SECTION I

Laboratory Medicine: Purpose and Practice

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CHAPTER 1

Introduction

The clinician uses the laboratory to assist in diagnosis and management of the patient. In effect, a test requisition is a request for consultative services which set in motion a vast array of manoeuvres to generate a laboratory report. Usefulness of the data in making clinical judgments depends upon prompt, accurate reporting of a result. Each procedure to generate a result consists of a series of steps, or processes. An adequate understanding of each process enables the laboratorian to achieve more nearly optional conditions and, consequently, to improve the accuracy and precision of each measurement. Collection, handling, and processing the specimen prior to analysis must receive prime consideration. Validity of data obtained on the specimen itself is highly dependent upon the excellence of laboratory technique, including proper manipulation of equipment, use of reagents of specified purity.

Table 1.1: Indications or reasons for ordering laboratory examinations

1. To confirm a clinical impression or establish a diagnosis
2. To rule out a diagnosis
3. To monitor therapy (management guide)
4. To establish prognosis
5. To screen for or detect disease

CHAPTER 2

Laboratory Management

The head of the laboratory should be conversant with many of the accreditation standards and governmental regulations. One should not only take up the job of managing a clinical laboratory attracted by the monetary benefits but also one should realise the vast responsibility as those laboratory reports have a direct impact on the healthcare of patients.

The clinical laboratory management requires expertise in medical scientific and technical areas. The person should have resources in the form of personnel, equipment, supplies, facilities and skills in organisation, management and communication.

A clinical laboratory should stand on firm goals and objectives. In order to achieve these objectives, the clinical laboratory should have adequate facilities, equipment and supplies and adequate number of personnel.

The objectives of a clinical laboratory are to assist the medical professionals with reliable reports of tests—the reliable test reports of clinical laboratory is a vital component of excellent healthcare delivery system (Fig. 2.1). Such being the case every effort is made at maintenance and improvement of quality of work.

Laboratory management can be well understood on the following heads:

1. **Space and design:** A laboratory should be spacious with good ventilation and lighting system. A well-designed workable avoids confusions.
2. **Equipment and facilities:** Basic instrument and equipment requirements should be made available. As far as possible some of the vital instruments should be in duplicates. Water and power supply should be adequate. For purposes of speedy communication, telephone should be provided. Safety measures like providing fire extinguisher, hoods or smoke chambers, first-aid kit and chart are essential.
3. **Procurement:** One should decide what supplies they need, when they need them and in what quantities. Much time can be wasted unless a systematic procurement system is developed. For this the addresses of various manufacturers must be procured. Equipment, chemicals and other supply market are so competitive that only through product investigations can the best product be selected within the. available funds. This is best done in consultation with several other clinical laboratories.
4. **Storage and maintenance:** Every personnel in a laboratory should be thoroughly patient familiar with storage specifications irrespective of chemicals, reagent or instruments.

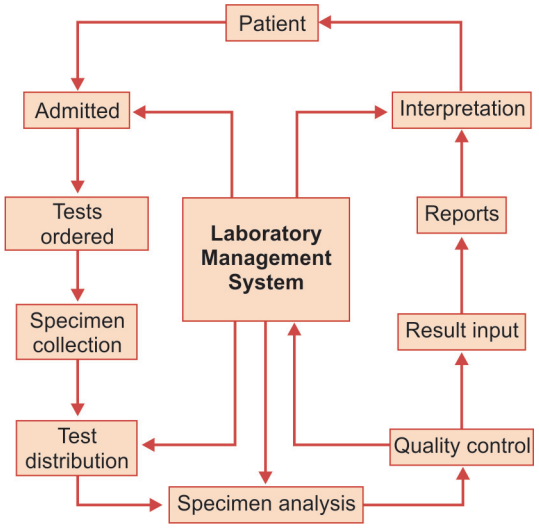

Fig. 2.1: Laboratory management system

A constant check is maintained on expiration dates. Chemicals and reagents after the expiration dates should be disposed of without delay to prevent impending hazards.

Chemicals and reagents. Storage specifications mentioned for the chemicals by the manufacturer and the expiration of dates are strictly followed:

i. Chemical balance should be checked for accuracy and sensitivity periodically. This is done with a sophisticated balance with high sensitivity, if available, or by comparing with a set of standard weights.
ii. pH meter should be checked monthly for correct performance. This is done with standard buffers prepared a fresh. The electrode is washed with organic solvent like alcohol occasionally.
iii. Colorimeters and spectrophotometers are checked periodically for correct performance at different wavelengths using different solutions of known OD. The phototube is replaced when it shows loss of sensitivity which is evidenced by *low* OD for the known standard solutions.
iv. Water-bath and oven temperatures should be checked often.
v. Distilled water should be checked for ions like chloride, sulphate, phosphate, calcium, etc.
vi. Voltage fluctuations in the mains are better overcome with the use of stabilizers.
vii. Perfect cleanliness and discipline should be maintained in the laboratory.

5. **Personnel**
   i. Qualified persons should be recruited.
   ii. A good rapport should be maintained between the person-in-charge and the working staff.
   iii. Basic needs and comforts should be provided.
   iv. The personnel is given the job description.
   v. Duty allotment schedule among the staff should be carefully prepared.
   vi. Opportunities should be provided for betterment of skills and position.

CHAPTER 3

Laboratory Planning

A pathologist having the opportunity to design a new laboratory or make major renovation in older laboratory, should think in functional terms about the laboratory operations and their facility needs.

Traditionally laboratories have been organised without due regard to the functional requirement. The whole concept of a laboratory has changed during the last decade. Unfortunately there has been a lot of proliferation of laboratories which are sub-standard and with no knowledge of quality control. The only way to curb such practices is to start good laboratories and provide facilities at reasonable costs, by qualified pathologists. Today, a planned laboratory can be started with a budget of rupees one lakh.

The accommodation initially, should be reasonably good to start the common tests that are in demand and should be properly planned so that it will be functional and convenient.

A few samples of plan are given below which are scientifically developed. Depending upon the area available, these plans may be applied suitably (Figs 3.1–3.3).

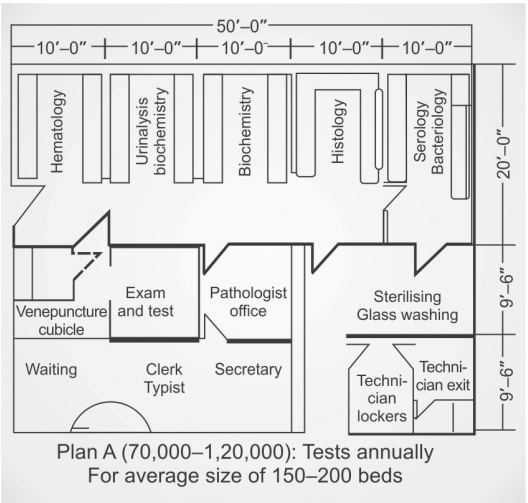

Fig. 3.1: Plan A: 150–200 beds

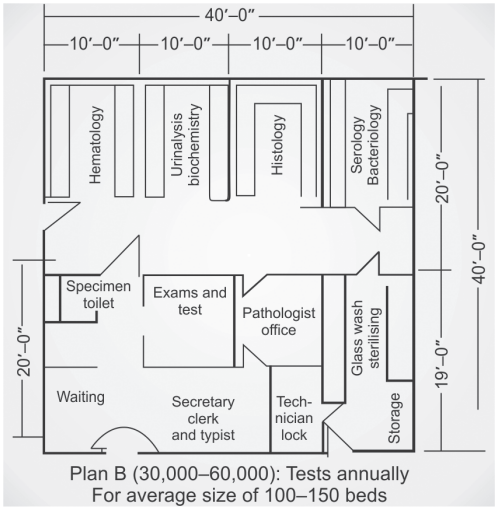

Fig. 3.2: Plan B: 100–150 beds

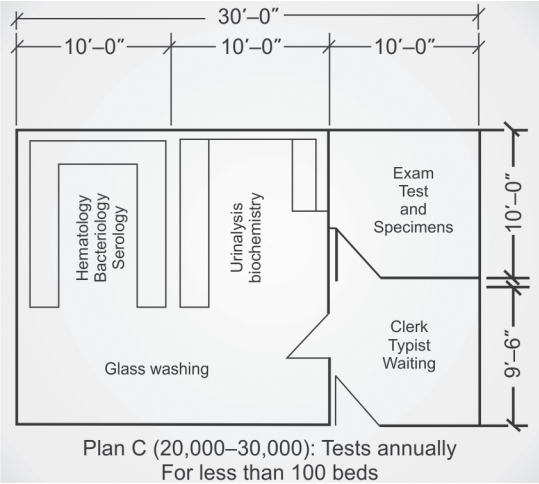

Fig. 3.3: Plan C: 20000–30000 beds

CHAPTER 4

Containers and Swabs for the Collection of Specimens

Specimens for bacteriological investigation should be forwarded as soon as possible to the laboratory in robust, leakproof, sterile containers. It is essential that each container should bear the name of the patient from whom the specimen is submitted and the accompanying form should be accurately completed. Relevant clinical data must indicate the probable clinical diagnosis, information regarding recent or current chemotherapy and, especially if a serological investigation is required, relevant details of previous immunization should be given.

Specimen containers, with adhesive labels, and printed request forms are usually supplied by the laboratory to its clinical users. Reusable glass containers are generally the most economical; after the specimens have been examined, they are sterilized with their contents, washed thoroughly, resterilized and again issued for use. Disposable plastic containers are more expensive, but make less work in the laboratory, they are merely sterilized with their contents; and then discarded or incinerated.

CHAPTER 5

Reusable Glass Containers

Universal containers: The screw-capped, cylindrical bottle known as the universal container is recommended for most types of specimens, including pus, blood, sputum, faeces and urine. It consists of a strong moulded glass bottle, 3¼ in. high × 1⅛ in. diameter, capacity 1 oz (28 ml), with a flat base and wide mouth. These containers are generally supplied with metal screw-caps containing rubber liners but wadless polypropylene caps, which are easier to clean, are also available.

Universal containers are sterilized by autoclaving with the caps loosely screwed on; after sterilization, the caps are tightened. They cannot be sterilized in the hot air oven, as the rubber liners or polypropylene caps will not withstand the higher temperature.

The screw-capped universal container has many advantages over a glass tube with a cork or rubber stopper. lt is stronger, and the screw cap keeps the mouth of the container sterile, whereas dust collects at the rim of a stoppered tube. The contents of a universal container cannot leak or become contaminated and, as it is quite stable on its base, it is particularly convenient when specimens are taken at the bedside.

For the collection of *serous fluids*, e.g. pleural fluid, the universal container is suitable. The addition of 0.3 ml of a 20 per cent solution of sodium citrate to the container prior to autoclaving (with the cap fitted) is recommended for the collection of fluids that may coagulate on standing. This avoids difficulty in performing cell counts or centrifuging procedures with such fluids.

Sputum may be collected in a universal container, but it may be difficult for the patient to expectorate into the container without soiling its outside. For this reason, squat, wide-mouthed disposable containers are preferred.

For *faeces*, the universal container should be supplied with a small metal or wooden spoon. The spoon is placed in the clean container and sterilized with it. The user removes the screw-cap, tips out the handle of the spoon and grasps it with his fingers. He picks up a single spoonful of faeces, drops it, with the spoon, into the container, and replaces the cap tightly. When there is likely to be a delay of several hours under warm conditions before laboratory examination, neutral glycerol saline should be added to the container.

For small quantities of *urine*, e.g. for the diagnosis of most cases of urinary tract infection, the universal container is used. For larger quantities, e.g. complete early morning specimens for the diagnosis of renal tuberculosis, 20 oz (250 ml) wide-mouthed screw-capped bottles are convenient.

Blood for serological examination may be submitted in a universal container. The blood clot remaining after withdrawal of the serum may be cultured by the addition of a selective liquid culture medium, e.g. for enteric organisms. But blood intended primarily for blood culture should be submitted in a special, broth-containing 'blood culture bottle'.

Blood culture bottle: This must be at least large enough to hold 50 ml liquid culture medium, with which it is issued from the laboratory, plus 5 to 10 ml of the patient's blood. A 4 oz (120 ml) 'medical' flat bottle with a special screw-cap is used. The metal cap has a central hole about 5 mm diameter, which is covered on the inside by an intact rubber liner. When the cap has been screwed on tightly after sterilization of the bottle containing the medium, a circular plastic disk of the same diameter as the cap is placed on top of it. A metal 'capeeler' is placed over the disk and down opposite sides of the neck of the bottle, and a moist cellulose collar is placed round the neck of the bottle, over the capeeler, and allowed to dry and shrink. For receipt of the blood, the capeeler is pulled to cut off the cellulose collar, and the plastic disk is removed. The blood is injected by insertion of the syringe needle through the hole in the metal cap and through the rubber liner beneath it. The metal cap *must not be removed* for introduction of the blood, and clinical users should be instructed accordingly.

CHAPTER 6

Disposable Specimen Containers

These are generally made of plastic (polystyrene or polypropylene), but some may be made of aluminium, glass or waxed cardboard. Waxed cardboard cartons must not be sent through the post.

Plastic containers of similar size and dimensions to the universal container are supplied, already sterilized, by a number of manufacturers. Particular types may have, e.g. a plastic spoon attached to the inside of the cap, for collection of faeces, added anticoagulant, for collection of serous fluid, or added boric acid, for bacteriostasis of urine specimens. Shallow, wide-mouthed 2 oz (60 ml) plastic containers with screw-caps are particularly convenient for the collection of sputum.

It should be noted that not all of these containers have been authorized for sending by post, as some, particularly those with push-on caps instead of screw-caps, have been known to leak during transmission. A separate standard covering specimen containers for microbiology is being prepared. Attention is drawn to the work of cash on a special type of double plastic envelope suitable for the transport of 'high risk' specimens such as blood for examination for hepatitis virus B.

All used disposable containers should be sterilized before discard, or be incinerated by responsible staff.

Swabs

Swabs suitable for taking specimens of exudate from the throat, nostril, ear, skin, wounds and other accessible lesions may be made in the laboratory. A 6 in. length of aluminium or tinned iron wire, 15 gauge, may be used as the handle, or 'swab-stick'. One end is made rough for about ½ in. by squeezing it in a small metal vice or the cutting edge of pliers. Around this end a thin pledget of absorbent cotton-wool is tightly wrapped for about ¾ in. The wire is placed in a narrow thick-walled test-tube, 5 in. × ½ in., and the top of the tube is plugged with cotton wool. Alternatively, and where swabs have to be sent by post, the wire should be 4½ in. long and the top inserted into a bark cork which stoppers the tube. The tube with swab should be sterilized in the autoclave and not in the hot-air oven, as in the latter the wool may char and give rise to tar-like products which may be inimical to bacteria on the swab. It is important to autoclave cork-stoppered tubes with the cork loose and to press in the cork after sterilization. Tubes plugged with cotton wool should be dried after autoclaving.

Instead of wire, swabs may be prepared from thin wooden sticks 6½ in. long that are specially made for the purpose, and are known as 'Peerless' wooden applicators. A cotton wool

pledget is wrapped round one end as above, and the tube is plugged with cotton wool. They cannot be used conveniently with a bark cork, but have the advantage that the stick can be broken off short when the swab has to be placed in transport medium in a screw-capped container.

Disposable Swabs

A variety of tapes of swab, e.g. plain, serum coated, charcoal coated, are available. These come in cardboard or clear plastic tubes, or plastic envelopes already sterilized. The swab in its tube or envelope is destroyed in a furnace after use.

The swabs may also be purchased loose and unsterile, for assembly and sterilization in tubes. This type of swab is also useful in the laboratory for other purposes, e.g. seeding of media for disk sensitivity tests.

Swabs for Special Purposes

Swabs differing from those described above are required to take specimens from less accessible areas:

1. **'Baby' swabs:** When taking specimens from babies and young children, it is often necessary to employ a very fine swab so that small orifices, such as the aural meatus, may be negotiated without gross contamination from the external surfaces. These are made in the same way as the swabs described above but with fine rigid wire. To avoid damage to the tissues, the end of the wire should be fused in a Bunsen flame before fireing a tiny pledget of cotton wool to it.
2. **Pernasal swabs:** These are used for the diagnosis of whooping-cough. The swab is passed along the floor of the nasal cavity to reach and sample the secretions in the nasopharynx. A small swab and a flexible swab-stick are required to minimize the risk of damage to the nasal tissues. The swab-stick may be made from 7 in. of flexible copper wire or nichrome SWG 25 (0.51 mm diameter), the terminal ¼ in. being bent back to take the pledget of cotton wool, a very thin layer of which is wound firmly round it. Readymade pernasal swabs with a finger loop may be purchased. The swab is contained in a 6 × ½ in. test-tube plugged with cotton wool.

   Disposable pernasal swabs in plastic tubes, which may or may not contain a transport medium, are commercially available.
3. **Post-nasal swabs:** These are used to sample nasopharyngeal secretion for the diagnosis of meningococcal carriage. The terminal ¾ in. of a long stiff metal swab-stick is bent through an angle of 45 degrees, so that when introduced through the mouth, it carries the swab up behind the soft palate into the nasopharynx. The swab is contained in a stoppered test-tube of sufficient width to admit the bent end.
4. **Laryngeal swabs:** These swabs are used to obtain a sample of bronchial secretion for the diagnosis of tuberculosis in patients who cannot expectorate sputum. Their construction resembles that of post-nasal swabs, but the bent, swab-bearing end should be longer, about 2 in., and be more sharply bent, through an angle of 60 degrees to its original direction. A very wide, stoppered tube is required to contain it. The swab is moistened with sterile water just before use, passed over the dorsum of the tongue and introduced into the larynx, where it stimulates coughing and collects the expelled secretion.
5. **High vaginal and cervical swabs:** For the diagnosis of gonorrhoea and puerperal fever, a swab should be taken from the uterine cervix and its lumen, rather than from the general area of the upper vagina. A swab on a specially long, rigid swab-stick, preferably about 9 in. long, is required.

CHAPTER 7

Transport Medium

When some time may elapse before the swab is examined, and especially where delicate pathogens, e.g. meningococcus or *Bord. pertussis*, may be present, it is advantageous to place the swab in a *transport medium*, such as Stuart's medium, which preserves the viability of the pathogens. The medium is non-nutrient, because the less delicate commensal bacteria present in the specimen would outgrow the pathogens on a nutrient medium.

The transport medium, which is solidified with agar, may be used in a screw-capped universal container or smaller bottle, or a hermetically stoppered test-tube. Immediately the specimen has been taken, the swab is plunged into the depths of the transport medium, the swab-stick is broken off and the cap or stopper is firmly applied. The container is then sent to the laboratory, and even delicate pathogens may remain alive for a day or two at ambient temperature.

For convenience, this method requires the use of swabs on wooden sticks that can be broken easily. If thin wire handles are used, they may be cut off or be pressed down into the container. If stiff wires are used, bark corks, through which the wires pass, are used as stoppers.

Charcoal impregnated swabs give the best survival of pathogens in transport medium.

The problem of bacterial survival is not confined to the neisseriae, since slow drying is known to be lethal to most bacterial species. As a result of comparative findings with many different pathogens, the use of a serum-coated cotton wool swab for long viability is recommended. The swabs are prepared using the cotton wool on a wooden spatula with undiluted or serum for 10–30 seconds spreading on sheets of blotting paper, drying in an incubator at 37°C for half an hour and finally sterilizing in an autoclave at 121°C for 20 minutes. The finished product is a compact honey-coloured pledget, 3–5 mm in diameter, in which the cotton wool fibres are firmly bound to each other and to the applicator. Clinicians may need to be warned about the unusual appearance of these swabs so that they will not think they have already been used.

CHAPTER 8

Choice of Diagnostics

As regards the choice of diagnosis, it depends upon the size of the laboratory, in terms of samples and the instruments in use. If the number of samples is small and the instrument is a colorimeter or spectrophotometer, then the kits that are to be selected should be of conventional methods. Otherwise, it will not work out economical as the reagent required is around 2.5 to 4 ml per test. Hence the choice of kit or the instrument should go with the demand. Any newly started laboratory should go with less sophisticated instruments, gradually adding better instruments as the demand increases.

So far as the choice of instruments is concerned, the section of Clinical Biochemistry is important, as other sections can be managed without much of a sophistication. The colorimeter and spectrophotometers are indigenously manufactured and are quite satisfactory. The other more sophisticated instruments are either imported and assembled or wholly imported. There are many firms in the market dealing with these instruments. Some instruments are more popular than others by virtue of their being in use in large numbers. Ultimately the choice of the instrument should weigh more on the manufacturer's capability in the after sales service. After sales service should be the most important criteria for selection of any instrument, for there should be minimum of breakdowns and the turn around time to attend to the instrument should be the shortest. The culture amongst majority of suppliers is not one of after sales service but of sales and no service. This is one of the most important problems faced by every buyer of medical equipment which should be discussed at the National Forum and a solution found to discipline the suppliers.

When you have properly planned the accommodation and the necessary equipment have been bought, it should not be difficult to start a laboratory, as the necessary kits are available for all methods of estimation. Before proceeding with kits available in the market, we should know something about the various methods of bio-chemical estimation. They may be enumerated as follows:

1. Old colorimetric methods—such as Folin-Wu, King, Orthotoludine for sugar.
2. New end point colorimetric methods mostly enzymatic as for sugar, cholesterol, triglycerides, etc.
3. Fixed time chemistry, as for blood urea nitrogen and creatinine.
4. Kinetic—as for enzymes.

Kits

A set of readymade reagents and method of estimation form a kit. Commercialisation of

technical skill resulted in the release of kits into the market. Some kits are well designed and reliable while others are not and should be avoided. "The purchase of a kit means that the laboratory buys not only whatever the expertise the manufacturer may possess in regard to the test, but also whatever errors, inaccuracies and general lack of quality that may exist in the kit."

A better kit should provide:

1. Detailed directions.
2. The principle involved in the estimation.
3. A description of calculations.
4. Calibration curve.
5. National value obtained with the kit.
6. Details of quality control.
7. Expiration dates and storage conditions of reagents.
8. A performance evaluation from a reputed laboratory.

As far as possible it is suggested to prepare the reagents in the laboratory not only for reasons of low costs but also maintain good quality of work.

In cases of tests requested infrequently or difficult to set up and maintain, tend to be more costly and inconvenient, they are the ones in which kits are used.

Finally, it is for the individual laboratory to select the kits and the instruments. It is for them to think over and select the best suited, keeping in mind the quality of service to be rendered to the patients. With the existing prices of various investigations there is always a margin of profit but that margin diminishes when a better kit is chosen, because of the increase in cost.

A quick personal attention to the patient when he comes to the laboratory and reliable report to the physician is all that is required to establish oneself, though it may take some time. The patient should properly be advised when they directly come for certain tests and not to be taken advantage of. This is one aspect. The other aspect is that the attending physician always expects a prompt and accurate report.

CHAPTER 9

Documentation of Specimens in the Laboratory

Different methods may be used to document the receipt ('booking-in') of specimens at the laboratory, record the results of each stage of the laboratory examination, formulate and issue reports, and keep records of the reports issued. The methods adopted will be influenced by the way in which the different tasks are distributed among the different categories of laboratory staff as well as by the need for economy in the use of expensive labour and the requirements of freedom from mistakes and speed in reporting results to the clinician.

Booking-in

Great care must be taken to identify specimens and Request forms on arrival at the laboratory so that when their future separation occurs, no mistake will arise. This is best carried out by having a responsible person label all specimens and corresponding forms with a duplicate serial number. For this purpose serial numbers printed on gummed paper, in duplicate, triplicate, etc., may be purchased.

It is preferable to record the patient's name and address, the type of specimen and its time of arrival in a day book and to affix a third label with the specimen number beside the entry. Reference to the day book is useful in revealing any delays in reporting on specimens received and in settling any questions raised as to whether a specimen has been delivered to the laboratory.

Specimen Numbers

Different colours of labels and different prefixes to the numbers may be employed to distinguish different types of specimens requiring different methods of examination, e.g. T for tuberculosis, U for urine examination. These serial numbers can be used for the chronological clerking of specimens. They should be cited on the reports of results issued to the clinicians, so that they may later be used in any reference that needs to be made to the laboratory records. They also provide an easily obtained measure of the laboratory's work-load, for the difference between the numbers used on the first and last days of any period, e.g. one year, indicates the number of specimens examined in that period.

Documentation on the Bench

In some laboratories the results of each stage of the examination are noted, as they are obtained, on the Request form, or on a copy of it. In others, the Request form is held centrally and the results are noted on a work sheet marked with the specimen number. All slides, culture plates and tubes should also be

marked with the specimen number slides with a diamond, and tubes and plates with a glass-writing pen. It is important that this marking should be done clearly, for one of the likeliest causes of error in clinical bacteriology is the inoculation of material into the wrong tube or plate.

Reports

In laboratories with sufficient staff, a senior bacteriologist may scrutinise the results of the various tests made on each specimen and write the report to be issued in a blank space on the request form. A typist can then type this report on a fresh Report form, copying the patient's name and other data from the Request form. Usually a more economical method has to be used.

Many laboratories issue duplicate Request forms with a space reserved for the laboratory report. Results obtained at the bench are recorded on one copy, and the final report is also written on it. The report is then typed, stamped or written fairly on the other copy, which is sent to the clinician. In this way the need to copy the patient data is avoided. About three quarters of all reports are 'negative' and these can readily be issued by standard stamps applied to the Request forms or by 'photo-copying' pre-printed reports beside the patient data areas of the Request forms.

Copies of all reports, e.g. the Request forms on which the laboratory findings have been noted, should be filed chronologically by type of specimen, and be preserved in the laboratory for one year.

CHAPTER 10

Ethics in Laboratory Practice

The main objective of any laboratory is to be a vital component in providing patient care environment of excellence. The ultimate goal of any laboratory, big or small, is to attend to the ailing patients when they come, carry out the investigations asked for by the doctor and prompt issue of accurate results to the doctor for favour of diagnosis and treatment. The only concern on the part of the laboratory and the attending physician should be care of the patient without any dubious understanding between themselves, as such an understanding will defeat the very purpose.

In any laboratory there are always some omissions and commissions. To err is human. One should be courageous enough to accept and rectify the mistake even if it were to be at the cost of one's own respect. It should never be manipulated. We have a great role in this as we are the ones to set an example for others in the laboratory to maintain certain code of conduct.

CHAPTER 11

Disinfection, Washing and Sterilisation

Definition

Sterilisation implies complete destruction of living microorganisms including spores. Disinfection means destruction of vegetative forms of organisms which might cause disease or spoilage of food, etc. It does not necessarily kill spores. The two terms are not synonymous.

Disinfection of used Laboratory Articles

Disinfection of both reusable and disposable glassware and articles contaminated with morbid or culture material is of utmost importance in the laboratory. All the specimens received in the laboratory should be considered as potentially pathogenic. The ideal method of treating such materials is to incinerate all the disposables and decontaminate the reusable articles by autoclaving. These facilities may not be available in every laboratory. For purpose of disinfection, disposal and recycling, all the articles may be divided into three categories:

i. Disposables
ii. Reusable articles contaminated with morbid material such as pipettes, slides, test tubes, etc.
iii. Material containing of contaminated with bacterial cultures.

Disposables

Soak the material overnight in a strong solution of disinfectant before disposing along with garbage. 10% solution of formalin or 3% Iysol may be used as disinfectant.

Reusable Articles Contaminated with Morbid Material

Discard the articles into a jar containing disinfectant solution. Let them remain in this solution overnight. Drain off the disinfectant. Transfer the material to a metal pot or tray with cover. Pour water and boil for 15 minutes. Cool and drain off the water. Pass on the articles for washing.

Glassware Containing Culture Material

Discard all the articles containing or contaminated with culture material directly in a metal box or a bucket. Place the box with material in the autoclave and decontaminate by autoclaving. Drain off culture medium and pass for washing.

Disinfection of Rooms

Seal all the windows, ventilators and fire places with brown paper and adhesive tape.

Pour 500 ml of formalin and 1000 ml of water in a pan or tray and boil with the help

of a spirit lamp. Spirit in the lamp should be just sufficient to boil off the formalin and the lamp extinguishes when there is a small quantity of liquid in the pan. Seal the door.

Open the door, next morning and spread a piece of lint soaked in ammonia on the table. This will neutralise excess of formalin present in the room.

WASHING OF LABORATORY GLASSWARES

New Glassware

Usually new glassware are slightly alkaline. Before washing these have to be neutralized. The method is as follows:

i. Prepare a 2% solution of hydrochloric acid in a big basin.
ii. Soak the new glassware in this solution for one day.
iii. Rinse twice with clean water and once with demineralized water and drain.

Dirty Glassware

i. Rinse twice in lukewarm or cold water, otherwise serum or blood may stick to them and may not be washed.
ii. Put the glassware in a bowl containing detergent solution and scrub the inside with a brush. After scrubbing soak the glassware in this solution for 2–3 hours.
iii. One by one, take out the articles and rinse under running tap water, then put all the glassware in a container containing tap water (no trace of detergent should be left, otherwise this may lead to false results).
iv. Drain the water by putting each article on a wall draining rack.
v. Place the articles in a wire basket and dry in a hot air oven at 60°C.
vi. Plug each article with non-absorbent cotton wool or aluminium foil and store in a cupboard to avoid dust.

Pipettes

i. Immediately rinse in running tap water to remove blood, urine, and serum reagent, etc.
ii. If the pipettes were used for infected materials, soak them in cylinder full of disinfectant solution (2% dettol or 2% phenol) for 24 hours, otherwise place in a large measuring cylinder full of water.
iii. Soak in detergent and rinse as in dirty glassware (ii).
iv. In case the pipettes are blocked, put them in dichromat solution for 24 hours. Next day clean under running tap water, check individually, rinse for a number of times.

Syringes and Needles

i. Immediately after use remove the plunger and rinse the barrel and plunger, syringe water through the needles forcefully. Finally remove the needle.
ii. If the piston is blocked, either soak the syringe for 2 hours in hot water or pipette with the syringe standing on its end, piston down. Alternatively, soak the syringe in a container of 1C vol. hydrogen peroxide.
iii. In case of blocked needles, use the stylet to remove the block.

Methods of Sterilisation

The common methods of sterilisation used in a microbiology laboratory can be broadly divided into three categories depending upon the materials to be sterilised:

i. Dry heat
ii. Moist heat
iii. Filtration

Dry Heat

The two commonly used methods of sterilisation by dry heat are:

a. Red heat or flaming
b. Hot air sterilisation

Red Heat or Flaming

Instruments such as inoculating loops and searing irons are sterilised by this techniques. For sterilisation of inoculating loop, hold the loop vertically on the blue cone of the flame for few seconds and slowly raise upwards till whole of the wire is red hot. Move the loopholder rapidly downwards through the flame so that several inches of the loopholder is also heated slightly.

Hot Air Sterilisation

Items to be sterilised: This is the best method for sterilisation of dry glasswares such as test tubes, flasks, pipettes, Petri dishes, assembled all glass syringes, throat swabs and other sealed materials which can withstand high temperature and where penetration by steam is not possible.

Equipment: Sterilisation by hot air can be conveniently carried out in an electrically heated oven. A thermostat is fitted to control the temperature. Larger units should be fitted with an air circulating fan to ensure uniform temperature in the different parts of the oven.

Moist Heat

Moist heat or steam sterilisation is one of the most efficient methods of sterilisation. Depending upon the material to be sterilised, moist heat can be applied:

i. Below 100°C: lnspissation
ii. At 100°C: Tyndallisation
iii. Above 100°C: Autoclaving

Inspissation

Media containing serum or egg are coagulated and sterilised by inspissation at 78°C.

i. Place the media contained in bottles, test-tubes or Petri dishes on the tray in the inspissator and hold at 78°C for one hour.
ii. Repeat this on three successive days. lnspissation may also be carried out at 82°C to 85°C for 40 minutes on one day for only coagulating the media.

Tyndallisation

This method is used for sterilisation of culture media containing easily hydrolysable carbohydrates or gelatin which might be spoiled by exposure to temperatures higher than 100°C. The sterilisation is done on three successive days. On the first occasion, vegetative bacteria are killed and spores present will germinate in the nutrient medium overnight, producing vegetative forms which are killed by subsequent steaming. Tyndallisation is carried out in a Koch's steamer. It is a metal box where steam is generated. The lid is conical in shape with a hole in the centre:

i. Place the material to be sterilised on a perforated rack just above the water level.
ii. Carry out steaming for 30 to 45 minutes on each of three successive days.

In the absence of a Koch's steamer, tyndallisation can be effectively carried out in an autoclave with steam outlet valve open.

Autoclaving

Principle: Water boils when its vapour pressure equals the pressure of surrounding atmosphere. The temperature at sea level is 100°C. When water is boiled within a closed vessel at increased pressure, the boiling point of water is increased and so is the temperature of steam produced. This principle is employed in sterilising material by steam at temperature higher than 100°C and the process is called autoclaving.

For autoclaving in the laboratory, the most agreeable and commonly used method is to use steam at 124°C for 15 to 30 minutes depending upon the particular material to be sterilised.

Items to be sterilised: Autoclaving is most suitable for culture media, aqueous solutions,

decontamination of discarded cultures and specimens, rubber items such as gloves, stoppers, metal stoppers with rubber liner, glassware with attached rubber tubings such as transfusion sets, glass metal syringes, etc. Autoclaves designed for laboratory work and capable of handling mixed loads should be used.

Filtration

Certain liquids containing serum, antibiotic solutions, toxins and toxoids cannot withstand heating and have to be sterilised by removing bacteria physically by filtration. For sterilising by filtration special filters are employed. Various types of filters available for this purpose are:

i. Asbestos filters (Seitz filters)
ii. Sintered glass filters
iii. Membrane filters

Asbestos Filters or Seitz Filters

These commonly used filters are economical and are available in different sizes and different porosity suitable for various purposes such as clarification and sterilisation. EK or EKS grades are suitable for sterilisation of liquid media in the laboratory. The filter is in the form of disk. One filter disk is suitable for use once only and should be discarded after use. These filters are suitable when large volumes are to be sterilised. They adsorb proteins from the material being filtered.

Sintered Glass Filters

These are made of finely ground glass fused sufficiently to make the small particles adhere and are available in various porosity grades numbered 1 to 5. Grade 5 is suitable for sterilising purposes. Since, these filters are made of glass, extreme temperatures should be avoided. After use these are washed by running water in the reverse direction. These may be occasionally cleaned with warm sulphuric acid to which small quantities of potassium nitrate are added and not with chromic acid. These filters have advantage over Seitz filters as they do not adsorb but are very slow.

Membrane Filters

These are made from cellulose acetate and are available in various sizes and porosities suitable for different applications. The filters suitable for sterilising filtration have pore size of less than 0.75 micron in diameter. The filters can be sterilised by autoclaving. For use the membrane is mounted on a supporting platform usually made of steel, glass or plastic, which is sealed together between upper and lower funnels. Filtration is achieved by applying either positive pressure to the entrance side or by applying vacuum to the exit side.

Filters for very small amounts are attached to syringe and the liquid to be sterilised is forced through.

These filters are most ideal for laboratory use as they do not adsorb and have a rapid flow rate.

QUALITY CONTROL

Introduction

Quality control is the process to ensure a test from being done wrongly. The primary aim of QC is to see that the very purpose for which a test is performed is not defeated due to unreliability of the result. The broad aim of QC is to fulfil the aim that results from one lab should be comparable to that from any lab provided the same method is followed.

Test results of laboratory are used for diagnosis and treatment of disease. Erroneous results may mislead in diagnosis resulting in unnecessary medical treatment and prolonged hospitalisation. Unnecessary hospitalisation puts extra-financial burden on the patient and brings a bad name to the medical man, the institution as well. Therefore, the quality of

such data must be controlled. The laboratory result must be fully reliable and to attain this every effort must be made for constant checking and search for error.

The International Federation of Clinical Chemistry's Expert Panel defines quality control as "The study of those errors which are the responsibility of the laboratory and the procedures used to recognise and minimise them. This study includes all errors arising within the laboratory between the receipt of the specimen and the despatch of the report; on some occasions, the responsibility of the laboratory may extend to the collection of the specimen from the patient, and the provision of a suitable container."

A high degree of quality control is achieved through cleanliness of glasswares, carefully designed requisition slips and report forms, identification and labelling of samples and recording the data and of course, high degree of precision and accuracy.

A careful and continuous check is necessary at:

1. Cleanliness of glasswares
2. Quality of glasswares—a grade pipettes
3. Instrument—maintenance
4. Chemicals—use of AR or GR grade
5. Method of determination
6. Clinical and other errors
7. Quality of work—precision and accuracy.

The quality of work done in a laboratory is assessed or evaluated by having a close look at the precision and accuracy, when a single sample is estimated several times and when these values are close together, it refers to good precision. Secondly, when these values are close together to the standard value of the same sample, it refers to good accuracy.

BRANCHES OF LAB MEDICINE COVERED BY QC

Some branches are more suited to QC because of mainly objective interpretation and numerical expression of results like chemical pathology. Others like histopathology with predominently subjective interpretation and non-numerical result expression are rather difficult to handle. In between the two extremes are branches in which some of the tests are quantitative and others not like in haematology, microbiology, serology and some of diagnostic immunology. QC of blood banking is an entirely different approach since no 'tests' are done for the purpose of reports, a consumable product is issued instead.

Quality Control Material

Usually pooled normal serum forms the material for quality control. Pooled lyophilised serum can be got from standard companies. It can also be prepared in the laboratory as follows:

1. Pool the left over serum after the analysis is complete. Collect 1–2 litres. Exclude hymolysed jaundice and lipemic (turbid) serum.
2. Filter the pooled serum through glass-wool taken in a funnel.
3. Mix the serum thoroughly.
4. Adjust the pH to 7.4. This is done by adding concentrated sulfuric acid carefully with continuous, vigorous mixing and checking the pH with a pH meter.
5. Distribute 10 ml portions of this into several plastic vials preferably, otherwise glass vials. Store in deep freezer. This lasts for three months.
6. Each day take out one vial for use.

Method

l. Remove one control serum vial from the deep freezer. Thaw it and allow to come to room temperature.
2. Perform the test treating it the same way as sample serum.
3. Determine the value.
4. Plot the value on the quality control chart.

Conclusion

1. The value on a particular day falls within ±2 SD. Standard deviation indicates all the reagents and standards are okay.
2. In case the value is above or below ±2 SD, same reagent or standard is deteriorated. Check, identify and replace the reagent or standard with a fresh one and again determine the value which falls within ±2 SD.
3. Sometime the control serum itself might be deteriorated. Replace with a fresh pool of serum.
4. Quality control chart indicates deterioration of reagent or standard or the quality or work.

Quality Control Chart

Levey-Jennings chart is almost universally followed in all the laboratories.

Levey-Jennings chart is prepared for each determination in a laboratory.

Before preparing the chart, a knowledge of standard deviation is a necessity.

Standard Deviation (SD)

Standard deviation indicates the average error or average deviation. A small deviation is tolerable, whereas a wide deviation shows loss of precision or error in reagent which has to be rectified.

It is calculated as follows:

1. The arithmetic mean ($\bar{x}$) of the value is calculated.
2. Find the difference between the individual value (x) and the mean ($\bar{x}$), i.e. ($\bar{x}-x$). If x is greater than $\bar{x}$, find the difference and drop the sign.
3. Square each of these deviations, i.e. $(\bar{x}-x)^2$.
4. Add these squares of deviations, i.e. $\Sigma(\bar{x}-x)^2$.
5. Divide the $\Sigma(\bar{x}-x)^2$ by the number of values minus one, i.e. if there are n number of values, then it is

$$\frac{\Sigma(\bar{x}-x)^2}{n-1}$$

6. Then the square root of this is SD, i.e.

$$\sqrt{\frac{\Sigma(\bar{x}-x)^2}{n-1}} = SD$$

The tolerance limit is ±2 SD. The chart is prepared on a graph sheet. The graph is drawn by plotting control serum values on y-axis and the days on x-axis.

The following is the composite quality control chart. The mean value for this is 20 and control limits are ± 2 SD, i.e. 16 to 24 (Fig. 11.1).

On 8th day and on 14th day the value is out of tolerance limits and hence problem exist. A search is made of reagent or standard and rectified.

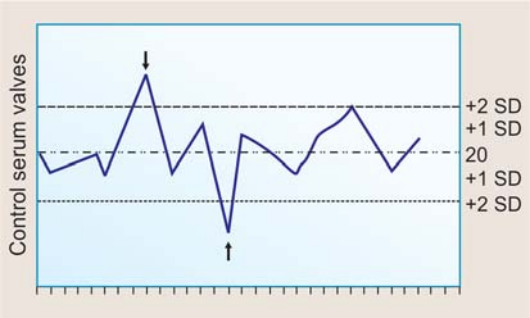

Fig. 11.1: Quality control chart

Technique of QC

QC is implemented under two broad heads:

1. *Internal QC (Intra-laboratory):* Performed by individual labs at their own levels. Forms the basis of day-to-day work quality assurance.
2. *External (Inter-laboratory):* Performed by many labs at the same time, monitored by one. Periodic monitoring of the lab's performance.

Internal QC

It is implemented in two phases, viz. prospective (preventive) and retrospective (corrective).

1. **Prospective phase QC:** All precautions that go into the preoperational period of any lab's working are covered in this phase. These involve planning of lab environment including layout electric installations, plumbing, waste disposal computer terminal and scope for future expansion. The equipment requires careful weighing of all aspects like cost, requirements, reputation, spares, after sales service, active life and job requirements against resources. The laboratory staff has to be selected carefully and a positive management staff relationship always pays. The workload has to be optimum, neither too much nor too little. The methods for analysis have to be selected after considering all aspects. All laboratory procedures should be documented, lab equipment has to be preventively maintained, reagents and kits and standards have to be assured against deterioration. Desired QC material should be procured in advance.
2. **Retrospective phase QC:** The approach here differs in case of quantitative procedures and others.

Retrospective QC for Quantitative Procedures

1. **Optimum conditions variance (OCV):** This is the lowest variance a lab can get on a known value QC material by a defined method. For this under the best available conditions for the lab, a QC sample is analysed consecutively 20 times and results plotted. Mean and SD are also calculated. A satisfactory performance is denoted by not more than 1/20 results falling outside 2 SD and an almost equal scatter on both sides of the mean.
2. **Routine conditions variance known values (RCVK):** For this the same QC material as used for OCV is analysed 20 times under 'routine' conditions, with no preferential treatment. The results are plotted like in OCV and mean and SD calculated. A satisfactory performance is reflected by a SD about twice of OCV with the rest remaining the same.
3. **Modified Levey (Jenning's graph):** QC material of values not known to analyst is introduced within the daily batches of analyses and results plotted against recommended values (mean and range). It is a very helpful visual guide for day-to-day monitoring of performance.
4. **Routine conditions variance (unknown values) (RCVD):** QC material of different levels of analytes is used. The values are unknown to the analyst. The rest is same as in RCV-K. Results are plotted as % variance (V):

$$\frac{\text{Observed value} - \text{Correct value}}{\text{Correct value}} \times 100$$

and are analysed both visually and statistically.
5. **Composite and long-term records:** Each day's performance on RCV-U can be plotted and/or fed into computer for review and future reference. Thus a monthly or yearly record becomes available. 2 SD is taken as 'Warning Limits' and 3 SD as action limits for purposes of QC.
6. **Retrospective QC using patients' material:** Under this the following methods are used:
   a. Randomised duplicate analysis using patient's specimen measures precision.
   b. Daily mean of all the cases for a particular test would be an index of accuracy.

   Cumulative sum (CUSUM). It is calculated from daily mean values and is an even more sensitive index.
7. **Computer aided checks:** When large amount of data in quantitative tests QC becomes available, it can be put to better use through computer programmes like Delta check, absurd value check and pattern identification based upon various established norms.

QC under Special Circumstances

1. **Automated analysers:** Apart from strict preventive maintenance and calibration, the QC of the kits, prevention of errors due to evaporation of switched samples have to be ensured. Daily QC sample plot may show 'Dispersion' (loss of precision) or a 'Trend' (loss of accuracy). Also, the automated analysers are most suited to computer aided checks.
2. **QC and RIA and ELISA:** Most of these techniques have now become quite automated and therefore the relevant QC principles apply. But some points have to be specifically kept in mind like short shelf like kit performance, strict bench discipline, running of test samples in duplicate and preparing a fresh standard curve with every run. Some other specific QC recommendations for RIA have also been recorded.

QC in Haematology

Quite a few tests in this branch also involve quantitative measurements and their QC is in no way different from that of chemical pathology described above. There are, however, some special aspects that deserve mention:

1. Difficulty regarding QC material and standards.
2. QC material for RBC enzymes and methemoglobin.
3. Recently, whole blood controls stabilised by special methods have become available including adenine and hydrocortisone. Also mixture of RBC with latex particles and a combination of preservatives at low temperature storage have helped prolonged the stability and, thereby utility of the QC material. With the help of these, total RBC and WBC counts, PCV, MCV, MCH, MCHC and platelet count can be reliably done and monitored through QC.
4. Due to the particulate nature and tendency to settle, whole blood suffers from improper mixing faults and extremely unreliable results.

Technique of QC

1. Scrutiny of individual reports: Values falling beyond 3 SD should be scrutinised with special reference to correlation with other related parameters.
2. Paired data evaluation: Results on the samples from the same patient analysed on different days on comparison should fall within a prescribed range of variance that is as low 2% for MCHC or as high as 50% for TLC.
3. Delta check
4. Setting up of normal and abnormal controls with each run: QC for coagulation and bleeding disorders: Indicated for diagnosis of bleeding and coagulation diseases as well as monitoring for anticoagulant therapy, the tests under this to be monitored most commonly are:
   1. Prothrombin time
   2. Activated partial thromboplastin time
   3. Assays of individual coagulation factors
   4. Fibrinogen degradation products (FDP) assay

QC Material

A normal person's blood collected at the same time and run with the test serves well. Also, lyophilised sera with normal or abnormal values for different constituents (sp. coagulation factors) have become available and have made QC more reliable.

Interpretation

For prothrombin time, a satisfactory performance is of % CV up to 15. In others, a mild, moderate or severe deficiency is reported compared to normal.

QC in Blood Banking

Here QC is quite different from other branches because no test results are issued.

Instead, a product for human consumption is produced in which no chance with safety can be taken. The keyword is faith and QC in blood banking revolves around the following: Technical staff, techniques, antisera, reagents, equipment and monitoring of blood constituents and sterility.

QC in Diagnostic Immunology

Diagnostic immunologic procedures involve perhaps the most variable collection of techniques under one banner. These include those based on clinical chemistry, immunochemical, antigen–antibody reactions of all kinds based upon complement fixation, chromatography, electrophoresis, immunodiffusion, immuno-fluorescence, immunohistochemistry, RIA and ELISA to name those well established. This is compounded by low amounts of substances to be measured on one hand and high cost and instability of antisera on the other.

The fundamentals of QC in all situations, however, remain the same. Stringent preventive maintenance of equipment, calibration of diluters and dispensors, monitoring of temperature, timing and RPM are but a few of the examples. The close check on antisera regarding their stability and reproducibility of results is even more important in view of cost and other factors enumerated above. Again, as an insurance against mistakes in the totally visual tests, a normal and abnormal control should be concomitantly numerically expressed and therefore, are subject to the QC technique described therewith.

QC in Urine Analysis

In spite of being the most commonly performed and also important screening test, QC here is the most neglected. Results are taken for granted and nobody seems to care simply because of ready availability of urine and relative low cost of repetition.

Three basics of QC are, however, recommended:

1. QC for reagent strips
2. Look for inconsistencies
3. Repeat test and split sample test

QC in Microbiology

It will include all branches, viz. bacteriology, parasitology, mycology, virology and serology. The basic approach in all remains the same:

1. Lab staff
2. Techniques
3. Proficiency testing
4. QC of items used:
   a. Media
   b. Stock cultures
   c. Antisera

QC for AFB

To act as positive QC material, irridiated slides of positive sputum are to be used. Other details regarding reagents, etc. remain the same.

QC of Syphilis Serology

New lots of sera and reagents should be tested in parallel with established ones. Check for contamination. Stick to recommended procedures regarding test conditions. Whenever possible try to report with more than one method. Inclusion of positive and negative controls is a must with every run.

QC for Parasitology

Fascal parasitology QC involves storage of wet preparation material of stool, mostly in formaline. More recently, polyvinyl alcohol based material has become available. Positive reference slide of all parasites must be provided since the commonest mistake in identification is due to misconceptions of size. For blood parasites also, positive slides must be available to prevent false positive or false negative reports.

QC for Mycology

Here QC is compounded by slow growth rate, selectivity of primary recovery medium, narrow range and slow biochemical reactions. Also special organisms are required for confirmation of media.

QC for Virology

Positive virus as particles demonstrated by electron microscopy need QC material of positive type like in herpes. QC for viral serology is similar to that described under diagnostic immunology. Measurement of antibodies to viruses by best giving results as positive/negative or in titres is done for which QC material is pooled human serum.

Thrombonised anti-HIV positive human plasma acts as QC material for AIDS. For hepatitis B surface antigen and other related antigens and markers, thrombinished human plasma acts as positive control. Similar controls are provided for rubella IgM antibodies and immune status.

QC for Antibiotic Sensitivity of Bacteria

The sensitivity testing material has to be stored under 20°C when concentrated solution are kept and at 14°C when discs have been made. Powder may, however, be stored at ordinary fridge temperature, when storing discs, allow the unopened container to come to room temperature before taking out the discs, otherwise water of condensation over discs reduce their activity fast. Never compromise on expiry date. Test with known organisms and recommended zone size of inhibition. If desired results are not obtained, try to find out 'why' before proceeding further.

QC for Antibiotic Assays

This involves monitoring of plasma and body fluids for therapeutic or toxic levels of gentamycin, tobremycin, netilmycin, chloramphenicol and vancomycin, QC depends upon the technique of estimation which in many instances may be quite automated.

QC of Histopathology and Cytology

The preparatory aspects of both these branches depend heavily upon the dexterity of technicians and good quality reagents and stains apart from following the recommended procedures closely. This involves thus, all imaginable aspects regarding fixation, dehydration and processing, etc. for which preventive maintenance of equipment is equally important. Wax needs special attention from melting point angle. Special stains must be performed in batches that include a positive and a negative control. One point from interpretational aspect is the training of 'sharp' technologists to act as persons to screen cytology slides. For that, positive control slides must be readily available. And number of randomly selected slides from negative ones reported be thoroughly scrutinised. The interpretative aspect QC is done by circulating either the same slide or slides from material amongst several pathologists. This is organised by one agency or person and the unbiased opinions of the whole group is then again reviewed by participants. Or a meeting or joint session may be called to discuss the difference of opinions.

Maintenance of Good Quality Control

The following points are essentially considered:

1. Complete methodology is made available at each working table.
2. A technician thoroughly trained is posted for each method.
3. A close continuous observation is he1d on storage specifications, expiration dates and details of preparation of reagents.
4. New batch of reagents are introduced after running through control serum and found satisfactory. This is done before the exhaustion of the present reagent.

5. Primary standards are used whenever feasible.
6. Instruments are checked frequently for correct performance.
7. Each batch of sample determination should include control serum and standard.
8. All glasswares are thoroughly cleaned.
9. Pipettes and volumetric flasks used must be of good quality.
10. Laboratory data are recorded separately for each test in separate books.
11. Experienced staff should be involved in the laboratory activities at all times.
12. The technicians should do the best job and also communicate observations and suggestions to their supervisors for appropriate action.
13. After careful scrutiny only the report forms are signed and despatched.
14. Complete honesty by the entire laboratory staff is very important since questionable results are potentially more harmful to the patient than no results at all.

It is recommended that each laboratory must develop a sound internal QC and then join some external QC too. It is evident and accepted by all experts in the field that if a lab introduces QC, the improvement is certain.

SAFETY PRECAUTIONS IN THE LABORATORY

Over 4000 laboratory associated infections have been reported and many more still go unreported. The infection can occur through ingestion, inhalation, injections or aerosols. In the laboratory acquired infections routes may be different than the natural infection and the symptoms may also be different due to different dosage received.

Biosafety in a microbiological laboratory is very essential and basically depends on three components:

a. Basic standards of laboratory design, operation and equipment.
b. Selection and use of essential biosafety equipment.
c. Safe laboratory procedures.

An exhaustive review of each component is beyond the scope of this manual but practical and easily achievable safe laboratory rules are listed here:

i. Avoid mouth pipetting as far as possible.
ii. Avoid eating, drinking, smoking and storing eatables in the laboratory.
iii. Decontaminate the working area at least once a day and more frequently after the spillage of potentially, infective material.
iv. Wash your hands with soap and water after handling the infectious material.
v. Wear laboratory coats/gowns in the laboratory and these should not be taken outside the working area.
vi. Use gloves for all those procedures that may involve accidental direct contact with blood or infectious materials.
vii. Decontaminate all liquid or solid waste materials before disposal.
viii. Perform all technical procedures in a way that minimise the aerosol formation.
ix. Provide adequate training to the staff in laboratory safety procedures.
x. As far as possible, actively immunize the workers against the diseases the materials of which they are handling.
xi. Employ medically fit staff only to work in clinical laboratories.
xii. Provide ample space and illumination for safe conduction of laboratory procedures.
xiii. Design smooth, easily cleanable walls, ceilings and floors which should be impermeable to liquids and resistant to chemical and disinfectants. Floors should be slip-resistant.
xiv. Ensure a dependable and good quality water supply.

xv. Make suitably equipped first aid rooms in the vicinity of the laboratories readily accessible.
xvi. Provide the staff safe laboratory equipment, e.g. pipetting aids, safety cabinets, screw cap tubes and bottles, incinerators and autoclaves, etc.
xvii. Carry out periodic health and medical check up of the workers to exclude the highly susceptible individuals.
xviii. Provide safety systems covering fire and electrical emergencies.
xix. Control rodents and insects in the laboratory so that the infectious material is not allowed to spread in the laboratory.
xx. Do not permit the entry of the experimental animals which are not to be used in the laboratory.
xxi. Laboratory workers on long-term steroid therapy, other immunosuppressives and in the pregnant stage should avoid working at places where there are increased chances of contracting infection.