable:

$$\frac{\text{Concentration (N)} \times \text{Volume (mL)}}{\text{Acid}} = \frac{\text{Concentration (N)} \times \text{Volume (mL)}}{\text{Alkali}}$$

Although more and more laboratories are switching towards molar expression rather than expressions based on equivalent weight (N), in developing countries, **normality** is still in use, particularly for acids, alkalis and electrolytes. Following the same pattern as any other metric system unit, remember the following two commonly used expressions:

1 g Eq. Wt/L = 1 N

1 mEq/L = 1 equivalent weight in milligram dissolved in 1000 mL of solution.

Thus 5 mEq KCl/L = 5 × 74.6 = 373 mg KCl/L = 5 mmol/L

(1 mmol = 1 molecular weight in mg).

Preparation of Reagent Solutions

Some kind of reagent solution is needed in every diagnostic test. Test results largely depend on the **reliability of the reagent solution** used. Hence the technician must take extra precaution in preparing dependable reagent solutions. Many laboratories use 'kits' supplied by various manufacturers and these may be more reliable. Nevertheless the technician must learn how to follow directions in preparing reagent solutions in case they are not available from the supplier or the laboratory cannot afford to buy them.

All original test procedures provide directions for preparing solutions. The kits supplied by the manufacturer, however, do not give details deliberately so that their 'trade secret' is not disclosed. Whether or not the laboratory should prepare all reagents depends on the cost analysis. In general, bigger laboratories will be better off by preparing their own reagents while smaller laboratories will find it more economical to purchase kits. There is another consideration, some of the chemicals are very difficult to make and the laboratory would be wise to purchase them. Thus the general comment that can be made here is that you must be prepared to make the solution, if you find it profitable.

General comments

Choose the right type of reagent for preparing solutions. Standard solutions are made from pure grade reagent while others might use laboratory grade reagent.

In preparing solutions, chemicals should be weighed. The type balance to be used, physical or analytical, depends on the accuracy desired. After weighing, the solute must be dissolved and made up to the desired volume. The significance of **quantitative transfer** cannot be overemphasized here. The kind of water used (tap water, distilled water,

deionized water) will also affect the result as water carries many undesirable substances when not specially purified. All of these principles have already been discussed earlier in Chapter 4.

Preparation of Standard Solutions of Acids and Bases

Accurately prepared standard solutions of hydrochloric acid, sulphuric acid and sodium hydroxide are often required in standard laboratory procedures. They cannot be prepared directly, but are made approximately and then standardized by titration. It is always more convenient to prepare solutions by dilution. In case one needs 5 N solution, prepare a 10 N normal solution and dilute it two-fold. This is more accurate than trying to prepare exactly the desired concentration.

In preparing the standard solution of an acid or base, one requires a **reference standard** or primary standard. In the case of hydrochloric acid, it is sodium carbonate. This is weighed carefully and titrated against the solution of unknown strength. The methods of weighing are described below:

'Add of weight' and 'loss of weight' methods in weighing

The '**add of weight**' method is a commonplace practice, used more often in everyday life than in the laboratory. Using this method, one adds the solute to a container resting on to the balance. The increase in weight according to the scale is the weight of the substance taken. This is needed in the preparation of a common solution where a definite quantity of solute is needed. However, for other procedures, this method is unnecessarily tedious and inaccurate.

In preparing primary standard, an accurate predetermined weight is unimportant as long as the **exact weight** taken for titration is known. In this situation, we use the '**loss of weight**' method. In this method the container is first weighed accurately with the substance inside. Then an approximate amount is removed from the container for use. When the container is reweighed, it naturally weighs less as some material was removed. This reduction in its weight is attributed to the substance that was removed.

Example Take anhydrous sodium carbonate (dried at 37°C overnight and cooled in a desiccator) in a watch glass. Weigh the watch glass accurately to four decimal places on an analytical balance (W_1). Transfer about 1 g of anhydrous sodium carbonate from the watch glass to a beaker and reweigh the container (W_2).

Note Keep your hands clean so that dirty hands do not add to 'weight error'. The difference of weight (loss) is the amount of carbonate added to the beaker ($W_2 - W_1$).

Preparation of 1.0 N hydrochloric acid solution

Pre-packaged 1 N hydrochloric acid (and other acids and bases) may be locally available, but its preparation in the laboratory is easy.

1. Dilute 100 mL of concentrated HCl (about 11.7 N) to about 800 mL in a graduated cylinder. This is 'too concentrated' solution of HCl which must be standardized.
2. Dry about 10 g of analytical grade reagent (AR) sodium carbonate in a porcelain dish at about 250°C in an oven. This usually takes 1 to 2 h. Cool in a desiccator.
3. Take about 1 g (0.8-1.2 g) of anhydrous sodium carbonate into a 250-mL beaker by the **loss of weight method**. Note the accurate weight of sodium carbonate taken in the beaker. (Take three replicates for better accuracy.)

4. Add about 40 to 50 mL of water into the beaker and two drops of methyl orange indicator solution (0.1% solution of methyl orange dye in distilled water). The solution will have a yellowish colour.
5. Fill a 50-mL burette with the well-mixed 'too concentrated' HCl solution.
6. Place a white paper or tile under the burette and place the beaker over it for titration.
7. Titrate the carbonate solution to the end point, an orange colour. The end point is the one when one more drop of acid solution turns the carbonate solution to a red colour.
8. Record the amount of acid solution required to titrate the carbonate solution. (Repeat the titration for each of the replicate.)
9. Calculate the concentration of the 'too concentrated' solution as follows: Strength (N) of 'too concentrated acid' (S_{ca}) =

$$\frac{\text{Weight of sodium carbonate} \times 1000}{\text{Eq.Wt. of sodium carbonate} \times \text{Volume of acid used in titration}}$$

Example Weight of sodium carbonate (Na_2CO_3) = 1.1821 g (Eq. Wt. 53).
Volume of acid used to titrate sodium carbonate = 19.2 mL

Strength of 'too concentrated' hydrochloric acid (S_{ca}) = $\frac{1.1821 \times 1000}{53 \times 19.2} = 1.16\,\text{N}$

10. Calculate the normality for each replicate and take the average strength of the 'too concentrated acid' for further dilution (Step 11).
    Note The results should be close, if not identical.
11. Calculate the amount of 'too concentrated acid' needed to yield the desired strength (1.0 N) and volume (1000 mL) of the diluted acid by the following steps.

$$S_{ca} \times V_{ca} = S_{da} \times 1000 \text{ mL (final volume)}$$

where S_{ca} = Strength of 'too concentrated acid' = 1.16 N
V_{ca} = Volume of 'too concentrated acid' = **unknown**
S_{da} = Strength of diluted acid (strength desired) = 1 N
V_{da} = Volume of diluted acid (volume desired) = 1000 mL
Thus, $V_{ca} = S_{da}/S_{ca} \times 1000 = 1/1.16 \times 1000 = 862$ mL

Hence the amount of water needed is 138 mL (1000 – 862). Take 138 mL of water in a 1-L volumetric flask and make it up to 1000 mL volume (q.s.) with 'too concentrated' hydrochloric acid.

Preparation of 0.1 N hydrochloric acid from 1.0 N strength

Take 100 mL of 1 N HCl in a 1-L volumetric flask and make it up to 1000 mL volume with distilled water. This dilutes 1 N HCl ten times that yields 0.1 N HCl.

Preparation of 0.1 N sodium hydroxide solutions

Method 1: Using 0.1 N hydrochloric acid solution

1. Prepare carbonate-free saturated solution of sodium hydroxide in the following way: In a 1-L beaker or wide-mouthed flask of resistance glass, dissolve approximately 110 g of best quality sodium hydroxide (pellets) in 100 mL of distilled water. Stir until all pellets have dissolved (the solution will be hot). The solution will be almost saturated and is approximately 27 N. Use a tight-fitting cover and allow to stand for 2 to 3 days until sodium carbonate settles out, leaving a clear solution of sodium hydroxide. Carefully separate the clear solution by decanting or by passing through

a fine-fritted glass filter. Transfer the carbonate-free solution of sodium hydroxide to a polyethylene bottle.

2. Dilute 5 to 7 mL of the strong solution of sodium hydroxide in about 1 L of freshly boiled and cooled distilled water (removes dissolved carbon dioxide).
3. Standardize the strong alkali against 0.1 N hydrochloric acid using methyl orange as indicator. The concentration of the alkali will be higher than 0.1 N.
4. After determining the concentration of the 'too concentrated' alkali, dilute to obtain 0.1 N **exactly** as done in the case of hydrochloric acid.
   Normality of strong alkali × Volume of strong alkali (x)
   = 0.1 N (desired concentration) × 1000 (volume to be made)

   Note If time is limited, pellets from a freshly opened bottle of reagent grade sodium hydroxide may be used. Dissolve 5.0 g of NaOH pallets in freshly boiled and cooled distilled water and make this to 1000 mL with boiled distilled water. This is more concentrated than 0.1 N.

Alternative Calculation

$$\frac{\text{Vol. of NaOH taken for titration}}{\text{Vol. of titrant (0.1 N HCl) used}} \times 1000 = \text{Vol. of too concentrated NaOH to be diluted to 1000 mL}$$

Method 2: Using potassium acid phthalate as primary standard

Potassium acid phthalate or KHP ($KHC_gH_4O_4$) is an excellent primary standard used to standardize the 'too concentrated' carbonate-free NaOH solution mentioned before.

1. Place 4 to 5 g of potassium acid phthalate in a clean weighing bottle and dry in an oven at 100°C for 2 h (no longer and not at higher temperature). Stopper the weighing bottle and transfer to a desiccator to cool.

   Note Molecular and equivalent weights of potassium acid phthalate (KHP) are the same, 204.228.
2. Take out about 0.4 to 0.45 g of KHP in a beaker or conical flask (250 mL, Figure 6.3) and calculate the loss of weight to the fourth decimal place (same way as the sodium carbonate primary standard).

   Note Prepare two other replicates in the same way as described above. Weigh the salt (primary standard) exactly in each case. The result of the three replicates must be close. The actual value will be the average of the three.
3. Add 20 to 25 mL of distilled water and two drops of Phenolphthalein indicator solution (1% alcoholic solution; 1 g Phenolphthalein in 100 mL of 95% alcohol) to each flask.
4. Take the 'too concentrated' carbonate-free sodium hydroxide in a burette and titrate the phthalate solution to the end point, a very light pink colour. Record the amount of NaOH solution required to neutralize each sample of KHP.
5. Determine the normality of the NaOH solution in each of three titrations as follows:

   Note Equivalent weight of KHP ($KHC_8H_4O_4$) = 204.228

$$\text{Normality (NaOH)} \times \text{Volume NaOH} = \frac{\text{Wt. of KHP (acid)} \times 1000}{\text{Wq. Wt. of KHP}}$$

Or, Normality of NaOH

$$= \frac{\text{Wt. of KHP} \times 1000}{204.228} \times \frac{1}{\text{Volume of NaOH}}$$

$$= \text{Wt. of KHP} \times 4.9 \times \frac{1}{\text{Volume of NaOH}}$$

6. Average the three normalities obtained from three titrations of KHP and calculate the correct dilution of the 'too concentrated' NaOH to be made.
   Normality of concentrated NaOH × Volume of concentrated NaOH required = 0.1 N (required) × 1000 (volume to be made).

$$\text{Or, volume of concentrated NaOH required} = 0.1 \times 1000 \times \frac{1}{\text{Normality of concentrated NaOH}}$$

7. Measure the amount of alkali in a 1-L volumetric flask and make it to the volume with distilled water. Alternatively, add the diluent first (volume will be less) and then fill to the mark with the '**too concentrated**' alkali.

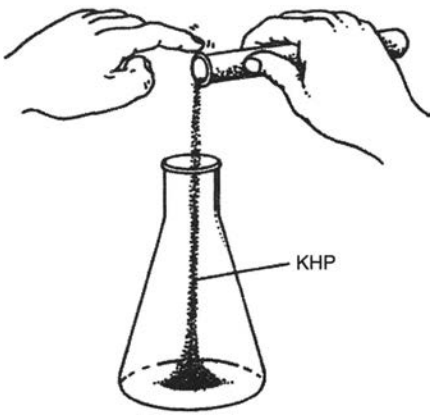

FIGURE 6.3 *Transfer of weighed quantity of potassium acid phthalate (KHP) into the titration flask*

LABORATORY CALCULATIONS

You will often be involved with a number of laboratory calculations related to the preparation of reagent or computation of diagnostic test results. A few of the problems related to the preparation of standard solutions are discussed in the previous pages. Specific computation problems concerning various diagnostic tests will be discussed at appropriate places. An attempt will be made here to expose you to the logic of laboratory calculation through selected examples.

Ratios and dilutions

A **ratio** is a relationship in number or degree between two things. **Dilutions**, which are ratios, express the relationship between a part of a solution and the total solution. Dilutions are used in haematology, serology and other fields. For example, if the instruction calls for preparing 10 mL of diluted serum (1:10), one has to mix 1 mL of serum with 9 mL of saline.

Note In the expression of 1:10, the second numeral is the final volume. Thus, 1:1 means no dilution.

In another example, the instruction calls for mixing 0.5 mL of blood with 9.5 mL of diluent for cell count. The dilution or the relationship between the aliquot (0.5 mL) and the total volume (0.5 + 9.5 = 10 mL) is expressed in the ratio of $\frac{10}{0.5}$ = 20 times or 1:20.

Weight relationships of hydrated and anhydrous salts

You will often face a dilemma when directions for preparing a reagent solution involve a chemical which is **hydrated** while your laboratory has only the **anhydrous** form, or vice versa. The following simple formula and example may help to find the solution.

$$\frac{\text{Molecular weight of hydrated form}}{\text{Molecular weight of anhydrous form}} = \frac{\text{Weight of hydrated form}}{\text{Weight of anhydrous form}}$$

Example The directions ask you to use 25 g of anhydrous copper sulphate ($CuSO_4$) whereas your laboratory has hydrated copper sulphate ($CuSO_4 7H_2O$). How much of the hydrated form would you weigh in order to meet the requirement of the directions?

Molecular weight of anhydrous form ($CuSO_4$) = 159.5 g
Molecular weight of hydrated form ($CuSO_4\ 7H_2O$) = 285.5 g
Apply the above formula:

$$\frac{285.5}{159.5} = \frac{x}{25}$$

$$x = \frac{285.5 \times 25}{159.5} = 44.7$$

Thus you should weigh 44.7 g of the hydrated form which is equivalent to 25 g of the anhydrous form.

Figuring out the weight of a liquid from its volume

Weighing of a solid is not a problem in a laboratory. But suppose you need 49 g of sulphuric acid to prepare 1000 mL of 1 N solution; the weighing of sulphuric acid can be dangerous, erroneous (the acid absorbs water when exposed to air) and might create a number of other problems. You can resolve this problem without weighing the sulphuric acid and the method is applicable to any liquid reagent.

The label on the bottle provides the **density** and **purity** of the chemical. If not, consult the appendix at the end of the third volume which gives normalities, purities and densities of commonly used acids and alkalis. Let us take the following figures and find out the volume of sulphuric acid that we need in order to meet the requirement of 49 g.

Specific gravity = 1.84
Purity = 98%
Weight needed = 49 g
Volume of the acid needed = *x* mL

$$\text{V (Volume)} = \frac{\text{M (mass)}}{\text{D (Density)}} = \frac{49}{1.84} \times \frac{100}{98} \text{ (ratio to compensate for impurity)}$$

Thus, V = 27.2 mL

If 27.2 mL of the given concentrated sulphuric acid is mixed with water and made to 1 L, it will be **about** 1 N. In order to get exactly 1 N, you have to titrate and then make the final dilution. Let us continue further in order to get exact 1.0 N sulphuric acid.

Note It is always easier to dilute than to add more concentrated acid to reach our goal.

Let us suppose the strength of the acid, determined by titration, came to 1.1 N. Then the new problem can be defined as follows: How much water should be added to the 1 L of 1.1 N sulphuric acid in order to get exactly 1.0 N acid solution?

Apply the basic formula:

$$V_1 \times C_1 = V_2 \times C_2$$

Where, V1 and V2 are the initial and final volumes, respectively, and C1 and C2 are the initial and final strengths of the acid. In other words, the above formula comes to: $1000 \times 1.1 = x \times 1.0$ (x is the final volume).

$$\text{Or, } x = \frac{1000 \times 1.1}{1.0} \text{ 1100 mL(the final volume)}$$

Thus, if we add 100 mL (1100–1000 mL) of water to the 1.1N acid solution, it results in exactly 1.0 N acid solution.

Mixing of two solutions of different concentrations

What will be the concentration of the final solution when two solutions of different strengths are mixed?

Problems

1. I have mixed 80 mL of 70% alcohol with 40 mL of 95% alcohol. What will be the concentration of the final mixture?
2. How much of 80% alcohol should be mixed with 30 mL of 95% alcohol in order to prepare 90% alcohol?

Solutions

Let us take the basic formula:

$$(C_1 \times V_1) + (C_2 \times V_2) = C_3 \times (V_1 + V_2)$$

Where, C and V respectively stand for concentrations and volumes of solutions 1 (C_1) and 2 (C_2). Solution 3 (C_3) is the concentration of the final solution (x).

Note It is not important for you to find out how this formula was derived. You are perfectly justified in applying the formula without any idea of mathematical derivations.

1. $(70 \times 80) + (95 \times 40) = x \times (80 + 40)$
   Or, $x = (70 \times 80) + (95 \times 40)/(80 + 40)$
   Or, $x = 78.3$
   Thus, the new solution will be of 78.3% concentration.
2. $(80 \times x) + (95 \times 30) = 90\ (30 + x)$
   Or, $80x + 2850 = 2700 + 90x$
   Or, $10x = 150$
   $x = 15$
   Thus, if 30 mL of 95% alcohol is mixed with 15 mL of 80% alcohol, the resulting mixture will be of 90% concentration.

Additional information

$$\text{mEq/L} = \frac{(\text{mg/100mL}) \times 10}{\text{Eq. Wt.}}$$

$$\text{mmol/L} = \frac{(\text{mg/100 mL}) \times 10}{\text{MW}}$$

$$\text{mmol/L} \times \text{Valence} = \text{mEq/L}$$

$$\text{Molarity} = \frac{\% \text{ Concentration} \times 10}{\text{MW}}$$

$$\text{Normality} = \frac{\% \text{ Concentraion} \times 10}{\text{Eq. Wt.}}$$

Review Questions

1. What are the basic metric units of length, weight, and volume?
2. Why is the metric system preferred over the English system in clinical laboratory?
3. Convert the following units: (a) 0.001 g into mg; (b) 300 mg into g; (c) 750 μg into mg; (d) 4000 mg into g; (e) 280 mg into μg; (f) 10 mg into pg
   Answers (a) 1 mg; (b) 0.3 g; (c) 0.75 mg; (d) 4 g; (e) 280000 μg or 2.8×10^5 μg; (f) 1×10^{10} pg.
4. Convert the following units: (a) 3 dL into mL and μL; (b) 0.3 L into dL and mL; (c) 45 cc into L and mL; (d) 4 dL into L; (e) 60 μL into L and mL; (f) 6700 mL into L
   Answers (a) 300 mL and 3×10^5 μL; (b) 3 dL and 300 mL; (c) 0.045 L and 45 mL; (d) 0.4 L; (e) 6×10^{-5} L and 0.06 mL; (f) 6.7 L
5. Convert 98.6°F (normal body temperature) to Celsius (C) degrees.
   Answer 37°C
6. Prepare 250 mL of a 2% solution of acetic acid using 10% acetic acid solution.
   Answer 50 mL 10% acetic acid mixed with 200 mL of distilled water. Apply the formula: $C_1 \times V_1 = C_2 \times V2$, where C_1 and V_1 are, respectively, the concentration and volume of first solution (10% acetic acid) and C_2 and V_2 are, respectively, the concentration and volume of the second solution (2% acetic acid). In other words: $10 \times V_1 = 2 \times 250$ or $V_1 = (2 \times 250)/10 = 50$ mL and the amount of distilled water will be 250 – 50 = 200 mL.
7. How much salt will you weigh to prepare 500 mL of saline (0.85%)?
   Answer $0.85 \times 500/100 = 4.25$ g
8. To prepare 100 mL of 10% formalin from the commercial grade of 37% strength, how much of the formalin would you measure?
   Answer $100 \times 10 = x \times 37$. Or, $x = 1000/37 = 27.03$ mL
9. To prepare 1 L of 2% acetic acid from concentrated acetic acid (glacial or 100%), how much of acetic acid do you need?
   Answer 20 mL
10. For preparing 4% suspension of red cells (to be used in blood bank) you centrifuged the whole blood and discarded the plasma. The volume of packed red cells was found to be 2.5 mL. How much saline would you add in order to make a 4% suspension of red cells in saline?
    Answer When 96 mL of saline is mixed with 4 mL of packed red cells it yields 4% suspension.
11. You are provided with concentrated sulphuric acid (specific gravity 1.98, 96% pure). In order to prepare 500 mL of 1 N H_2SO_4 (approximately), what quantity of concentrated sulphuric acid would you need?
    Answer Eq. Wt. of sulphuric acid = 49; hence, 24.5 g needed for 500 mL solution. In order to get 24.5 g weight of sulphuric acid, the volume of sulphuric acid that has to be measured = 24.5/1.98 (V = M/D or volume is equivalent to mass divided by density) = 12.37 mL. Finally the purity is to be compensated: $12.37 \times 100/96 = 12.88$ mL.

12. In a certain acid-base titration 6 mL of 1.5 N acid neutralized 4 mL of the alkali. What is the strength of the alkali?
    Answer $6 \times 1.5 = 4 \times x$ or $x = 2.25$ N
13. You have 1.15 N HCl solution ('too concentrated'). How much water would you mix in order to obtain 1 Lof 1 N HCl solution?
    Answer 130 mL
14. One litre of 70% alcohol is needed. How much 95% alcohol is required to make that?
    Answer 736.8 mL
15. How much KCl would you require for preparing 1 L of 5 mEq K/L?
    Answer 1 mEq KCl = 39.1 + 35.5 = 74.6 mg KCl = 1 mEq K. Hence, for preparing 1 L solution of 5 mEq K/L you should weigh $74.6 \times 5 = 373$ mg or 0.373 g of KCl.

Chapter 7

Good Laboratory Practices and Statistical Quality Control

Aloka Chakravarty

Chapter Outline

"Quality is everyone 's responsibility. It is not enough to do your best; you must know what to do, and then do your best. "

—W. Edwards Deming
Statistician and Father of Total Quality Management (1900–1993)

Laboratory findings must be dependable so that accurate diagnosis can be based on them. Medical laboratory personnel must be able to identify sources of variation and control for them. For example, if a patient has a battery of laboratory chemistry tests, there is a possibility that some tests will be abnormal, the so-called false positive results, purely due to chance alone. It is important to identify and control by stringent margins each source of variation so that we can minimize, control and quantify such errors.

As the laboratory also depends on external agencies, an effective quality control programme will not only monitor internal laboratory procedures but will also communicate needs and requirements of the clinical laboratory to other departments of the hospital. Strict enforcement of laboratory policies creates an environment conducive to producing reliable, reproducible and high-quality results. A relaxed approach toward quality control leads to an equally relaxed approach toward the handling and testing of specimens and should be avoided at any cost.

Areas where common errors are likely to occur and can be prevented are discussed further.

Sources of Common Errors in Laboratory

Laboratory test request

All laboratory test requests should be made in writing on the appropriate request form as indicated in Chapter 5. It may be worthwhile to insist on three time slots:

- when request was issued,
- when specimen was obtained,
- when results were reported (with the name of the technician who reported the result).

Specimen collection and processing

The technologist, trained nurse or the physician collects the specimen. Proper patient preparation is an important component of quality control. **Laboratory results are only as good as the specimen.** Hence, quality control is a joint responsibility and the cooperation of the attending nurse is critical. If the specimen collected is not promptly delivered to the laboratory, the specimen becomes useless for analysis.

The person who collects the specimen must report to the nursing station for entering the information about specimen collection and identification of the patient; and must collect the specimen according to standards laid down by the laboratory. A frequent source of error has been identified to be the failure to observe basic precautions and laboratory rules. **Identification** of the patient is extremely important—identify the patient before taking the specimen, check the name on the requisition slip, check also the name on the patient's wristband (if it is there) or ask the nursing staff as a double check. Specimens must be in **appropriate containers** which must be carefully labelled. It is a good practice to label the container before taking the specimen and use only waterproof ink so that identification does not get 'washed away'.

Proper collection **procedure** and specimen processing after its collection are both important and must be strictly adhered to if meaningful results are to be expected. Haemolysed blood is of little value in most laboratory tests. Also, if the serum is not promptly separated from the cells, the results may not be accurate. When the specimens are directed to other laboratories for tests and follow-ups, care must be taken to ensure proper identification and re-labelling of each specimen. Mix-up is more likely to occur within the laboratory.

Laboratory records

Each specimen arriving in the laboratory must receive the **laboratory accession number** recorded in the laboratory register or the log book. The technician's record must refer to the accession number and when reports are dispatched by the laboratory, the results must be noted in the accession register, with the time of reporting and the name of the technician who reported the results. The technician's register must indicate the quality control procedure followed. Many advanced laboratories maintain an alphabetized test report card system for each patient; these cards are further classified under different specimens and laboratories. This helps to trace back the previous records of the patient.

Error in procedure

Well established laboratory procedures must be adopted and documented for the physician's information. The laboratory must maintain a **procedure manual** for each test performed. Failure to adhere to established rules and procedures may provide results that are not reproducible or reliable. Technicians must be discouraged from inventing individual methods without appropriate documentation. A good technician keeps

abreast of newer techniques, is aware of drug interactions and is alert within limitations. A method that is fast, economical, reliable and reproducible and can be done by personnel without specialized training is optimal.

Reagents must be stored properly and outdated reagents must be discarded. **Instruments** must be properly maintained. A regular maintenance programme is essential for the proper running of the laboratory. All instruments, at regular intervals, must be checked for their performance—wavelength of colorimeter, efficiency of the autoclave, water bath temperature, oven temperature, refrigerator temperature, cold room temperature, etc.

Calculation and **transcription errors** can occur at any point and may lead to wrong diagnosis. The technician must record the actual instrument reading, indicate calculations and tally the report at least twice before sending it out of the laboratory.

Proficiency Testing

Only properly **trained personnel** should be appointed by the laboratory. The training must be provided by a recognized institution and should not be only On the job training'. The employee should be required to submit evidence of continuing education every year. The technician must maintain daily plotting of the **quality control chart** explained in the following pages and must participate in the proficiency testing programmes organized by various agencies, including the World Health Organization (WHO).

Proficiency testing is an important component of quality control. An external agency may send **blind samples** to the laboratory that have been assayed multiple times. The laboratory then performs the assay and sends results back to the agency for comparison and a detailed report on each component.

Quality Control Issues by Laboratory Type

Each laboratory has its own characteristic features and hence the quality control approach varies. There are, however, some common features such as specimen collection, identification and processing, laboratory records, maintenance of instruments, procedure and reagents and supplies. The manufacturer also plays an important part in providing reliable data by supplying reliable instruments and reagents. There should be close cooperation between the manufacturer and the laboratory so that the former can meet needs of the laboratory effectively on a continuous basis.

Haematology laboratory

Specimens must be processed correctly and tests must run within the specified time. Some of the tests must be done within 2 h (ESR), within 4 h (WBC count) or can be delayed for 8–10 h (RBC count, PCV or HCt). Fixed smears can be examined after several days.

The maintenance of the equipment must include the checking of centrifuge speed, volume dispensed by diluters, temperature of the water bath, etc. Keep a record of the maintenance plan. Manual RBC count is highly erroneous. Automated counters have their own settings; run the control every day and set controls according to the manufacturer's directions.

Correlation of results is a good way to check on the results. For example, a normal haematocrit should have normal haemoglobin; a stained smear must be correlated with the count (Table 7.1) and the same holds for the platelet count (Table 7.2); a slide with hypochromic red cells must have a low haemoglobin value; presence of macrocytes and megaloblasts should correspond with increased mean cell volume (MCV). It has been found that indices stay close to the normal values, hence, for standardization use indices.

Table 7.1 *Correlation of number of leucocytes under high-power objective (400X) and WBC count*

Number of WBC under high power	Estimated WBC count per mm^3
2–4	$4–7 \times 10^3$
4–6	$7–10 \times 10^3$
6–10	$10–13 \times 10^3$
10–20	$13–18 \times 10^3$

Table 7.2 *Correlation between average number of platelets under oil-immersion objective (1000X) and actual platelet count*

Number of platelets per oil-immersion field	Comparable platelet count
Less than 1	Less than 2×10^4 (thrombocytopenia)
6–15 (several platelets with occasional clumps)	3×10^5 (normal)
More than 15	$>5 \times 10^5$

Coagulation laboratory

The reagent is the biggest source of error in coagulation. Temperature of the water bath must be checked regularly. All tests run must be accompanied by a control run with normal plasma. Due to lack of appropriate facilities, lyophilized normal plasma is not yet very commonly used in laboratories of developing countries.

Blood bank laboratory

Technical error is still high and all possible precautions should be adopted in order to avoid wrong transfusion. Properly functioning equipment is equally important and so also is the reagent supply. All reagents must be stored and frequently checked according to the manufacturer's directions. Check the Coombs reagent with Coombs control (sensitized cells). Keep a semi-quantitative report of the haemagglutination reaction.

Before the matched blood leaves the laboratory for a particular patient, it must be re-checked and countersigned by the technician and receiving nurse in order to minimize clerical mistakes.

A complete record of every unit of blood received by the blood bank must be kept in a log book that indicates: date of receiving donor's blood, source, code number, blood type, result of compatibility testing, name of recipient, date of transfusion and result of transfusion. In case the blood is supplied by any outside agency, the blood type must be rechecked.

Microbiology laboratory

Collection of the specimen by recommended procedures and its proper transportation to the laboratory are crucial for getting reliable results from the microbiology laboratory. Performance of the equipment and reliability of reagents are the first steps in quality control, followed by appropriate culture technique or direct microscopic examination. In case of bacteriology and mycology, testing of media and stains against type-cultures is extremely important. In selecting the type-organisms, both 'positive' and 'negative' must be chosen. Table 7.3 gives the type of organisms used in testing the common media. Table 7.4 gives the procedure for maintaining important test organisms. Sensitivity testing must also be checked with appropriate organisms and the zone formed must tally with the suppliers' specifications.

Table 7.3 *Tests of performance of common media and biochemical reactions*

Medium	Test organism	Result
Blood agar	*Streptococcus pyogenes*	Beta-haemolysis
Chocolate agar	*Neisseria gonorrhoeae*	Growth in 5% CO_2
McConkey	*Escherichia coli*	Red colonies
S-S agar	*E. coli*	Pink colonies
TSI agar	*E. coli*	Acid butt, acid slant, gas, no H_2S
Sab-dextrose	*Candida albicans*	Growth
Citrate agar	*E. coli* *Klebsiella*	Negative
Urease agar	*E. coli* *Proleus*	Positive
Bile solubility	*S. pneumoniae*	Loss of turbidity negative
Indole oxidase	*E. coli* *E. coli* *Pseudomonas aeruginosa*	Positive Negative Positive (purple)

Table 7.4 *Maintenance of important test organisms*

Organism	Medium	Incubation temperature	Interval between subcultures
E. coli *Shigella*	Nutrient agar	37°/18 h	6 months
Klebsiella *Proteus* *Staphylococcus*	Nutrient agar	37°/18 h	3 months
Pseudomonas	Peptone water	37°/18 h	3 months
Salmonella	Dosett egg	37°/18 h	12 months
Streptococcus	Cooked meat	37°/18 h	3 months

Note Keep all type cultures at 5°C in the refrigerator.

Parasitology laboratory

The stool specimen is one of the most frequently neglected materials submitted to the laboratory. Only fresh, properly collected and uncontaminated stool specimens can yield a proper diagnosis. In following the technique of faecal preparation, you must follow the established procedure without unscientific modifications. A parasitological reference book must be available in the laboratory for identifying unusual eggs and other parasite-related objects. An experienced technician must be consulted in case of doubt. Every tenth negative report must be checked by the supervisor and every positive specimen must be tallied by the supervisor. A set of prepared slides should be kept in the laboratory for reference.

Serology laboratory

All serodiagnostic tests must have a positive and a negative control. Most manufacturers supply them along with the kit. The technician should have sufficient experience to recognize the positive reaction and whenever possible should grade the reaction (1+ to 4+). The borderline reactions specially need personal judgement.

Clinical pathology laboratory

A urine specimen must be processed within two hours. Quick chemical screening tests done with the urine sample must be checked with the positive and negative controls. If necessary, add an appropriate reagent to normal urine in order to prepare the positive urine. Do not add an excessive amount so that the borderline specimens can be recognized. Follow the same criteria for other body fluids as is done in case of urine.

Urine culture must be done with well-mixed specimens and not with the sediment. This gives the correct picture. If the bacterial population is less than 1×10, the specimen is rejected. The counting procedure must be standardized.

Laboratory findings of **histology and cytology** are highly subjective to the decision made by the pathologist during the examination of the specimen. Hence, selection of a representative specimen and procedures of fixing, cutting, embedding and staining are all variable factors that may result in false findings and artefacts. Stains must be checked with known sections. Permanent slides may guide the technician towards ideal staining. In case of poor quality, re-staining may have to be done. In most cases, staining can be partially repeated.

Clinical biochemistry laboratory

The biochemistry laboratory requires quantitative analysis. All factors mentioned earlier, e.g., specimen collection, instrument handling and procedures adopted, affect the reliability of the findings. Specimen-related errors must be minimized. For example, if the blood specimen arrives with haemolysis for potassium determination, it must be rejected; a specimen submitted for the determination of blood gases must be in ice and under anaerobic conditions; a specimen for bilirubin assay must not be exposed to strong light; and so on. At regular intervals prepare a new calibration curve of the test in order to find out whether the assay region is in the linear range. Each morning the analysis of the control serum should be done and the result plotted on the Quality Control (QC) chart which will be explained in the following section.

Quality Assessment and Quality Assurance

Quality assessments are designed so that reliable laboratory results can be obtained and reported as early as possible, by minimizing systematic sources of error. It evaluates pre-analytical, analytical and post-analytical factors that can affect the results before, during and after the test.

Pre-analytic factors include proper patient identifiers, specimen collection and handling, specimen rejection criteria, maintaining proper laboratory setup including calibration. Selecting right test methods, personnel and maintaining updated procedure manuals also are important components.

Post-analytic factors are primarily in reporting and charting of the results, including transcription and clerical errors. Use of computers as well as proper patient identifiers, laboratory request forms, labels and specimen, containers can greatly aid in minimizing these errors.

Analytic factors that can affect laboratory tests are:

- Laboratory preparation of samples
- Instrument calibration and maintenance
- Standards and procedural control
- Test procedure logistics (reagents, pipetting, timing, etc.)
- Interfering conditions or substances
- Statistical analysis of control results

Statistical Quality Control of Quantitative Data

It is difficult to apply statistical tools in evaluating data which are of a qualitative or subjective nature. **Quantitative laboratory data** must be subjected to rigorous **'quality control'** procedures in order to obtain dependable information for clinical diagnosis. The commonly followed procedure of quality control in handling quantitative data will be discussed in this section. Before we focus on the quality control procedures of laboratory findings, it may be worthwhile to explain some of the commonly used terms in quality control.

Commonly used terms in quality control

Control: Controls are solutions that contain the same constituents as the patient sample. The control sera must be analysed with patient sample using identical methods, test conditions and reagents. At least two levels of controls should be used and these have to be run at least daily. Records of the control assay must be documented for any future inspection.

Standard solution: It is a carefully made solution of the test substance whose concentration is known.

Calibration and Standards: A **standard** or **reference material** is a substance that has an exact known composition and that, when accurately weighed or measured can produce a solution of exact concentration.

Calibration refers to process of checking, standardizing, adjusting a method or equipment so that it yields accurate results.

Precision: Precision refers to reproducibility of results or the closeness of obtained results to each other.

Accuracy: Accuracy refers to closeness of the result to the true value.

Dependability: A combination of precision and accuracy that implies reproducibility and accuracy (close to the true value).

Basic Statistics

Quality Control programs use Statistics, the branch of Mathematics that deals with collection, classification, analysis and interpretation of numerical data. The entire collection of observations is called a **population,** while a group of specimens realized from this bigger domain is called a **sample.**

A few common statistical measures used in Quality Control (QC) are mean, standard deviation, coefficient of variation and tolerance range.

Mean

The **arithmetic mean,** often simply called the 'mean', is a measure of central tendency of the dataset and denoted by $\overline{X}$. It is calculated by summing up value of each observation, divided by the number of observations. In mathematical notations,

$$\overline{X} = \frac{\sum_{i=1}^{n} X_i}{n}$$

where Σ is the summation symbol, X_i = individual observation and n is the number of observations. For example, the arithmetic mean of six values: 34, 27, 45, 55, 22 and 34 is obtained as

$$\overline{X} = \frac{34 + 27 + 45 + 55 + 22 + 34}{6} = \frac{217}{6} = 36.167$$

Standard deviation (SD)

It is a commonly used measure of dispersion of the data and is measured by variability from the mean. It is defined as

$$SD = \sqrt{\frac{\sum_{i=1}^{n}(X_i - \bar{X})^2}{n-1}}$$

where Σ = summation sign; X_i = individual observation; $\bar{X}$ = mean and n = number of observations.

This can be simplified for easy calculation, often referred to as the calculator method for determining standard deviation:

$$SD = \sqrt{\frac{n\sum_{i=1}^{n}X_i^2 - \left(\sum_{i=1}^{n}X_i\right)^2}{n(n-1)}}$$

where, $\sum_{i=1}^{n}X_i^2$ = sum of squares of individual values; $\left(\sum_{i=1}^{n}X_i\right)^2$ = square of the sum of all values; and n = number of observations. Using the calculator method for the dataset above, we note that $\sum_{i=1}^{n}X_i^2 = 8575$ and $\left(\sum_{i=1}^{n}X_i\right)^2 = 47089$. Substituting in the formula, we get

$$SD = \sqrt{\frac{6 \times 8575 - 47089}{6 \times 5}} = 12.0568$$

These two methods can give slightly different numerical results; however, the numerical difference is expected to be minimal for datasets with at least 20 observations.

Coefficient of variation

For some purposes, the standard deviation is expressed as a percentage of the mean value. This is called the **coefficient of variation** (CV) or relative standard deviation (RSD); the latter is a better term. Coefficient of variation is calculated as follows:

$$CV(\%) = 100 \times \left(\frac{SD}{Mean}\right)$$

Tolerance range

It is the acceptable range of variation in quality control. This is equivalent to ±2SD.

Use of Standard Deviation in Laboratory

It is expected that the data for most situations in a laboratory will be from a bell-shaped curve, also called **normal distribution** or **Gaussian distribution** (Figure 7.1). This curve is symmetric about the mean, with half of the values greater than the mean and half less than the mean (Figure 7.1a). Frequency of values closer to the mean is higher than that away from it.

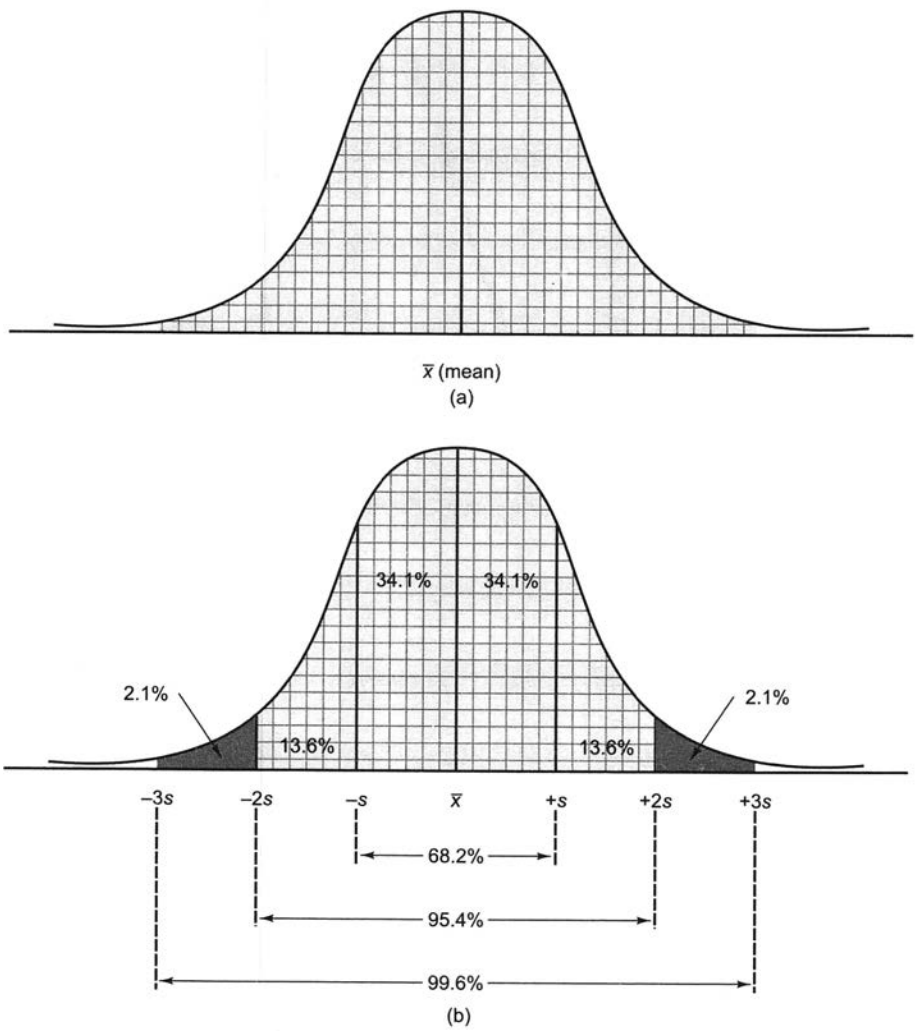

FIGURE 7.1 *Normal distribution curve showing (a) frequency distribution around the mean, (b) proportion of population falling between mean and ± 1s (SD), ±2s (SD) and ±3s (SD)*

Normal distribution can be divided into percent divisions in terms of its mean and standard deviation. If *s* refers to its standard deviation, then we note that 68.2% of the values are expected to lie between $x \pm s$; 95.4% between $x \pm 2s$; and 99.8% between $x \pm 3s$ (Figure 7.1b). As only 0.4% of the values are expected to be greater than $x + 3s$ or less than $x - 3s$, special attention should be paid to values exceeding these thresholds and double checked to ensure that they are not due to any systematic errors. Values outside $x \pm 3s$ threshold are called **outliers.**

Clinical laboratories must establish allowable standard deviation for each analysis method. A common choice is two-standard deviation limit, often called **confidence limit.** As noted before, it is expected that 95.4% of the values lies between this confidence limit.

Preparation of Quality Control Chart

Quality Control (QC) charts or **Levey–Jennings charts** demonstrate a method's precision and allow problems to be easily detected. This will be further illustrated with the help of an example.

Suppose for a particular method, the mean is 75 and the standard deviation is 8. In Figure 7.2, thus, mean line is 75 and lines on either side of the mean line denote ±2*s*. For each day, the control serum is plotted (Figure 7.3).

A **trend** is observed when a series of control values consistently increase or decrease (moves away from the mean in the same direction) for consecutive days (Figure 7.4). It is a signal of a systematic error that the laboratory technician should further investigate and locate the root cause. Some of the common sources of such an error are the instrument, the technique, the reagents or the control serum. Once the source is located, a new lot of control serum can be analysed. If the error is still present, it may call for recalibrationof instruments.

Control serum is expected to randomly fluctuate above and below the mean (Figure 7.3). When the control serum stays either above or below the mean for several consecutive days, but at a constant level, a **shift** is observed (Figure 7.4). This may signal a systematic error and should be investigated for root cause.

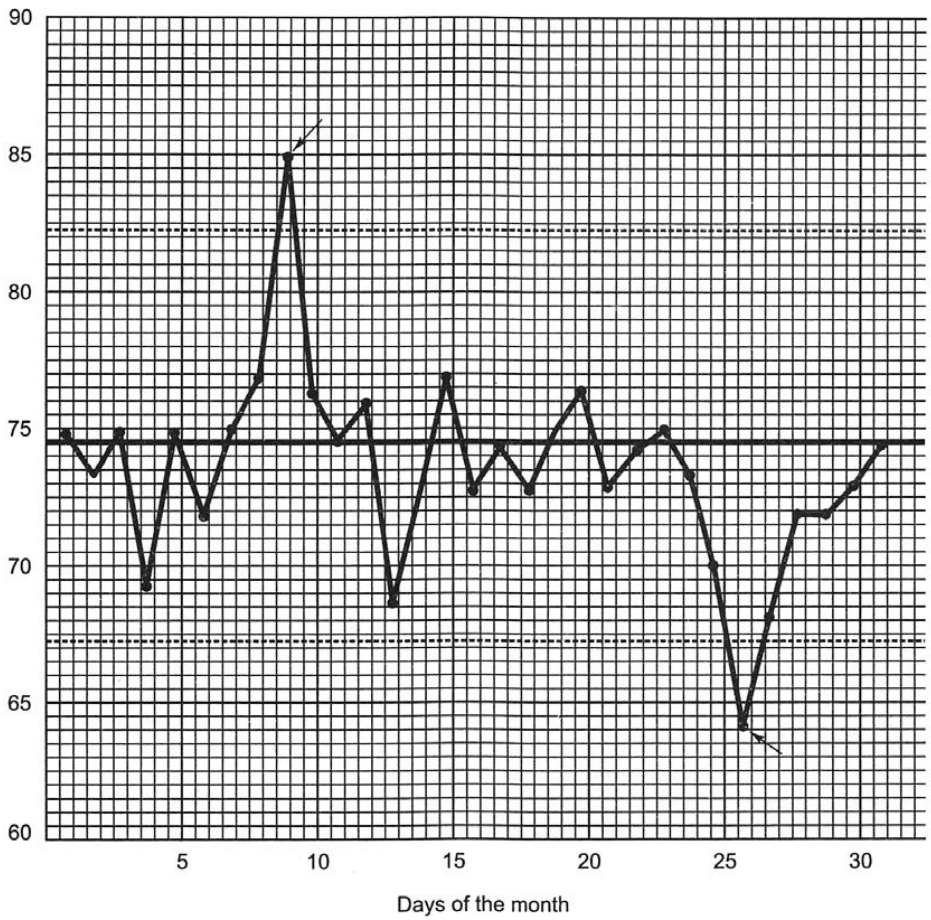

Figure 7.2 *Quality control (QC) chart of glucose analysis prepared from Table 7.6*

Step 1: Repeated analyses of control serum

The goal of repeated analyses with the control serum is to establish **accuracy** and **degree of variation** (CV) which depends on the type of test and analytical skill. The analysis of control serum for a specific test (e.g., glucose) should be performed for at least 20 times. The control

serum used in repeated analyses is preserved in the freezing compartment of the refrigerator (in small vials) for **'daily analysis'** of control and for plotting the quality control (QC) chart (Table 7.6 and Figure 7.3).

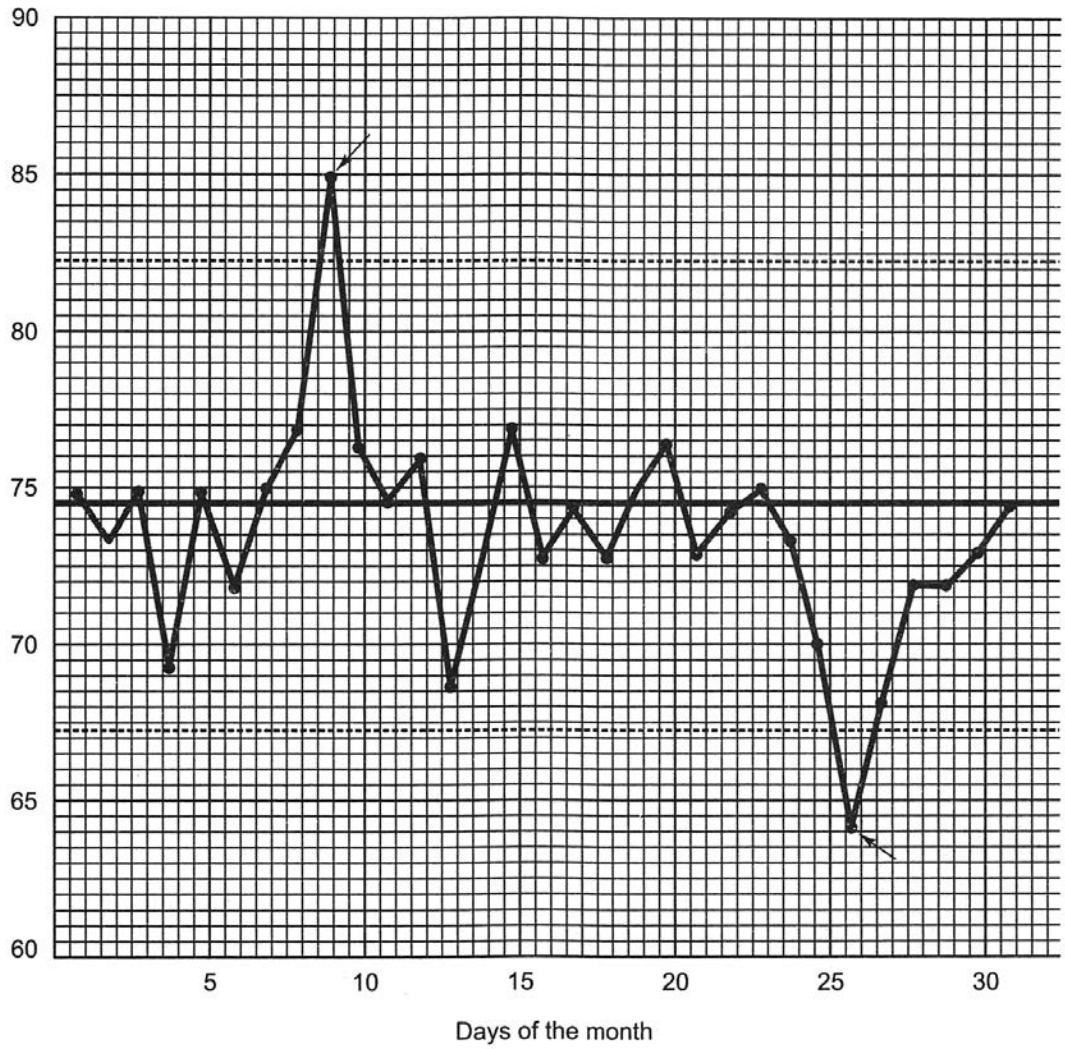

Figure 7.3 *Quality control chart for daily plotting. An acceptable quality control chart should indicate equal distribution of points on both sides of the mean and the points should stay within the tolerance range (±2SD). Note the out of range plots shown by arrows.*

Step 2: Calculation of standard deviation and coefficient of variation

From the data obtained in Step 1, determine mean, SD and CV. The SD provides the tolerance range (±2SD). Table 7.5 provides an example, but for convenience, only 10 observations are shown. The following calculations are used to determine the mean and tolerance range for the data in Table 7.5.

$$\bar{X} = \frac{745}{10} = 74.5$$

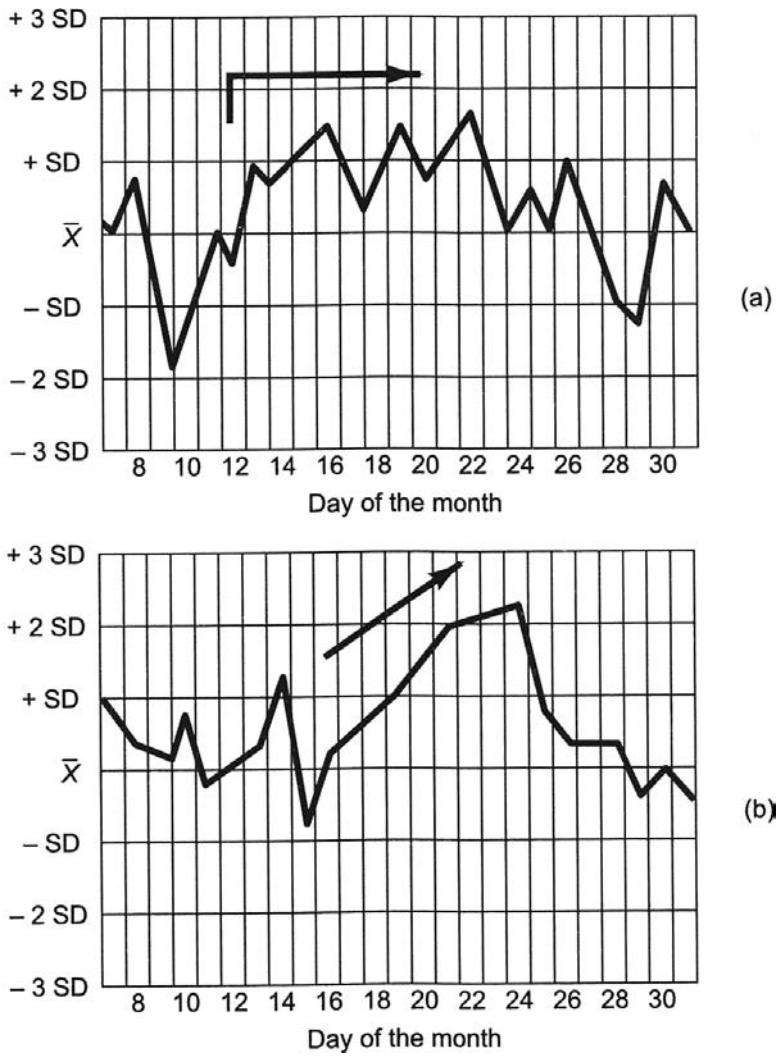

Figure 7.4 *Quality control chart showing shift (a) to one side of the mean, and (b) trend of unidirectional move. Both should be investigated. A shift is usually due to defect in control and trend is due to deterioration of chemical.*

$$\text{SD by the manual method} = \sqrt{\frac{141.50}{9}} = \sqrt{15.70} = 3.9$$

$$\text{SD by calculator method} = \sqrt{\frac{556440 - 555025.00}{10 \times 9}} = 3.9$$

For the above example:

$$\text{CV (\%)} = 100 \times \left(\frac{3.9}{74.5}\right) = 5.23\%$$

This expression of CV, an indicator of precision, should desirably be less than 5%.

TABLE 7.5 *Determination of mean and tolerance range of control serum*

Observation	Serum glucose (mg/dL, X)	Mean ($\overline{X}$)	Difference ($X - \overline{X}$)	Square of the Difference
1	78.1	74.5	3.6	12.96
2	70.0	74.5	4.5	20.25
3	77.0	74.5	2.5	6.25
4	79.0	74.5	4.5	20.25
5	71.2	74.5	3.3	10.89
6	72.3	74.5	2.2	4.84
7	76.0	74.5	1.5	2.25
8	74.5	74.5	0.0	0.00
9	79.0	74.5	4.5	20.25
10	67.9	74.5	6.6	43.56
Sum	**745.0**			**141.50**

Note

- Mean + 2 SD = 74.5 + 7.8 = 2.3; mean – 2 SD = 74.5 – 7.8 = 66.7
- The control specimen can be prepared in the laboratory by pooling normal serum or can be purchased from commercial companies that provide the true value (manufacturer's analysis). The latter helps to establish the accuracy of the procedure followed in the laboratory.

Establishing the tolerance range

The tolerance range accepts the normal variation expected in the analytical procedure. The higher the SD, wider will be the tolerance range and lower will be the precision. For clinical laboratories, that includes 95.4% of the data, ±2SD is calculated for the tolerance, which comes to ±2 × 3.9 = ±7.8. Therefore, the tolerance range for the given data is 66.7 to 82.3, with the mean of 74.5 (mg/100 mL) for the serum glucose value.

Drawing the quality control chart

After the mean and tolerance range are determined, a quality control (QC) chart is prepared on a linear graph paper (Figure 7.2). The analytic values are on the Y-axis and dates on the X-axis. The QC chart should show the mean (solid central line) and the tolerance range (dotted lines on two sides of the mean).

Daily plotting (use of QC chart)

Run the first analysis of the day with the control serum. The control serum used daily is an aliquot of the same serum that was used for preparing the quality control chart. For convenience the control serum is kept in small individual vials in frozen state and one vial is thawed each day for running the 'control'.

Take one of the frozen vials of control serum, bring to room temperature and analyse in the same way as done in Step 1. Plot the result on the quality control (QC) chart (Table 7.6, Figure 7.3). If the result of the control serum falls within the tolerance range, proceed with the analysis of the specimens. An ideal QC chart should show plots uniformly distributed on two sides of the mean. Deviation from this will be interpreted in the following way.

TABLE 7.6 *Daily analysis of serum glucose with control serum (Data plotted on QC chart, Figure 7.3)*

Date	Serum glucose (mg/dL)	Date	Serum glucose (mg/dL)	Date	Serum glucose (mg/dL)
1	75.0	11	74.5	21	73.0
2	73.5	12	76.0	22	74.5
3	75.0	13	69.0	23	75.0
4	69.5	14	73.0	24	73.5
5	75.0	15	77.0	25	70.0
6	72.0	16	73.0	26	64.5
7	75.0	17	74.5	27	68.5
8	77.0	18	73.0	28	72.0
9	85.0	19	75.0	29	72.0
				30	74.5

Interpretation of Quality Control Chart

The following are important principles in interpreting the quality control chart (Figure 7.4). These are the set of considerations to be noted, as well as Westgard's Rule that formalizes the course of action to define if a process is in control (Figure 7.4).

1. Inclination of the curve toward increase or decrease indicates a **'trend',** which may be caused by deterioration of reagent or a similar factor. The technician receives the warning from the quality control chart and corrects the source of variation.
2. A drift of the curve toward one side indicates **'shift',** which may be caused by inappropriate operation of equipment or a similar factor. The technician should investigate and continue specimen analysis only after the shift is corrected.
3. When the daily analysis of the control specimen crosses the tolerance range, an immediate correction is necessary and no specimen should be analysed until the **variable factor** is controlled.

Westgard's rule

A set of guidelines, called Westgard's Rule, gives laboratory staff insight as to whether a quality control program is **in control** or not. It delineates **acceptable variation** in control

before patient test results should be rejected. Two different levels of control sera (normal and abnormal) should be analysed, along with each set of patient samples. **A run** (set of patient samples) is considered out of control if any of the following scenarios happen:

- Both controls are outside the +-2SD limit
- The same control level is outside the ±2SD limit on two subsequent runs
- Controls in four consecutive runs have values greater than ±1s all in same direction
- Ten consecutive control values fall on one side of the mean

Patient test results cannot be reported until the method is considered in control.

Summary

The overall goal of good laboratory practices is to provide reliable results with utmost precision. Programs that assess quality should be part of every good laboratory's daily operations. Providing highest standards with well trained personnel and adherence to good statistical principles ensure reliable test results and lead to optimum patient care.

Review Questions

1. What is the significance of quality control? Explain use of standards and controls in a laboratory's daily operations.
2. What is the significance of coefficient of variance? How can it be used to compare methods of analysis?
3. Prepare a QC chart from the following data of total protein (g/dL) in 10 samples: 6.59, 7.14, 8.00, 6.82, 7.55, 7.00, 7.43, 7.60, 6.91 and 7.44
   Answer SD = +0.429
4. Plot the following daily results on the above chart.

Date (Feb)	1	2	3	4	5	6	7	oo	9
Total protein (g/dL)	7.21	6.73	7.69	7.95	8.26	7.01	5.91	6.56	7.24

5. Interpret the curve of Question 4.
6. What precautionary measures are to be taken in a laboratory in order to maintain the reliability of results?
7. What are the differences between accuracy, precision, and reproducibility?
8. What are the guidelines for Westgard's Rule?