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Guidelines for Peripheral and Intermediate Laboratories in

Quality Assurance

IN

BACTERIOLOGY AND IMMUNOLOGY

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FOREWORD

Health services are utilizing laboratories more extensively now than ever before. The demand for quality results from laboratories has been echoed by all the health care professionals. Unreliable laboratory results are known to have serious consequences for the health of the individual as well as the community. The sole objective of quality assurance is to provide reliable laboratory data in all health care activities and to ensure inter-laboratory comparability of results, particularly in epidemiological investigations, health surveys, environmental monitoring, medical research and other public health activities.

The importance of quality in the functioning of health care laboratories in developing countries has also been universally recognized. Laboratories practicing the principles of quality assurance generate relevant, reliable and cost-effective results.

The mainstay of quality assurance in health laboratories is the result of practice of internal quality control. Participation in the external quality assessment schemes ascertains whether or not internal quality control is in place. Quality assurance covers a wide spectrum of activities which

include collection, storage and transportation of appropriate specimens; performance of test by correct technique with suitable controls, and data processing such as reporting , recording and interpretation of results and feedback.

Improvements in the quality of laboratories can be done by the application of good laboratory practices and commitment of all echelons of personnel. Quality is never due to an accident, it is a result of concerted planned activities. One of the major constraints in applying quality assurance tools is the nonavailability of comprehensive and practical guidelines, especially in peripheral and intermediate laboratories. This manual has been designed to meet the needs for ensuring quality in the field of bacteriology and immunology in health laboratories. I am sure the laboratories for which these guidelines have been developed will find these appropriate and useful.

Dr Uton Muchtar Rafei
Regional Director

PREFACE

Communicable diseases continue to be major public health problem in developing countries. Control of these diseases include better management of individual patient and adequate prevention and control measures at community level. Both these components require establishment of precise diagnosis so that disease-specific control activities can be initiated promptly. The microbiological laboratories play a vital role in confirming the diagnosis as well as indicating most suitable intervention measure.

During the past few years there has been an increasing dependence of patient care, and of clinical and epidemiological research on laboratory investigations. The more and more recognition of need of laboratory services has resulted in building up of significant infrastructure With growing network of laboratories, it has become mandatory to check results to make them reliable, cost-effective as well as comparable with those obtained by other laboratories. This has generated the demand of quality assurance in the functioning of these laboratories so that authentic results are be obtained. The concept of quality assurance in health

laboratories acquires greater importance because of the continuous addition of new methods, introduction of sophisticated instruments in day to day working and high cost of laboratory investigations.

Whereas many big laboratories have instituted measures to assure quality of their results by integrating essential internal quality control steps in their day to day working and also participating in appropriate external quality assessment schemes, the intermediate and peripheral laboratories need suitable support and inputs for assuring quality. These guidelines have been developed to assist member countries in strengthening laboratory activities in microbiology so as to ensure better primary health care delivery. These guidelines dwell upon the basic concepts of quality assurance in microbiology and also describe essential steps of assuring quality in various activities that a microbiology laboratory is expected to undertake in its support to primary health care system.

ABBREVIATIONS AND ACRONYMS

AFB	acid fast bacilli
AR	analytical reagent
ASO	antistreptolysin O
ATCC	American Type Culture Collection
CAMP	Christie Atkins Munch-Peterson
cm	centimeter
CSF	cerebrospinal fluid
EIA	enzyme immunoassay
ELISA	enzyme linked immunosorbent assay
EMB	eosin methylene blue (agar)
EPEC	enteropathogenic *Escherichia coli*
EQA	external quality assessment
EQAS	external quality assessment scheme
FAT	fluorescent antibody test
GLP	good laboratory practices
H_2S	hydrogen sulphide
HA	haemagglutination
HBsAg	hepatitis B surface antigen
HEPA	high efficiency particulate air
HIV	human immunodeficiency virus
IgG	immunoglobulin G
IgM	immunoglobulin M
IQC	internal quality control
mL	millilitre

mm	millimeter
NCCLS	National Committee on Clinical Laboratory Services (USA)
°C	degree centrigrade
ONPG	o-nitrophenyl-β-D-galactopyranoside
OPD	outdoor patient department
p.a.	pro analysis
PHA	passive haemagglutination
p.p.a.	purissimum pro analysis
QA	quality assurance
QC	quality control
RBCs	red blood cells
RPR	rapid plasma reagin
SOP	standard operating procedures
STA	standard tube agglutination test
TQM	total quality management
VDRL	venereal diseases research laboratory
XLD	xylose-lysine-desoxycholate (agar)
ZN	ziehl neelsen (staining)

GLOSSARY

Accuracy

The degree to which a measurement or an estimate based on measurements, represents the true value of the attribute that is being measured.

Accession list

This list records all specimens that are received in the laboratory for processing.

Accreditation

This is the process of inspection of laboratories and their licensing by a third party to ensure conformity to predefined criteria.

Audit

An examination or review that *establishes the extent to which a condition, process or* performance conforms to predetermined standards or criteria.

Bias

Deviation of results or inferences from the truth, or processes leading to such deviation.

Coefficient of Variation

The standard deviation expressed as a percentage of the mean

Control Serum

Serum with known concentration used to measure the accuracy and precision and sensitivity and specificity of a procedure. They are used to determine, verify and document performance.

Dilution

The ratio of the volume of serum or other medium of the total volume produced by combining it with diluent. The dilution 1:10 denotes one part serum plus nine parts diluent for a total volume of 10 parts. A dilution of 1:1 indicates no dilution. The same notation is used in areas other than serology to indicate volume of material to volume of diluent. So the two methods must be carefully differentiated.

Efficiency

The ability of a test to give a positive result on positives and negative results on negatives.

$$\textit{Efficiency} = \frac{\text{True Positive} + \text{True Negative}}{\text{True Positive} + \text{False Positive} + \text{True Negative} + \text{False Negative}} \times 100.$$

Error

The difference between an observed or measured value and the best obtainable estimates of its true value.

Internal Quality Control

Internal quality control is a set of procedures undertaken by the staff of a laboratory to ensure quality from the collection of specimens, the performance of the test upto analytical results, and the procedure being planned, ordered and followed up by the staff itself.

Limit of detection

The limit of detection is the lowest level of analyte that can be detected, but not necessarily determined in a quantitative fashion, using a specific method under the required experimental conditions.

The limit of detection is usually defined as 3 s.d. of the mean of results obtained from the measurement of specimens that do not contain the analyte under investigation.

Precision

The closeness of the relationship between replicate determinations with no regard for bias error. Freedom from variation. Reproducibility. An inverse function of variance; greater the precision smaller is the variance (ISO/DIS 3534-1; 1990,3.14)

Predictive values

The predictive value of a positive test result is defined as the percentage of positive results that are true positives in a given situation. It indicates the probability that a patient with a positive test result has, in fact, the disease in question. The predictive value of a negative test result is the percentage of negative results that are truly negative for the same given situation. The predictive value of test results is directly dependent on the prevalence of the condition in the population being tested. This also implies that predictive values are not constant but change with the prevalence of the situation.

$$\text{Predictive value of a positive test} = \frac{\text{True positive}}{\text{True positive} + \text{False positive}} \times 100$$

$$\text{Predictive value of a negative test} = \frac{\text{True negative}}{\text{True negative} + \text{False negative}} \times 100$$

Probability

Quantitative measure of chance. The ratio of the number of outcomes that produce a given event to the total number of possible outcomes.

Range

The difference between the maximum and minimum of a set of values.

Reference Material

A material of a substance with values of measurable quantities sufficiently well established to be used for calibration of a measurement system, the assessment of measurement procedures or for assigning values to materials (ISO Guide 30:1981, 2.1).

Sample

A subset or group of objects or things selected from a larger set or population. Usually information obtained from samples is used to make inferences about the population.

Sensitivity

Diagnostic sensitivity: The incidence of true positive results obtained when a test is used for patients known to have the disease or condition.

$$\text{Sensitivity} = \frac{\text{True Positive}}{\text{True Positive + False Negative}} \times 100$$

Analytical sensitivity: The ability to detect small differences in concentration in a series of specimens.

Specificity

A measure of the ability of a test to indicate the absence of a component in a specimen when it is truly negative for that component, or a measure of the ability of a test to measure accurately one component in a specimen without interference by other components.

(1) A measure of a ability of a test to give a negative result in the absence of a disease.

$$\text{(2) Specificity} = \frac{\text{True Negative}}{\text{True Negative + False Positive}} \times 100$$

Standard material (calibration standard or calibrator)

A substance of known concentration (usually determined by comparison to a reference material) used as a basis for determining the concentration of a substance in unknown specimens. (WHO LAB/81.3)

Titer

The *reciprocal* of the highest dilution of a constituent which leads to a positive reaction. For example, if a serum produces a positive reaction in the tube which contains 1:80 dilution but not in the next higher dilution, then it has a titer of the *reciprocal* of 1:80, or 80. The dilution is 1:80; the titer is 80. A titer of 80 means that the serum is positive in a 1:80 dilution.

Validation

Confirmation by examination and provision of evidence that specific requirements for specific intended use are met.

1
INTRODUCTION

QUALITY IS *the degree of congruence between expectation and realization*. In other words it is the matching of what you wanted with what you got i.e. *expectations versus fulfillment.* The simplest definition which encompasses the soul of quality is:

> ***Quality*** *means meeting the pre-determined requirements of the users for a particular substance or service.*

In recent times concept of quality has been elaborated further and an international consensus has been obtained through the efforts of International Organization for Standards (ISO).

According to ISO quality is defined as totality of characteristics of an entity that bear on its ability to satisfy stated and implied needs.

Quality has also been appropriately referred to as the characteristics of a product or service that make it suitable for the purpose for which it is intended (fitness for purpose).

Quality has been shown to be synonymous with consistency which denotes providing the same product or service time after time thus making the outcome more predictable.

In health laboratory services, the product is the report of analysis of the material received by the laboratory for processing. The physician or the public health professional for the benefit of the patient or the community utilizes this report. Hence they are considered as customers for the health laboratory.

1.1 BENEFITS OF QUALITY

There are many benefits of a good quality assurance (QA) programme. Some of these are listed in Table 1.1.

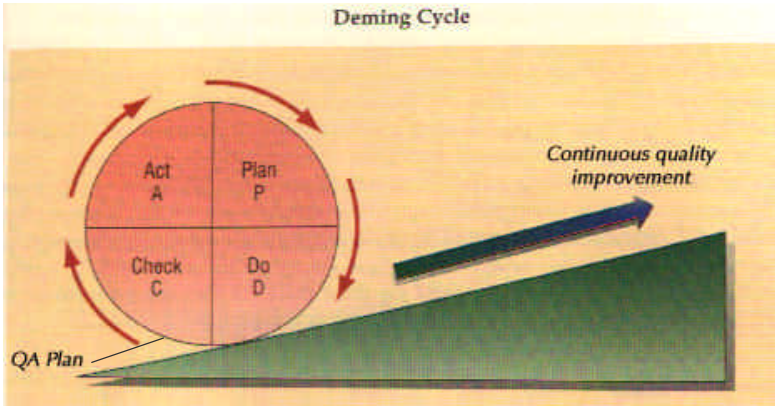
Table 1.1: ***Benefits of a quality assurance program***

➢ Production of a quality product or generation of a reliable service
➢ Helping the physician in establishing proper diagnosis rapidly, thus generating confidence and better health care for the patient
➢ Creation of a good reputation for the laboratory
➢ Motivation factor for staff to work better
➢ Mandatory requirement for accreditation
➢ Prevention of legal suits and associated complications

Doing things in a similar manner, time after time ensures consistency.

Doing things right first time means reduced rework and product failure, resulting in cost savings.

Various measuring tools can be used for monitoring operational, customer or product problems. Once problems are identified action can be taken for improving either the service or product. This action should be such that it prevents further such problems from re-occurring in the future (Preventative action).



1.2 CONTINUOUS QUALITY IMPROVEMENT

The Deming cycle illustrates the important principle of continuous improvement. Quality is not a static thing. The cycle of continually planning (P), doing (D), checking (C) and acting is essential to ensure that quality cycle keeps turning and that quality improves.

The important point about the Deming cycle is that it illustrates the need for continual momentum to go forwards and upwards. If there is no progress forward the pressure is to fall backwards - down the slope - with the consequent loss of quality. The only way to prevent the backwards movement is to continually support the cycle - this is the function of the quality system that has been put in place.

Initially the quality of the final product was all that was looked at. This soon changed to include the processes that gave the product. From that a broader view of quality developed and concepts such as good laboratory practices (GLP) arose followed by customer satisfaction. This has now been taken further to the approach of total quality management in which the focus is on the satisfaction of customers, suppliers, staff and society, and even consideration of environmental issues.

1.3 USERS' PERCEPTION OF HEALTH LABORATORY SERVICES

The perception of laboratory users of the quality of bacteriological and immunological services can be manifold. An overview of the diversity of this perception is given in Table 1.2:

Table 1.2: ***Users' perceptions of quality in laboratory services***

➢ Courteous personnel who are readily available, cooperative and willing to help
➢ Test results are consistent; repeats usually confirm the first test
➢ Sample mix-ups are rare and reports reach the right persons
➢ Results are made available as per the requirements of the individual
➢ Health care workers rarely have difficulty in knowing what specimens to collect and what to do to resolve problems with the laboratory
➢ Needs are understood, met and exceeded
➢ Complaints and problems are swiftly addressed, resolved and rarely repeated.

Though users' perception of quality is important, this is only a small part of a comprehensive quality assurance program.

1.4 GOOD LABORATORY PRACTICES

All activities that are performed by the laboratories can be grouped under a common term **good laboratory practices (GLP).** It indicates performance of all the activities of the laboratory in the best possible way so that the results obtained are of the highest possible accuracy. The GLP (Table 1.3) encompasses various factors.

Table 1.3: ***Good Laboratory Practices***

➢ Proper collection of samples
➢ Appropriate identification of specimens with special labels on hazardous specimens
➢ Prompt transportation to laboratory at appropriate temperature.
➢ Collection and storage under conditions which prevent deterioration of the sample before the performance of test
➢ Accurate performance of test
➢ Release of reports after proper scrutiny
➢ Delivery of reports to the correct destination in the shortest possible time
➢ Cordial relationship with the users

An indirect but important effect of quality assurance systems is the improvement in scientific technology. Such assessments frequently detect deficiencies in laboratory kits, which can be remedied through the efforts of both

laboratory scientists and manufacturers of kits and equipment.

The needs and benefits of quality assurance have been abundantly demonstrated in those countries which have built up national programmes for quality assurance.

1.5 INTERNATIONAL STANDARDS ORGANIZATION

The International Standards Organization (ISO) is one of the leading international bodies that has brought together international community in developing uniform standards for quality in manufacturing and service sectors. Some of the documents that may be pertinent to laboratories are summed up in box below.

ISO No	Refers to
ISO 9000 series (9000, 9001, 9002, 9003, 9004 etc)	Design/development, production, installation, servicing and final inspection and testing as applied to manufacturing processes
ISO Guide 25 (ISO Guide 17025)	Performance of objective measurements, use of reference material and calibration as well as test methodology
ISO Guide 43	Design and operation of external quality assessment scheme
ISO Guidelines 15189	Quality management of medical laboratories

1.6 TRACEABILITY

Traceability is an important concept in quality assurance programme. An effective QA procedure should allow an audit trail to be followed back from the laboratory report. This should allow access to a complete documented history from receipt of the specimen to the issue of the report.

This helps in identifying the cause of error and application of remedial measure.

2
FACTORS INFLUENCING QUALITY

THE RELIABILITY of laboratory results depends on many para-analytic factors as well as analytical procedures. Traditionally, concern has been expressed regarding various intralaboratory technical aspects that may affect the analytical process. The analytical cycle, however, is more complex and includes pre-analytical and post-analytical elements, which may be sources of error that are beyond the control of laboratory staff. This important fact should be borne in mind both by the laboratory personnel and the clinicians-that quality assurance is a mutual responsibility which cannot be achieved through improving and controlling the analytical process alone without parallel improvement and control of pre-analytical and post-analytical factors.

The factors affecting analytical and para-analytical quality control are summarized in Table 2.1.

Table 2.1: ***Factors influencing quality***

Analytical Quality Control

- ➢ Equipment reliability
- ➢ Reagent stability, integrity and efficiency
- ➢ Adequate calibration
- ➢ Procedure reliability in terms of
  - Precision
  - Accuracy
  - analytical specificity

Non-analytical Quality Control (Para-analytical Factors)

- ➢ Patient preparation
- ➢ Proper sample selection
- ➢ Proper collection and transportation
- ➢ Details of the patient and specimen identification
- ➢ Proper recording of results and their interpretation
- ➢ Knowledge of normal range of results and abnormalities

The analyses performed by health laboratories are subject to biological and technical influences, professional skills and environmental effects. Pre-analytical, analytical and post-analytical factors influencing the quality of the laboratory results (Table 2.2 and Figure 2.1) are discussed here.

2.1 PRE-ANALYTICAL FACTORS

The range of reference values as well as the results obtained from measurements in a patient's specimen are prone to a number of pre-analytical influences such as:

2.1.1 Age-dependent variations

Age dependent changes of concentration or activities occur in a number of haematological and chemical analytes.

Normal bacteriological flora also change with age. Hence laboratory results should be interpreted keeping in view the age of the patient.

2.1.2 Incorrect specimen identification

An incomplete specimen identification will obviously give wrong information despite all the precautions taken for a proper analytical procedure.

2.1.3 Prolonged transportation and storage

Prolonged and improper storage prior to processing of the specimen can affect the results especially since many organisms do not survive for long unless they are subcultured or kept in an environment which is rapidly changing. Urethral discharge containing gonococci, specimens for anaerobic bacteriology, etc are some examples.

2.1.4 Selection of appropriate samples

An appropriate sample should be collected in an appropriate way, e.g. for diagnosing an acute intestinal amoebiasis, it is essential to examine the stool sample within 30-60 minutes since trophozoites survive for that much time only. Similarly examining stool samples for the eggs of *Enterobius vermicularis* is not as effective as a perianal swab. Microfilaria showing nocturnal periodicity should be sampled at night only.

2.1.5 Selection of right test method

Selection of right test method is of paramount importance. For example, the serodiagnosis of dracunculiasis or filariasis is not well established, and attempting such techniques will be of little use.

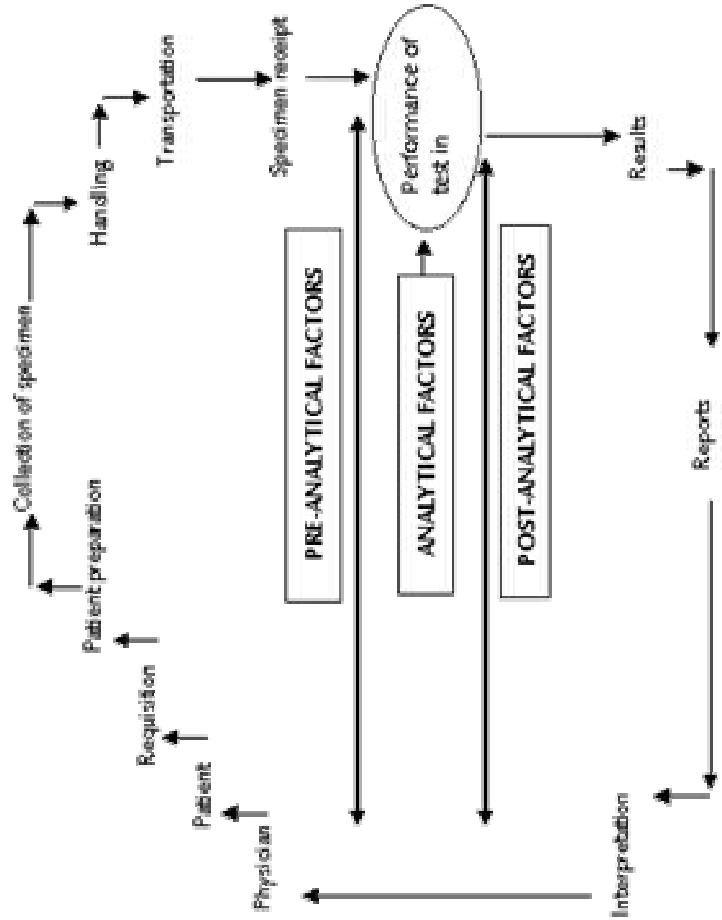


Figure 2.1 *Factors influencing quality*

2.1.6 Sending the sample to the right laboratory

The sample for analysis of a particular analyte should be sent to the right laboratory undertaking that particular investigation. A laboratory not doing a test and yet receiving the samples for analysis will cause inordinate delay as well as a decline in the quality of the sample.

2.1.7 Collecting the right specimen

Collecting the right specimen is of critical importance. For example if a diagnosis of meningitis is expected, obtaining a throat swab or a sputum sample (instead of CSF) would not be the best sample for this purpose.

Table 2.2 *Factors influencing quality*

Analytical	Para-Analytical	
	Pre-Analytical	Post-Analytical
Equipment reliability	RIGHT investigation	Accurate recording
Reagents stability, integrity and efficiency	RIGHT sample	Range of normal values
Adequate calibration	RIGHT collection	Age and sex related variations
Correct interpretation	RIGHT technique	Turnaround time
Procedural reliability using SOP	RIGHT laboratory	Availability of guidance
Proficiency of personnel	RIGHT background mileu	
Right technique for available reagents	RIGHT transportation	
Internal quality control	RIGHT quantity	
External quality assessment	RIGHT labeling	

2.2 ANALYTICAL FACTORS

Analytical quality control depends upon the following factors:

- Equipment reliability
- Reagent stability, integrity and efficiency
- Adequate calibration
- Procedure reliability using standard operating manuals
- Specificity, precision and accuracy of the test of the high order
- Proficiency of personnel and continuous updating of their knowledge
- Selection of the right technique of proven efficacy for which reliable reagents are available
- Good internal quality control
- Participation in external quality assessment programmes

All these factors are discussed in various chapters of this document in greater detail.

2.3 POST-ANALYTICAL FACTORS

After the proper analytical process is over, it is important to record the findings on the right requisition slip in a clear way. Any possible remarks on the results obtained should also be entered, as well as the normal range of the results. The significance of the results obtained should be highlighted wherever required. There should be frequent dialogue between the laboratory personnel and the physicians for appropriate use of the facilities and the right interpretation of results obtained in the laboratory.

3

OVERVIEW OF QUALITY ASSURANCE

HEALTH CARE laboratories are being utilized more often now than in the past. Unreliable laboratory results may have serious consequences for the health of an individual as well as the community. The main objective of quality assurance is to provide reliable laboratory results in all health care activities and to ensure inter-laboratory comparability of results, particularly in epidemiological investigations, health surveys, environmental monitoring, medical research and other public health activities.

In the direct clinical care of patients, the objectives are to improve the accuracy of clinical diagnoses, to reduce health care costs (through avoidance of the necessity to repeat tests and of needless or inappropriate treatment) and to provide a basis for the continuing education of physicians and laboratory scientists in scientific methods of investigations, laboratory organization and management and analytical techniques.

3.1 QUALITY ASSURANCE

Quality assurance is a wide ranging concept covering all matters that individually or collectively influence the quality of a product. It is the totality of the arrangements made with the objective of ensuring that the product is of the quality required for its intended use. It denotes a system for continuously improving reliability, efficiency, and utilization of products and services.

Quality assurance (QA) in bacteriology and immunology spans a wide spectrum from monitoring the performance of equipment and reagents to examining the clinical value of services and information.

- ➢ QA is cost effective
- ➢ QA is an aid to productivity
- ➢ QA is a means of getting it right the first time, every time
- ➢ QA is good management sense, and its responsibility is shared by all.

3.2 QUALITY ASSURANCE PROGRAMME

A quality assurance programme is concerned with sampling, specifications and testing as well as with organization, documentation and release procedures that ensure that the necessary and relevant steps have been taken to ensure satisfactory quality.

The bacteriology and immunology laboratory is responsible for providing accurate and relevant information that is of use for clinical diagnosis of a patient or in support of a public health activity. The accuracy and clinical value of the laboratory analysis of the clinical specimen and

microbial isolates are dependent upon a QA programme that

- assesses the quality of the specimen
- documents the validity of the test method
- monitors the performance of test procedures, reagents, media, instruments and personnel
- and reviews test results for errors and clinical relevance

3.2 OBJECTIVES OF QUALITY ASSURANCE

Murphy's law states that if anything can go wrong, it will. This law is applicable to laboratories also where virtually every conceivable process has the capability of giving erratic results. The purpose of quality assurance is to prevent as many errors as possible and to detect those that do occur. ***Total quality management*** means that every variable that could possibly affect the quality of the test results has been *controlled.* This ideal situation may not be possible, but a level of quality assurance that can control most of the factors which are likely to affect the test results can be attained. Determining which controls are important components of a quality assurance system, how often and how many controls to include, and how to use control results requires knowledge and experience.

3.3 COMPONENTS OF A QUALITY ASSURANCE PROGRAMME

An effective quality assurance programme should cover all aspects of the laboratory. These have been shown in Table 3.1.

Table 3.1: ***Components of a quality assurance programme***

➢ Personnel with adequate training and experience
➢ Proper specimen collection
➢ Employment of techniques with precision and accuracy
➢ Proper performance of tests
➢ Efficient processing of results
➢ Reagents and equipment of good quality
➢ Methods for detecting errors
➢ Corrective steps when analyses go out of control
➢ Preventive maintenance of equipment
➢ Continuous training of staff
➢ Documentation
➢ Coordination
➢ Timely feedback

The basic aim of quality assurance is to generate the confidence of the user in the final report. This can happen only if both the product and the laboratory personnel have performed in accordance with good laboratory practices and approved standards, and tests or analyses have been validated.

3.4 COMPUTERS IN QUALITY ASSURANCE

Computers and information technology have come in a big way in microbiology laboratories also. Many laboratories utilize a laboratory information management system (LIMS)

to improve efficiency of the laboratory and to assure quality of its services (Table 3.2).

Table 3.2 ***Role of computers in improving efficiency of laboratory***

➢ Report generation
➢ Data analysis
➢ Traceability
➢ Validation checks
➢ Mandatory data checks
➢ Code checks
➢ Data entry devices
➢ Detection of abnormal/unusual results
➢ Test controls
➢ Appropriate interpretation of test results
➢ Quality assessment of specimens
➢ Stock control
➢ Maintenance schedules

In health laboratories the quality assurance is the total process by which the quality of laboratory reports can be guaranteed. This may be summarized as the right result at the right time on the right specimen from the right patient, with result interpretation based on correct reference data and delivered at right price.

4
QUALITY SYSTEM

IN AN industrial unit, the raw materials (input) are processed and transformed into a product (output). A process is defined as the sum total of activities which use resources to transform inputs into outputs. The process may comprise of a number of procedures. A procedure is a specified way to carry out an activity. Each procedure is undertaken in such a way that it delivers the desired result through a system approach. Quality is ensured through a well defined quality system.

Input (Raw material) → **Processing** → **Output (Product)**

The health laboratories also work on the similar principles. The clinical (or environmental) material constitutes the raw material (inputs) which is processed in the laboratory to generate an output in the form of a report. The health laboratories also strive to assure quality of their product (report). However, quality does not just happen on its own. Systematic efforts through organizational structure

and efficient utilization of resources are needed to implement all the steps that will assure generation of quality reports by the laboratories. Quality system is a part of overall quality management that aims at ensuring consistency, reproducibility, traceability and efficaciousness of the products or services.

Accordingly quality system is defined as the organizational structure and resources needed to implement quality requirements. ISO defines quality system as the organizational structure, responsibilities, procedures, processes, and resources for implementing quality management.

4.1 KEY ELEMENTS

Quality system has following five key elements:

- ➢ Organizational management and structure
- ➢ Referential (quality) standards
- ➢ Documentation
- ➢ Assessment (Monitoring and evaluation)
- ➢ Training

Organizational management and structure

The overall responsibility for the design, implementation, maintenance and improvements in quality system rests with the laboratory management. Quality is the responsibility of all the staff members of the organization. However, the top management needs to make a firm commitment to ensure quality and allocate adequate resources. Quality policy reflects the intention and commitment of the organization to attain quality. The policy can be translated into implementation through a quality plan which, along with

policy, need to be documented in the form of a quality manual.

Laboratory management delegates responsibility and authority to appropriate individuals who are directly responsible for implementing the quality policy and quality system and makes them available adequate resources to efficiently discharge their duties. The inter-relationship between various staff members and their job description are decided by the management.

The management also makes all decisions and strategies with quality as the overarching theme. If the size of the organization requires and resources permit a Quality Manager can be appointed to supervise and guide all activities related to implementation of quality. In smaller laboratories one of the staff members can be given the additional responsibility of a "quality manager".

Referential (quality) standards

The referential standards are an integral part of the quality system. These aim at ensuring safety and consistency. These need to be followed to meet the regulatory requirements as well as monitoring of the functioning of the laboratory.

Both management and technical standards need to be followed to ensure quality. These must also conform to the local laws.

Documentation

Document is a record of any information or instructions including policy statements, quality manuals, procedures, specifications, calibration tables, reports, job description, documents of external origin such as regulations, standards

and examination procedures etc. These may be on various media, whether hard copy or electronic.

The quality system of the laboratory shall define, document and maintain procedures to control all documents and information (from internal and external resources). The current version of relevant documents shall be available at all locations where operations needed for effective functioning of the quality system are performed.

Monitoring and evaluation

The laboratory management shall develop and implement quality indicators to systematically monitor and evaluate the laboratory's contribution to patient care. When the programme identifies opportunities for improvement within the system, the laboratory management shall take appropriate steps to address them. Error management shall be vigorously implemented.

Assessment of quality through audits (internal or external) and participation in external quality assessment schemes are other tools, the results of which should guide the management in further improving the quality.

Training

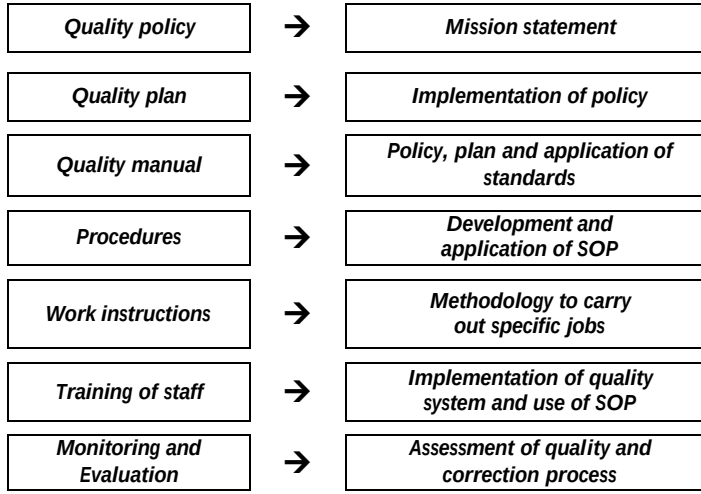
The quality system is only as good as the staff who actually work with it. No matter how good the quality system is on paper, if the theory cannot be translated into practice, quality cannot be achieved. Training must also include an understanding of why quality is important. Training should be competency based and must be followed by a post-training support to provide a continuous support.

Existence of a quality system demonstrates that the laboratory has:

- ➢ commitment to quality
- ➢ a definite programme for quality and its continuous improvement
- ➢ methods for processing laboratory specimens in the form of approved written SOP
- ➢ evidence based control systems
- ➢ appropriate documentation
- ➢ trained human resource
- ➢ mechanism for error management under which it can detect when and where things have gone wrong and take necessary actions to prevent recurrence of such episodes

4.2 DEVELOPMENT OF A QUALITY SYSTEM

The development of a quality system can be done in a step-wise approach as shown in Figure below.



5

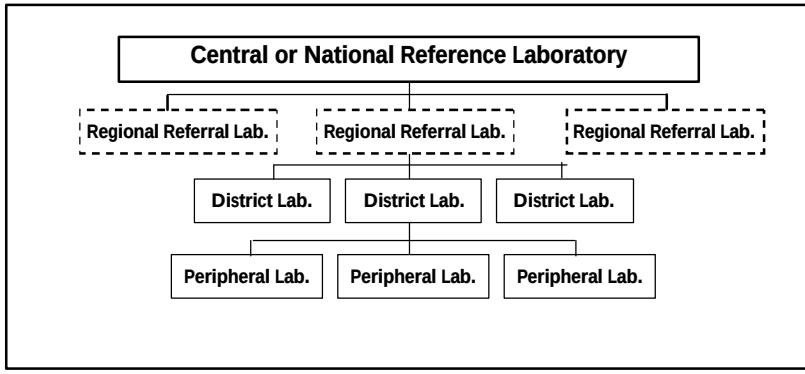
ORGANIZATION AND FUNCTIONS OF LABORATORIES

THE ORGANIZATION of laboratories in any country is usually a three or four tier system with various possible functional linkages between them. One possible way of networking of laboratories is shown in Figure 5.1.

5.1 PERIPHERAL LABORATORY SERVICES

Peripheral laboratories are located at the point of first contact of patients with the health care services. In most developing countries these are available only at primary health centre or community health centre (upgraded primary health centre) level. These laboratories provide technical support for preventive, curative and promotive services for the individual as well as the community.

Figure 5.1: *Networking of laboratories*



5.1.1 Staff

The staff in peripheral laboratories should include at least one technician and one laboratory assistant/attendant.

5.1.2 Space

Space available in peripheral laboratories should include at least one laboratory-cum-office/record room (approx. 5 metres x 3 metres) and one store-room which can be used for other services also (approx. 5 metres x 3 metres).

5.1.3 Other facilities

Other necessary facilities include:

- supply of safe water
- reliable source of energy (battery, electricity solar or kerosene)
- sterilization/disinfection facilities
- waste disposal facilities

There must also be transport and communication facilities between the peripheral and intermediate laboratories for referral of samples and patients, procurement of supplies and personal discussion.

5.1.4 Equipment and supplies

Necessary equipment and supplies include good microscopes, centrifuges, autoclaves, refrigerators, balances, pH meters, incubators, water bath, transport media, glassware, sterile swabs, reagents for staining (eg. Gram, Albert, Ziehl Neelsen, Romanowsky), reagents for chemical examination of urine, kits and reagents for rapid diagnostic tests, sterilized syringes and needles, micropipettes and tips as well as sterile collection bottles for blood/serum and water analysis.

5.1.5 Tests to be performed

Peripheral laboratories are expected to undertake tests of public health as well as clinical relevance. Amongst the tests of public health relevance, diseases of greater epidemiological importance should be accorded priority. Testing of environment samples (especially water) also falls into the priorities of public health relevance. Certain rapid serological tests may be of use in studying epidemiological patterns of important diseases and the same can also be performed at peripheral laboratories.

The tests to be performed by peripheral laboratories are subject to the availability of resources, manpower, technology and prevalence of various diseases in the area catered to by the laboratory. A suggested list is provided in Table 5.1.

*Table 5.1**: Suggested tests to be performed at peripheral laboratories***

Procedure/Specimen	For detection/diagnosis of
Urine examination	Pus cells, RBCs
	Albumin Sugar
Stool examination	Ova and cysts
Stained smears	
- Throat specimen	Diphtheria
- Sputum	Tuberculosis
- CSF	Meningitis (pyogenic and tubercular)
	Malaria, filariasis
Peripheral blood smear	
Rapid diagnostic tests	HIV Ab.
	Hepatitis B surface Ag
	Syphilis Meningococcal disease

As far as possible, these tests should be reliable, sensitive, specific, rapid, easy to perform and cost effective and should be easily undertaken in the peripheral laboratories. Tests such as dip-stick ELISA, latex agglutination, etc. are useful in this kind of situation. However, before these tests are introduced in peripheral laboratories, a central or regional laboratory must evaluate the same and assess their efficacy and feasibility in a peripheral laboratory.

5.2 INTERMEDIATE LABORATORY SERVICES

In most developing countries, intermediate laboratories are located at district or the regional headquarters and may act as clinical as well as public health laboratories. The following functions are expected to be performed by these laboratories:

- ➢ Laboratory support to clinical diagnosis/public health
- ➢ Quality assurance
- ➢ Logistic and technical support to peripheral laboratories
- ➢ Training of staff for peripheral laboratories

Intermediate laboratories help in the diagnosis and treatment of the individual patient and are also used as public health laboratories for epidemiological surveillance and control of diseases in the community. These laboratories also serve as links between peripheral laboratories and the state/central laboratory for the following:

- ➢ Collection, storage and analysis of data
- ➢ Distribution of reagents, media, laboratory manuals
- ➢ Guidelines for purchase of equipment
- ➢ Supervision of peripheral laboratories
- ➢ Organization of EQAS for peripheral laboratories
- ➢ Participation in EQAS organized by the state/ central laboratories
- ➢ Referral to higher/reference laboratories for characterization of isolate/confirmation of diagnosis

5.2.1 Staff

Qualified pathologist/ microbiologist (Doctor of Medicine/diploma in clinical pathology)	1
Technicians - DMLT (diploma in medical Laboratory technology) with experience	2
Laboratory Assistants (DMLT)	1
Laboratory attendants	2
Cleaner	1
Clerk-cum-storekeeper	1

Since it may not be possible to have a full-time epidemiologist, services of an epidemiologist should be available at least on part time basis.

5.2.2 Space

Microbiology/Serology laboratory (approx. 8 meters x 5 meters)	1
Sterilization, media preparation laboratory (approx. 6 meters x 4 meters)	1
Store-room (approx. 3 meters x 5 meters)	1
Office (approx. 3 meters x 5 meters)	1

5.2.3 Equipment

Binocular microscope	2
Dark-field microscope	1
Inoculating chamber	2
Centrifuge	2
Autoclave	2
Incubator	2
Hot air oven	1

Water bath	2
VDRL shaker	1
Colorimeter	1
Refrigerator	1
Balance	2
pH meter	1
Inspissator	1
Distilled water apparatus	1
Micropipettes	as per workload
Tips for pipettes	as per workload

The tests expected to be performed at the intermediate laboratories are listed in Table 5.2.

Table 5.2: ***Suggested microbiological tests to be performed at an intermediate laboratory***

Procedure/Specimen	For detection/ diagnosis of
Unstained wet preparations	
Urine deposit Dark-field illumination	Pus cells, RBCs Treponema pallidum
Stained smears (Gram, Albert, Ziehl Neelsen)	
Nasopharynx and throat swab	Diphtheria, Vincent's angina
Sputum	Tuberculosis, Pneumonia
CSF	Meningitis (pyogenic & tuberculous)
Peripheral blood smear	Malaria, Filariasis
Gastric washing	Tuberculosis

Procedure/Specimen	For detection/ diagnosis of
Urethra/vaginal exudate	Gonorrhoea
Wounds/pus	Clostridia/other organisms
Cultures	
Nasopharyngeal specimen	Corynebacterium diphtheriae, Streptococcus pyogenes
Sputum	AFB, cocci, others
CSF	AFB, cocci, Haemophilus influenzae
Exudate/pus	Bacterial infections
Blood	Salmonella typhi, other salmonellae; Brucella Streptococci, Meningococci, Haemophilus influenzae
Gastric washing	AFB
Urethral/vaginal exudate	Neisseria gonorrhoeae Chlamydia Haemophilus ducreyi
Faeces	Salmonella and Shigella Vibrio cholerae Escherichia coli and others Food poisoning bacteria

Procedure/Specimen	For detection/ diagnosis of
Urine	Pyogenic organisms and AFB
Antibiotic sensitivity	For various pathogens
Serological tests	
Widal	Enteric fever
Tube agglutination	Brucellosis
ASO	Rheumatic fever
VDRL	Syphilis
Rapid diagnostic tests for	Detection of HBsAg for hepatitis B, HIV antibody, Meningococcal antigen
KOH preparation of skin/nail/hair	Fungi
Environmental samples	Bacteriology of water and food

5.3 RAPID DIAGNOSTIC TESTS AT PERIPHERAL AND INTERMEDIATE LABORATORIES

Since rapid tests for the detection of antigens and antibodies are now available, the relevant tests that can be considered for use in peripheral laboratories are listed in Tables 5.3.

*Table 5.3**: Bacterial infections Antigen detection***

Disease/syndrome	Sample	Test
Sore throat (Streptococcus A)	Throat swab	Latex agglutination Coagglutination ELISA dipstick
Meningitis *(H.influenzae b, S.pneumoniae, Neisseria meningitidis,* Group B streptococci)	CSF	Latex agglutination Coagglutination
Gonorrhoea	Exudate/pus	Coagglutination test
Cholera	Stool	Coagglutination
Salmonellosis	Blood	Dipstick ELISA
Chlamydial infections	Urethral exudate genital swab	Dipstick ELISA

Antibody detection

Typhoid fever	Serum	Slide agglutination
Syphilis	Serum	RPR
Rheumatic fever	Serum	ASO, latex agglutination

6

DOCUMENTATION IN THE LABORATORY

MAINTENANCE OF detailed records for all aspects of laboratory activities is an absolute requirement for validating any activity. In the absence of such records it can very well be surmised that no work has been done. *If it is not recorded, it has not been done.*

Document is a record of any information or instructions including policy statements, quality manuals, procedures, specifications, calibration tables, reports, job description, documents of external origin such as regulations, standards and examination procedures etc. These may be on various media, whether hard copy or electronic. Documentation is an essential part of quality system and carries many advantages.

6.1 VALUE OF DOCUMENTATION

Documentation is valuable in that it:

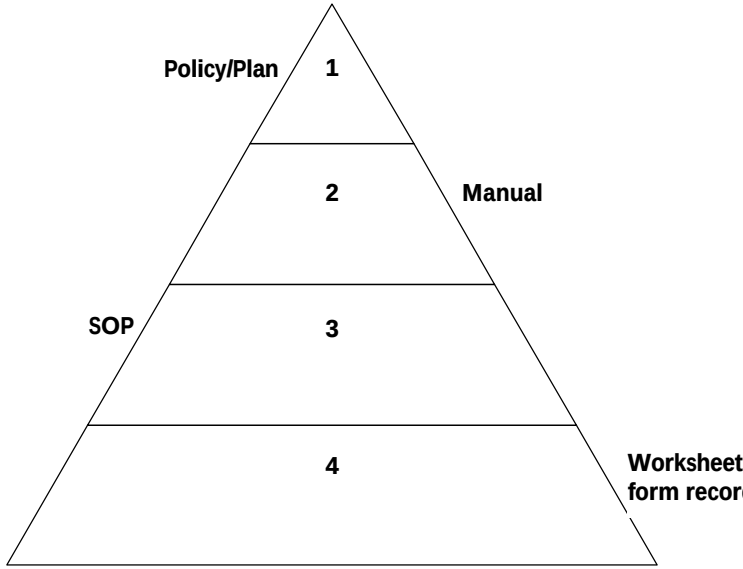
- Defines responsibilities and authorities and interrelationships
- Ensures processes and outcomes are traceable e.g. a process may refer to the addition of a reagent to complete the laboratory test and outcome refers to the report meeting the product specifications
- Proves that the job was done according to referential standards
- Helps external assessors measure the compliance to the standards
- Facilitates training and makes it easier to train the staff to approved procedures than to ad hoc information
- Reminds one what next to be done
- Assists in making decisions
- Helps in investigation of problems
- Helps in improving efficiency

6.2 TYPES OF DOCUMENTATION

Various types of documents are used in the quality system of the laboratories. These include:

- Policy and Plans
- Manuals
- Standard Operating Procedures
- Work instructions
- Data sheets
- Specifications
- Forms
- Standards
- Records
- Labels
- Reports

6.3 LAYERS OF DOCUMENTATION



Quality system documentation is made up of four levels. These levels have been defined in ISO standards as Level 1 to 4 (ISO 9000, 1994) or Levels A to C (ISO 10013, 1995). According to ISO 9000, Level 1 documents describe the quality policy and the outline of the quality system. Level 2 includes the quality manual and the general standard operating procedures (SOP) that are common to whole organization. The specific SOP are categorized in Level 3 and the work instructions are classified under Level 4 along with forms, records, reports which may be annexed with specific SOP. Under ISO 10013 (1995), Layer 2 and 3 have been combined to form Level B.

6.4 DOCUMENTATION STRUCTURE

There is no universal rule for creating a documentation structure. Guidelines for preparing SOP are, however, available to ensure uniformity and ease of understanding. The laboratory management has to decide about it. The structure includes format, style, document number and distribution list.

6.5 REVIEW AND REVISION

Most of the documents need periodic review which is usually one year in most instances. The review may lead to revision of the document to reflect current practices. The revisions have to be authorized by the top management of the laboratory and the new number of the document should exhibit revision number and date.

6.6 DOCUMENT CONTROL

The laboratory management must ensure that only current version of relevant documents shall be available at all locations where operations needed for effective functioning of the quality system are performed.

The quality system of the laboratory shall define, document and maintain procedures to control all documents and information (from internal and external resources).

The basic objectives of document control are threefold:

(1) The latest version is at the work place. This means that the latest information/procedure can be used/followed by the staff performing a specific activity;

(2) Previous version of the same procedure must be destroyed to prevent the possibility of staff having access to old procedures that may have a negative effect on the quality of the results released by the laboratory, and

(3) No unauthorized changes are permitted on controlled documents.

The controlled documents are issued to specific members of staff who are responsible for ensuring that the staff at the workplace have access to them, that they do not disappear and that copies of these are not made. Accordingly, the distribution list of the controlled documents is also documented in a distribution register which provides traceability of the individuals who were issued the document. Proof of issuing and the evidence of the recipient having received the document are also documented. Similarly, destruction/removal of the previous version of the document is also properly documented.

To identify the controlled copies of the document, these must either be superscribed with "CONTROLLED" word or should be printed on paper with different colour.

Documents that need to be controlled

Not all documents that are used in the laboratory need to be controlled. Some examples of the documents that must be controlled include:

- ➢ Quality manual
- ➢ SOP

- ➢ Work sheets
- ➢ Reagent specifications
- ➢ List of approved suppliers

Documents such as forms, patients records etc. need not be controlled.

6.7 DANGERS OF OVERDOCUMENTATION

One must document only to the extent necessary to ensure quality of the results. Quality is not measured by the number of documents, but by their quality and utility in continuous quality improvement. Staff have to use, produce and manage the document to achieve the overall goals of the organization and must not document only for the sake of documentation or to satisfy the auditors.

6.8 LABORATORY RECORDS

Laboratory records serve a large number of purposes, the important amongst them being:

- ➢ As documentation of whatever has occurred
- ➢ As point of reference for establishing facts regarding an incident
- ➢ As an aid in the recognition of trends and resolution of problems
- ➢ As a way to establish credibility of the laboratory
- ➢ It is mandatory to maintain records in a uniform pattern. The uniformity is to be ensured by the top management of the laboratory. The recordings are made in pretested and standardised proformae which will vary according to requirements.

Format

Most records are paper forms that are manually completed by personnel. However, modern technology has also brought in the use of computers for better storage and retrieval of data. Computers can also be used for generation of hard copies for sending to the user as well as compiled data in the form of reports to supervisors or regulatory agencies.

There are various methods by which records can be stored, e.g. numerical, chronological and alphabetical systems. All systems are acceptable provided they are capable of generating data and reports without error, in the least possible time and that they are cost-effective.

Retention of Records

There are no hard and fast rules regarding the period for which records should be retained in laboratories. In general, records are kept for two years, except for recording and reporting instruments and equipment which have to be kept and maintained for the life of the equipment. Irrespective of the period of retention, the storage should be such that it permits easy access for review, as required.

Necessary Laboratory Records

Various types of records are required to be maintained in the clinical microbiology laboratory. Of these, a few are briefly described hereunder:

Accession List

This list records all specimens that are received in the laboratory for processing. The information to be included in this list is given below:

- Name of patient
- Identifying number (OPD No. / Admission No)
- Specimen type and source
- Name of test requested
- Date of collection (if possible time of collection also)
- Time and date of receipt in the laboratory
- Specimen accepted or rejected. If rejected, reason for same, e.g.
  - improper labelling
  - improper collection
  - improper transport of specimen

Requisition Form

The requisition form is the record on which the test of the specimen requested by the patient's attending doctor is recorded. A suggested list of information in the requisition form, which obviously has to be separate for each specimen, is provided below:

- Name, age and sex of patient
- Identifying number (OPD and/or admission number)
- Location of patient (Ward and bed number)
- Presumptive diagnosis with clinical notes

- Antimicrobial therapy, if any, prior to collection of specimen
- Specimen type and source
- Name of tests requested
- Date of collection of specimen
- Date of transportation of specimen
- Name of physician

Work Card

A work card is assigned to each specimen so that procedures performed on the specimen, notes by the laboratory personnel, results obtained and interaction between the physician and the laboratory staff can be recorded. A properly completed work card can be used to reconstruct and assess the accuracy of the final report. It should contain the following information:

- Name, age and sex of patient
- Identifying number (OPD and/or admission number)
- Specimen type and source
- Name of test requested
- Initials of laboratory personnel performing procedures
- Procedure, date performed and media used
- Preliminary results with
  - direct microscopy
  - culture
  - colony characters
  - biochemical and other tests for identification

- Final diagnosis
- Antimicrobial susceptibility and resistance
- Any discussion with physician

Test Report

Test reports convey the laboratory data to the doctor requesting the test. These must be unambiguous and precise and become part of the permanent hospital record of the patient. The desired information in these reports include:

- Name and location of patient
- Identifying number (OPD and/or admission number)
- Name of physician requesting the test
- Specimen type and source
- Name of test requested
- Date specimen received
- Date specimen processed
- Name of laboratory personnel performing the test and reporting

There are four types of reports that can be sent out from the laboratory.

Telephonic report: Critical information that may be urgently required by the physician should be reported by telephone, followed by a proper report on proforma. Positive microscopic smears, antigen tests, detection of growth from normally sterile sources or detection of highly infectious diseases should be communicated by telephone.

Preliminary report: This indicates the status of test results within 24 to 48 hours of receipt of the specimen. If a

tentative diagnosis has been established by this time, this may be reported.

Final report: This conveys results after the test is completed. Results are to be reviewed for erroneous information before the final report is placed in the patient's chart.

Corrected report: In case an erroneous report has been sent to the physician, he should be immediately contacted and informed about it. A corrected report, duly labeled as such, should be sent.

All reports should be concise, unambiguous, relevant and should include normal values as far as possible. A few lines regarding the clinical importance of the findings may also be useful to the physician, e.g. isolation of less than significant number of bacteria in a sample of urine from a patient who has been bedridden for a long period, from a pregnant woman or from a paraplegic may have diagnostic importance.

Quality Assurance Cards

These records of control values for procedures, tests, and equipment are used to identify problems and trends within the laboratory. These cards help identify the problems and trends associated with the application of laboratory data by the end user. A list of relevant information is provided below:

- Date
- Name of person performing the test
- Acceptable range of values
- Control results
- Lot number of reagents used

- Expiration date of reagent used
- Corrective action
- Name of person reviewing results

Various types of quality assurance records that need to be reviewed ***monthly*** include the followings:

- Tests and procedures
- EQA (depending upon the periodicity of participation)
- Temperature records for incubators and water baths
- Procedural manuals
- Equipment preventive measures and repair records
- Equipment function checks

Incident Reports

Incidence reports document problems related to the performance of care givers (e.g. collection, labeling or transport of specimen; complaints; uncooperative behaviour of laboratory personnel in processing of specimens, safety violations). The information that has to be incorporated in these is as follows:

- Identification of the problem
- Date
- Names of persons involved
- Corrective action taken
- Name of reviewer
- Date of review

Safety Records

Safety records document that all employees with a potential occupational exposure to hazardous chemicals or potentially infectious material have participated in a training programme at the time of employment and annually thereafter. This document should have following information:

- Dates of training sessions
- Contents of training sessions
- Names of persons conducting training
- Names of persons attending training

Reportable/Notifiable Disease Record

This record reports cases of infectious diseases as required by the Government. The report may be based on a clinical syndrome, the isolation of a microorganism or positive serological test. The information to be included is:

- Patient demographics
  - Name
  - Address
  - Hospital registration number
  - Occupation
- Disease
- Test results
- Name and address of physician

Similarly personnel record which includes information on the ongoing personnel training and the level of certification and training should also be maintained.

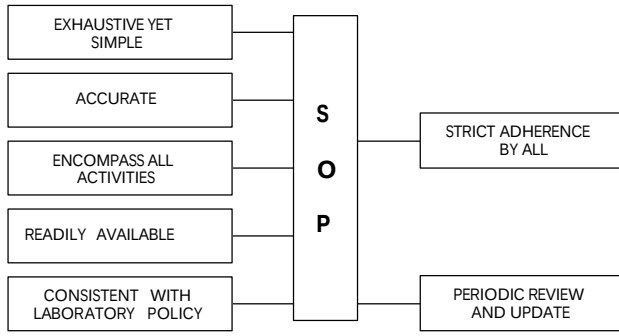
Documentation is the key to a quality system. It helps to ensure consistency of the processes and procedures. Documentation facilitates traceability, helps in identifying the problems and assists in decision making to improve quality. Good documentation indicates a good functional quality system.

7

STANDARD OPERATING PROCEDURES

THE STANDARD operating procedures (SOP) is the most important document in a laboratory. It describes in detail the complete technique for performing tests. This is extremely important in microbiological work to ensure that consistent and reproducible results are generated. The factors that are extremely important with respect to SOP are shown in Figure 7.1 and Table 7.1.

Figure 7.1



The instructions given in SOP must be strictly adhered to by all those who are related with the functioning of the laboratory. A few important points about SOP are:

- ➢ Each section of the laboratory should have a copy of SOP which should be easily accessible to all
- ➢ SOP should be available on the work bench area
- ➢ It should be reviewed annually
- ➢ SOP should contain only those procedures which are currently in use
- ➢ Any change in the SOP must be documented by recording it and having it duly signed by the Chief of the Laboratory

***Table 7.1**: Essential components of a SOP*

Abbreviated administrative structure diagram Indicating the line of authority in the laboratory.
Laboratory safety instructions Including emergency measures.
Specimen collection Including a statement on the condition under which the sample is to be collected properly from the patient, or environment including techniques for selection, collection, and transportation of specimens. It should also include criteria for the rejection of a specimen and the action to be taken in case the sample is rejected.
Inoculation procedures Enlists media required for each specimen, type and plating or inoculation technique used for this purpose. The temperature, duration and environment in which the samples are to be incubated should be indicated.

Details of procedures

Gives in detail the examination procedure indicating differential tests, flow charts or keys as well as identification criteria.

Differential tests

Need description in detail regarding

- Title
- Principle
- Material (e.g. colony to be tested)
- Reagents
  - (a) source
  - (b) preparation technique
  - (c) storage technique
- Standards and controls
- Directions for performing the test
- Interpretation of results
- Commonly encountered problems and their solution

Antimicrobial susceptibility testing

Organism that can be tested and the antimicrobial agent which is to be used for testing.

Serological testing

Includes details as given above under heading differential tests.

Reference to higher laboratories

How to use the reference laboratories

All staff must understand the limitations of his laboratory and quickly despatch relevant samples to the Reference Laboratory with a request for appropriate tests for which the forwarding laboratory is not well-equipped.

Quality control

Including the laboratory's written policy stating time and frequency for performing quality control steps for media, reagents, antibiotic discs, sera. Instructions must state what control results are acceptable and what results are not. It is also important to specify how these results are to be recorded and the actions to be taken when deviations occur.

Reporting

Including clear cut instructions about reporting results.

Some of the tests take a longer time for completion. In such cases, the preliminary reports can be issued. The details of such reports must be indicated in the SOP, e.g. if acid fast bacilli are detected under the microscope in sputum from a patient clinically suspected to have tuberculosis, the report should be "acid fast bacilli seen". The report of *Mycobacterium tuberculosis* can be given only after the culture and the biochemical tests have confirmed the identity of the isolate. Finally, the SOP must also include steps for preventive maintenance of various equipment and materials used in the laboratory.

7.1 STRUCTURE OF SOP

Each SOP should have two broad parts: one giving information about the SOP and the other describing the procedures *per se*. Followings are the essential information that must be provided as a part of the SOP:

- ➢ The laboratory or the section that can use it
- ➢ Name and title of SOP
- ➢ Unique identification code
- ➢ Version or issue number
- ➢ Date issued
- ➢ Name of person who has issued the document
- ➢ Name of the person who has authorized the document
- ➢ Page number with a reference to total page numbers

The part of SOP that describes the procedure should have following format:

- ➢ Introduction
- ➢ Scope

- Definitions
- Culture media and reagents/material
- Equipment
- Sampling
- Test procedure
- Results
- Calculation
- Quality assurance
- Reporting
- References

7.2 DOS AND DON'TS ON SOPS

Following guidelines will help in drafting better SOP.

- There must be no gaps or duplications
- Must have no conflicting statements
- SOPs must be distributed to each workplace to ensure easy access. Well fingered SOPs indicate that these are being accessed and used
- SOPs are references to the standardized ways of doing things in an organization
- Procedures must only be written if the procedure may have an effect on the quality of product or service
- Write an SOP on SOP. This SOP outlines the format of the written document
- Train staff to write SOPs and then get people to identify SOPs to be written

SOP needs to include the followings:

- The Name of the Organization
- The SOP title
- Revision number e.g 0 for initial then consecutive numbers 1, 2, 3, etc.
- Paginate each page e.g. Page 1of 3, Page 2 of 3, Page 3 of 3
- Date on which this document becomes the operating standard within the organization (Effective date)

SOPs should be written by the people doing the work. The draft SOP should be reviewed by, and comments made by those who are performing same kind of work. In addition, persons who will be directly or indirectly affected by the SOP must review the draft procedure and suggest the necessary changes.

Validation of SOPs is done to ensure that the activity, outlined in the SOP, has been investigated and proven to be reliable. SOPs are essential part of a quality system which must have following characteristics:

- SOPs should be written for all the procedures in the laboratory
- SOPs must be clear, concise and easy to follow
- SOPs should be used for staff training
- SOPs should be living documents
- Staff must have easy access to the SOPs
- SOPs must be followed by all staff members all the times

8
VALIDATION

A VARIETY of equipment, processes and software are utilized by the health laboratories in processing the clinical material. Generation of quality report depends upon the performance of all these on acceptable line. Before commencement of processing the clinical specimen one must ask the question: does this thing (equipment/process/software) do what it is supposed to do? In simpler words, equipment, processes and software need to be validated before these are used to demonstrate that these will do what they are expected to do (incubator set at 37°C must be shown to have really 37°C in its chamber to qualify as an validated equipment).

8.1 DEFINITION

ISO 9001 defines validation as the attaining and documenting of sufficient evidence to give reasonable assurance, given the current state of science and the art of

manufacture, that the process, system and test method under consideration consistently does and/or will do what it is expected to do.

Validation is that part of a quality assurance system that evaluates in advance the steps involved in operational procedures or product preparation to ensure quality, effectiveness and reliability.

8.2 PROCESS OF VALIDATION

Equipment, processes, software and *in-vitro* devices need to be validated before their use in the laboratory analysis. These need to be revalidated periodically as well as whenever there is any change in these.

Planning a validation is essential. The aim of the validation and the user requirements must be clear (what does the user want it to do and what does it need to have to be able to do it?). In complex systems, the simple approach is to validate the smallest component parts first and then gradually validate by putting the parts back together.

Responsibility for validation usually rests with the head of the department but this can vary in different organisations. Quality staff may be involved or review the final documents

Process of validation

- ➢ Plan and define aims of validation
- ➢ Develop protocol for the process of validation
- ➢ Execute the process and collect data
- ➢ Compare results against agreed requirements
- ➢ Consider any other issues: e.g. health and safety

- ➢ Accept or reject
- ➢ Document
- ➢ Implement
- ➢ Review

Validation is that part of a quality system that evaluates in advance the steps involved in operational procedures or product preparation to ensure quality, effectiveness and reliability. This is a tool that controls changes. Validation ensures that when new process/equipment or software is introduced it performs correctly thus ensuring that the quality of the product is not compromised. In some instances this may be necessary for regulatory requirements.

9
ASSESSMENT OF QUALITY

MONITORING and evaluation are two important and vital components of a quality system. Though both are tools for judgment of quality, there are a few essential differences between the two (Table 9.1).

Table 9.1: ***Essential differences between monitoring and evaluation***

Monitoring	Evaluation
Concurrent	Retrospective
Continuous	Periodic
In-house	External or internal
Part of supervision	Assessment

9.1 MONITORING

Main objectives of monitoring are

- ➢ to confirm consistency
- ➢ to alert to change
- ➢ to assess the impact of changes to processes or procedures
- ➢ to identify opportunities for improvement
- ➢ to provide objective measurements

The decision as to what to monitor depends upon the work-area and the activities. It is really common sense what is monitored, but it is usually best to start with parameters that are easily measurable and have profound influence upon the quality of the laboratory results.

This may mean starting with the final output of processes rather than the intricate details, but that is acceptable as long as the factors that influence the final outcome are known.

Once the decision has been made to monitor and what to monitor, the question is how to collect the appropriate data and analyze the same.

9.2 ASSESSMENT OF QUALITY

The retrospective and periodic assessment of quality can be undertaken by an independent external agency or internally by the designated staff on behalf of the laboratory management. Quality can be thus assessed by an on-site inspection by trained professionals (viz. auditors) or by processing of the material sent by a designated institution. Accordingly, assessment of quality can be man-driven or material driven (Table 9.2).

Table 9.2: ***Types of assessment of quality***

Man-driven	Material-driven
On site inspection and observation • Internal audit • External audit	Internal quality assessment External quality assessment

The main objective of external quality assessment (EQA) is to establish inter-laboratory comparability. This will influence the reliability of future testing. In contrast, the main objective of continuous monitoring of quality is to ensure day-to-day consistency. Hence, *both monitoring of quality in its implementation and quality assessment are complementary in ensuring the reliability of procedures, their results and finally the quality of the product*.

Important differences between monitoring and EQA are shown below in Table 9.3

Table 9.3: ***Essential differences between monitoring and EQA***

Feature	Monitoring	EQA
Nature	Concurrent and continuous	Retrospective and periodic
Performed by	Laboratory staff	Independent agency
Objective	Release of reliable results on day to day basis	Ensure inter-laboratory comparability and improve performance

Quality assurance (QA) is a broad term encompassing both monitoring and quality assessment. The term quality

control is now restricted to an activity which includes, apart from others, quality control of stains, media, reagents etc. It is not synonymous with quality assurance.

9.2.1 External Quality Assessment Scheme

The assessment of quality in a schematic way through an external agency using material of known but undisclosed results is called as external quality assessment scheme (EQAS). This is considered a powerful tool that challenges the internal quality control measures that are being adopted by the laboratory. EQAS is a tool by which entire testing process including the quality of results generated by a particular laboratory is assessed.

External quality assessment scheme compares the performance of different testing sites and is the challenge of other components of the quality assurance system (internal quality control). This assessment is achieved through processing of specimens of undisclosed but known contents. It measures the accuracy of the results.

EQAS was earlier known as proficiency testing. It is not correct to consider EQAS and proficiency testing as synonymous since in current terminology proficiency testing denotes competence assessment of individuals and not material-driven external assessment.

Objectives of external quality assessment scheme

EQAS are organized to achieve the following objectives:

- Monitor laboratory performance and evaluation of quality control measures
- Establish inter-laboratory comparability
- Influence reliability of future testing

- Ensure credibility of laboratory
- Stimulate performance improvements
- Promote high standards of good laboratory practices
- Encourage use of standard reagents/ methodology and trained personnel
- Identify common errors
- Provide mechanisms to remedy identified deficiencies
- Facilitate information exchange
- Support accreditation
- Education through exercises, reports and meetings

Benefits of EQAS

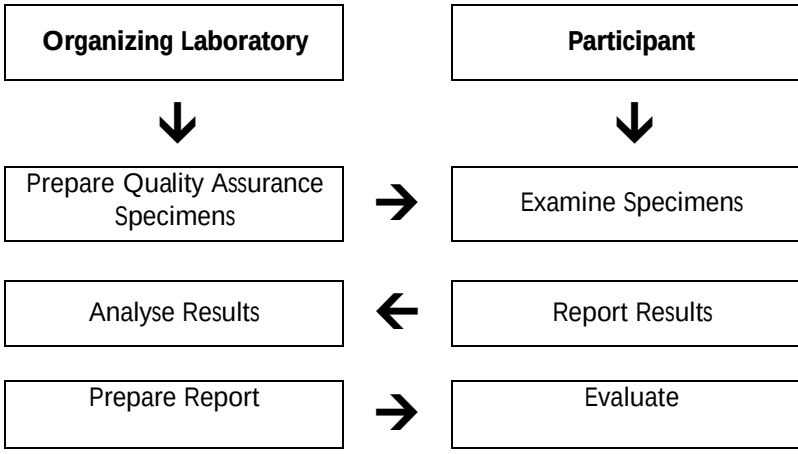
- Helps laboratories in comparing their results with other laboratories
- Acts as an educational stimulus to laboratory staff
- Participation provides credibility to the laboratory
- Helps the health administrators and regulatory agencies to have an insight into the status of quality across the country, identifying the problems and devising methodology to overcome these

Process of EQAS

EQAS requires a well equipped, experienced laboratory at intermediate or central level to act as the organizing laboratory and a fairly reasonable number of laboratories as the participating laboratories. The process of EQAS with

important functions of organizer and the participating laboratories has been shown in Figure 9.1

Figure 9.1: *Steps involved in EQAS*



Basis of success of EQAS

Various factors that may influence the success of any EQAS are:

- Voluntary participation
- Confidentiality of individual reports
- Avoiding provocative statements about the performance
- Identifying unsatisfactory performers in groups or individuals
- Providing educational opportunities
- Organizer acting as adviser rather than enforcer

The EQAS are targeted for participating laboratories who are its customers. Their needs have to be met. The

participating units should aim to gain on following accounts with participation in the EQAS:

- Comparison of performance and results
- Minimisation of errors
- Self appraisal
- Objective evidence of quality/accreditation
- Identification of training needs

Desirable features of EQAS

The organization of EQAS is a complex task that requires considerable resources and expertise. One must aim to integrate following features into such schemes:

- Clinical relevance and match with the mandate of microbiology laboratories
- Comprehensiveness: should cover a large number of tests to satisfy the needs of diverse laboratories
- Versatility: may provide a combination of tests that laboratories can pick up
- Frequency of distribution should be adequate to allow participants to regularly assess their functioning
- Material distributed should be derived from clinical source to provide realistic and relevant challenge to IQC and must be of required quality
- Availability of repeat specimens
- Timeliness of feedback
- Availability of individual performance data
- Number of participants should be large enough to ensure reliability of consensus results

- Independence of the scheme: should not be influenced by any commercial or industry interest

Requirements of EQAS

EQAS includes submission of samples to participating laboratories; analysis by them and returning of the results to the EQA organizer who performs the statistical analysis and sends feedback to the participants so that they may judge their individual performances. The essential requirements are briefly discussed below:

The Material Supplied

The material supplied should be homogenous so that all the participating laboratories should receive the same material. All specimens of a quality assessment material that are sent out in a survey should be prepared from one source to assure maximum equality. The stability of the material after preparation (e.g. reconstitution) should be stated. The material must be stable over a period of time necessary for its transportation from the organizing laboratory to the participating units.

Documentation of Accompanying Material

Unequivocal documentation should accompany the specimens regarding the analyses to be included in the survey. The provision of a scheme to deal with different types of units should also be clearly stated. The address to which results should be returned, the manner of returning the results, and the last date on which results could be accepted for inclusion in the statistical analysis should all be stated.

Manner of Performing the Test

Laboratories should be encouraged to carry out testing of the specimens in the same routine manner as that is being employed for other similar samples.

Number of Participating Laboratories

In general, the greater the number of participating laboratories, the greater is the usefulness of the scheme in terms of subdividing the results according to the analytical technique. In addition, this provides knowledge about the performance of a large number of laboratories. However, there is obviously a limit to the size depending on the organization required and the funds available.

Statistical Analysis of Results

The statistical analysis of the results and the methods of displaying them should be easily understood by all participating laboratories. The performance of each participating laboratory should be assessed for individual determinations as well as for all determinations collectively. This should be done for each particular distribution and also over a period of time for several distributions. Results shall be disclosed to show whether quality is improving or deteriorating over a number of distributions.

Turn-Around Time and Frequency

The turn-around time for a programme is the time between the materials being sent out from the central agency to the time that the statistical calculations are available in all participating laboratories. For the programme to have maximum usefulness, this time should be as short as possible. In some schemes this has been accomplished in

days, in others it takes months. The shorter time resulting in faster feed back of information enables more relevant action to be taken if the quality is unsatisfactory. Equally, the frequency and number of the different types of analyses also have considerable importance.

It is of little value to survey clinical microbiological laboratories once a year. At the same time it is difficult to do it weekly. But the more frequently it is done, the better it is. Depending upon available resources and feasibility, a quarterly organization of EQAS is considered ideal. Continuous reminders of poor quality or continuous assurance of good quality and improvements are essential features of modern external quality assessment schemes.

Anonymity of Participating Laboratories

To make available the results of the performance of individual laboratories to other laboratories or agencies, or to preserve anonymity, is a choice which will have to be made in each individual country according to the policy decided by health authorities. In some schemes the purpose is to provide a service to laboratories for their own information, in others the purpose is one of surveillance for legislative objectives.

9.3 ORGANIZATION OF EQAS

Organizers

The organizers of external quality assessment schemes (EQAS) should be scientists or experts authorized to do so by the national health authorities. Administrative training alone is not sufficient since expert knowledge of microbiological techniques is essential for the success of the programme.

Principles

There can be different principles guiding the organization of EQAS. In some schemes, specimens are sent out every month to the participants; in other schemes they are sent out every three or six months. Some schemes check the precision only; others check for precision and accuracy. Some schemes send out two specimens (usually one specimen with values in the normal range and the other in the pathological range). Whatever principles are adopted by the organizers should be related to the participants in unambiguous terms.

Selection of Laboratories and Number of Participants

It is recommended that all laboratories should take part in EQAS. In general, it is said that an EQAS should not be started with too small a number of participants, since analyses of results will have no statistical validity. However, it is possible to commence EQAS with only a few participants, even less than 10 laboratories. Furthermore, if the organizers do not have previous experience, it is also inadvisable to start the programme with too large a group, since this would not permit the analysis of results and feedback to the participants in a timely manner.

Identification of the Laboratories

It is essential to employ a code system for identification of participating laboratories, i.e. the identity of the laboratory should be known solely to the person or group of persons who are analysing the results. The organizer should guarantee that he will not reveal the identity of any laboratory or give information on the results of one laboratory to other participating laboratories. The

participants will be able to compare their results with those of other laboratories but will be unaware of the identity of the laboratories. Anonymity, however, should not render aid to the laboratory impossible.

Frequency of Distribution

The frequency of distribution of quality assessment specimens in an EQAS is a matter of policy. There are schemes in Europe that have only two surveys per year, each survey with two specimens for investigation. Other EQAS have 3,4,6 or 12 surveys per year. Each of these has its advantages and disadvantages (from the educational, organizational and economical point of view). EQAS with 12 surveys are usually more expensive. A frequency distribution should be chosen which makes possible good preparation of the materials to be distributed and careful analysis of the results from the laboratories.

Criteria for Assessment of Results

Clear definitions of the criteria on which results are to be based should be established by the organizer. The limits of acceptable results can be fixed as a percentage deviation from the expected values or as a standard deviation. The important point is that the participants should be fully aware of these criteria and of the calculations the organizer will make.

With regard to the time which should be given to the laboratory for analysing the material it received, it has been observed that a period of one week is usually suitable. If the time allowed is too long, the laboratory will forget to process the sample. Once the organizer receives the results he should communicate with the laboratories as soon as possible so that they can relate the analysis of the results to

any possible errors they may have made. In this way the EQA will also have an educational function.

The presentation of the analysis of results should be clear and precise so that the participants can easily judge the quality of their work and can correct errors wherever necessary.

Selection of Investigations and Help to Laboratories

An EQAS should incorporate as many investigations as are representative of the total work performed by the laboratories. At the same time, it is not recommended to commence a quality assessment scheme if there is no possibility of helping laboratories that produce poor quality results. To identify sources of errors and suggest solutions, laboratory experts with considerable experience are needed.

9.4 SCORING SYSTEM IN EQA

The objective of any EQA scheme is to stimulate performance and bring interlaboratory concordance. Thus participants require a clear demonstration of whether their results are in consensus, i.e. whether or not any corrective action is needed. Many participants experience difficulty in comparing their results with the target data, whether they be in the form of designated values, histograms or statistical parameters classified according to method etc. In addition, experience indicates that it is usually just those laboratories which have the least time, inclination and ability to devote to this task, which also have the most need to understand the system.

Semi-Quantitative Scoring Systems

In this system, results are classified on a semi-quantitative scale, e.g. the allocation of points according to how close the results approach the designated results A three -point rating system is in use in EQAS for clinical microbiology. For the identification of unknown organisms, a score of two is awarded for a correct answer, one for a partially correct answer and zero for a totally wrong answer or failure to answer. Similarly for antibiotic susceptibility testing, a distinction is made between minor and major errors. A minor error is when a resistant or susceptible organism is reported as intermediate or vice versa, while a major error is the substitution of resistant with susceptible result and vice versa.

Scoring as a Stimulus to Laboratory Improvement

Scoring systems are a viable means of data reduction, to assist participants in assessing their performance relative either to other laboratories or to a standard. This objective applies to the individual laboratory situation.

The most primitive systems give information in qualitative form, e.g. pass or fail which gives only the crudest reflection of performance. Most sophisticated systems yield information as a numeric score, retaining the potential for easy interpretation and being amenable to graphical presentation. These have been of great assistance in enabling laboratories to recognise the existence of suboptimal performance and in stimulating them to improve.

An analyst receiving a report needs to make decisions on a series of questions which are usually self terminating when a negative answer is given:

- ➢ Do I have a major overall problem?
- ➢ Which factors are contributing most to this?
- ➢ Are these problems significant?
- ➢ What is the source of errors in each case?

A well designed combination of a scoring system and a report format can assist considerably in this process, and thus contribute to patient care not only through stimulation of improvement where this is indicated but also through removing the need for unnecessary investigation.

Assessment of Progress - Comparison Over Time

A well designed scoring system should be able to delineate changes in the performance of an individual laboratory over a period of time. The main requirement for this is that the score should be independent of performance of other laboratories. Such scoring systems can enable assessment of trends in interlaboratory agreement, and provide means to judge the overall success of the scheme in stimulating improvement.

EQAS process

A summary of the process from when the decision is made to set up an EQAS to the time a report is produced and distributed to participants involves not only planning, research, evaluation and validation etc, but the actual process of designing, manufacturing, distributing the panel then collecting, collating and analysing the results and sending out a report before considering the appropriate feedback.

The steps involved include:

- ➢ Forwarding an initial questionnaire to determine which tests are used by prospective participants and other information
- ➢ Seeking potential participants
- ➢ Panel preparation
- ➢ Panel distribution
- ➢ Collection of results from each laboratory
- ➢ Forwarding of preliminary report containing reference results to the participants
- ➢ Collation and analyses of results
- ➢ Communication of final report to the participant
- ➢ Recommendations for performance improve-ment to participants
- ➢ Award of EQAS certificates

9.5 INTERNAL QUALITY ASSESSMENT

Internal quality assessment (IQA) is similar to EQAS except that the material is prepared, distributed, evaluated and results assessed internally. IQA can be designed to meet the need of the laboratory and larger number of specimens can be provided as a challenge to IQC.

Clinical material is split in two and one is allowed to be processed in routine. The other carries same medical information that may be needed to interpret the results. These specimens are labelled as QA specimens.

The discrepancies are observed, recorded and analysed by a senior professional in consultation with quality manager and possible solutions suggested to prevent recurrence of such discrepancies.

Applications of IQA

The applications of IQA include

- ➢ Assessment of variability in tests where subjective interpretation plays an important role
- ➢ Statistical analysis and confidence limits on repeat testing
- ➢ Assessment of effect on changes in procedures or introduction of a new technique

For internal quality assessment (IQA), the specimen is split in the laboratory and one half is processed as a patient's specimen and the other half by the same protocols as a known IQA sample. This provides a measure of precision and throws light on the effectiveness of the quality system.

Re-examination of specimen in another laboratory can be done by normal referral procedures when results obtained with specimens submitted to reference laboratory for confirmation are checked against the sending laboratories original results. An alternative that is usually employed for initial assessment of validity of data is to select fixed percentages of negative and positive specimens reported by the testing laboratory and re-examine them in a reference laboratory. This is an expensive method and usually not feasible in routine.

10
QUALITY AUDIT AND ACCREDITATION

10.1 QUALITY AUDIT

QUALITY AUDIT is the process of critical review of the laboratory. Once a quality assurance programme has been developed and implemented the only possible way a laboratory can verify its effectiveness is to carry out regular audits.

Definition

Quality audit is defined as a planned and documented activity performed in accordance with written procedures and check-lists to verify by investigation, and the examination and evaluation of objective evidence, that applicable elements of a quality assurance programme have

been developed, documented and implemented. Stated more simply, internal audit is a way to establish whether all activities that affect quality are being carried out.

Internal audits are also called **first party audits** i.e. those audits which are performed by the staff of laboratories themselves to inspect their own system. It is better if internal audits are carried out by staff members who are trained in audit techniques, and who are objective as well as competent.

The basic principle behind internal audit is that no quality system will be developed and continue to be maintained without regular testing by the use of audit techniques. Internal audit is a mechanism which allows managers to maintain a better understanding of the day to day work of their department and, through the review process, gives them the opportunity to make management decisions based on the information obtained. The objective of the drill should be to audit the quality of the system and not the staff. The deficiencies observed should form part of a noncompliance report which should be submitted to top management.

Each laboratory should nominate its own auditors. Internal audit is not easy and requires some training. The auditors obtain information by

- Interviewing staff
- Checking documents (SOP, work sheets etc)
- Observing processes
- Checking status of equipment
- Observing organization of laboratory area
- Examining biosafety measures and waste disposal mechanism

Internal audit has been accepted as a valuable tool that helps to maintain standards in working environment, keeps management better informed about the performance of the laboratory and to provide a mechanism for continuous quality improvement (CQI).

Internal audits are normally the most frequent, they are performed by internal staff from other departments/divisions and are normally part on an ongoing program. Internal audit should be performed at least once a year.

External audits **(2nd party audits)** are normally supplier audits performed to ensure that goods supplied are of the required standard. This is a GMP requirement and they are especially needed when suppliers change and on a planned basis for regular suppliers. This also depends upon the quality of the service provided by the supplier.

External audits **(3rd party audits)** are normally those performed by regulatory/statutory bodies and are usually mandatory; they are on a planned basis as required by the overall responsible body (often the government or government authorised body.

Benefits of Quality Audit

The benefits of audits must be emphasised all the time. The term 'improvement opportunities' is very useful in helping some overcome the fear of audits. The 'independent view' is very valuable as often people 'too close' to the work cannot see the need for change/improvement

- ➢ Quality audits can help work areas make changes when they know that changes are needed but are

constrained in some way - the audit can act as an additional lever

- A well performed quality audit can help boost the confidence of staff in what they do
- Quality audits can help focus everyone on the real issues rather than just what people think are the issues
- Quality audits are essential to maintain and improve quality levels
- Quality audits must be performed and received as positive events
- Quality audits should be viewed as 'improvement opportunities'
- All staff should become involved and be able to contribute to the quality audit and its outcome

Factors Influencing Quality Audit

Auditors need to observe and inspect following factors to assess the quality in overall functioning of the laboratory

- Overall approach of the organization to quality
- Management support to the quality system
- Status of quality system
  - Documentation
  - Practice according to agreed procedures
  - Awareness of staff of the need for quality and their understanding of the implications of poor quality
- Is the quality system actually functional

10.2 ACCREDITATION OF LABORATORIES

Accreditation is an approved procedure by which regulatory authorities or an authorized body accord formal recognition to a laboratory to undertake specific tasks provided that predefined standards are met by the laboratory. Standards for laboratory accreditation have been developed by the International Standards Organization.

This system is in progress in various developed countries where it is mandatory for laboratories to be accreditated before commencing their functions. It may be possible to introduce it in developing countries also in the near future. In essence this is a process of inspection of laboratories and their licensing by a third party to ensure conformity to pre-defined criteria pertaining to various aspects of infrastructure and functioning of laboratory.

The process of accreditation starts with the request of the laboratory for accreditation. The accreditating authority deputes inspector(s) to assess the laboratory on the basis of specific criteria. The report of the inspectors may recommend accreditation of the laboratory or may suggest some corrective measures. In either situation, the laboratory is informed of the decision of the authorities. The deficiencies pointed out by inspectors are to be rectified before formal recognition is accorded.

This system helps in ensuring good quality laboratories, since those which do not conform to the pre-defined criteria are not given licenses to undertake laboratory activities.

Standards for inspection of laboratories

A. Organization and Administration: The laboratory should have a document describing its scope of work, defined objectives, adequate financial strength and adequate interaction between the management and the technical staff.

B. Staffing and Direction: The number of staff members, their educational qualification and experience in similar settings should be adequate. Each staff member should have written description of his job profile. Continuous education after an initial induction training should be mandatory

C. Facilities and Equipment: The space for reception of patients, collection clinical materials, performance of tests, disposal and sterilization as well as storage of material should be adequate. The working environment should be safe. Sufficient data storage, retrieval and communication facilities should exist. Appropriate equipment and provision for their maintenance should be ensured and there should be a suitable system of maintenance of records and issuing of reports.

D. Policies and Procedures: A standard operating procedures (SOP) for each technique should be available to all the technical staff. This must include instructions for collection, storage and transportation of specimens and their disposal. Written instructions should also be available to field staff regarding this.

E. Staff Development and Education: A continuing education programme for all staff members should form a part of the policy of the laboratory and adequate resources must be made available for in-house as well as outside

training activities. A system for appraisal of staff members should also be in position.

F. Evaluation: The laboratory must have a formal policy for internal quality control and must participate in relevant external quality assessment scheme.

Accreditation is not a one-time affair. A periodicity of (may be of 2-3 years) should be decided to reassess the laboratory to ensure that standards are maintained.

11
SAFETY IN THE LABORATORY

LABORATORY safety is a vital part of any total quality control programme. The basic concept is the use of *common sense* within the laboratory and in the use of all techniques. There is clearly a potential risk of infection to workers who continuously come in contact with pathogenic organisms. Even experienced workers are at risk and there is no microbiological laboratory where the potential hazard of acquisition of infection has not been recognized.

Laboratory-acquired or laboratory-associated infections are preventable only if a strict safety policy is enforced. The laboratory is staffed with safety-minded people and written safety instructions as required by each employee are made available and understood.

The brief description given below is intended to describe some of the hazards that are constantly present in a microbiology laboratory. Awareness of these hazards and biohazards is the first step towards establishing a safe laboratory environment. The attitudes and skill necessary for controlling these hazards and the continual reinforcement of good laboratory practices are essential to sustain a safe and healthy microbiology laboratory.

11.1 PRACTICE OF LABORATORY SAFETY

As a basic rule, microbiological laboratories should have sufficient space, equipment, and facilities for the performance of the required volume of work with optimal accuracy, precision, efficiency and **safety**. Safety is emphasized because of hazards inherent in many phases of laboratory operations. These include fire, chemical hazards, microbiological hazards, electrical hazards, and various other physical and radioiostopic hazards.

Fire

Fire is a potential hazard in almost any environment. Instructions for emergency action in case of fire must be prominently posted and must be required reading by all the employees. Some of the important preventive measures are described below.

- ➢ Prominent display of emergency directions
- ➢ Fire drills, some of which may be without warning
- ➢ Distinct marking of fire exits
- ➢ Fire exits kept unblocked
- ➢ Fire extinguishers: Available, functional and staff trained in their use

- ➢ Installation of fire alarm
- ➢ Declaration of the laboratory as a no smoking zone
- ➢ Proper storage of flammables and explosives

Open ether cans must not be stored in refrigerators which are not explosion-proof. Bulk storage of flammables must be arranged to minimize the hazards of explosion and fire.

Unbreakable containers (polyethylene or metal) should be used for safe bulk storage of flammables. The storage area for flammables should be provided with explosion-proof electric switches and fixtures and suitable fire extinguishers.

Chemical Hazards

The use of hazardous chemicals in the laboratory can cause serious injury to workers if they are not instructed and trained in how to handle them safely. Overt exposure can result in serious tissue damage and acute adverse effects. Safety goggles should be worn when there is a potential hazard of chemical splashes. Various other preventive measures which should be instituted are given below:

- ➢ Provide facilities for rapid flushing of chemicals from clothes, skin and eyes
- ➢ Label chemicals properly
- ➢ Prohibit mouth pipetting

- ➢ Provide emergency shower
- ➢ Provide step-on eye washer
- ➢ Provide safety goggles or full-face respirators as needed

Electrical Hazards

Some electrical units can cause electric shock and fire hazards. Explosions in electrophoretic units causing fires have occurred frequently. The microwave oven has become a common piece of laboratory equipment, but its use has introduced a significant new hazard to the laboratory. Careless operating procedures have resulted in violent release of superheated fluids and explosions caused by rapid pressure build up in accidentally sealed containers. Serious injury is always a potential consequence of the misuse of this equipment. To prevent these all electrical equipment should be earthen (grounded); information regarding location of master switch be prominently posted and uninterrupted source of power should be provided.

Microbiological Hazards

Microorganisms will invariably be found in microbiology laboratories. The greatest risk of occupational infection in these laboratories is associated with the use of pathogenic microorganisms or the handling of contaminated material. The ability to prevent laboratory-acquired infections requires skill and knowledge which can best be acquired through training and careful guidance. It is necessary that proficiency in microbiological techniques be acquired through practice with nonpathogenic microorganisms before higher-risk microorganisms are introduced into the

laboratory routine. The salient preventive measures (Table 11.1) must be practised by all the workers.

Table 11.1: ***Preventive measures against laboratory-acquired infections***

➢ Protect workers, patients and cultures
➢ Perform adequate sterilization before washing or disposing waste
➢ Provide receptacle for contaminated glassware
➢ Provide safety hoods
➢ Ensure that tissues are handled and disposed of properly
➢ Promote regular handwashing and cleaning of bench tops
➢ Ensure use of gloves
➢ Provide mechanical pipetting devices
➢ Protect patients from laboratory personnel with skin or upper respiratory tract infections
➢ Provide special disposal containers for needles and lancets

Apart from these, there are numerous other hazards in the laboratory. **Lacerations** are common laboratory injuries. They are most frequently caused by accidents involving the handling of razor blades, scalpels, scissors and other cutting instruments, glassware and Pasteur pipettes. The considerable use of **flammable solvents** creates potential fire hazards associated with storage, use and disposal practices. The storage of samples in **liquid nitrogen** creates the potential for injury from accidental skin contact and from explosion when improperly sealed vials are removed from storage.

11.2 BIOHAZARD LEVELS

Safety begins with the collection of the specimen. The approach is not only to protect the specimen from contamination, but also to protect the laboratory and other personnel. Specimens should be collected in sturdy containers with adequate closure to prevent spillage or leakage. Clinical information must be available for instituting adequate precautions and proper handling. Specimens suspected of containing highly infectious agents should not be placed in a container with numerous routine specimens. The laboratory worker must treat each specimen as a potential hazard to his health and that of his colleagues in the laboratory.

Good laboratory practices protect the specimen and the worker. Rules of good technique and hazard awareness are especially important in the clinical laboratory because most highly infectious agents isolated from clinical specimens are a surprise to the clinician as well as to laboratory personnel.

Level 1 Biosafety

Level 1 biosafety is not appropriate for work with pathogenic bacteria. This is applicable to basic practices appropriate for undergraduate and secondary educational training and teaching laboratories for which introductory microbiological protocols would involve only defined and characterised strains of viable microorganisms not known to cause disease in healthy adult humans. Emphasis is placed on:

- ➢ the use of mechanical pipetting aids
- ➢ handwashing

- ➢ not eating, drinking or smoking in the work area
- ➢ daily decontamination of the work surface

Examples of agents that can be handled at biosafety level 1 are *Bacillus subtilis, and Staphylococcus epidermidis*. Many agents not associated with disease process in humans, however, may prove to be opportunistic pathogens capable of causing infection in the very young, in the aged and in immunodeficient or immunosuppressed individuals.

Level 2 Biosafety

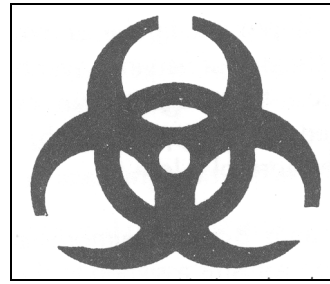
Level 2 biosafety practices are obligatory for most laboratory activities involving known pathogens and for experiments involving either the introduction of recombinant DNA into pathogenic bacteria or the introduction of DNA from pathogenic bacteria into nonpathogenic prokaryotes or lower eukaryotes.

These practices, equipment and facilities are applicable to clinical, diagnostic, teaching and other facilities in which work is done with a broad spectrum of indigenous, moderate risk agents present in the community and associated with human diseases of varying severity. With good microbiologic techniques, these agents can be used safely in activities conducted on the open bench, provided the potential for producing aerosol is low. Hepatitis B virus, salmonellae and *Toxoplasma* species are microorganisms assigned to this containment level.

Primary hazards to personnel working with these agents are accidental autoinoculation or ingestion of infectious materials. Procedures with high aerosol potential that may increase the risk of exposure of personnel should

be conducted in primary containment equipment or devices such as biological safety cabinets.

Figure 18.1 *Universal biohazard symbol*



Level 3 and Level 4 biosafety are not meant for activities expected to be performed by district laboratories.

11.3 ADMINISTRATIVE RESPONSIBILITY

Though the essentials of biosafety have been described, from the administrators point of view following salient features and checkpoints should be given top importance.

Laboratory Access

- ➢ Appropriate signs should be located at points of access to laboratory areas directing all visitors to a receptionist or receiving office for access procedures.
- ➢ The universal biohazard symbol (Figure 11.1) should be displayed at specific laboratories handling pathogenic microorganisms. Only authorized visitors should enter a laboratory displaying the universal biohazard sign. Doors

displaying a biohazard symbol should not be propped open, but should remain closed.

Clothing

- All employees and visitors in microbiological laboratories should wear protective laboratory clothing and shoe covers.
- Disposable gloves should be worn wherever microbiological work of moderate to high risk is undertaken (e.g. processing of blood samples for HBsAg and HIV testing).
- Laboratory clothing including shoes should not be worn outside the work area.
- Clothing from laboratories in which moderate and high risk virus manipulations are being performed should be autoclaved before sending these to laundry.

Eating, Drinking and Smoking

There should be no eating, drinking or smoking in any working area of a microbiology laboratory. Food should not be stored in a laboratory containing high, moderate or low risk materials.

Pipetting

There should be strictly no mouth pipetting in any laboratory.

Use of Containment Equipment

- Adequate precautions, with consideration for both equipment and operating procedures should be

taken to reduce the potential hazards of aerosol generating processes such as centrifugation, and grinding for both low and moderate risk materials.

- Pathogenic microorganisms should be handled in safety cabinets/hoods only.

Transportation of Infectious Material within Laboratory

Care should be taken while moving breakable containers of biological agents from work sites to cold boxes, incubators, centrifuges, etc. and in moving contaminated glassware etc. to a sterilizer prior to cleaning and discard. Pans with solid bottoms and side walls and/or instrument carts or trolleys should be utilized for the transfer.

Use of Liquid or Gaseous Disinfectants

- All contaminated laboratory ware and similar items to be removed from a laboratory to a sterilization site should be placed in containers and immersed in appropriate chemical disinfectant. The containers should be covered during transport and autoclave staging.
- Contaminated items too large for autoclaving or hot air sterilization should be hand wiped with disinfectant.

Housekeeping

- Dry contaminated wastes from laboratories should be collected in impermeable bags which should be sealed at the collection site before removal to the autoclave or incinerator. Metal cans with tight sealing covers may be used in lieu of bags. The

seals of both containers should be loosened appreciably during autoclaving to ensure sterilization of the contents.

- ➢ No dry mopping of the work area should be allowed. Vacuum cleaners may be used provided they are equipped with HEPA filters.
- ➢ Laboratory floors should be wet mopped with a disinfectant or detergent solution.

Sterilization of Used Material

- ➢ All contaminated materials should be decontaminated or sterilized before disposal or recycling. This should preferably be done before these materials leave the laboratory area, unless these can be moved in sealed or covered containers.
- ➢ Tissue culture or other virus containing liquid wastes should be decontaminated, either chemically or by heat, before being discharged to the community sanitary sewer system. Water from toilets, hand wash basins and personnel showers in a change room requires no special treatment.

Testing of Containment Systems

All biosafety equipment should be tested annually and certified as fit for use.

Good laboratory safety is a matter of education and training. One person should be incharge of all safety procedures and equipment who should carry out all educational and training services so that all the staff members are equally involved in ensuring safety in laboratory. There should be enforcement, discipline and

awards for safety; and there must be adequate funds allocated in the budget for safety. Good and efficient safety management is important because it achieves good quality.

11.4 ACCIDENTS IN THE LABORATORY

Most laboratory associated injuries and accidents result from inadequate standards of practice, ignorance and inexperience as well as thoughtlessness. Overcrowding, badly maintained equipment and poorly designed premises also contribute. Despite the prominence given in the literature to the risk of transmission of hepatitis B virus and human immunodeficiency virus (HIV) and the serious sequel of infections by these organisms in laboratory personnel, they represent only a small proportion of potential hazards.

11.5 CATEGORIES OF PATHOGENS

Microorganisms are classified into hazard groups on the basis of:

- ➢ pathogenicity for man
- ➢ hazard to laboratory personnel
- ➢ transmissibility in the community and
- ➢ availability of effective prophylaxis and treatment against that organism

There are four hazard groups:

Group 1 includes organisms which have no known or minimal potential hazard to laboratory personnel or the community (eg *Bacillus subtilis*).

Group 2 includes organisms which are of moderate potential hazard to personnel, but of limited risk to the community. Laboratory exposure rarely produces infection and effective prophylaxis or treatment is available (e.g. Salmonellae, *Staphylococcus aureus*, hepatitis B).

Group 3 includes organisms that cause severe human disease and which are a serious hazard to laboratory personnel. There may be risk of spread in the community. Effective prophylaxis or treatment is normally available (e.g. *Mycobacterium tuberculosis, Yersinia pestis*)

Group 4 includes organisms causing severe human disease and serious hazard to laboratory workers. There is a high risk of spread in the community and usually no effective treatment or prophylaxis is available (e.g. Ebola virus, yellow fever virus)

Each hazard group requires a defined containment level. "Containment" describes a safe method for handling infectious agents in the laboratory. From time to time variations in codes of practice are recommended for certain organisms because of changes in immune status of the individual or the community, or because of the dose, route and site of infection.

11.6 LABORATORY-ACQUIRED INFECTIONS

Of major concern is the infected specimen from a patient whose infection has not been recognized clinically. Generally high standards (good laboratory practice) are required to minimize the risk and "universal precautions" should be taken; that is, each sample should be tested as if it is potentially infectious. The minimum requirements which have already been outlined above should suffice. The risk of acquiring hepatitis B and HIV infections is well

publicized and they are probably the most frequent risk outside the microbiological laboratory. However, in the microbiological laboratory bacterial infections pose the most frequent risk. The important organisms/diseases are:

- Hepatitis B
- Tuberculosis
- Leptospirosis
- Brucella
- Mycobacteria
- Histoplasmosis
- Meningitis
- *Shigella species*
- *Salmonella species* including *Salmonella typhi*
- Anthrax
- Plague
- Coccidiomycosis
- Relapsing fever

Haemorrhagic fever specimens which may have Lassa, Ebola and Marburg viruses require containment level 4 facilities.

Accidents and Spills

The order of priorities is as follows:

- protection of personnel
- confinement of contamination
- decontamination of personnel
- decontamination of area involved

Decontamination of skin: The area should be washed thoroughly with soap and water. Detergents or abrasive materials must not be used and care must be taken not to damage the skin.

Decontamination of cuts/eyes: These should be irrigated with water, taking care to prevent spread of contamination from one area to another.

Decontamination of clothing: Contaminated garments should be removed immediately and placed in a container. They should not be removed from the spill location until contamination has been monitored.

Decontamination of work surfaces: The total spillage area including the broken container should be flooded with disinfectant and left undisturbed for 10 minutes prior to mopping with cotton wool or absorbent paper. Disposable gloves, apron and goggles should be worn. If a dustpan and brush or forceps have been used, these too require disinfection.

For most organic matter and bacteria clear soluble phenolics, diluted according to the manufacturers' recommendations are suitable. For blood or viruses, hypochlorites should be used. Blood spillage requires a concentration of 10 gm/L. Hypochlorite solution should not be used in centrifuges. Activated gluteraldehyde (20 gm/L) should be used on surfaces for viral decontamination.

All potentially contaminated materials should be placed in a separate container and retained until monitored. Entry to such areas should be restricted until contamination monitoring has been carried out.

Management of Laboratory Accidents

An adequately equipped first aid box should be kept in the laboratory in a place that is known and accessible to all staff members. The box must be clearly marked and preferably be made of a metal or plastic to prevent its being damaged by the pests. A medical officer should be consulted regarding the contents of the box. A first aid chart giving the immediate treatment for cuts, burns, poisoning, shock and collapse should be prepared and displayed in the laboratory.

11.7 GENERAL LABORATORY DIRECTIONS

No worker should be allowed to work alone and all laboratory workers must be familiar with the continuous practice of aseptic techniques. It is important to avoid any risk of contaminating oneself or one's neighbours with culture material. It is also important to prevent contamination of the work place itself with microorganisms from the environment. Some of the general laboratory directions are provided below:

- ➢ Bind back long hair neatly away from shoulders
- ➢ Do not wear any jewellery to laboratory work place
- ➢ Keep fingers, pencils, bacteriological loops, etc. out of your mouth
- ➢ Do not smoke in the laboratory
- ➢ Do not lick labels with your tongue (use tap water)
- ➢ Do not drink from laboratory glassware

- ➢ Do not wander about the laboratory; uncontrolled activities cause accidents, distract others, and promote contamination
- ➢ Do not place contaminated pipettes on the bench top
- ➢ Do not discard contaminated cultures, glassware, pipettes, tubes or slides in the wastepaper basket or garbage can
- ➢ In general, all safety procedures and precautions followed in the microbiology laboratory are designed to:
- ➢ restrict microorganisms present in specimens or cultures to the vessels in which they are contained and
- ➢ prevent environmental microorganisms (normally present on the hands, hair, clothing, laboratory benches or in the air) from entering specimens or cultures and interfering with results of the studies

11.8 WASTE MANAGEMENT

Waste is defined as any solid, liquid or gaseous material that is no longer used and will either be recycled, disposed of or stored in anticipation of treatment and/or disposal.

All laboratories should develop a comprehensive waste management programme that ensures the safe handling and disposal of all laboratory wastes. The programme must be tailored to meet the specific needs of the individual laboratory and should incorporate all applicable legal requirements. The basic goals of this programme are:

- ➢ to operate the laboratory in compliance with all applicable legal requirements and good laboratory practices and
- ➢ to manage the wastes generated in a manner that protects laboratory workers, the environment and the community.

These goals should be part of an SOPM which must also delineate the steps required to meet these objectives. In addition, the policies and procedures should be formalized and documented to the extent practical, and incorporated in the laboratory's operating manuals and training programmes.

Biohazardous Waste Management

General guidelines for the proper disposal of biohazardous waste are provided below:

Storage

Prior to disposal, all biohazardous waste should be maintained and stored separately from the general waste stream and from other hazardous wastes. The containers used to store biohazardous waste should be leak-proof, clearly labeled with a red or orange universal biohazard symbol and sealed tightly when transported. In certain cases it may be necessary to double-bag the waste to prevent leakage. Any biohazardous sharps, such as infectious needles and scalpels, must be placed in containers that are puncture resistant, leak proof on all sides and the bottom, and closable. These containers can then be placed in a standard biohazard bag.

In addition, waste containers should be compatible with the planned treatment process. For example, biohazardous waste that is to be incinerated should be stored in lined durable boxes, while waste that is to be autoclaved should be placed in the appropriate heat resistant containers.

If a primary waste container has become damaged or its exterior contaminated beyond decontamination, then its contents should be placed in a secondary container that meets the same requirements as the first.

Disposal options

There are three main disposal options:

- ➢ render waste noninfectious by autoclaving and dispose it in the general waste stream
- ➢ on site incineration, if possible
- ➢ transportation of locally generated waste to a distant appropriate facility

Incineration is the preferred disposal option. Not only does this method render the waste noninfectious but it also changes the form and shape of the waste. Sterilization is an effective method for decontaminating waste, but it does not alter the appearance of the waste. Steam sterilization in an autoclave at a temperature of 121°C for at least 15 minutes destroys all forms of microbial life, including high numbers of bacterial spores. This type of complete sterilization can also be accomplished using dry heat which requires a temperature of 160-170°C for 2-4 hours. However, it must be ensured that heat comes in contact with the material to be rendered sterile. Therefore, bottles containing liquid material should have loosened caps or cotton plug caps to

allow for steam and heat exchange within the bottle. Biohazard bags containing waste should be tied loosely. Once sterilized, biohazardous waste should be sealed in appropriate containers, labeled as disinfected waste and disposed of in an approved facility.

Biohazardous waste can also be sterilized by immersing the contaminated materials in a chemical sterilant. However, this method is not usually recommended since the chemical application is not always effective. Also, treated waste must still be incinerated or landfilled. Chemical disinfection should be used only if it is impossible to sterilize with a heat process.

Biological waste should be clearly labeled prior to disposal and complete records should be maintained.

12
QUALITY ASSURANCE IN CLINICAL LABORATORY

QUALITY assurance in clinical microbiology should be comprehensive and must cover all aspects from the decision to collect the specimen to the interpretation of the report. Errors at any stage in the investigation of a patient can affect the outcome. Any break in the chain can ultimately lead to the generation of a faulty report.

12.1 QUALITY ASSURANCE PARAMETERS

Quality assurance (QA) programmes ensure that the information generated by the laboratory is accurate, reliable and reproducible. This is accomplished by assessing the quality of specimens; monitoring the performance of test procedures, reagents, media, equipment and personnel; reviewing test results and

documenting the validity of the test method. Guidelines for various QA parameters have been summarized in Table 12.1.

Table 12.1: ***Guidelines for quality assurance parameters***

Parameter	Guidelines
Specimen collection and transportation	Provide instructions for collection and transport Establish criteria for acceptable specimens Establish rejection criteria for unacceptable specimens
Procedural manual	Define test performance, tolerance limits, specimen acceptability, reagent preparation, QA calculations and reporting Review annually Make available in work area
Personnel	Use sufficient qualified personnel depending upon volume and complexities of work Provide continuous technical education Provide written performance standards Evaluate annually
QC records	Record all QC results on prescribed forms Report all out-of-control observations to supervisor Note corrective actions on QC form Review QC records monthly
Patient reports	Report only to authorized personnel Notify test requester of important values immediately Provide normal ranges where appropriate Correct errors in patient's reports in timely fashion Retain records for at least two years
Referral specimens	Use only authorized referral laboratory Include name of reference laboratory on patient's reports
EQAS	Participate in appropriate external quality assessment scheme Consider adopting internal quality assessment programme
Equipment performance	Document function checks of equipment Perform as frequently as recommended by manufacturer

Parameter	Guidelines
	Document routine preventive maintenance Retain maintenance records for life of equipment
Commercially prepared media	Inspect each shipment for cracked media or petri dishes, haemolysis, unequal filling, excessive bubbles and contamination Document deficiencies, take corrective action, inform manufacturer Perform in house QC testing
User prepared media	Record amount prepared, source, lot numbers, sterilization method, preparation date, pH, expiration date. Check medium for colour, consistency, depth or slant, smoothness, haemolysis, contamination, bubbles Test media with QC microorganisms of known characters
Stains, reagents and sera	Label containers as to contents, concentration, storage requirements, date prepared, received/ placed in service, and shelf life Store as per recommendations Test with positive and negative controls prior to use Discard outdated materials and reagents that fail to perform
Commercial kits	Test each batch as per recommendations of manufacturer

12.2 MONITORING AND EVALUATING TESTS AND USE OF TEST RESULTS

The most important goal of quality assurance programme is to ensure generation of reliable laboratory result. To achieve this goal, laboratories must ensure that testing is purposeful and efficient. Improvement in test utilization has traditionally been measured by the degree to which the number of tests performed is reduced (Table 12.2). However, an association between reduced testing and clinical outcome is difficult to document. It can be argued that one might rather test a little too much than risk missing

an important clinical finding. In some cases, however, certain tests that are costly or have limited value should be restricted.

In many cases, such as solitary blood cultures or solitary sputum cultures for mycobacterium, under-utilization may be a problem.

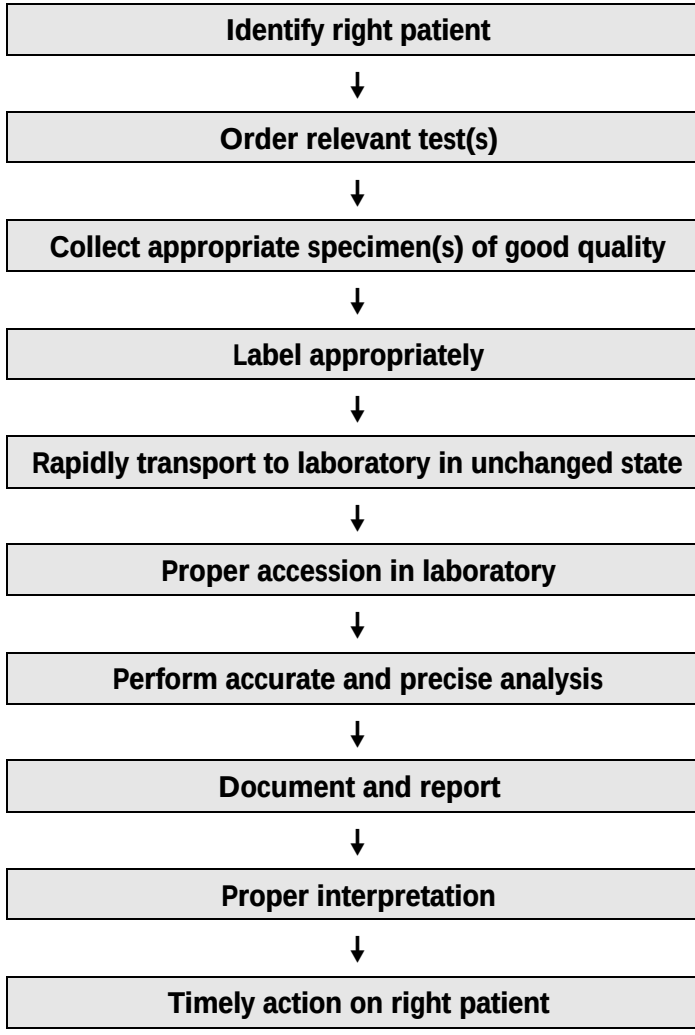
While restriction policies may help cut down unnecessary testing (and work load as well as expenditure), they probably provide little direct benefit to the patient. On the other hand, methods to ensure that appropriate tests are ordered when indicated (as opposed to preventing inappropriate tests from being ordered when they are not indicated) as well as proper use of test results are quality improvement objectives that directly benefit the patient, especially if corrective action can be simultaneously linked to the monitoring and evaluation process.

Table 12.2 ***Examples of restricting microbiology testing***

➢ Routine bacterial culture and parasite examination on patients hospitalized for more than three days
➢ CSF cultures for mycobacterium of specimens with normal cell counts and glucose and protein levels
➢ Urine cultures for asymptomatic patients receiving antibiotics
➢ More than one specimen from same site by same method of collection received on same day (except blood cultures, CSF and faeces)
➢ Bacterial cultures of poor-quality specimens: mouth, bowel contents, perirectal abscess, pilonidal abscess, lochia, vomitus, placenta
➢ Hepatitis A virus -IgM testing in patients with normal liver enzymes

A simplified algorithm of quality assurance in clinical microbiology is depicted in Figure .12.1.

Figure 12.1 *Algorithm of quality assurance in clinical microbiology*



12.3 STATISTICAL CHALLENGES IN QUALITY ASSURANCE PROGRAM

There are a wide range of statistical methods which can be used as a part of QA program in the laboratory. Not all the tools need to be applied every time a test is put up or a new technique is introduced in the laboratory. It is, however, important to use the most important technique(s). Some of these are:

- Setting acceptable limits for QC sample results
- Plotting the results on a Levey Jennings or Shewhart plot on a day to day basis
- Recognizing violations of Westgard Rules
- Standard deviation index
- Cumulative sum (CUSUM)
- Patient precision testing

Setting Acceptable Limits

Mean, standard deviation (SD) and acceptable range are used to calculate expected range of value for a control. The quality control samples demonstrate a normal, stochastic distribution of results. Accordingly, 68.3% of values are within +/- 1 SD of the target value, 95.5% are within +/- 2 SD and 99.7% are within +/- 3 SD. To ensure high probability of error detection and low frequency of falsely rejected assay runs, the acceptable limits are set at +/- 3 SD.

Levey Jennings and Schewhart Plots

These monitor quality control results. The target values and the limit values of +/- 1SD, +/- 2SD and +/- 3SD are

delineated on these charts on which values obtained with the assay controls are plotted as a series of points. Inferences are drawn by the position of the points indicating the location of values within acceptable limits.

Westgard Rules

These detect both systematic and random errors. These rules also define the specific performance limits. There are six Westgard Rules of which three (1_{2SD}, 2_{2SD} and 4_{1SD}) are known as warning rules and three (1_{3SD}, R_{4SD} and 10_x) as mandatory rules. The violation of warning rules suggests a review of test procedures, reagent performance and equipment calibration. Any deviation from the mandatory rules requires rejection of results on patient's material in that assay. These have been summed up in Table 12.3.

Table 12.3 ***Interpretation of Westgard Rules***

Westgard Rule	*Observation*	*Interpretation*
Warning Rules	1_{2SD} is violated	If control value exceeds mean value by +/-2 SD
	2_{2SD} is violated	If two consecutive control values exceed mean value on the same side of mean by +/-2 SD. This indicates systematic error.
	4_{1SD} is violated	If control value exceeds the same mean value by +/-1 SD for four consecutive control values. This may indicate need for instrument maintenance or reagent calibration.
Mandatory Rules	1_{3SD} is violated	If control value exceeds mean value by +/- 3SD. The results based on this assay are rejected.

Westgard Rule	Observation	Interpretation
	R_{4SD} is violated with the use of controls in duplicate	When the difference in SD between duplicates exceeds 4SD. The results based on this assay are rejected.
	10_x mandatory rule is violated	When the last ten consecutive control values are on same side of the mean. The results based on this assay are rejected.

Standard deviation index

The standard deviation index (SDI) is a statistical indicator that can be used with the data generated by quality control samples. It determines the occurrence of a trend. SDI is calculated by dividing the mean of suspect data points, minus the QC cumulative mean, by the QC cumulative SD. An SDI greater than 1 indicates a possible problem with the assay.

Cumulative sum

Cumulative sum (CUSUM) is the method for identifying shifts or trends occurring in an assay. It indicates systematic errors which may include deterioration of reagents or poor equipment calibration. The established target value and SD of the QC samples are used to determine the upper and lower threshold limits for the controls and set at +/- 1SD. Any value outside this range should trigger calculation of CUSUM.

Patient precision testing

Patient precision testing is used to distinguish between assay performance, including reagents, equipment and

operator, and the performance of the QC sample. A patient's sample, previously tested and having a value within the clinically significant range, is chosen for inclusion in the next assay run. The coefficient of variation (CV) is applied to the initial result obtained with the patient's sample to determine an acceptable replicate range. Sample is retested along with the QC samples. If the patient's sample and QC samples are within acceptable range, the result indicates no problem with the control or assay performance.

Efficient management of quality assurance activities in bacteriology and serology requires use of appropriate statistical tool. The inclusion of QC samples, setting acceptable limits, plotting the results on a Shewhart plot on day-to-day basis, recognizing violations of the Westgard rules and taking remedial action should be an integral part of quality assurance activities. These should be described in all standard operating procedures. SDI and CUSUM may be used in quality assurance activities to confirm problems detected through violation of Westgard rules. All these are intended to ensure quality of results generated by the laboratory.

13
QUALITY CONTROL OF LABORATORY MATERIALS

THE QUALITY of clinical laboratory determinations depends a great deal on the quality of the materials used. Even the best technologist cannot produce good results with poor quality materials.

In the vast majority of cases, the reason for bad analysis is the poor quality of one or more of the materials used. Contaminated or deteriorated standards or reagents, glassware that is not properly cleaned, a pipette that is not calibrated before being put to use, an instrument that is not calibrated or maintained properly, a specimen that is not collected properly or preserved adequately can all cause erroneous analytical results. Any of these items may be categorized as poor quality material.

13.1 PIPETTES

Pipette, the most frequently used volumetric glassware, play a crucial role in determining the degree of accuracy and precision in a great many analyses performed in the laboratory. The greater the number of manual test methods used in the laboratory, the greater the role played by the pipettes. Completely automated laboratories do little or no pipetting at all. In any case, if pipettes are used in the laboratory, these should be properly selected and used with care.

Volumetric, or transfer pipettes are made to deliver specified volumes of liquid. They are usually marked "TD," meaning "to deliver." They are used for the transfer of a specified amount of a nonviscous, dilute, aqueous solution. Viscous solutions do not drain properly; therefore, these pipettes cannot be used in the quantitative transfer of viscous solutions. Pipettes and burettes are calibrated for water, and an error is introduced whenever they are used to handle solutions having viscosity and surface tension significantly differing from those of water.

Every laboratory should have a written procedure for pipetting and see that there are no personal variations in the standard procedure. The precision of the analysis can be improved if all technicians adhere to a uniform and careful procedure. It must be universally accepted that the practice of mouth pipetting must be prohibited. The following procedure is recommended:

(a) Inspect the pipette.

Is it of the right size?
Is it free of water spots?
Is it free of chipping?

Is it free of cracks or a broken tip?
If the answer to any of these questions is "no," do not use it.

(b) Introduce the pipette about 2 inches into the liquid.

Apply suction slowly to withdraw the liquid into the pipette to about 2 inches over the calibration mark, using a rubber ball fitted to the upper end of the pipette.

(c) Do not directly introduce the pipette in a reagent or standard bottles.

Use a separate beaker.

(d) Keep the pipette in the vertical position.

Empty the pipette slowly, and bring the lower meniscus in line with the calibration mark. The calibration mark should be at the eye level.

(e) Hold the mouthpiece tightly.

Wipe the lower outside of the pipette that was in contact with the liquid during suction.

(f) Do not hold the wiping gauze directly under the orifice.

It may absorb some liquid from the pipette.

(g) Deliver the contained liquid into suitable glassware.

There are four important points to remember during this delivery:

- ➢ Hold the pipette as vertically as possible.
- ➢ Do not force the liquid out of the pipette to finish the job faster.

- ➢ Do not place the tip of the pipette on the bottom of the glassware in order to prevent obstruction of the free flow. When the level of the liquid reaches the lower end of the pipette, touch the tip of the pipette to the side of the receiving container until the flow is complete.
- ➢ At the end of the delivery, do not leave the tip of the pipette in contact with the liquid. The liquid may rise back into the pipette by capillary action.

13.2 CLEANING GLASSWARE

There are several methods for cleaning laboratory glassware. The liquids usually used for cleaning glassware are sodium dichromate sulphuric acid cleaning solution (commercially available from laboratory supply houses) nitric acid, fuming sulphuric acid, alcohol, and water. The choice of cleaning agent to be used depends on the nature of the contaminant. After being washed with the cleaning solution and thoroughly rinsed with tap water, the vessel should be rinsed with distilled water. Following tests can be performed to check the cleanliness of glassware:

- ➢ Fill the glassware with reagent grade water, drain it, and see whether the glassware is covered by a continuous film of water. Unclean vessels usually leave little drops of water behind on the interior surface instead of a continuous film.
- ➢ Observe water spots inside or outside the vessel. These spots might have originated from inadequate rinsing of the glassware with reagent grade water.

13.3 REAGENTS AND STANDARDS

Next to water, reagents and standards are the most important laboratory supplies; which can directly affect the quality of analyses. Therefore, proper selection, use, and storage of reagents and standards are a vital part of a good quality assurance programme.

Reagents and standards used in the clinical laboratory are complex in nature. These may be aqueous solutions of pure chemicals, solutions in organic solvents, lyophilized substances, or even simulated materials. It is, therefore, very difficult for one person to know or one professional organiza-tion to establish standard requirements for the quality of all these diverse substances. Consequently, standards have been specified by a variety of professional organizations and governmental agencies.

13.4 CHEMICALS

American Chemical Society's **(ACS)** Committee on Analytical Reagents has established specifications for chemicals to qualify as analytical reagent **(AR)** grade. AR chemicals are of very high purity and, whenever available, should be used in all analyses in the clinical laboratory. Manufacturers of these chemicals analyze their product and label them AR or ACS only if they meet ACS specifications. AR chemicals are analyzed for purity, which is usually marked on the bottle. The impurities are also analyzed and reported along with the purity. The purity of the chemicals is also indicated by terms such as **p.a**. (pro analysis) and **p.p.a.** (purissimum pro analysis).

The use of AR chemicals in the laboratory cannot be overemphasized. No one wants to analyze chloride in serum with a reagent already containing chloride or magnesium with a chemical containing magnesium.

13.5 REFERENCE SERA

Reference sera are pools of sera in which the concentrations of various constituents have been determined. These are available from international centres and should be made use of in calibrating the national and subsequently local standard sera. These are of great use in all kinds of serological studies and especially so when the titres of antibody (or antigen) are to be established.

13.6 PROPER USE OF REAGENTS AND STANDARDS

The following are necessary precautions to be taken in the storage and preservation of reagents and standards:

- ➢ Store all aqueous solutions in plastic bottles that can be tightly closed
- ➢ Concentration of standards by evaporation and air oxidation of reagents are some of the causes of bad analyses. These can be minimized if one takes care to see that the bottles are tightly closed. Colored aqueous solutions are preferably stored in amber-colored plastic bottles. Colored solutions absorb light in the visible region and may undergo photochemical reactions if stored in white plastic bottles
- ➢ Never store organic liquids and solutions in organic solvents in plastic bottles

  Most of them either dissolve or soften plastic material. Organic liquids and solutions may be stored in amber-colored glass bottles.
- ➢ Keep all reagents and standards requiring refrigeration in the refrigerator

Except during the time of actual use. All such bottles should be labeled "REFRIGERATE" so that even an

inexperienced technician would not leave the bottle out at room temperature for any length of time. The labeling may be conveniently done with self-sticking adhesive tape rolls with "REFRIGERATE" printed on each label.

➢ Never introduce a pipette, a glass rod, or any other substance into the reagent or standard bottle

This could contaminate the reagent or standard and no one would be aware of it. Such contamination could result in small or gross errors in the analyses. Small errors can bring about a decrease in precision. Gross errors can bring about a waste of time in repeated analyses and a waste of money in discarded reagents. To avoid these difficulties, it is best to first transfer a small amount of the material into a suitable beaker for immediate use. The remainder in the beaker should not be poured back into the original container but should be discarded

➢ All reagents and standards perform best when they are freshly prepared

They deteriorate slowly for several reasons, including air oxidation, decomposition, contamination, evaporation, and microbial growth. Some remain usable for a few months while others go bad in a few days. For this reason, all reagents, standards, and general chemicals should have labels indicating the date received, date opened, and the expiration date. If a reagent or standard is prepared in the laboratory, the label should also indicate the date of preparation and the initials of the person who prepared it

14
QUALITY CONTROL OF MEDIA AND STAINS

CULTURE media are used in the laboratory for a variety of purposes. These are used to support the growth of microorganisms showing typical colonial and morphological appearance. Media are also used to demonstrate many other properties of organisms, e.g. production of acid and gas in carbohydrate fermentation media or haemolysis on blood agar. Variations in the composition of the medium may alter these characters.

14.1 QUALITY CONTROL OF MEDIA

14.1.1 Sources of Media

A few years back media used to be prepared from basic chemical ingredients, but laboratories are no longer required to do this now.

Dehydrated Media

These are commercially available and require only the addition of water to be reconstituted for use. The responsibility for quality control lies with the manufacturer. However, it has to be tested for its quality, after preparation, because of changes that can be brought about by the process of reconstitution and sterilization.

Dehydration with Additive

For isolation of fastidious organisms, certain additives need to be used when media are prepared in the laboratory. The additives usually are unstable materials such as blood, serum or other growth factors. Hence, quality control needs to be maintained.

Commercially Prepared Media

Ready to use media are commercially available. In these media also the responsibility for quality control maintenance lies with the manufacturer but laboratories need to keep a watch on their behaviour.

14.1.2 Sources of Error

Inappropriate Medium

Since dehydrated media are usually arranged alphabetically on a shelf, one may select the wrong bottle inadvertently, or an improper additive might be selected, making the medium unsuitable for use. It is always important to read the label, particularly when a new lot of medium has been received in the laboratory.

Water

Measure carefully the amount of water that is added when reconstituting media. Since impurities render tap water unsuitable for the preparation of most biological media, laboratories should use either distilled water, deionized water, or water that has been treated in both ways.

Weighing

Accurate balances should be used for weighing dry materials. Weighing errors significantly alter the composition of the final product.

Dispensing

Media should be dispensed accurately and aseptically in plates and tubes. Failure to measure the amount accurately may result, for example, in too shallow or too deep agar medium, either of which may make the medium unsuitable for use.

Proper Sterilization

A common error in media preparation is sterilizing media at too high a temperature or for too long a period, or both. This may result in deterioration or decomposition of some constituents of the media, which will render the media useless for the intended purpose.

Glassware

Care should be taken to use clean glassware, since residues on glass may be inhibitory to some fastidious micro-

organisms, particularly viruses grown in cell culture, or to the cells themselves.

14.1.3 Quality Control

Any quality programme for culture media must in the final analysis assure that a medium will support the growth of the organisms likely to be in the specimen. It must, if specified inhibit the growth of commensal organisms, exhibit a typical biochemical response, be stable and have a reasonable shelf life. Because laboratories usually have no control over the preparation, shipping or storage of these products it is very important that they document the information that is available for each.

Physical Appearance

If the medium is stored for an excessively long time under adverse conditions or has been improperly prepared, the following signs may develop and these should be documented.

- Presence of turbidity or a precipitate indicates that some constituent has come out of the solution.
- Colours darker than normal may indicate overcooking of sugar containing media, incorrect pH or incorrect mixture of ingredients.
- Colour lighter than normal may also indicate incorrect mixture of ingredients or a wrong pH.
- Prolonged storage of medium after pouring in plates causes its dehydration and makes it unfit for use. Dehydration of the medium can be reduced by preparing only required number of plates of

media and storing them by sealing plates in plastic bags.

Sterility

A few media are used without terminal sterilization, but these are exceptions; most media must be sterile when they are inoculated. Each batch of medium, whether prepared in the laboratory or received from a commercial source, should be sampled for sterility. This is best done by removing 1-5% of the batch and placing it in a bacteriologic incubator at 35°C for 48 hours. If contaminants appear in the medium as a result of inadequate sterilization, a new lot should be obtained. Those containers that are used for sterility testing should be discarded at the completion of the test, since they are unsuitable for inoculation because of the dehydration that occurs after up to 48 hours in the incubator.

Growth

Determine the ability of the medium to support the growth of suspected organisms by inoculating the medium with a typical stock culture isolate. A frequent quality control error is the use of a heavy inoculum for this purpose. For most media, inoculating with a stock culture that is too heavy may result in misleading growth. In a specimen, the organism may be much more fastidious or present in very small numbers; therefore, the medium may not support its growth. When testing for the ability to support growth, it is good to prepare a dilute suspension to use as the inoculum. This suspension will give greater assurance that the medium is adequate for the growth of a small number of organisms in a patient's specimen. In selecting an organism for testing, one should select from among the more fastidious species

of organisms that one may be looking for in specimens received from patients.

Biochemical Response

When inoculating media used to identify a specific reaction, such as fermentation or H_2S production, it is necessary to use only a species or strain of organism that will produce the desired reaction.

Selective Media

Since selective media are designed not only to support the growth of organisms but to inhibit the growth of others, it is necessary to inoculate the medium with representatives of both groups of organisms. To demonstrate the inhibitory effect, one can challenge the medium with a heavy inoculum, since, if the medium will prevent the growth of a large inoculum, it will inhibit the small number of organisms that may be present in the primary specimen. The medium must also support the growth of the selected organisms.

As a matter of general principle, each batch of culture medium should be checked before use with control strains to ensure that it supports the growth of bacteria and, in the case of selective media, inhibits the growth of undesirable organisms. However, if economics does not permit this approach, those media which are known from experience to be trouble free and reliable need not be subjected to such a regular quality control regimen. The laboratory has to identify such reliable media and accordingly establish quality control schedules. This concept must be periodically reviewed. However, whenever a new batch of medium, new supplier or a new product is to be used it is prudent to

subject it to rigorous quality control measures until confidence in the quality of the product is established.

A "batch" of the medium refers to all the tubes, plates or containers of medium prepared at the same time in the laboratory, or all the plates, tubes or containers having the same lot number that are received in a single shipment from an outside supplier.

Spectrum of Quality Control

The frequency of performing quality control procedures needs to be determined from the experience of the laboratory. To meet certification requirements, laboratories need to perform quality control procedures according to a prescribed pattern. Careful records of quality control procedures should be made and maintained which should be reviewed periodically to determine the stability of media so that corrective measures can be taken in time. Quality control of culture media should not be a blind procedure, but should be approached in a rational and disciplined manner.

14.2 PERFORMANCE OF PLATED MEDIA

Samples of plates from each batch are selected for performance testing and are inoculated with the appropriate stock cultures. For each type of medium, at least two or three microorganisms having growth characteristics with 'positive' and 'negative' results for the medium should be used. The size of inoculum and method of inoculating the test plates must be standardized as closely as possible. In general, control organisms should be selected from an actively growing broth culture and a

standard loopful of culture seeded directly onto the test medium, which is then streaked so as to obtain isolated colonies. After appropriate incubation, the results of the performance test are recorded. The medium is released for use in the clinical laboratory only if the results indicate satisfactory performance. In initiating a quality control programme, one must establish some priorities, such as beginning by testing those media that are most likely to demonstrate deficiencies. Top priority should be given to blood agar, chocolate agar and Thayer Martin agar media. Secondary priority should be accorded to selective enteric media such as MacConkey agar, EMB, XLD and bile salt agars.

A quantitative approach may be more useful for testing of performance of selective or inhibitory media such as Thayer Martin agar. *N gonorrhoeae* and *N.meningitidis* usually grow on Thayer Martin agar when the inoculum is heavy, but when a fairly light inoculum is used, the pathogens might be inhibited. Consequently, a somewhat quantitative performance test could detect deficiencies that would be overlooked if one simply inoculated test plates with undiluted stock cultures.

14.3 QUALITY CONTROL OF STAINS

Test all stains at appropriate intervals for their ability to distinguish positive and negative organisms and document the results. The performance standards for some of the commonly used stains in the bacteriology laboratory alongwith their desired frequencies of testing so as to have continuous reliable results have been shown in Table 14.1.

Table 14.1: ***Performance standards for stains***

Stain	Control organism/ material	ATCC No	Expected result
Ziehl-Neelsen	*Mycobacterium sp.* *Esch. Coli*	25177 25922	Pink red bacilli Blue bacilli
Acridine orange	*Esch. coli* *Staph.aurues*	25922 25923	Fluorescent bacilli/cocci
Giemsa	Thin film blood smear		Distinct staining of WBCs and RBCs
Gram	*Esch. coli* *Staph.aureus*	25922 25923	Gram -ve bacilli Gram +ve cocci
Iodine solution	Formalin treated stool specimen with cysts		Visible cyst nuclei
Spores	*Bacillus species*		Spores stain one colour and bacillus stains with counterstain

Quality control of stains should be performed on a weekly basis and also as and when a new lot of reagents for staining are procured.

15
QUALITY CONTROL OF BACTERIOLOGICAL TECHNIQUES

VARIOUS biochemical tests are performed in the laboratory on the isolates obtained from the clinical specimen. These tests help in identification of the organism. Quality control procedures (Table 15.1) are essential for these tests to avoid generation of wrong results which may lead to erroneous diagnoses.

In microbiological assays one must control the density of the inoculum which should be prepared by a standard method and checked through turbidity standards

Any laboratory test will give quality results if it is performed by

- ➢ Using a pure culture on a medium that supports the expression of full phenotypic characteristics
- ➢ Using fully quality controlled media and reagents
- ➢ Using controlled test conditions, i.e. incubation atmosphere, temperature and duration of incubation
- ➢ Concurrent use of appropriate positive and negative control organisms. For critical key tests, these should be implemented as single test and not as commercial test strips.

Organisms known to give positive or negative reactions with various biochemical tests have been identified. These must be used frequently in the laboratory to assess the authenticity of results of biochemical reactions.

Table 15.1: ***QC procedures for commonly used tests***

Procedure/ Test	Control organism	Expected result	Expected reaction
Catalase	*Staph aureus* *Streptococcus species*	+ –	Bubbling reaction No bubbling
Coagulase	*Staph aureus* *Staph epidermidis*	+ –	Clot formation in 4 hours No clot
Indole	*Esch coli* *Enterobacter aerogenes*	+ –	Red ring at surface Yellow ring at surface
Methyl red	*Esch coli* *Ent aerogenes*	+ –	Instant red colour No colour change
Oxidase	*P. aeruginosa* *Esch. Coli*	+ –	Purple colour in 20 seconds No colour in 20 seconds
Voges Proskauer	*Enterobacter aerogenes* *Esch. Coli*	+ –	Red colour No colour change

Procedure/ Test	Control organism	Expected result	Expected reaction
Bacitracin disc	*Streptococcus group A* *Enterobacter faecalis*	+ –	Zone of inhibition No zone of inhibition
Optochin disc	*Strept. Pneumoniae* *Strept. Viridans*	+ –	Zone of inhibition No zone of inhibition
ONPG disc	*Esch. Coli* *Proteus vulgaris*	+ –	Yellow colour No change in colour
Oxidase disc	*P aeruginosa* *Esch. Coli*	+ –	Purple colour in 30 seconds No change in colour

It is also essential to undertake quality control procedures at regular intervals. These should be performed:

- With each new batch of reagents
- With each new vial of reagent
- Daily for catalse, oxidase, and coagulase
- Weekly for bacitracin, optochin and ONPG

A test procedure not giving anticipated results with the control organisms should not be used till such time that remedial steps have been taken to correct the problem.

16
PRESERVATION OF STOCK CULTURES

STOCK cultures are used in microbiology for the purpose of employing a quality control procedure that can prevent avoidable mistakes. This chapter pertains mainly to the use of cultures for checking culture media, reagents, and equipment, and not for checking the proficiency of personnel.

There is no single ideal method that can be applied universally for the preservation of all microorganisms. The method may be selected on the basis of needs and availability of resources or infrastructure for the same. Various factors that may influence this decision include capability of the method to

- cause minimum loss of microorganisms
- ensure stability of characters

- ➢ prevent contamination
- ➢ ease of supply and transport of preserved microorganisms
- ➢ duration of preservation
- ➢ cost

Various methods of preservation and their advantages vis a vis the factors described earlier are shown in Table 16.1

Table 16.1: ***Methods of preservations***

Method	Survival period	Stability of characters	Chances of contamination	Cost	Ease of Supply and transport	Duration
Subculture	+	+	+ + + +	+	+	+
Gelatin discs	+ +	+ +	+	+	+ +	+ +
Lyophilization	+ + +	+ + +	+	+ + +	+ + +	+ + +
Liquid nitrogen	+ + + +	+ + + +	+	+ +	+	+ + +

16.1 PRESERVATION OF STOCK CULTURES

There are many means of preserving living microorganisms, varying from highly sophisticated to very practical and inexpensive methods. Some methods are ideal for long-term preservation of cultures (measured in years), but when a culture is restored to its normal active state it is no longer in a preserved state. Other methods hold the organisms in a state of reduced metabolism and are available continuously for subculturing but require transferring at intervals of months or years. The choice of method for maintaining a

stock culture collection depends upon the equipment available and the intended use for the cultures. Recommended methods of preserving some bacterial isolates at the intermediate and peripheral laboratories are summarised below:

Enterobacteriaceae

Escherichia coli (ATCC 25922) is required as one of the test organisms in performing antibiotic susceptibility testing by the Kirby-Bauer technique. Many members of family enterobacteriaceae may also be employed for checking various differential media such as MacConkey, eosin-methylene blue agar (EMB), xylose-lysine-desoxycholate agar (XLD), triple sugar iron agar (TSI), potassium cyanide medium (KCN), and methyl red/Voges-Proskauer broth (MR/VP), as well as different biochemical tests. If the laboratory is required to test for enteropathogenic *Esch. coli*, it is also desirable to maintain some of the cultures of the recommended standard strains for checking the activity of the immune serum to the most commonly encountered types.

All members of the family enterobacteriaceae are readily maintained on heart infusion agar slants under oil at room temperature with yearly transfers.

Pseudomonas

Pseudomonas aeruginosa and *P. maltophilia* are useful for checking oxidation-fermentation (OF) media and Seller's medium. The organisms may be maintained on heart infusion agar slants under oil at room temperature with yearly transfers.

Staphylococcus

Staphylococcus aureus (ATCC 25923) is required as one of the test organisms for performing antibiotic susceptibility testing by the Kirby-Bauer technique. In addition, it can be used for checking plasma for the coagulase test; for checking culture media for the production of indole, catalase, and DNase; for the reduction of nitrates, and for checking the Gram stain. *Staph. epidermidis* is used for a negative control in the coagulase and DNase tests.

A strain of *Staphylococcus* that produces ?-haemolysin and is suitable for the CAMP test for identifying group B ?-hemolytic streptococci is desirable. Staphylococci may be grown on slants of heart infusion agar, placed under oil, and held at room temperature; transfers can be made at intervals of 1 or 2 years.

Streptococcus

Streptococcus pyogenes is necessary for checking bacitracin discs, the quality of group A antisera, the ability to produce β-haemolysis on blood agar plates, and to grow on blood agar containing colistin and nalidixic acid.

Streptococcus group B is needed for positive controls in the CAMP test and sodium hippurate test, for a negative control with bacitracin discs, and for checking the quality of group B antisera.

From the group D streptococci, *Streptococcus faecalis*, a representative of enterococci is needed for checking bile-aesculin medium, as a negative control in the starch hydrolysis test, and for checking the quality of group D antisera. *Strept. salivarius* is needed as a representative of the α haemolytic streptococci.

Streptococcal cultures can be maintained in 10-mL heart infusion broth containing 10% defibrinated sheep's blood. After overnight incubation at 36°C, they can be stored at 4°C. Transfers need to be made every 3-4 months since some strains begin to die after 5 or 6 months.

Vibrio

Vibrio cholerae will grow easily on heart infusion agar. Good growth takes place on heart infusion agar slants containing 1.5% NaCl, and the slants may be held under oil at room temperature.

Fungi

Fewer and less complex culture media are required for fungi than are necessary for bacteria in a clinical diagnostic laboratory, and so there are fewer requirements for quality control. The necessary cultures of fungi may be maintained on slants of Sabouraud's agar in tubes with screw caps at room temperature and in the dark. Transfers need to be made every 2 or 3 months. Sterile mineral oil may be added to the slants after the cultures have attained their optimum growth, and then transfers may be made.

17
QUALITY ASSURANCE IN ANTIBIOTIC SUSCEPTIBILITY TESTING

ANTIBIOTIC SUSCEPTIBILITY testing has become a very essential step for properly treating infectious diseases and monitoring antimicrobial resistance in various pathogens. The choice of antibiotic needs to be made taking into consideration the susceptibility profile of the pathogen, pharmacology of the antibiotic, the need for antibiotic therapy, and its cost effectiveness.

17.1 INDICATIONS FOR ROUTINE SUSCEPTIBILITY TESTING

A susceptibility test may be performed in the clinical laboratory for two main purposes:

- ➢ To guide the clinician in selecting the best antimicrobial agent for an individual patient
- ➢ To accumulate epidemiological information on the resistance of microorganisms of public health importance within the community.

17.2 SUSCEPTIBILITY TEST AS A GUIDE FOR TREATMENT

Susceptibility tests should never be performed on contaminants or commensals belonging to the normal flora, or on other organisms that have no causal relationship to the infectious process. These should be carried out only on pure cultures of organisms considered to be causing the infectious process. The organisms should also be identified since not every microorganism isolated from a patient with an infection requires an antibiogram.

Routine susceptibility tests are not indicated when the causative organism belongs to a species with predictable susceptibility to specific drugs. This is the case for *Streptococcus pyogenes* and *Neisseria meningitidis*, which are still generally susceptible to penicillin. If resistance of these microorganisms is suspected on clinical grounds, representative strains should be submitted to a competent reference laboratory.

17.3 SUSCEPTIBILITY TEST AS AN EPIDEMIOLOGICAL TOOL

Routine susceptibility tests on major pathogens (e.g. *S.typhi*, shigellae) are useful as part of a comprehensive programme of surveillance of enteric infections. These are essential for informing the physician of the emergence of resistant strains

(chloramphenicol resistant *S.typhi,* co-trimoxazole resistant and ampicillin resistant shigellae) and indicate a need to modify standard treatment schemes.

Continued surveillance of the results of routine susceptibility tests is an excellent source of information on the prevalence of resistant staphylococci and Gram-negative bacilli that may be responsible for cross-infections in the hospital. Periodic reporting of the susceptibility pattern of the prevalent strains is an invaluable aid to forming a sound policy on antibiotic usage in the hospital by restriction and/or rotation of life-saving drugs, such as the aminoglycosides and cephalosporins.

17.4 CHOICE OF DRUGS

The choice of drugs used in a routine antibiogram is governed by considerations of the antibacterial spectrum of the drugs, their pharmacokinetic properties, toxicity, efficacy, and availability, as well as their cost to both the patient and the community. Among the many antibacterial agents that could be used to treat a patient infected with a given organism, only a limited number of carefully selected drugs should be included in the susceptibility test.

Table 17.1 indicates the drugs to be tested in various situations. These agents are divided into two sets. Set 1 includes the drugs that are available in most hospitals and for which routine testing should be carried out for every strain. Tests for drugs in set 2 are to be performed only at the special request of the physician, or when the causative organism is resistant to the first-choice drugs, or when other reasons (allergy to a drug, or its unavailability) make further testing justified.

17.5 DIRECT VERSUS INDIRECT SUSCEPTIBILITY TESTS

In the standardized method, the inoculum is prepared from colonies on a primary culture plate or from a pure culture. This is called an "indirect sensitivity test". In certain cases, where a rapid answer is important, the standardized inoculum may be replaced by the pathological specimen itself, e.g. urine, a positive blood culture, or a swab of pus. For urine specimens, a microscopic examination of the sediment should first be made in order to see if there is evidence of infection, i.e. the presence of pus cells and/or organisms. The urine may then be used as the inoculum in the standard test. Likewise, susceptibility tests may be performed on incubated blood cultures showing evidence of bacterial growth, or a swab of pus may be used as a direct inoculum, when a Gram stained smear shows the presence of large numbers of a single type of organism. This is called a "direct susceptibility test"; its advantage over the indirect test is that a result is obtained 24 hours earlier. The disadvantage is that the inoculum cannot be properly controlled. When the susceptibility plate shows too light or too heavy growth, or a mixed culture, the results should be interpreted with caution or the test repeated on pure cultures.

Table 17.1: ***Basic sets of drugs for routine susceptibility tests***

	Set 1	**Set 2**
Staphylococcus	Benzylpenicillin Oxacillin Erythromycin Tetracycline Chloramphenicol Ampicillin	Gentamicin Amikacin Co-trimoxazole Clindamycin - Norfloxacin

	Set 1	Set 2
	Chloramphenicol	
	Co-trimoxazole	
Intestinal	Nalidixic acid	
	Tetracycline	
	Sulfonamide	Norfloxacin
	Trimethoprim	Chloramphenicol
Enterobacteriaceae	Co-trimoxazole	Gentamicin
Urinary	Ampicillin	
	Nitrofurantoin	
	Nalidixic acid	
	Tetracycline	
	Ampicillin	Cefuroxime
	Chloramphenicol	Ceftriaxone
	Co-trimoxazole	Ciprofloxacin
Blood and tissues	Tetracycline	Piperacillin
	Cefalotin	Amikacin
	Gentamicin	
	Piperacillin	Amikacin
Pseudomonas aeruginosa	Gentamicin	
	Tobramycin	

17.6 GENERAL PRINCIPLES OF ANTIMICROBIAL SUSCEPTIBILITY TESTING

Antimicrobial susceptibility tests measure the ability of an antibiotic or other antimicrobial agent to inhibit bacterial growth *in vitro*. This ability may be estimated by either the dilution method or the diffusion method.

17.6.1 The dilution method

For quantitative estimates of antibiotic activity, dilutions of the antibiotic may be incorporated into broth or agar medium, which is then inoculated with the test organism. The lowest concentration that prevents growth after overnight incubation is known as the minimum inhibitory concentration (MIC) of the agent. The MIC value is then compared with known concentrations of the drug obtainable in the serum and in other body fluids to assess the likely clinical response.

17.6.2 The diffusion method

Paper discs impregnated with a defined quantity of antimicrobial agent are placed on agar medium uniformly seeded with the test organism. A concentration gradient of the antibiotic forms by diffusion from the disc and the growth of the test organism is inhibited at a distance from the disc that is related among other factors to the susceptibility of the organism.

The recommended method for intermediate and peripheral laboratories is the modified Kirby-Bauer method, the methodology of which is given below: This method has been recommended by National Committee on Clinical Laboratory Services (NCCLS-USA) Subcommittee on Antimicrobial Susceptibility Testing. This is the most thoroughly described disc diffusion method for which interpretive standards have been developed and which is supported by laboratory and clinical data.

The Modified Kirby-Bauer Method

Reagents

Mueller-Hinton agar

(1) Mueller-Hinton agar should be prepared from a dehydrated base according to the manufacturer's recommendations. The medium should be such that control zone sizes within the standard limits are produced. It is important not to overheat the medium.

(2) Cool the medium to 45-50oC and pour into the plates. Allow to set on a level surface, to a depth of approximately 4 mm. A 9 cm diameter plate requires approximately 25 ml of the medium.

(3) When the agar has solidified, dry the plates for immediate use for l0-30 minutes at 36oC by placing them in an upright position in the incubator with the lids tilted.

(4) Any unused plates may be stored in a plastic bag, which should be sealed and placed in the refrigerator. Plates stored in this way can be kept for 2 weeks.

To ensure that the zone diameters are sufficiently reliable for testing susceptibility to sulfonamides and co-trimoxazole, Mueller-Hinton agar must have low concentrations of the inhibitors thymidine and thymine. Each new lot of Mueller-Hinton agar should therefore be tested with a control strain of *Enterococcus faecalis* (ATCC 29212 or 33l86) and a disc of co-trimoxazole. A satisfactory lot of medium will give a distinct inhibition zone of 20 mm or more that is essentially free of hazy growth or fine colonies.

Antibiotic Discs

Any commercially available discs with the proper diameter and potency can be used. Stocks of antibiotic discs should preferably be kept at -20°C; the freezer compartment of a home refrigerator is convenient. A small working supply of discs can be kept in the refrigerator for upto 1 month. On removal from the refrigerator, the containers should be left at room temperature for about l hour to allow the temperature to equilibrate. This procedure reduces the amount of condensation that occurs when warm air reaches the cold container.

Turbidity Standard

Prepare the turbidity standard by pouring 0.6 mL of a 1% (10 g/L) solution of barium chloride dihydrate into a l00-mL graduated cylinder, and filling to l00 ml with 1% (10 ml/L) sulfuric acid. The turbidity standard solution should be placed in a tube identical to the one used for the broth sample. It can be stored in the dark at room temperature for 6 months, provided it is sealed to prevent evaporation.

Swabs

A supply of cotton wool swabs on wooden applicator sticks should be prepared. These can be sterilized in tins, culture tubes, or on paper, either in the autoclave or by dry heat.

Procedure

➢ To prepare the inoculum from a primary culture plate, touch with a loop the tops of each of 3-5 colonies of similar appearance of the organism to be tested.

- When the inoculum has to be made from a pure culture, a loopful of the confluent growth is similarly suspended in saline.
- Compare the tube with the turbidity standard and adjust the density of the test suspension to that of the standard by adding more bacteria or more sterile saline. Proper adjustment to the turbidity of the inoculum is essential to ensure that the resulting lawn of growth is confluent or almost confluent.
- Inoculate the plates by dipping a sterile swab into the inoculum. Remove excess inoculum by pressing and rotating the swabs firmly against the side of the tube above the level of the liquid.
- Streak the swab all over the surface of the medium three times, rotating the plate through an angle of 60° after each application. Finally, pass the swab round the edge of the agar surface. Leave the inoculum to dry for a few minutes at room temperature with the lid closed. The antibiotic discs may be placed on the inoculated plates using a pair of sterile forceps.
- A sterile needle tip may also be used to place the antibiotic discs on the plate. Alternatively, an antibiotic disc dispenser can be used to apply the discs to the inoculated plate.
- A maximum of seven discs can be placed on a 9-10 cm diameter plate. Six discs may be spaced evenly, approximately 15 mm from the edge of the plate, and 1 disc placed in the centre of the plate. Each disc should be gently pressed down to ensure even contact with the medium.
- The plates should be placed in an incubator at 35°C within 30 minutes of preparation. Temperatures above 35°C invalidate results for oxacillin/methicillin.

- Do not incubate in an atmosphere of carbon dioxide.
- After overnight incubation, the diameter of each zone (including the diameter of the disc) should be measured and recorded in mm. The results should then be interpreted according to the critical diameters by comparing them with standard tables.
- The measurements can be made with a ruler on the under surface of the plate without opening the lid.
- The endpoint of inhibition is judged by the naked eye at the edge where the growth starts, but there are three exceptions.
- With sulfonamides and co-trimoxazole, slight growth occurs within the inhibition zone; such growth should be ignored.
- When beta-lactamase producing staphylococci are tested against penicillin, zones of inhibition are produced with a heaped-up, clearly defined edge; these are readily recognizable when compared with the sensitive control, and regardless of the size of the zone of inhibition, they should be reported as resistant.
- Certain *Proteus* species may swarm into the area of inhibition around some antibiotics, but the zone of inhibition is usually clearly outlined and the thin layer of swarming growth should be ignored.

17.7 CLINICAL DEFINITIONS OF TERMS RESISTANT AND SUSCEPTIBLE: THE THREE-CATEGORY SYSTEM

The result of the susceptibility test, as reported to the clinician, is the classification of the microorganism in one of two or more categories of susceptibility. The simplest

system comprises only two categories, susceptible and resistant. This classification, although offering many advantages for statistical and epidemiological purposes, is too inflexible for the clinician to use. Therefore, a three-category classification is often adopted. The Kirby-Bauer method recognizes three categories of susceptibility and it is important that both the clinician and the laboratory worker understand the exact definitions and the clinical significance of these categories.

Susceptible: An organism is called "susceptible" to a drug when the infection caused by it is likely to respond to treatment with this drug at the recommended dosage.

Intermediate susceptibility: This term covers two situations. It is applicable to strains that are "moderately susceptible" to an antibiotic that can be used for treatment at a higher dosage because of its low toxicity or because the antibiotic is concentrated in the focus of infection (e.g. urine). The term also applies to those strains that are susceptible to a more toxic antibiotic that cannot be used at a higher dosage. In this situation this category serves as a buffer zone between susceptible and resistant.

Resistant: This term implies that the organism is expected not to respond to a given drug, irrespective of the dosage and of the location of the infection.

For testing the response of staphylococci to benzylpenicillin, only the categories 'susceptible' and 'resistant' (corresponding to the production of ?-lactamase) are recognized. Factors influencing zone size and common problems encountered in performing susceptibility test are shown in Tables 17.2 and 17.3.

Table 17.2: ***Factors influencing zone size in antibiotic susceptibility testing***

Factor	Influence
Inoculum density	Larger zones with light inoculum and vice versa
Timing of disc application	If after application of disc, the plate is kept for longer time at room temperature, small zones may form
Temperature of incubation	Larger zones are seen with temperatures < 35°C
Incubation time	Ideal 16-18 hours; less time does not give reliable results
Size of the plate	Smaller plates accommodate less number of discs
Depth of the agar medium	Thin media yield excessively large inhibition zones and vice versa
Proper spacing of the discs	Avoids overlapping of zones
Potency of antibiotic discs	Deterioration in contents leads to reduced size
Composition of medium	Affects rate of growth, diffusion of antibiotics and activity of antibiotics
Acidic pH of medium	Tetracycline, novobiocin, methicillin zones are larger
Alkaline pH of medium	Aminoglycosides, erythromycin zones are larger
Incubation in the presence of CO_2	Increases zone size of tetracycline and methicillin
Addition of thymidine to medium	Decreases activity of trimethoprim
Addition of defibrinated blood	Decreases activity of sulfonamides
On chocolate agar, decreased activity of	Sulfonamides, trimethoprim, aminoglycosides
Reading of zones	Subjective errors in determining the clear edge
Chelating agents such as calcium, magnesium and iron	Decrease diffusion of tetracycline and gentamicin

Table 17.3: ***Troubleshooting guide for disc diffusion test in antibiotic susceptibility testing***

Aberrant results	Probable cause
Tetracycline zone too small	pH of medium too low
Aminoglycoside zone too small	pH of medium too high
Aminoglycoside zone too large	Ca^{2+} and/or Mg^{2+} level too high in medium
	Ca^{2+} and/or Mg^{2+} level too low in medium
Too large zone on control plates	Inoculum too light
	Nutritionally poor medium
	Slow growing organisms (not seen with controls)
	Improper medium depth (too thin)
Zone universally too small on control plates	Inoculum too heavy
Methicillin zone indeterminant in disc test	Methicillin degraded by strong α lactamase producing staphylococci
Carbenicillin zone disappears with *Pseudomonas* control	Resistant mutant has been selected for testing
Single disc result above or below control limits	Error in reading, fuzzy zone edge, transcription error, bad disc
	Disc may not be pressed firmly onto agar surface
Colonies within zone of inhibition	Mixed culture Resistant mutants within zone
Zones overlap	Discs too close together
Zones indistinct	Poorly streaked plates
Zone within zone phenomenon	Swarming *Proteus species*
	Feather edge of zones around penicillin or ampicillin discs usually with α lactamase negative strains of *Staph.aureus*
Enterococcus appears sensitive to aminoglycoside discs	Assessment of aminoglycosides inaccurate in disc test

17.8 NEED FOR QUALITY CONTROL IN SUSCEPTIBILITY TEST

The final result of a disc diffusion test is influenced by a large number of variables. Some of the factors, such as the inoculum density and the incubation temperature, are easy to control, but a laboratory rarely knows the exact composition of a commercial medium or the batch-to-batch variations in its quality, and it cannot take for granted the antimicrobial content of the discs. The results of the test must, therefore, be monitored constantly by a quality control programme which should be considered part of the procedure itself.

The precision and accuracy of the test are controlled by the parallel use of a set of control strains, with known susceptibility to the antimicrobial agents. These quality control strains are tested using exactly the same procedure as for the test organisms. The zone sizes shown by the control organisms should fall within the range of diameters given in Table 18.4. When results regularly fall outside this range, they should be regarded as evidence that a technical error has been introduced into the test, or that the reagents are at fault. Each reagent and each step in the test should then be investigated until the cause of the error has been found and eliminated.

Table 17.4: ***Quality Control – Susceptibility of Control Strains****

Antibiotic	**Disc potency**	**Diameter of zone of inhibition (mm)**		
		***Staph.aureus* (ATCC 25923)**	***Esch.coli* (ATCC 25922)**	***P.aeruginosa* (ATCC 27853)**
Amikacin	30 µg	20-26	19-26	18-26
Ampicillin	10 µg	27-35	16-22	–

Antibiotic	Disc potency	Diameter of zone of inhibition (mm)		
		Staph.aureus (ATCC 25923)	*Esch.coli* (ATCC 25922)	*P.aeruginosa* (ATCC 27853)
Ceftriaxone	30 µg	22-28	29-35	17-23
Cephalothin	30 µg	29-37	15-21	–
Chloramphenicol	30 µg	19-26	21-27	–
Ciprofloxacin	5 µg	22-30	30-40	25-33
Clindamycin	2 µg	24-30	–	–
Erythromicin	15 µg	22-30	–	–
Gentamicin	10 µg	19-27	19-26	16-21
Nalidixic acid	30 µg	–	22-28	–
Nitrofurantoin	300 µg	18-22	20-25	–
Norfloxacin	10 µg	17-28	28-35	–
Oxacillin	1 µg	18-24	–	–
Penicillin G	10 units	26-37	–	–
Piperacillin	100 µg	–	24-30	25-33
Tetracycline	30 µg	19-28	18-25	–
Tobramycin	10 µg	19-29	18-26	19-25
Trimethoprim	5 µg	19-26	21-28	–
Trimethoprim-sulfamethoxazole	1.25/23.75	24-32	24-32	–

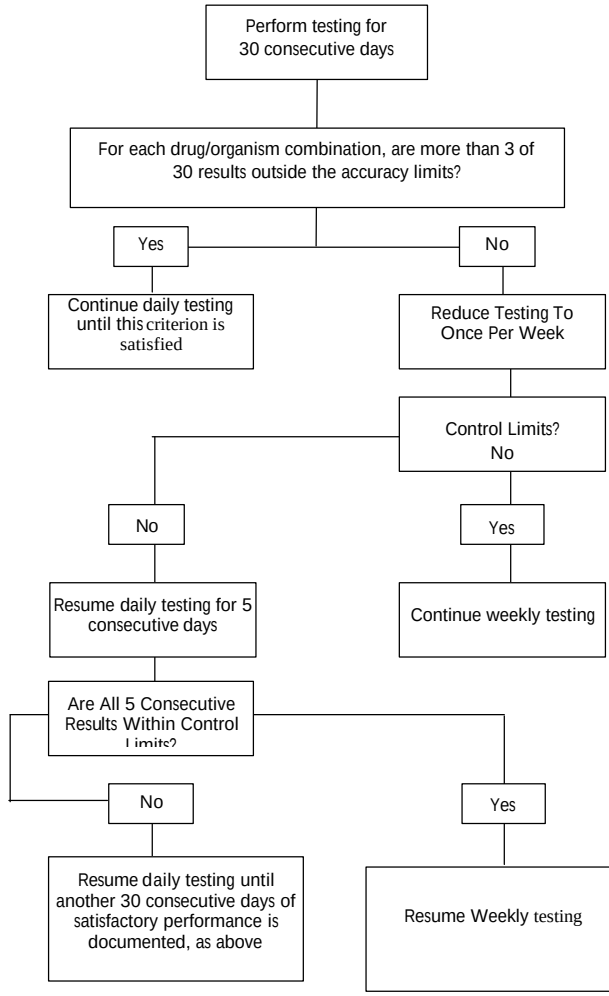
*NCCLS: 1995

17.9 STANDARD PROCEDURE FOR QUALITY CONTROL

The quality control programme should use standard reference strains of bacteria that are tested in parallel with the clinical culture. They should preferably be run every week (Figure 18.1), or with every fifth batch of tests, and in

addition, every time that a new batch of Mueller Hinton agar or a new batch of discs is used.

Figure 17.1: *Frequency of QC Testing in Antimicrobial Susceptibility Testing*



Standard Strains

These are:

- *Staphylococcus aureus* (ATCC 25923)
- *Escherichia coli* (ATCC 25922)
- *Pseudomonas aeruginosa* (ATCC 27853)

Culture for day-to-day use should be grown on slants of nutrient agar (tryptic soya agar is convenient) and stored in the refrigerator. These should be subcultured onto fresh slants every 2 weeks.

17.10 FREQUENCY OF QUALITY CONTROL TESTING

Salient features of quality control in antibiotic susceptibility testing are summarised below:

- Use antibiotic discs of 6 mm diameter
- Use correct content of antimicrobial agent per disc
- Store supply of antimicrobial discs at -20°C
- Use Mueller-Hinton medium for antibiotic sensitivity determination
- Use appropriate control cultures
- Use standard methodology for the test
- Use coded strains from time to time for internal quality control
- Keep the antibiotic discs at room temperature for one hour before use
- Incubate the sensitivity plates for 16-18 hours before reporting
- Incubate the sensitivity plates at 35°C

- ➢ Space the antibiotic discs properly to avoid overlapping of inhibition zone
- ➢ Use inoculum size that produces 'near confluent' growth
- ➢ Ensure even contact of the antibiotic disc with the inoculated medium
- ➢ Measure zone sizes precisely
- ➢ Interpret zone sizes by referring to standard charts

The zone size produced by an antimicrobial agent indicates its activity against the organism. However, zone sizes of two agents to which the organism is sensitive are not comparable and should not give an erroneous impression that the test organism is more sensitive to the drug which has yielded a bigger zone size.

18
QUALITY CONTROL IN SEROLOGY

SEROLOGICAL tests are performed to demonstrate antigens in the serum, or the response of the human body to these infectious agents to establish its contact with the immune system. Their diagnostic importance stems from demonstration of a rising titre of antibodies to the agent which *inter alia* indicates a progressive infection. In rare instances is the presence of antibody in a single sample indicative of infection and disease. Serological tests are of importance in epidemiological studies and to ascertain the response of the population to vaccines and other immunopotentiators.

Serological tests are also useful for the *in vitro* detection of microbial infections, and for the classification and subclassification of infectious agents (e.g. *Salmonella, Shigella, Streptococcus,* etc.).

Serological reactions detect either a specific antigen produced by the microorganism or a specific immune response of the human body. Serological tests may detect:

- ➢ an immunological principle (antigen-antibody reaction: ELISA, Widal)
- ➢ a non-specific reaction (VDRL test)
- ➢ a reaction mediated by complement (complement fixation test)

There are many advantages with serological methods. Some of these are:

- ➢ Rapid identification of agent
- ➢ High specificity of detection of antigen
- ➢ Simplicity of performance
- ➢ Safe procedures
- ➢ Diagnostic aids
- ➢ Epidemiological tools
- ➢ Retrospective confirmation of diagnosis

A wide variety of serological tests are now available and every day new ones are added to an already impressive list. Every laboratory must define a policy for conducting these tests because some may be expensive, all require certain reagents (sera or antigens etc.) which have limited shelf life, and all require standardized techniques which must be documented in SOPM.

18.1 PROCEDURE MANUAL

An important element in maintaining day-to-day uniformity in laboratory results is an established procedure manual (SOPM) which details all phases of the laboratory's

operation (including safety precautions) and is used by all laboratory personnel. It should include instructions for collecting, transporting, and storing specimens, for preparing and storing reagents, and for performing tests. In addition, the controls and calibrators to be used should be listed along with directions for their use, expected results, and instructions for corrective measures if the expected results are not obtained.

18.2 SELECTION OF TEST OR PROCEDURE

As new tests and methods are developed for various analytes (antibodies or antigens), the most appropriate must be chosen for each laboratory's needs. A number of factors must be considered, including bias, specificity, sensitivity, precision, cost and ease of performance. Bias, specificity and sensitivity may be related. Frequently the more sensitive a test, the less specific it is. Bias may result from low specificity or sensitivity. The population to be tested may influence the decision of what test to use, e.g. screening essentially normal patients does not necessarily require the same methods which are appropriate for following patients with a confirmed disease.

To determine the presence of **bias**, the proposed method should be compared with other reliable methods, preferably with a standard method or clinical data. The same specimens should be run with both methods in the same laboratory and the results compared, although interlaboratory comparisons are also useful. If the results from the different methods do not agree, one must determine the reason for the difference and then decide which result is more useful.

The **clinical specificity** of a method is evaluated by testing negative samples and samples containing substances which might cause interference. Closely related or cross-reacting substances frequently found in clinical specimens should be included.

The **clinical sensitivity** of a method being evaluated should be compared to that of other methods, but the purpose of the test must also be considered. In general, a definitive test need not be as sensitive as a screening test. The test should distinguish between normal and abnormal levels of analyte.

The **precision** of a quantitative or semiquantitative test must be evaluated in light of the precision required for the clinical application of the test results. Many factors affect precision, but one that is frequently overlooked in serologic tests is the size of the dilution increments. If all other variables are held constant, serologic tests tend to become less precise as the size of the dilution increment increases. For example, it should be expected that a test based on a four fold dilution would be less precise than the same test with a two-fold dilution.

A test with maximum possible sensitivity is desirable when a disease is serious and its diagnosis should not be missed when the disease is treatable, and when false-positive results do not lead to serious problems. Similarly a test with maximum specificity is desirable when a disease is serious but is not treatable, the knowledge that the disease is absent has psychological or public health value, and false-positive results can lead to serious problems. A high predictive value of a positive test result is desirable when treatment of a false positive might have serious consequences.

18.3 COLLECTION OF SPECIMEN

There must be a system for the orderly and efficient requesting of tests; collection and identification of specimens; and transporting, preparation, and storage of specimens. Nothing is more important than having an adequate amount of an appropriate specimen in good condition for examination. If each specimen is not properly collected, labeled, and handled, or is not representative, the laboratory may do more harm than good by testing it.

Haemolysed Blood

Haemolysed blood specimens are not suitable for serological studies. It is always advisable to avoid factors which cause haemolysis (Table 18.1). Specimens containing precipitates should be centrifuged prior to testing.

Table 18.1: ***Avoidable causes of haemolysis***

- ➢ Blood sampling through too small bore of a needle
- ➢ Forced suction of blood in the syringe during blood collection
- ➢ Vigorous shaking of blood from the syringe, especially through a needle
- ➢ Centrifuging blood sample at a high speed before clotting
- ➢ Freezing and thawing of blood
- ➢ Unclean tubes with residual detergents
- ➢ Water in syringe or tube

18.4 CONTROL SERA

Source

Some control sera are available commercially. Small volumes are generally available as components in kits but are intended to be used only with a single kit. A few may be available in larger quantities.

Preparation

Sera to be used as controls should be kept sterile to avoid deterioration. In general each procedure should have a normal control serum (negative), a strong positive control serum, and another positive control serum which is reactive at the critical concentration (borderline positive). With some tests, controls with a low concentration of analyte should be included. Controls recommended by the manufacturer of a particular test should always be used and additional control sera can be included if a test involves special problems.

Storage

Sera to be used as controls should be standardized against international reference materials when they are available. "Standards" included in commercial kits are not calibrated with each other and often are not interchangeable. These should be stored in aliquotes in frozen forms. Repeated freezing and thawing should be avoided.

18.5 REAGENTS

Quality reagents are necessary for quality performance. A record should be kept of any changes in reagents in case

the performance of a test changes. Before new reagents are introduced into a system they should be tested in parallel with the old reagents against a panel of appropriate reference sera to be sure that consistent reactions are obtained. The results obtained with the panel should reflect the sensitivity and specificity of the reagents being compared.

Reagents should be clearly labeled to indicate their identity, hazards involved in their use, recommended storage conditions, and preparation and expiration dates.

18.6 EQUIPMENT AND INSTRUMENTS

All glassware used in immunologic tests must be clean and free of detergent. Chipped or etched glassware should be discarded. Calibrated glassware should be checked for accuracy.

The user's accuracy and precision requirements should be met or exceeded when equipment is tested under working conditions. The manufacturer's specifications for performance should be checked and met. Instruments and equipment should be monitored routinely. The temperature of water baths, incubators, refrigerators, and freezers should be checked periodically and records maintained. Maintenance should be performed and records kept on a regular basis by individuals who are trained and are familiar with the equipment.

Instruments used for measurements including spectrophotometers, spectrometers, dilutors, and automatic pipettors should be calibrated on a regular basis. Quality control procedures of various tests are shown in Tables 18.2 and 18.3.

18.7 PERFORMANCE OF TESTS

The performance of tests is monitored with controls. Antigenic serum panels as well as sera with known quantities of antibodies are available and should be routinely used. Correct performance of reagents is reflected by the expected reaction in tubes which lack one or more of the components necessary for the reaction. For example, the presence of anti-streptolysin O reagent is demonstrated by haemolysis in the tube containing the reagent buffer and cells but no antibody to inhibit haemolysis.

Table 18.2: ***Quality control of tests detecting antibodies****

Antibody test	Control procedures required	Expected results
Flocculation test (RPR)	Nonreactive serum control Weakly reactive serum control Reactive serum control	No clumping Clumping of graded activity Clumping of graded activity
Latex agglutination test (ASO)	Negative control serum Positive control serum	No clumping Clumping
Direct agglutination (Widal test, STA for Brucellosis)	Antigen control Negative control serum Positive control serum	No clumping No clumping Clumping
Passive haemagglu-tination (ASO)	Streptolysin control Red cell control	Haemolysis No haemolysis

** Quality control procedures have to be carried out every time samples are tested*

18.8 REPORTING AND RECORD KEEPING

Complete and accurate records must be maintained in a good quality assurance programme. These records should include personnel information; details of equipment, preventive maintenance, service, and repair; copies of reports to physicians or other clients; accession records; records of reagents and materials used; records of observations made concurrently with the performance of each step in the examination of specimens; proficiency testing results; and internal quality control results.

Table 18.3: ***Quality control procedures for tests detecting antigens***

Antigen test	Control material	Expected result
Capsular Quellung reaction (Omni serum, *H.influenzae* type b)	Pneumococci Haemolytic streptococci *H.influenzae* type b *Acinetobacter anitratum*	Capsular swelling No reaction Capsular swelling No reaction
Coagglutination test (Haemolytic streptococci meningitis antigens)	Group A,B,C streptococci *N.meningitidis*	Agglutination with corresponding serum,

The accessioning and reporting system should minimize the possibility of clerical errors. Precautions should be taken to prevent reporting results on the wrong specimen and transposing digits in reporting quantitative data. The system should be so designed that the history associated with a sample can be reconstructed in detail if necessary. Who performed which tests, what reagents and lot numbers they used, what the control results were for that run, and how and when the results were reported should also be documented.

19
QUALITY CONTROL IN STERILIZATION

STERILIZATION AND disinfection are part of the daily routine in microbiological laboratories and constitute vital activities which ensure that cultures, containers, media and equipment are treated in such a way that only the desired organisms that are inoculated will grow and all others will be eliminated. These are accomplished by the use of heat, chemicals, radiation or filtration. A few important definitions which are frequently used are provided below:

Sterilization is the destruction or removal (by filtration) of all microorganisms, including bacteria, viruses, fungi and prions. It is conceived as an absolute.

Disinfection describes a procedure of treatment to render a contaminated item into a state that it will be no more infectious. The procedure implies the destruction of bacteria, fungi and viruses but not necessarily of spores.

Antisepsis is a process involving the destruction or inhibition of micoorganisms in living tissue thereby limiting or preventing the harmful effects of infection.

Over the years, heat has proved to be the most popular method of sterilization. It is the most economical, safe and reliable method. Heat is believed to kill microorganisms by denaturation and coagulation of their vital protein systems. Oxidation and other chemical reactions are also greatly accelerated as the temperature is increased, roughly doubling for every rise of 10°C. There are principally two methods of thermal sterilization: **moist heat** (saturated steam) and dry heat (hot air) sterilization. Moist heat has the advantage of acting more rapidly and requiring lower temperatures.

Relatively few chemicals are capable of performing sterilization and have the additional properties of stability, safety, lack of colour etc that make them acceptable. Some of these may be dispensed as gases which gives them the ability to penetrate deep into materials (eg ethylene oxide, formaldehyde) whereas others are used as liquids in applications where the volatility of gases would not be suitable (e.g. glutaraldehyde, hydrogen peroxide).

19.1 INDICATORS OF THE STERILIZATION PROCESS

The wide application of sterilization processes makes it mandatory to impose strict control measures to validate the results obtained (Table 19.1). These processes are of three broad types: physical, chemical and biological. In addition, sterility tests on the treated products are necessary. Of various indicators, the biological indicators have gained maximum popularity.

Table 19.1: ***Methods of validating sterilization processes***

Process	Physical methods	Chemical methods	Biological test organism
Dry heat	Temperature recording charts	Colour change indicator	*B.subtilis var niger*
Moist heat	Temperature recording charts	Colour change indicator	*B.stearothermophilus*

Biological Indicators

Bacillus stearothermophilus was earlier considered ideal for monitoring because this organism lacks pathogenicity, pyrogenicity and toxicity. Biological indicators manufactured today are generally impregnated with a spore population to meet a performance requirement of surviving a certain period of time in a sterilizing atmosphere but being killed in a longer period of time at the same sterilizing conditions. The number of spores that should be present when sterilization is being monitored is 10^4 to 10^6 for *B.stearothermophilus* and around 10^6 for *Bacillus subtilis var niger*.

19.2 GENERAL PRINCIPLES FOR TESTING STERILIZING AGENTS

It is necessary to know whether or not the sterilizing agent is effectively doing the job intended for it. Each method of sterilization is different and each requires its own test procedure.

Heat Sterilization

For heat sterilization, the first requirement is knowledge that the temperature is recording correctly. Furthermore, the operator must know that the temperature is reaching all the parts of the load and is maintained for the desired length of time. Recording thermometers and barometers for steam sterilizers should be employed for the chambers and thermocouples can be buried inside the load. Paper strips treated with chemicals that change colour at the required temperature may be used. If, in a steam autoclave, a container is tightly closed and receives no steam, it may reach the correct temperature, but this will not ensure that sterilization will occur. To give this assurance requires the use of biological testing in the form of heat-resistant spores. With moist heat, spores of *B. stearothermophilus* are used, and with dry heat sterilizers, spores of *B.subtilis* var *niger* are selected. The spores are dried on paper treated with nutrient medium and chemicals. After the sterilization treatment, they are incubated for germination and growth and a colour change indicates whether they have or have not been activated. This method may take several days of incubation, whereas physical and chemical methods are immediate, but the biological tests are more dependable.

Chemical Sterilization

For chemical sterilization, there are colour indicator tapes for ethylene dioxide and formaldehyde, which show whether or not these gases have penetrated in sufficient quantity at the prescribed temperature to provide sterilization. But here, as with other methods, biological methods are preferable. With ethylene oxide, strips treated with *B.subtilis* var *niger* are employed, whereas with formaldehyde *B.stearothermophilus* is used.

In the case of liquid sterilants, spores of *B.subtilis* var *niger* and *Clostridium sporogenes* are both tested. The spores are dried onto carriers that may be porcelain or stainless steel or suture loops. They are exposed to the solution of chemical sterilant at the desired concentration, given a specified temperature and time of immersion, after which they are incubated in a rich medium for germination and growth. These tests are replicated many times and, if any of the replicates show growth, the candidate sterilant fails the test.

Sterilization by Filtration

With filtration sterilization, membrane filters may be readily tested for passage of microorganisms of different sizes. Spores of *B.subtilis* var *niger*, cells of *P. diminuta*, bacteriophages and other viruses give a range of sizes. The bubble point test can also be used. This correlates the pore diameter with the air pressure required to cause the first bubble to break through a filter. Depth filters may be tested by the passage of selected organisms or by the penetration of aerosols made up of chemical dusts of known particle size. For example, dry particles of sodium chloride can be detected and quantitatively determined with a hydrogen flame photometer.

Combined Treatments

Enhanced sterilizing activity can take place if two or more processes, chemical or physical, are employed together. There are various kinds of such treatments as discussed below:

Thermochemical Treatment: With an increase in temperature the antimicrobial activity of various

compounds increases. Use of ethylene oxide at 60°C and low temperature steam with formaldehyde are salient examples of these combined treatments.

Chemical Treatment and Irradiation: During radiation if certain chemicals are also present, spores get sterilized and respond better to the action of irradiation. These findings have yet to find application.

Thermoradiation*:* Simultaneous use of heat and ionizing radiation can provide good results provided the temperature is carefully selected to avoid paradoxical inversion of thermorestoration which may occur at certain temperatures.

Table 19.2: ***Preferred methods of sterilization for common use articles***

Autoclaving	Hot air oven	Ethylene oxide	Filtration
Animal cages	Glass ware	Fabric	Antibiotics
Sugar tubes	Beakers	Bedding	Sera
Lab. coats	Flasks	Blanket	Vaccines
Cotton	Petri dishes	Clothing	
Filters	Pipette	Mattresses	
Instruments	Slides	Pillows	
Culture media	Syringes	**Disposables**	
Rubber	Test tubes	Blades	
gloves	Glycerine	Knives	
stopper	Needles	Scalpels	
tubing	Oils	Scissors	

Autoclaving	Hot air oven	Ethylene oxide	Filtration
Glass		**Paper**	
slides		cups	
syringe and needles		plates	
test tubes		**Plastics**	
Enamel metal trays		flasks	
Wire baskets		petri dish	
Wood		tubes	
tongue depressors		**Rubber tubings**	
applicator		**catheters**	
Steel tumbler		drains	

20
QUALITY CONTROL FOR EQUIPMENT

EQUIPMENT IN a laboratory is used for measurement and for the preparation and storage of materials and reagents. Comprehensive information on the specifications is essential for the purchase of appropriate equipment and its maintenance.

Equipment play critical role in assuring quality of results of laboratory. These must be properly installed and maintained to ensure satisfactory working. Following factors influence optimal utilization of equipment:

- Proper installation
- Calibration
- Validation
- Regular maintenance
- Training to operator

20.1 PURCHASE OF EQUIPMENT

Various parameters should be considered before decision to purchase equipment is finalised. Some of the important check-points are summarised below:

- The instrument specifications should fit the intended purpose with comparative cost-effectiveness
- The specifications should conform to local conditions such as power supply, humidity and climate
- Prompt and preferably local, cost-effective maintenance service should be available
- Should not be a very complex instrument and must be assessed for technical safety and moderate running cost
- An operation and maintenance manual must be supplied
- A trouble shooting list should also be provided by the manufacturer
- Infrastructure and basic services required for the instrument should be available
- Should be safe for operator
- Must be compatible with availability of reagents on long term
- Should be serviceable and easy to maintain

Autoclave

An autoclave is an integral part of any microbiological laboratory. Various steps that can be taken to ensure its proper functioning are provided below:

- ➢ Record temperature in each run
- ➢ Record pressure once during each run
- ➢ Use properly placed colour indicators in each run
- ➢ Use peak temperature thermometers weekly
- ➢ Use spore strips or spore suspensions monthly
- ➢ If evidence of contamination is found, make sample cultures frequently (daily or weekly) until the cause is determined and eliminated.

Incubators

Incubators should be subjected to continuous recording of temperature. However, if it is not possible, the temperature must be recorded every day and before opening of the incubator.

pH Meter

A pH meter needs to be standardised before *each run* with a standard buffer of pH 7.0. However, in instances when the work is related to a pH range of less than 6.0, it is advisable to use a standard buffer of pH 4.0. The buffer solution should be checked *monthly* with another pH meter and discarded if the pH deviates more than ± 0.4 or if the buffer is contaminated with microorganisms.

Centrifuge

A centrifuge should be evaluated often enough to assure proper performance. The rheostat control should be checked with a tachometer at various loadings and at frequent intervals (e.g. six monthly) to assure proper gravitational fields.

Volumetric Glasswares

All volumetric glassware such as flasks and pipettes, should be checked for proper calibration before being used. All glassware must be clean and free of detergents. Chipped or etched glassware should be discarded.

Pipette

Each pipette, whether manual, semiautomated or automated, must be tested periodically to determine if it is delivering the correct volume.

Timers

Mechanical timers should be tested on a regular basis to determine their accuracy by comparing them with electronic or electric clocks.

For the following equipment, daily monitoring of the temperature is recommended

- water baths
- refrigerators
- hot air ovens
- freezers

In addition, indicators of sterility (chemical or biological) should be used with hot air ovens. The details of these are given in Chapter 15 (Quality Control in Sterilization).

20.2 FUNCTION CHECKS

It is essential that laboratory personnel know and document that all equipment is in good working condition each day of use. This can be accomplished by undertaking function checks, often referred to as calibration and validation.

Calibration: That process which is applied to quantitative measuring or metering of equipment to assure its accurate operation throughout its measuring limits

Validation: The steps taken to confirm and record the proper operation of equipment at a given point of time in the range in which tests are performed.

Temperature monitoring of incubators, water baths and refrigerators

Temperatures should be checked daily and recorded. If the readings are beyond tolerance limits, staff should know what needs to be done. Some of the suggested acceptable temperature ranges, as recommended in national standards of few developed countries, are shown in Table 20.1

Table 20.1: ***Temperature checks***

Temperature	Acceptable range ^{o}C	Purpose
Refrigerator	4-8	Storage of media, reagents and stock cultures
Freezer at -20^{0}C	+/-5^{o}C	Storage of specimens, sera, reagents and stock cultures
Freezer at -70^{o}C	+/-10^{o}C	Storage of reference culture and sera

20.3 DOCUMENTATION

The assurance that a piece of equipment is operating properly can best be judged by examining its performance over time. Records of performance parameters, therefore, are a vital element in the proper operation of laboratory equipment. Some suggested information is provided below:

- Name and serial number of instrument
- Elements to be checked and kind of data to be collected
- Frequency of checking
- Record of data
- Comments on data
- Changes made to restore accuracy and precision, if any
- Signature with date of the person performing these tasks

20.4 PREVENTIVE MAINTENANCE

Maintenance of equipment is an extremely important function in the microbiology laboratory. Unfortunately, this is often grossly neglected because of indifference on the part of laboratory workers and on the erroneous belief that it is too costly. The expense of such maintenance policies as inspection, lubrication and adjustment of instruments is insignificant when compared with the cost of emergency repairs, rebuilding or overhauling equipment, and the additional personnel time and materials involved in producing test results when equipment is down.

Preventive maintenance is defined as a programme of scheduled inspections of equipment and instruments

resulting in minor adjustments or repairs for the purpose of delaying or avoiding major repairs and emergency or premature replacements. It provides the following advantages over breakdown maintenance.

- Better quality results
- Identification of components showing excessive wear
- Greater safety
- Fewer interruptions in services
- Lower repair costs
- Less standby equipment requirements

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