SEARO Regional Publication No. 47

Quality Assurance in Bacteriology and Immunology

Third Edition

World Health Organization
Regional Office for South-East Asia

SEARO Regional Publication No. 47

Quality Assurance in Bacteriology and Immunology

Third Edition

World Health Organization
Regional Office for South-East Asia

WHO Library Cataloguing-in-Publication data

World Health Organization, Regional Office for South-East Asia.

Quality assurance in bacteriology and immunology. - 3rd ed.

(SEARO Regional Publication No. 47)

1. Quality Assurance, Health Care. 2. Laboratory Techniques and Procedures-methods. 3. Laboratories – organization and administration. 4. Waste Management. 5. Quality Control. 6. Bacteriological Techniques – Standards.

ISBN: 978-92-9022-415-0 (NLM classification: QY 100)

© World Health Organization 2012

All rights reserved.

Requests for publications, or for permission to reproduce or translate WHO publications – whether for sale or for noncommercial distribution – can be obtained from Publishing and Sales, World Health Organization, Regional Office for South-East Asia, Indraprastha Estate, Mahatma Gandhi Marg, New Delhi 110 002, India (fax: +91 11 23370197; e-mail: publications@searo.who.int).

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

All reasonable precautions have been taken by the World Health Organization to verify the information contained in this publication. However, the published material is being distributed without warranty of any kind, either expressed or implied. The responsibility for the interpretation and use of the material lies with the reader. In no event shall the World Health Organization be liable for damages arising from its use.

This publication does not necessarily represent the decisions or policies of the World Health Organization.

Printed in India

Contents

Acknowledgements v

Foreword vii

Abbreviations ix

Glossary xi

1. Introduction 1
2. Factors influencing quality 10
3. Overview of quality assurance 15
4. Quality management system 19
5. Organization and functions of laboratories 25
6. Documentation in the laboratory 33
7. Standard operating procedures 47
8. Validation 52
9. Assessment of quality 54
10. Quality audit and accreditation 67
11. Safety in the laboratory 77
12. Practice of quality assurance in clinical laboratory 98
13. Quality control of laboratory materials 106
14. Quality control of media and stains 111
15. Quality control of bacteriological techniques 117
16. Preservation of stock cultures 119

17. Quality assurance in antibiotic susceptibility testing 123

18. Quality control in serology 137

19. Quality control in sterilization and disinfection 144

20. Quality control for equipment 153

21. Suggested further reading 160

Annex 1: Contributors 162

Index 164

Acknowledgements

The first draft of the third edition of this document was developed by Dr Sudarshan Kumari, former Regional Adviser, WHO Regional Office for South-East Asia, New Delhi, We gratefully acknowledge her contribution as well as inputs from several experts (Annex 1) in finalization of this document.

Foreword

Health services are using laboratories for clinical purposes as well as public health more than ever before. The demand for quality results from laboratories has been echoed by all health care professionals as unreliable results can have serious consequences for the health of the individual as well as the community. The main objective of quality assurance is to provide reliable laboratory data for 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, particularly in developing countries, has been universally recognized. Laboratories practising the principles of quality assurance generate relevant, reliable and cost-effective results, delivered timely and in the best interest of the patient.

The mainstay of quality assurance in health laboratories is the sound practice of internal quality control. Participation in external quality assessment schemes tells us whether or not internal quality control is in place. The concept of quality assurance has now been expanded to the Quality Management System, which covers not only pre-analytic, analytic and post-analytic aspects of treatment but also policies, programmes, processes and procedures. It encompasses a wide spectrum of activities that includes the collection, storage and transportation of appropriate specimens; the performance of tests using

the right technique with suitable controls; and data processing, such as the reporting, recording and interpretation of results as well as their timely feedback to the right user.

The quality of laboratories can be improved by the application of good clinical laboratory practices and commitment. Quality is never an accident, it is the result of concerted planned activity. Constraints include lack of awareness, inadequate financial resources, low level of information about the positive impact of improved laboratory quality, as well as the nonavailability of comprehensive and practical guidelines, especially in peripheral and intermediate laboratories. Laboratories of good quality must be prepared for accreditation so as to achieve a healthier world through quality laboratory services.

This manual has been designed to ensure quality assurance, including biosafety, 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.

Samlee Plianbangchang

Dr Samlee Plianbangchang
Regional Director

Abbreviations

AFB	acid fast bacilli
AR	analytical reagent
ASO	antistreptolysin O
ATCC	American type culture collection
CAMP	Christie Atkins Munch-Peterson
cm	centimetre
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	millimetre
CLSI	Clinical and Laboratory Standards Institute, USA
^{o}C	degree celsius
ONPG	O-nitrophenyl-®-D-galactopyranoside
OPD	outdoor patient department
PA	pro analysis
PHA	passive haemagglutination
PPA	purissimum pro analysis
QA	quality assurance
QC	quality control
RBCs	red blood cells
RPR	rapid plasma reagin
SOP	standard operating procedure
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 by which an authoritative body gives formal recognition that a body or person is competent to carry out specific tasks.

Analytical Phase

Activities and steps related to performing a laboratory test.

Audit

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

Auditor

A person with demonstrated personal attributes and competence to conduct an audit.

Auditee

The organization being audited.

Bias

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

Biohazard

An infectious agent, or part thereof, that presents a real or potential risk to the well-being of man, animals or plants and environment.

Biological Reference Interval

An arbitrary but common convention that defines the reference interval as the central 95 % interval. This supersedes the term “normal range”.

Biosafety

The application of knowledge, techniques and equipment to prevent personal, laboratory and environmental exposure to potentially infectious agents or biohazards. Biosafety defines the containment conditions under which infectious agents can be safely handled.

Competency

A demonstrated ability to apply knowledge and skills.

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, sensitivity and specificity of a procedure. It is used to determine, verify and document performance.

Continual Quality Improvement (CQI)

This is part of a quality management system focused on increasing the ability to fulfill quality requirements. It is a philosophy and attitude for analyzing capabilities and processes and constantly improving them to achieve quality test results. This is possible through small, incremental changes using scientific methods.

Corrective Action

The implementation of solutions resulting in the reduction or elimination of an identified problem.

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.

Document

Information and its supporting medium (may be paper-based or electronic). ISO identifies five types of documents: specifications, quality manuals, quality plans, records, and procedure documents.

Document Control

A system for maintaining and ensuring the proper use of time or version-sensitive documents.

Efficiency

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

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

Error

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

External Controls

Controls not included in test kits and used in addition to internal controls.

External Quality Assessment (EQA)

EQA is a system of objectively assessing laboratory performance by a designated laboratory (organizing laboratory).

False Negative

A negative test result for a person who is actually infected.

False Positive

A positive test result for a person who is actually not infected.

Form

A paper or electronic document on which information or results are captured. Once completed, a form becomes a record.

Internal Audit

Internal quality audits are audits carried-out by laboratory personnel (first party audit) who examine the elements of a quality management system in their laboratory in order to evaluate how well these elements comply with quality system requirements.

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 as well as the analysis of results, with the procedure being planned, ordered and followed up by the staff itself.

Inter-laboratory Comparison

The organization, performance and evaluation of tests for the same analyte by two or more laboratories in accordance with predetermined conditions. Laboratories may participate in inter-laboratory comparison for testing of those analytes where formal EQAS is not easily accessible.

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 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.

Measuring Equipment

An instrument that shows the extent or amount or quantity or degree of something.

Medical Laboratory

A laboratory for the biological, microbiological, immunological, chemical, immunohaematological, haematological, biophysical, cytological, pathological or other examination of materials derived from the human body for the purpose of providing

information for the diagnosis, prevention and treatment of disease, or assessment of the health of human beings, which may provide a consultant advisory service covering all aspects of laboratory investigation including the interpretation of results and advice on further appropriate investigation.

Pre-analytical Phase

In chronological order, steps that begin with the clinician's request, examination requisition, preparation of the patient, collection of the primary sample, transportation to and within the laboratory, and ends with the beginning of the examination phase.

Analytical Phase

The term used to describe everything that happens during the handling and analysis of the sample in the laboratory.

Post-analytical Phase

The processes following the examination phase, including systematic review, formatting and interpretation, authorization for release, reporting and transmission of the results, and storage of samples for examinations.

Precision

The ability of a measurement to be consistently reproduced as well as the number of significant digits to which a value has been reliably measured.

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.

Predictive value of a positive test = True positive / True positive + False positive x 100

Predictive value of a negative test = True negative / True negative + False negative x 100

Probability

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

Preventive Action

Proactive actions taken to remove or improve a process to prevent potential future occurrences of non-conformity.

Preventive Maintenance

Scheduled periodic work on a piece of equipment that is not a result of malfunction or failure and is intended to avert such failure.

Procedure

A detailed procedure which defines the work that should be done, explains how it should be done, who should do it, and under what circumstances.

Quality

The degree to which a set of inherent characteristics fulfills requirements.

Quality Assurance

A planned and systematic set of quality activities focused on providing confidence that quality requirements will be fulfilled.

Quality Control

A set of activities or techniques to ensure that all quality requirements are being met. Simply put, it examines "control" materials of known substances along with patient samples to monitor the accuracy and precision of the complete examination process.

Quality Management System (QMS)

A system which establishes a quality policy and quality objectives and which achieves those objectives.

Range

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

Record

A document stating the results achieved or providing evidence of activities performed.

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, and for assigning values to materials (ISO Guide 30:1981, 2.1).

Root Cause Analysis (RCA)

A tool designed to help identify not only what and how an event occurred, but also why it happened.

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 populations in question.

Sensitivity

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

Sensitivity = True Positive / True Positive + False Negative x 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.

(2) Specificity = True Negative / True Negative + False Positive x 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)

Standard Operating Procedure (SOP)

A document which describes the regularly recurring operations relevant to the quality of the investigation. The purpose of a SOP is to carry out the operations correctly and always in the same manner.

Test

A technical operation that consists of the determination of one or more characteristics of a given product, process or service according to a specified procedure.

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.

Total Quality Management

The management approach of an organization centered on quality, based on the participation of all its staff and aiming at long-term success through customer satisfaction and benefits to staff and society.

Traceability

The ability to trace the history, application or location of that which is under consideration.

Validation

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

Work Environment

A set of conditions under which a person operates.

1

Introduction

Quality is the degree of congruence between expectation and realization. In other words, it is the matching of what you want with what you get. The simplest definition is:

> **Quality** is the meeting of pre-determined user requirement for a particular substance or service.

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

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

It has been rightly described as the characteristics of a product or service that makes it suitable for the purpose for which it is intended (fitness of purpose).

It has also been associated with consistency. This means providing the same product or service time after time, thereby making the outcome more predictable.

In health laboratory services, a report is the analysis of the material received by the laboratory and processed by the the physician or public health professional, for the benefit of the patient or community – the customers of 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 programme

- Delivering a quality product or generation of a reliable service
- Helping the physician establish proper and rapid diagnosis, 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
- Efficient utilisation of resources
- Cost-saving on account of wrong/over/under treatment
- Assuring safety of patients, staff, visitors, community and environment

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

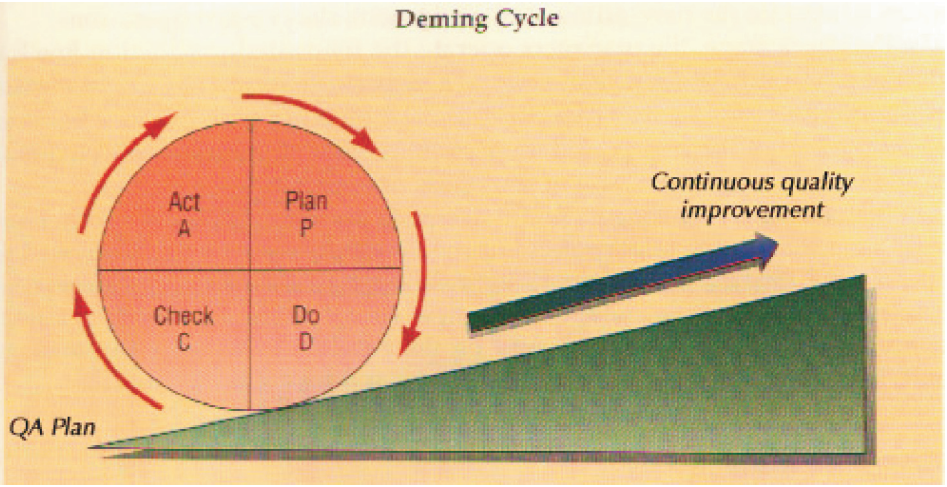
Doing things right first time means reduced rework and product failure, resulting in cost savings.

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 prevent further problems from re-occurring in the future.

1.2 Continual quality improvement

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

The important point about the Deming cycle is that it illustrates the need for continual momentum to go forward and upward. If there is no progress



forward, the pressure is to fall backwards -- down the slope – with consequent loss of quality. The only way to prevent backward movement is to continually support the cycle. This is the function of the quality system that has been put in place.

Continuous quality improvement, therefore, comprises a set of activities that organizations should routinely carry out in order to enhance quality. It emphasizes the need for continuous improvement rather than only error correction All aspects of laboratory operations are explicitly targeted for improvement in a sequential manner.

Continual quality improvement can be achieved by :

- Monitoring of quality indicators & analyzing data
- Identification and control of nonconformities
- Undertaking root cause analysis, corrective and preventive actions and improvement processes
- Assessment of customer satisfaction and complaints
- Carrying out internal audits
- Performing management reviews

In the early years, the focus of quality improvement was limited to the final product. This was soon expanded to incorporate the processes that made

up the product. From there a broader view of quality developed and concepts such as good clinical laboratory practices (GCLP) and customer satisfaction came into being. Now we talk of total quality management which focusses on the satisfaction of customers, suppliers, staff and society, as well as the consideration of environmental issues.

Quality indicators

Quality must be measured if it has to be managed. Quality indicators (QI) help the health laboratory to define and measure progress. The measurement of quality indicators leads to early detection of system failure which includes all aspects of service – pre-analytic, analytic and post-analytic – so that remedial actions can be taken promptly.

Table: Examples of indicators

Pre-analytic indicators:

- Incomplete requisitions
- Phlebotomy efficiency
- Specimen acceptability/rejection rates
- Accuracy of sample accessioning
- Specimen transport times

Analytic indicators:

- Internal and External Control failures
- Performance in EQAS
- Frequency of unscheduled service and repairs of equipment
- On-time performance – Calibration and Maintenance of equipment
- Vendor evaluation (supplier performance – Backorders, delays, incorrect or damaged supplies)
- Inventory – emergency orders, Outdating
- Misinterpretations of results
- Availability of back-up services

Post-analytic indicators:

- Compliance to turnaround times (TAT)
- Errors/ Incomplete test reports
- Availability of archived samples

Other Quality Indicators:

- Number and type of laboratory accidents (Needle sticks)
- Training and competency evaluation of personnel
- Document problems discovered – outdated, incomplete, incorrect
- Customer feedback and complaints – physicians, other health care staff, patients.
- Numbers and types of non-conformances
- Physician satisfaction with report format and content

Bench-level staff must be engaged in the development of quality indicators as they are key players in laboratories. Fewer staff are better than too many, as tracking becomes difficult. Moreover, quality indicators should only be used as long as they provide useful information. Once they indicate a stable and error-free operation, select a new quality indicator.

1.3 Users' perception of health laboratory services

There are many perceptions by laboratory users of the quality of bacteriological and immunological services. An overview of this diversity 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 available according to the requirements of the individual
- Health care workers are well aware of the kind of specimens to collect and how to resolve problems
- Needs are understood, met and exceeded
- Complaints and problems are swiftly addressed, resolved and rarely repeated.

Though users' perception of quality is important, it is only a small part of a comprehensive quality assurance programme. Better assessments are obtained by getting active feedback from customers.

1.4 Good clinical laboratory practices

Good Clinical Laboratory Practices (GCLP) deal with the organization, process and conditions under which laboratory studies are planned, performed, monitored, recorded and reported. GCLP practices are intended to promote the quality and validity of test data. They point towards the best laboratory activities that deliver the most accurate results. The components of the GCLP are given in Table 1.3.

Table 1.3 Major components of GCLP

Infrastructure	• Plan infrastructure of laboratories according to the services provided by the laboratory.
Personnel, training and development	• Designate a laboratory Head and a Quality Manager • Clearly define the roles and responsibilities of the staff • Develop a programme for continuous technical training and updating of skills • Maintain a personal file of all technical and non-technical staff

Equipment	• Prepare a list of equipment required according to scope • Place equipment suitably and ensure that it is in good working condition • Calibrate and validate equipment before putting it to use and regularly thereafter • Train staff in the correct use and calibration of equipment
Reagents and Materials	• Use certified standard quality reagents. • Validate quality of newly purchased reagents prior to use. • Store reagents, chemicals and consumables under appropriate environmental conditions with appropriate labels
Specimen collection	• Use the "primary specimen collection manual" which contains information on how to prepare the patient before specimen collection, the exact methodology of collection, labeling the specimen as well as its handling, transportation and storage.
Requisition Form	• Use the requisition form delineating the patient's identity, age, gender, location, date, time of specimen collection and its receipt as well as the investigations requested along with relevant clinical and treatment history.
Accession list	• Develop an accession list recording all the specimens received along with patient's identity
Specimen rejection log	• Prepare specimen rejection criteria • Maintain a record of specimens which were rejected prior to analysis • Use specimen rejection statistics to identify training needs for technical staff
Worksheet	• Use standard worksheet for each test/ assay

Reporting test results	• Ensure that authorized signatory has verified test results • Report results clearly, without any errors, specifying measurement procedures where appropriate and units of measurement signed by authorized signatory • Ensure predetermined turnaround times • Ensure release of results to authorized persons only
Data Management	• Record laboratory data including patient details, findings of analyses, reporting of results • Archive the data for future reference.
Standard Operating Procedures (SOP)	• Prepare and use SOP containing detailed, written instructions which describe the step-wise process and technique of performing a test or procedure in the laboratory. Perform test as per the SOP
Laboratory safety	• Document and practice laboratory safety policies and procedures. • Train all laboratory personnel about laboratory safety policies and procedures.
Ethical	• Comply with the ethical codes of the laboratory/ institution
Quality Management System (QMS)	• Develop and implement QMS encompassing preanalytic, analytic and post analytic components of testing • Involve higher management at each step of QMS

Countries which have built up national programmes for quality assurance have shown promising results as judged by international organizations.

1.5 International Organization for Standardization

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

ISO No	Refers to
ISO 9000 series (9000, 9001, 9002, 9003, 9004, etc)	Design/development, production, installation, servicing, 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
ISO Guidelines 15190	Medical laboratories — Requirements for safety

1.6 Traceability

Measurement traceability is the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties.

Specimen traceability is an important concept in quality assurance programmes. 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 measures.

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 intra-laboratory 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 include sources of error that are beyond the control of laboratory staff. Laboratory personnel and clinicians should bear this important fact in mind, that quality assurance is a mutual responsibility which cannot be achieved only through improving and controlling the analytical process without simultaneous 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.

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 are discussed in Table 2.2 and Figure 2.1.

2.1 Pre-analytical factors

The range of reference values as well as the results obtained from measuring specimens are prone to a number of pre-analytical influences such as :

Table 2.1: Factors influencing quality

Analytical Quality Control

- Equipment reliability
- Reagent stability, integrity and efficiency
- Temperature control
- Adequate calibration of measuring equipment
- Procedure reliability in terms of :
  - Precision
  - Accuracy
  - Analytical specificity

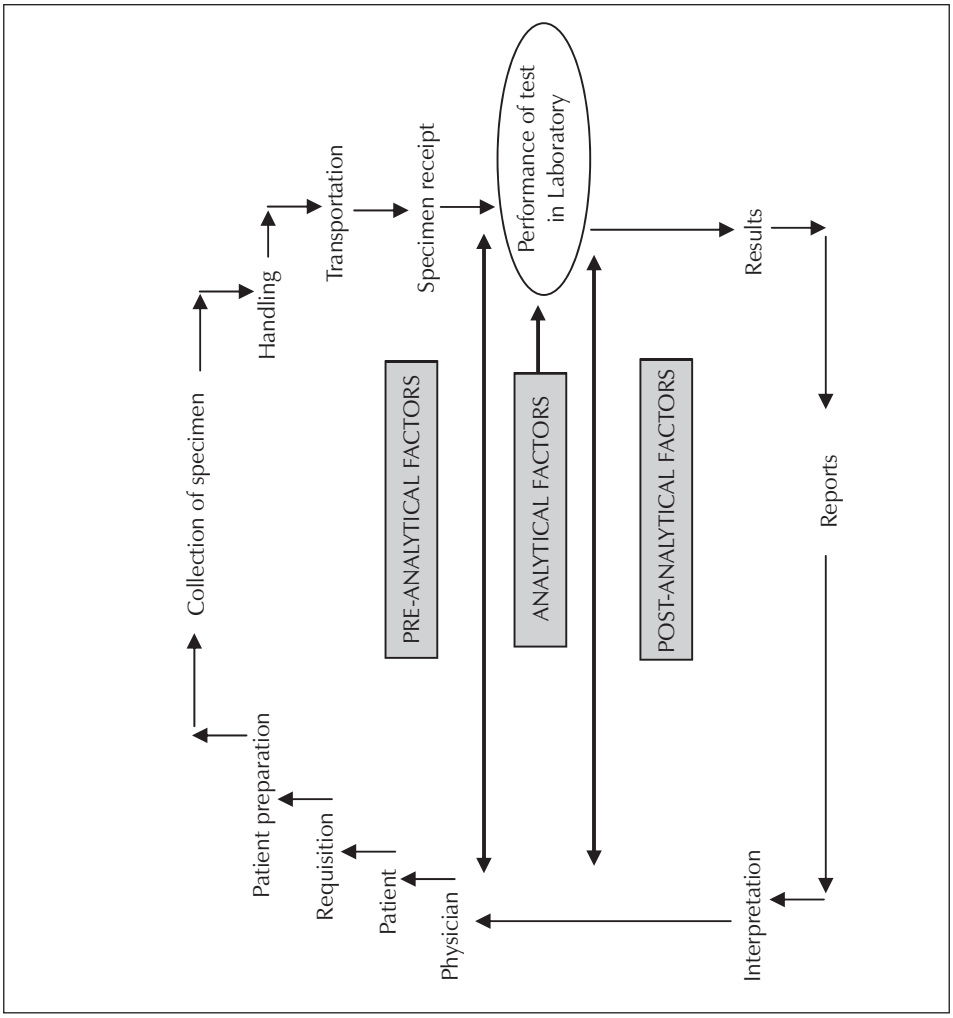
Non-analytical Quality Control (Para-analytical factors)

- Patient preparation
- Proper sample selection
- Proper collection of specimen and transportation
- Details of the patient and specimen identification
- Proper recording of results and their interpretation
- Knowledge of biological reference interval of the analyte measured

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	Biological reference intervals
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 milieu	Authorized release of results
Right technique for available reagents	Right transportation	Archiving of specimen
Internal quality control	Right quantity	Transcribing results from worksheet to report forms
External quality assessment	Right labelling	

Figure 2.1 Factors influencing quality



Age-dependent variations

Age dependent changes of concentration or activities occur in a number of hematological and chemical analytes. Normal bacteriological flora also changes with age. Hence laboratory results should be interpreted keeping in view the age of the patient.

Incorrect specimen identification

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

Prolonged transportation and storage

Prolonged and improper storage prior to processing of the specimen can affect results, especially since many organisms do not survive for long periods unless they are sub-cultured or kept in a rapidly changing environment. Urethral discharge containing gonococci, specimens for anaerobic bacteriology, etc are some examples where improper storage and transportation can give false results.

Selection of appropriate samples

An appropriate sample should be collected in an appropriate way. For example, for diagnosing acute intestinal amoebiasis, it is essential to examine the freshly passed stool sample within 30-60 minutes, since trophozoites only survive for that period. Similarly, stool examination for the eggs of *Enterobius vermicularis* is not as effective as a perianal swab. While, blood samples for microfilaria showing nocturnal periodicity should be sampled at night only.

Selection of right test method

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

Sending the sample to the right laboratory

The sample for analysing a particular analyte should only be sent to the laboratory which undertakes that investigation. Failure to do so will only mean delays as well as a decline in sample quality.

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.

2.2 Analytical factors

Analytical quality control depends upon the following factors:

- Equipment reliability through periodic, scheduled maintenance, both routine and preventive
- Reagent stability, integrity and efficiency
- Adequate calibration of measuring instruments
- Procedure reliability using standard operating procedures
- Specificity, precision and accuracy of the test of high order
- Proficiency of personnel and continuous updation of their knowledge
- Selecting the right technique for which reliable reagents are available
- Good internal quality control
- Participation in external quality assessment programmes

All the above factors are discussed in greater detail in the chapters below.

2.3 Post-analytical factors

After the proper analytical process is over, it is important to record the findings on the right report form in a clear way. Any possible remarks on the results obtained should also be entered, as well as the biological reference interval of the results. The significance of the results obtained should be highlighted wherever required. There should be frequent dialogue between laboratory personnel and physicians so that laboratory facilities are correctly used and the right interpretation of results obtained.

The reports should be signed by an authorized signatory after duly verifying that all quality control requirements have been met and no transcription errors have occurred. The results should be promptly made available to the right requester within an agreed-upon interval.

3

Overview of quality assurance

Health care laboratories are being utilized more often now than in the past. Unreliable laboratory results are likely to have serious consequences for individual as well as community health. 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 in clinical laboratories, epidemiological investigations, health surveys, environmental monitoring, medical research and other public health activities.

The objectives for patients in direct clinical care are to improve the accuracy of clinical diagnosis, to reduce health care costs (through the avoidance of repeat tests and needless or inappropriate investigations and treatment) as well as 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 encompasses all planned and systemic actions and programmes that are developed and practiced and provide the confidence that a product or service meets customer expectations. It denotes a system for continuously improving reliability, efficiency and utilization of products and services.

Quality assurance (QA) in bacteriology and immunology spans the wide spectrum from monitoring to evaluation of various aspects of services or facilities to ensure that standards of quality are met.

- QA is cost-effective
- QA is an aid to output and efficiency

- QA is a means of getting it right the first time and 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 necessary and relevant steps have been taken to ensure satisfactory quality.

The bacteriology and immunology laboratory is supposed to provide accurate and relevant information that is useful in the clinical diagnosis of a patient or in support of a public health activity. The accuracy and clinical value of the laboratory analysis 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 also applies to laboratories where every part of the process could give 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. **Total quality management** or **TQM** is an integrative philosophy of management for continuously improving the quality of products and processes. TQM functions on the premise that the quality of both products and processes is the responsibility of all those involved with their creation and consumption. In other words, TQM

capitalizes on the involvement of management, workforce, suppliers, and even customers, in order to meet or exceed customer expectations.

Even if an ideal situation is not possible, a level of quality assurance that controls most factors affecting test results should be the aim. Determining the kind of controls to be used, how often, how may and how control results are to be used requires knowledge and experience.

3.3 Components of a quality assurance programme

An effective quality assurance programme should cover all aspects of the laboratory. (Table 3.1.)

Table 3.1: Components of a quality assurance programme

- Personnel with adequate training and competence
- Proper specimen collection, storage and transport
- Use of techniques with high precision and accuracy
- Proper performance of tests
- Efficient processing of results
- Good quality reagents and equipment
- 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 only happen if laboratory personnel have used the product 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 processes are being utillised in a big way in microbiology laboratories as well. Many laboratories utilize a laboratory information management system (LIMS) to improve the 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
- Inventory management
- Maintenance and calibration schedules
- Monitoring trends and providing early warning signals during outbreaks

4

Quality management system

Quality Management System (QMS) is a management approach which requires the management as well as the laboratory administration to be fully on board so that the entire testing process, including its technical aspects, meet quality requirements. The development of QMS in all aspects of a laboratory's working and organization, such as quality planning (QP), quality assurance (QA), quality control (QC), as well as continual quality improvement (CQI) processes requires total focus.

Quality planning ensures the control of quality. It ensures that policies, infrastructure, equipment, environment and personnel requirements are met before a testing procedure is undertaken.

Quality assurance stresses on the anticipation and detection of errors before a test report is released. Two key principles characterize QA: "Fit for purpose" (the test is suitable for intended purpose) and "right first time and right every time" (errors are eliminated before they occur).

It incorporates all three phases – works that need to be done before testing (pre-analytical), during testing (analytical) and after completion of a test procedure (post-analytical). In all three phases, QA should be planned and implemented without any deviation. In the pre-analytical phase, collection, transportation and storage of specimens ; In the analytical phase, the quality of diagnostic kits and reagents, maintenance and calibration of laboratory equipment, environmental control, testing procedures and technical skill; and in the post-analytical phase, the correct interpretation of results, their transcription and communication to the appropriate person within a stipulated time (turnaround time).

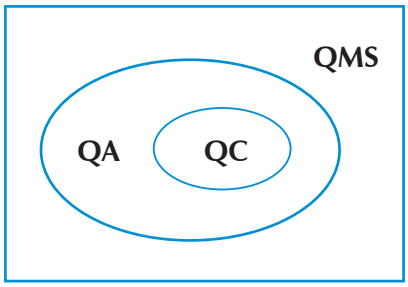
It is generally seen that maximum effort is applied on the analytical phase of quality assurance, even though it is well known that most errors occur during the pre and post-analytical phases. Nevertheless, QA alone cannot guarantee the quality of test results. It can be best summarized as the right result, at the right time, on the right specimen, from the right patient, with the right result interpretation based on correct reference data, and delivered at the right cost.

Quality control refers to the institution of appropriate checks during the testing procedures and constant monitoring of testing processes. While QA is process-oriented and focuses on error prevention, QC is test-oriented and focuses on error identification. It primarily concerns the control of errors in the performance of tests and verification of test results, that is, the analytical phase of QA. Internal quality control (IQC) and external quality assessment (EQA) are the two key pillars of QC. They enable the laboratory to evaluate itself with both internal and external resources and expertise. IQC is performed with both internal controls provided by the manufacturer of test kits, as well as external controls procured or prepared by the laboratory. The results of the tests performed on patient specimens are validated on the basis of the results of these controls. EQA provides validation of test results between laboratories. It allows a comprehensive analysis of results and discrepancies. Root cause analysis of discordant results ensures that errors are corrected.

Continual quality improvement is aimed at the creation of an attitude which continuously analyses capabilities and processes with the objective of delivering quality test results. It is based on the premise that it is possible to achieve continual improvement through small, incremental changes using scientific methods. Documentation at all levels is the essence of a good QMS. If the procedure or process is not documented; it is presumed to have not been performed.

A diagrammatic representation (Figure 4.1) explains the relationship between QC, QA and QMS.

Figure 4.1: Relationship between QC, QA and QMS



4.1 Quality system

In an industrial unit, 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 consist 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 systems approach. Quality is ensured through a well defined quality system.

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

Health laboratories also work on 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. Health laboratories also strive to assure quality of their product (report). However, quality does not just happen on its own. Systematic efforts in the 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 needed for implementing quality management.

4.2 Key elements

Quality system consists of the following five key elements:

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

Organizational management and structure

The overall responsibility for the design, implementation, maintenance and improvements in the 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, needs 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 available adequate resources to efficiently discharge their duties.

The management also makes all decisions and strategies with quality as the overarching theme. Resources and size of the organization permitting, a Quality Manager can be appointed to supervise and guide all activities related to implementation of quality. In smaller laboratories a staff member can be given the additional responsibility of a "quality manager".

Referential (quality) standards and validation

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

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

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.

Documentation

Documentation is information that provides directions or instructions, including policy statements, quality manuals, procedures, specifications, calibration tables, reports, job description, documents of external origin such as regulations, guidelines and examination procedures and user manuals of equipment. These may be deliverd through various media, such as hard copy or electronic copy.

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. Records should be archived for the chosen period following national and specific regulations and must be easily retrievable.

Assessment, 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 post-training support. The objective of training is to close the gap between theory and practice.

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 to detect how, when and where things have gone wrong and take necessary actions to prevent their recurrence

4.3 Development of a quality system

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

Quality policy	→	Mission statement
Quality plan	→	Implementation of policy
Quality manual	→	Policy, plan and application of standards
Procedures	→	Development and application of SOPs
Work instructions	→	Methodology to carry out specific tasks
Training of staff	→	Implementation of quality system and use of SOPs
Monitoring and evaluation	→	Assessment of quality and correction process

5

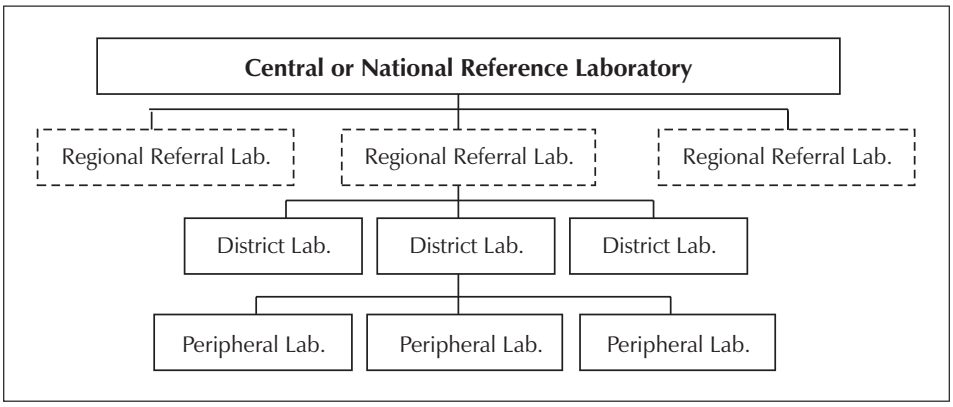
Organization and functions of laboratories

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

Figure 5.1: Networking of laboraotries



Staff

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

Space

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

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

Besides, clinical sample collection and transport facilities must also exist, along with communication facilities between peripheral and intermediate laboratories for the referral of samples and patients, procurement of supplies and personal discussions.

Equipment and supplies

Necessary equipment and supplies include good microscopes, centrifuges, autoclaves, refrigerators, balances, pH meters, incubators, water bath, transport media, glassware, sterile swabs, and reagents for staining (e.g. Gram, Albert, Ziehl Neelsen, Romanowsky's), 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.

Tests to be performed

Peripheral laboratories are expected to undertake tests of public health as well as clinical relevance. Among the tests of public health relevance, diseases of greater epidemiological importance should be accorded priority. Testing of environment samples (especially water) also falls in this category. Certain rapid

serological tests which may be useful in studying the epidemiological patterns of important diseases can also be performed at peripheral laboratories.

Tests at peripheral laboratories are subject to 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 • Sputum • CSF	 Diphtheria Tuberculosis Meningitis (pyogenic and tubercular)
Peripheral blood smear	Malaria, filariasis
Rapid screening/ 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. 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 and assess their efficacy and feasibility.

5.2 Intermediate laboratory services

In most developing countries, intermediate laboratories are located at district or 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 EQAS (Organization and participation)

- 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 as well as for the 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

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, its services should be available at least on a part time basis for the efficient utilization of laboratory services.

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. 5 meters x 3 meters)	1

Equipment

Binocular microscope	2
Dark-field microscope	1
Innoculating 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
Elisa Reader	1
Distilled water apparatus	1
Micropipettes	as per workload
Tips for pipettes	as per workload
Generator/ UPS (in case of power outages)	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 beperformed 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
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
Urine	Pyogenic organisms and AFB

Procedure/Specimen	For detection/diagnosis of
Antibiotic sensitivity	For various pathogens
Serological tests	
Widal	Enteric fever
Tube agglutination	Brucellosis
ASO	Rheumatic fever
VDRL/RPR	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, 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

A document refers to any information that provides direction. This may be internal documents, such as policies, quality manuals, processes, procedures, or external documents, such as specifications, plans, regulations and standards.

Documentation includes the maintenance of records. A record refers to any information that produces evidence, such as requisitions, examination results and reports, quality control and EQA records, incident/accident reports. laboratory forms, when filled, become records. These may be available on various media, both hard copy or electronic.

Maintenance of detailed records for all kinds of laboratory activities is absolutely necessary 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.

6.1 Value of documentation

Documentation is valuable because it:

- Defines responsibilities and authorities and inter-relationships
- Ensures processes and outcomes are traceable e.g. a process may refer to the addition of a reagent to complete the laboratory test, while outcome refers to the report meeting the product specifications
- Proves that the job was done according to referential standards
- Helps external assessors measure compliance to standards

- Facilitates training and makes it easier to train staff to approved procedures than to ad hoc information
- Reminds one what needs to be done next
- Assists in making decisions
- Helps in the investigation of problems
- Helps in improving efficiency

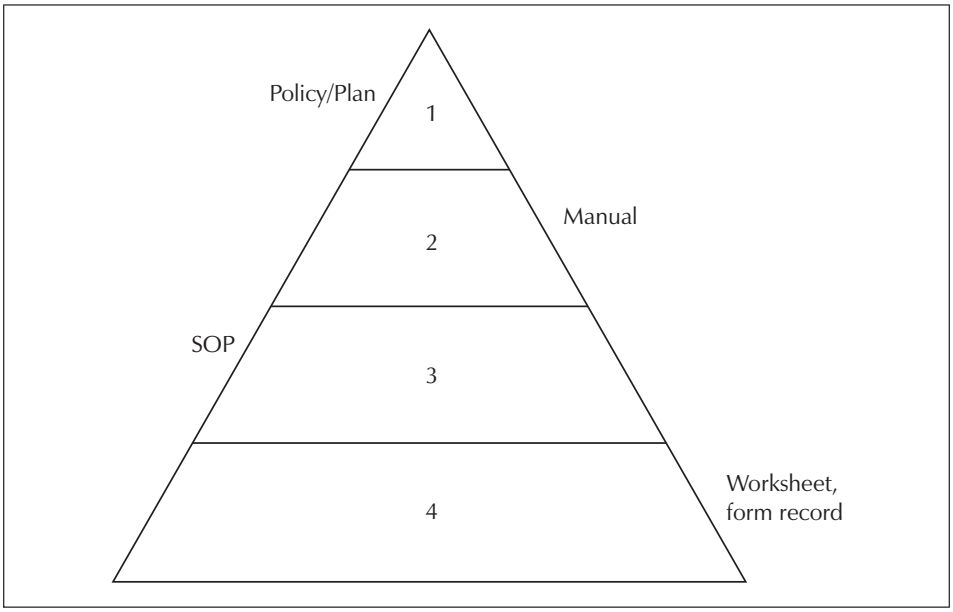
6.2 Types of documentation

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 or Level A documents describe quality policy and the outline of the quality system. Level 2 includes the general standard operating procedures (SOP) that are common to the whole



organization. The specific SOP are categorized in Level 3 and work instructions are classified under Level 4, along with forms, records and reports which may be annexed with the specific SOP. Under ISO 10013 (1995), Layer 2 and 3 have been combined to form Level B. Level 4 of ISO 9000, 1994, corresponds to Level C of ISO 10013, 1995.

Quality manual

Quality Manual describes the QMS and the structure of documentation used in the laboratory. It is the first level document describing the laboratory, its legal identity and purpose of work.

Procedural documents specify details of processes and procedures. Quality System Procedure (QSP) describes major processes undertaken by the laboratory to implement quality measures. Standard Operating Procedure (SOP) is a document that describes how a particular activity is to be performed.

Work instructions are a set of detailed, sequential, step-wise instructions for performing a task. These are specified for each activity and should be clear, understood and implemented by the staff.

Forms are used to record data arising from all activities, e.g. test requisition forms, report forms, indent forms, etc. Procedures and instructions should relate forms with the relevant activity for which they are to used and annexed to them.

Records preserve specific information in a form arising from procedures and tasks that the laboratory performs and needs to maintain.

6.4 Documentation structure

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

6.5 Review and revision

Most documents need periodic review, usually one year long. The review may lead to the revision of the document to reflect current practices. Revisions have to be authorized by the laboratory's top management and the document's new number should show revision number and date.

6.6 Document control

Document control is a system to regulate the handling and management of documents, including the archiving, storing and destruction of obsolete documents. The quality system of the laboratory shall define, document and maintain procedures to control all documents and information (from internal and external resources).

The laboratory management must ensure that only current versions of relevant documents are available at all locations so as to ensure effective functioning of the quality system.

The basic objectives of document control are threefold:

(1) The latest version of the document at the work place ensures that only the latest information/procedure is used/followed by the staff performing a specific activity;

(2) The previous version of the same procedure must be destroyed to prevent staff from having access to old procedures which may have a negative effect on the quality of the results. However, a copy of the same must be archived.

(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 has access to them, that they do not disappear and that no copies are made. Accordingly, the distribution list of the controlled documents is also part of a distribution register which can trace the individuals who were issued the document. Proof of issuing and evidence of receipt are also documented, as is the destruction and removal of its previous version.

To identify controlled copies of the document, each must have a unique ID number, title, date of issue, edition and/or current revision date and/or revision number, page numbering, including total pages, and issuing authorities. These documents should be superscribed with the word, "CONTROLLED", or printed on paper with different colours.

6.7 Key points about documentation

- All documents should be legible and uniquely identifiable
- All new documents should be prepared, created, reviewed and approved by authorized personnel prior to issue
- A document control log is a document register or master index of documentation.
- Only currently authorized versions of appropriate documents should be available for active use at relevant locations
- Review and approval of changes to approved documents and periodic review of unchanged documents should be carried out by authorized personnel. The purpose of regularly reviewing documents is to ensure that they remain updated for their intended purpose
- Invalid or obsolete documents should be promptly removed from all points of use, or otherwise insured against inadvertent use

- Archiving, storage, and retention of obsolete documents for a fixed duration must be appropriately identified to prevent their inadvertent use
- The laboratory's documentation control system should allow for the amendment of documents by hand, and pending the re-issue of documents, the procedures and authorities for such amendments should be defined
- All amendments should be clearly marked, initialled and dated, and a revised document formally re-issued as soon as possible
- Procedures should be established to delineate how changes to documents in computerized systems are to be maintained and controlled so that they are protected against unauthorized access or amendments. Electronic records should be backed-up periodically.

6.8 Dangers of overdocumentation

Documentation should be carried out only to the extent necessary to ensure quality of results. Quality is not measured by the number of documents, but by their quality and utility in continuous quality improvement. Staffs have to use, produce and manage documents to achieve the overall goals of the organization, and not document only for its own sake or to satisfy the auditors.

6.9 Laboratory records

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

- Documentation of whatever has occurred helps establish points of reference for any incidents that may take place
- Serve as an aid in the recognition of trends and resolution of problems
- Helps to establish the credibility of the laboratory

Management of documentation

It is mandatory to maintain records in a uniform pattern. Uniformity is to be ensured by the laboratory's top management. Recordings should be 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, several laboratories now use computers for better storage, compilation and retrieval of data, as well as use this data to create reports for supervisors and other 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 are cost-effective. Records must be archived in a suitable environment to prevent damage, deterioration, loss or unauthorized access.

Retention of records

There are no rigid rules regarding the period for which records should be retained. However, laboratories must ensure that records conform to national, regional and local requirements and regulations, and that related documents are also available.

In general, records are kept for two years, except for the record of 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, whenever required.

Necessary laboratory records

Various types of records are required to be maintained in the clinical microbiology laboratory. They include :

- Request forms
- Examination results and reports;
- Instrument printouts

- Examination procedures
- Laboratory work-books or sheets;
- Accession records;
- Calibration functions and conversion factors;
- Quality control records;
- Complaints and action taken;
- Records of internal and external audits;
- External quality assessment records/inter-laboratory comparisons;
- Quality improvement records;
- Instrument maintenance records, including internal and external calibration records
- Lot documentation, certification of supplies, package inserts;
- Incident/accident records and action taken;
- Staff training and competency records.

Details of some of these are given below :

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, gender and age
- Identifying number (OPD No. / Admission No)
- Specimen type and source
- Name of test requested
- Date and time of primary sample collection
- The date and time of receipt of samples, as well as the identity of the receiving officer
- Unique Laboratory sample ID number

- Specimen accepted or rejected. If rejected, reason for same, e.g.
  - improper labelling
  - improper collection
  - improper transport of specimen
  - damage/leakage

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, 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
- Anti-microbial therapy, if any, prior to collection of specimen
- Specimen type and source
- Name of tests requested
- Date and time of primary sample collection
- Date of transportation of specimen
- Name of physician or requester of test

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
- Anti-microbial susceptibility and resistance
- Any discussion with physician

Test report

Test reports convey 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 includes:

- Identification of the laboratory that issued the report;
- 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 and time of primary sample collection, when available and relevant to patient care, and time of receipt by the laboratory
- date and time of release of report

- Name, designation and signature of personnel performing the test and identification and signature of the person authorizing the release of the report
- Biological reference intervals, where applicable
- Interpretation of results, where appropriate

There are four types of reports that laboratories send out :

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 any wrong information before the final report is placed in the patient's chart.

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

All reports should be concise, unambiguous, legible, without mistakes in transcription and should include biological reference intervals as far as possible. A few lines regarding the clinical importance of the findings may also be useful to the physician. For example, the. 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. Laboratory management must ensure that reports are received by the appropriate individuals within an agreed-upon time interval.(turnaround time)

Quality assurance cards/records

Records of control values for procedures, tests, and equipment are used to identify problems and trends within the laboratory. These cards help identify

problems and trends associated with the application of laboratory data by the end user. A list of relevant information is provided below:

- Date
- Name and signature of person performing the test
- Acceptable range of values
- Control results
- Lot number of reagents used
- Expiration date of reagent used
- Corrective action
- Name and signature of person reviewing results
- Instrument printouts, if applicable

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

- Tests and procedures
- EQA (depending upon the periodicity of participation)
- Temperature records for incubators, refrigerators and water baths
- Procedural manuals
- Equipment preventive and maintenance, service and repair records, including internal and external calibration records
- Equipment function checks

Other quality and technical records that must also be reviewed periodically are

- Request forms
- Accession records;
- Complaints and action taken;
- Records of internal and external quality audits;
- External quality assessment records/interlaboratory comparisons;

- Lot documentation, certificates of supplies, package inserts;
- Incident/accident records and action taken;
- Staff training and competency records.

Incident reports

Incidence reports document problems related to the performance of care-givers through the workflow path, including pre-analytical, analytical and post-analytical phases. The information that needs to be incorporated is as follows:

- Identification of the problem
- Date and time
- Names of persons involved
- Corrective action taken with timelines along with identity of personnel responsible to execute corrective action
- Name of reviewer
- Date of review

Safety records

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

- Dates of training sessions
- Contents of training sessions
- Names of persons conducting training
- Names of persons attending training along with records of the periodic, scheduled safety audit conducted by the designee safety officer using a checklist.

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 records which includes information on ongoing personnel training as well as the level of certification and training should also be maintained.

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

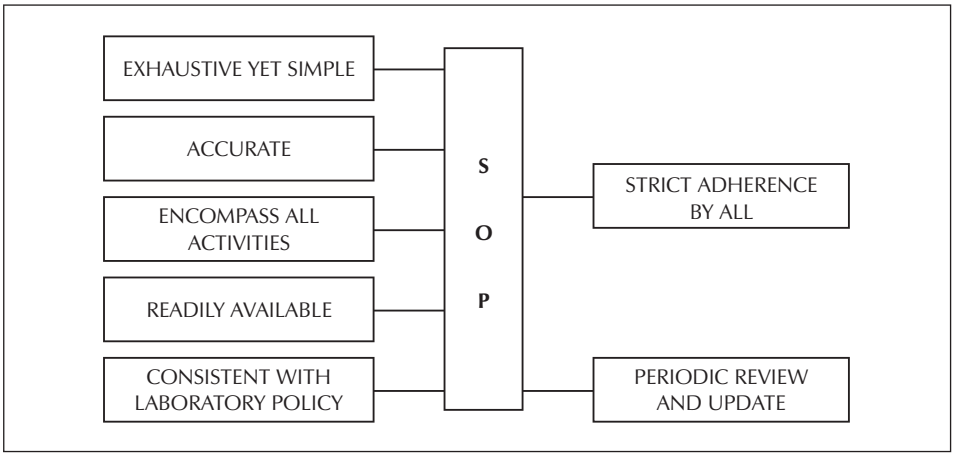
7

Standard operating procedures

Standard operating procedures (SOPs) are the most important documents in a laboratory. They describe in detail the complete technique of the routine or repetitive activity followed by the organization or a testing laboratory.

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

Figure 7.1: Essential features of SOP



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

- The SOP should be preferably prepared by users, or in a language clearly understood by users

- The SOP should include all pre-analytic, analytic and post-analytic requirements for performing the test, keeping the quality issue in view.
- SOPs describe both technical and fundamental programmatic operational elements. They document the way activities are to be performed to facilitate consistent conformance with technical and quality system requirements.
- The SOP is a controlled documents and needs a regulated system for Preparation, Approval, Distribution, Revision, and Training
- The SOP should have a defined structure. Such as, the name of the organization, title, purpose, scope, responsibilities/authorities, description activity, and resources
- Each section of the laboratory should have a copy of the SOP which should be easily accessible to all
- The SOP should be available on the work bench area
- It should be reviewed at least annually or whenever the procedure is changed
- The 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
- SOPs that are no longer in use should be removed from the area of activity and master copy archived for future reference.

The essential components of a typical SOP are summarised in Table 7.1.

Table 7.1 Essential components of a SOP

1.0	Purpose and scope
	Purpose refers to the document and Scope refers to the procedure
2.0	Definitions and abbreviations
3.0	Responsibility: Technical and supervisory,
4.1	Principles usually given in kit inserts
4.2	Method
4.3	Primary sample collection: with details of Type of specimen (e.g anticoagulant used), Volume required, Collection of specimen, How container should be labeled, Criteria of specimen rejection.
4.4	Sample Transportation: Stability and Temperature
4.5	Equipment; Material needed (Pipette, incubator, water bath…)., Daily-start up Machine, Daily/Weekly/Monthly maintenance, Temperature monitoring
4.6	Reagents: How to store reagents, Monitoring (temperature, precipitation etc.), How to prepare the reagent, Check expiry date, Shelf life of reagent (opened/unopened)
4.7	Quality Control: QC everyday (1 level, 2 level or 3 level); Serology Positive and Negative controls; Record control results and sources of control
4.8	Procedure: (In chronological order, optimum detailing, special instructions, flowcharts can be part of the procedure)
4.9	Performance Characteristics: (Accuracy, Precision
	Analytical Measurement Range (AMR), Clinical Reportable Range (CRR), Sensitivity, Specificity, Interference
4.11	Result and Interpretation: Validating the test (whether or not controls are in the correct range), Unit , Biological reference interval, Panic Value, Cross check
4.12	Limitations: false positive/negative, other conditions
5.0	Troubleshooting/Errors: Monitoring all steps, Specimen collection
6.0	References
7.0	Appendices and forms: including Records to be maintained

Some of the tests take a longer time for completion. In such cases, 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 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 be broadly divided into parts: one giving information about the SOP and the other describing procedures per se. The essential information that must be part of the SOP is given below:

- The laboratory or the section that can use it
- Name and title of SOP
- Unique identification code
- Version or issue number
- Revision number and date
- Date issued
- Name of person(s) to whom the document has been distributed
- Names, designation and signatures of individuals who have prepared, reviewed and authorised the document
- Page number with a reference to total page numbers

7.2 Do's and Don'ts on SOPs

The following guidelines will help in drafting better SOPs.

- There must be no gaps or duplications
- There must be 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 it has 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 understand, use and follow SOPs
- The use of SOPs should be reviewed and re-inforced periodically

SOP must be written with sufficient detail so that someone with even limited experience or knowledge of the procedure can successfully reproduce the procedure when unsupervised.

The draft SOP should be reviewed by and commented upon by those performing the same kind of work. In addition, all those directly or indirectly affected by the SOP must review its draft procedure and suggest necessary changes.

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

- SOPs should be written for all procedures in the laboratory
- SOPs must be made specific for each 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 SOPs
- SOPs must be followed by all staff members at all times

8

Validation

A variety of equipment, processes and software are used by health laboratories in processing clinical material. The generation of quality reports depends upon how well they have performed. Before beginning to process the clinical specimen one must ask the question: Does this thing (equipment/process/software) do what it is supposed to do? In other words, equipment, processes and software need to be validated before they are used to demonstrate that they 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 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.

The laboratory should use only validated procedures for confirming that the examination procedures are suitable for intended use. The validations should be as extensive as necessary to meet the needs in the given application or field of application. The laboratory should record the results obtained and the procedure used for the validation.

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 user requirements must be clear (what does the user want to validate and what is required for the same ?). 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 organizations. Quality staff may be involved or in the review of 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. Both are tools for measurement of quality.

9.1 Monitoring

Main objectives of monitoring are

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

The decision on to what to monitor depends upon the work-area and related activities. A common-sense approach should be used to identify areas that need to be monitored. Areas chosen should have profound influence upon the quality of the laboratory results.

This may mean starting with the final output of processes rather than its 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, 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 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 9.1).

Table 9.1: 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, monitoring of both types is crucial since they 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 9.2

Table 9.2: Essential differences between IQC and EQA

Feature	IQC	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 quality control of stains, media, reagents etc. It is not synonymous with quality assurance.

Types of external quality assessment schemes (EQAS)

EQAS techniques vary depending on the nature of the item or material under test, the test method in use and the number of participating testing laboratories. Most possess the common feature that allows you to compare test results between laboratories. In some programmes, one of the participating laboratories may be a controlling, coordinating, or reference function. The major types of proficiency testing programmes are:

Inter-laboratory testing

In this programme, participating laboratories are provided with sub-samples from a source offering a suitable degree of homogeneity which they are expected to test at comparable levels of competence.

Split sample testing

This program involves samples of a product or a material being divided, into two or more parts with each participating laboratory testing one part of each sample.

Measurement comparison

Measurement comparison schemes involve the test item to be measured or calibrated and circulated successively from one participating laboratory to the next.

Qualitative schemes

Qualitative schemes do not need the involvement of multiple laboratories or inter-laboratory comparisons to evaluate a laboratory's testing performance.

Known-value schemes

Involving test items with known amounts of the measure under test. It is then possible to evaluate the capability of an individual laboratory and provide numerical results for comparison with the assigned value.

Partial – process schemes

This scheme evaluates the laboratory's abilities to perform parts of the overall testing or measurement process. For example, some existing proficiency schemes evaluate laboratories' abilities to transform and report a given set of data rather than conduct the actual test or measurement.

9.3 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 an external quality assessment scheme (EQAS). This is considered a powerful tool that challenges internal quality control measures that are being adopted by the laboratory. EQAS is a tool by which the entire testing process, including the quality of results, generated by a particular laboratory is assessed.

External quality assessment schemes compare 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. However, it is not correct to consider them synonymous as in current terminology proficiency testing denotes the 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

- Establish the effectiveness and comparability of new test or measurement methods and monitor established methods
- Encourage use of standard reagents/ methodology and trained personnel
- Assign values to reference materials (RMs) and assess their suitability for use in specific test or measurement procedures
- Determine the performance characteristics of a method – often known as collaborative trials
- 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 health administrators and regulatory agencies to get an insight into quality conditions across the country, enabling them to identify problems and devise methodology to overcome them

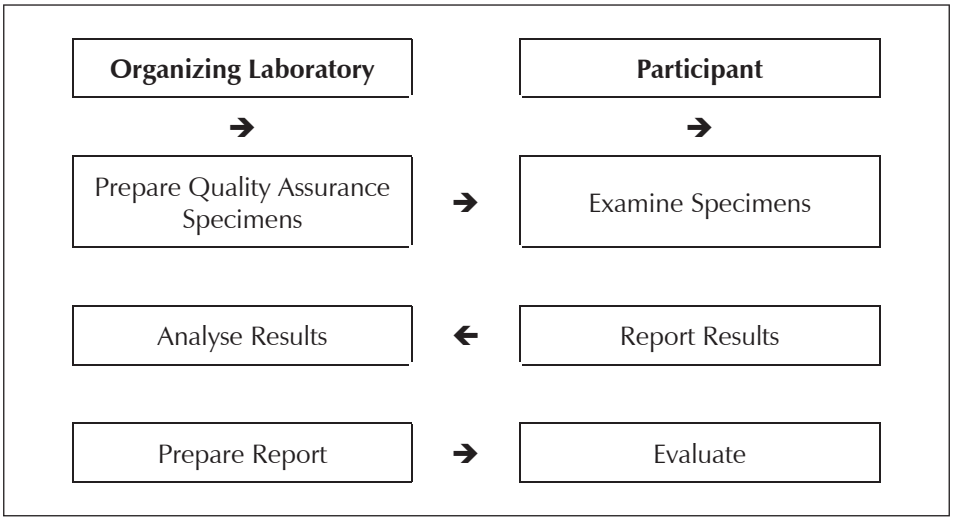
Process of EQAS

EQAS requires a well equipped, experienced laboratory at the intermediate or central level to act as the organizing laboratory and a reasonable number of laboratories as participating laboratories. The EQAS process demonstrating its key function as organizer as well as those of its participating laboratories has been shown in Figure 9.1

Various factors may influence the success of an EQAS :

- Voluntary participation
- Confidentiality of individual reports

Figure 9.1: Steps involved in EQAS : Basis of success of EQAS



- Avoiding provocative statements about performance
- Identifying unsatisfactory performers in groups or individuals
- Providing educational opportunities
- Organizer acting as adviser rather than enforcer

EQAS are targeted for participating laboratories who are its customers. Their needs have to be met. The participating units should aim at the following gains :

- Comparison of performance and results
- Minimization 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 the 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 sources to provide realistic and relevant challenges 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 the submission of samples to participating laboratories; their subsequent analysis and their return 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:

Material supplied

The material supplied should be homogenous so that all participating laboratories receive the same material. All specimens of 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 with regard to the analyses that should 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 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 this way, a large number of laboratories are kept under the radar.

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 as well as collective determinations. This should be done for each particular distribution, and 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 (TAT) 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 feedback 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 ? This is a choice which each country will have to make according to its health policies. In some, the purpose is to expand the information base of the laboratory in question, in others surveillance for legislative objectives predominates

Evaluation of EQAS results

Where an EQAS is performed, the coordinator should be responsible for ensuring that the method of evaluation is appropriate to maintain the credibility of the scheme. Along with technical advisors, the coordinator may provide comments on performance with respect to :

- Overall performance versus prior expectations (taking uncertainties into account)
- Variation within and between laboratories
- Variation between methods or procedures, if applicable
- Possible sources of error and suggestions for improving performances
- Any other suggestions, recommendations or general recommendations

- Conclusions
- EQAS must be looked upon by participating laboratories as opportunities for continual improvement. Review helps to identify problems in laboratories and initiate remedial actions which may be related to, for example, to individual staff performance or calibration of instrumentation, etc

Scoring system in EQA

The objective of any EQA scheme is to stimulate performance and enhance inter-laboratory concordance. Participants, therefore, need to be clearly told 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 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. points are allocated depending how close the results are with the designated score. In clinical microbiology, a three -point rating system is used for EQAS.. 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 wrong answer or failure to answer. Similarly for antibiotic susceptibility testing, a distinction is made between minor and major errors. A minor error occurs when a resistant or susceptible organism is reported as an intermediate, or vice versa. A major error is the substitution of a resistant or susceptible organism with susceptible results, and vice versa.

Scoring as a stimulus to laboratory improvement

Scoring systems are a viable means of data reduction, used 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 recognize 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 inter-laboratory agreement, and provide means to judge the overall success of the scheme in stimulating improvement.

EQAS process

A summary of the process, from the time the decision is made to set up an EQAS to the production and distribution of the report, involves not only planning, research, evaluation and validation etc, but the actual process of designing and manufacturing, collecting, collating and analysing the results.

The steps involved include:

- Forwarding an initial questionnaire to determine which tests are to be 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 improvement to participants
- Award of EQAS certificates

9.4 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 needs of the laboratory. An increase in the 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 a 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 the effect of 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 laboratories for confirmation are checked against the sending laboratory's 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 systematic examination of a quality system carried out by an internal or external quality auditor or an audit team. It is an important part of an organization's quality management system and is a key element in the ISO quality system standard. ISO 9001quality audits are typically performed at predefined time intervals and ensure that the institution has clearly defined internal system monitoring procedures linked to effective action. This can help determine if the organization complies with the defined quality system processes and can involve procedural or results-based assessment criteria.

Definition

Quality audit is defined as a planned and documented activity performed in accordance with written procedures and check-lists to verify by investigation, as well as 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.

There are two types of audits, internal (also called first party audits) and external. External audits are also of two types, namely, second party audits and third party audits (also called accreditation). Internal audits are performed by laboratory staff to inspect their own system. Since this may open them to the accusation of being partisan, it might be best for the staff not to audit their own activities, and that this is done by members who are either trained in audit techniques, objective as well as competent.

The basic principle behind internal audit is that quality systems cannot otherwise be developed and maintained. The audit allows managers to better understand the day-to-day work and, through the review process, allows them to make better decisions. The objective of the drill should be to audit the quality of the system and not the staff. The deficiencies that are observed should form part of a non-compliance report that is submitted to the higher management.

Each laboratory should nominate its own auditors. Internal audit should be well structured and only be carried out by a trained auditor. Auditors may obtain information by the following methods :

- When interviewing staff, ask open-ended questions which clarify documentation and observations. Ask questions like, "Show me how…" or "Tell me about…"
- Review laboratory records to verify that the laboratory quality manual, policies, logs, SOPs and other manuals are complete, current, accurate, and regularly reviewed.
- Observe laboratory operations to ensure that practice matches written policy or procedure in all phases of testing, that processes are appropriate and that identified problems have been adequately investigated and resolved.
- Check equipment especially for calibration, routine and preventive maintenance
- Observe the organization of laboratory area for smooth, efficient uni-directional path of workflow
- Examine biosafety measures and waste disposal mechanisms
- Follow a specimen through the laboratory from collection through registration, preparation, aliquoting, analyzing, result verification, reporting, printing, to post-test handling and storage, to determine the strength of a laboratory's systems and operations.
- Review any previous Assessment reports (internal and external), noting deficiencies and their resolution.

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

Internal audits are most frequently performed, usually by internal staff from other departments/divisions and are normally part of ongoing programmes. Internal audit should be performed at least once a year.

When deficiencies are seen or noted, the laboratory should take appropriate corrective or preventive actions, which are documented and carried out within a specified time-frame. A complete record of the audit should be maintained. The nature and likely causes of each non-conformity should be recorded, along with the kind of corrrective action required and time required for removing it.

Details on how to conduct an internal audit as per ISO 15189:2007 requirement are available on the websites of various accreditation bodies. For example, National Accreditation Board for Testing and Calibration Laboratories (NABL) in India describes internal audit guidelines in the document no. 161. Countries should access guidelines from bodies from which they plan to seek accreditation.

A typical audit cycle is described in Table 10.1.

External audits **(second party audits)** are normally supplier audits performed to ensure that goods supplied are of the required standard. This is a GMP requirement. They are especially needed when suppliers change and also on a planned basis for regular suppliers.

External audits **(third party audits also called accreditation)** are normally those performed by regulatory/statutory bodies and can be voluntary or mandatory. Details of accreditation or third part audits are described in section 10.2.

Benefits of quality audit

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

- Quality audits can help make changes in work areas, especially when workers know that changes are necessary but some or other constraint keeps coming in the way. The audit can act as an additional lever
- A well-done quality audit can help boost the confidence of staff

Table 10.1: Phases of an Audit Cycle

Phase of internal Audit	Steps
Phase 1: Planning and Preparation	• Develop and document processes and procedures or review current ones • The compilation of an audit/agenda/plan • The development of an effective checklist • Selection of auditors independent to the area being assessed
Phase 2: Conduct of audit	• Hold an opening meeting • Conduct the audit • A review of previous audit records • Hold a closing meeting
Phase 3: Recording and reporting the audit findings	• Audit plan and agenda • Records of the opening and closing meetings • Audit report • Corrective and preventive action report that includes an assessment of the root cause(s), the corrective action(s) required, appropriate time frames for completion, and the responsible person for carrying out the action • Audit follow-up report that includes evidence of review by laboratory management and evidence of verification of effective corrective action
Phase 4: Follow up and confirmation of corrective action	• Document and address non-conformities, take corrective and preventive measures for each non-conformity • Validate the effectiveness of action taken • Maintenance of records: A complete record of the audit must be retained even when no non- conformities are found during the audit

- Quality audits can help focus 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 the following factors to assess quality in the 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
- Whether the quality system actually functional

10.2 Accreditation of laboratories

Accreditation is an approved procedure by which regulatory authorities or an authorized body accords 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 Organization for Standardization (ISO).

This system is in progress in various developed countries where it is mandatory for laboratories to be accredited before they can begin work. In developing countries accreditation of laboratories is increasingly becoming popular, though on a voluntary basis. 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 the laboratory.

The process of accreditation starts with the laboratory's request. The accrediting authority deputes assessor(s) to assess the laboratory on the basis of specific criteria. Their report may recommend accreditation or suggest corrective measures. The deficiencies, also called non-conformities, must be rectified within a specified time-period before formal recognition is accorded. This process ensures top quality laboratories.

Benefits of accreditation:

- National and international recognition
- Public acceptance
- Assurance to customers of good laboratory practices
- Provides global equivalence
- Provides comparability in measurements
- Decision-makers can rely on test results
- Improves staff motivation
- Ensures better support in the event of legal challenges
- Saves money by getting it right first time.

Preparing for accreditation

It is important for a laboratory to make a concrete plan for seeking accreditation and nominate a quality manager to co-ordinate all activities related to the accreditation process.

The laboratory must undertake the following tasks:

- Obtain documents from the accreditation body in preparation for the accreditation, study them and undertake a gap analysis
- Train laboratory personnel on the quality management system and internal audit as per the requirements of the accreditation body
- Prepare standard operating procedures for each investigation carried out in the laboratory.

- Prepare the quality manual and supplement it with next-level documents such as quality system procedures.
- Ensure appropriate conditions such as temperature and humidity, storage facilities.
- Ensure calibration of instruments/equipment only from an accredited agency.
- Ascertain the status of the existing quality system and technical competence with regard to accreditation standards (such as ISO15189:2007).
- Ensure proper implementation of all aspects that have been documented in the Quality Manual and other supporting documents.
- Document and review IQC data.
- Satisfactory participation in external quality assessment schemes (EQAS) for the examination procedures to be included in the scope of accreditation.
- Document corrective actions on IQC/EQA outliers.
- All laboratory personnel should adhere to the use and application of the quality manual and all referenced documents as well as the requirements for their implementation.
- Conduct and record internal audit and management review.
- Apply to the accreditation body along with appropriate fee.

Management review

In order to ensure continuing suitability and effectiveness in support of patient care and introduce necessary changes or make improvements, the laboratory management should review the laboratory's quality management system and all of its medical services, including examination and advisory activities. The management review should be conducted at least annually.

The management review should take account of, but not be limited to:

- Follow-up of previous management reviews
- Status of corrective actions taken and required preventive action

- Reports from managerial and supervisory personnel
- The outcome of recent internal audits
- Assessment by external bodies
- The outcome of external quality assessment and other forms of inter-laboratory comparison
- Any changes in the volume and type of work undertaken
- Feedback, including complaints and other relevant factors, from clinicians, patients and other parties
- Quality indicators for monitoring the laboratory's contribution to patient care
- Non-conformities
- Monitoring of turnaround time
- Results of continuous improvement processes
- Evaluation of suppliers

All actions arising from the review should be carried out as required. Their impact should be monitored at regular (perhaps monthly) management meetings. All management reviews should be documented, in the form of minutes clearly indicating the actions that need to be taken, by whom and in what time limit.

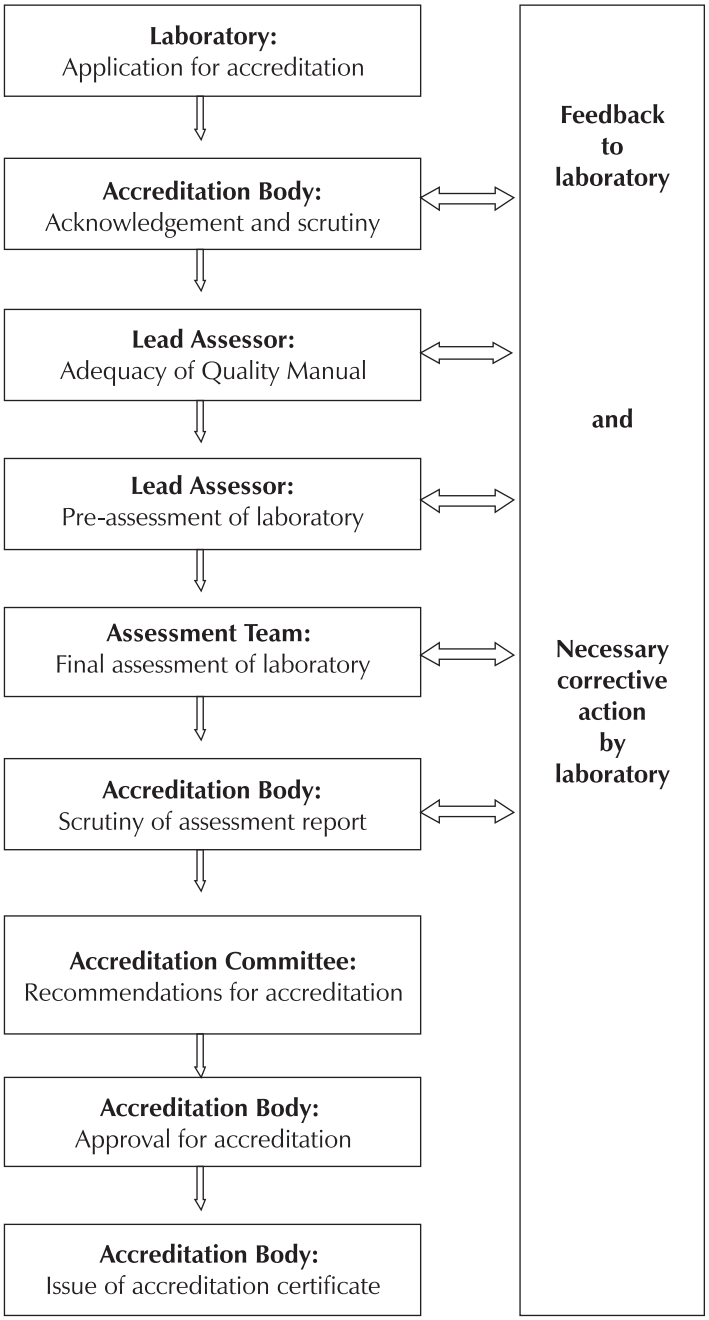
Standards for inspection of laboratories

(1) **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.

(2) **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

(3) **Facilities and equipment:** The space for receiving patients, collection of clinical materials, performance of tests, disposal and sterilization as well as storage of material should be adequate.

Flowchart depicting a typical accreditation process



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 for maintening records and issuing of reports.

(4) **Policies and procedures:** A standard operating procedure (SOP) for each technique should be available to all 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 in this regard.

(5) **Staff development and education:** A continuing education programme for all staff members should form part of laboratory policy and adequate resources available for in-house as well as outside training activities. A system for staff appraisal should also be in position.

(6) **Evaluation:** The laboratory must have a formal policy for internal quality control and participate in relevant external quality assessment schemes.

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. Basically, this revolves around the use of common sense within the laboratory and in the use of all techniques. Clearly there exists a potential risk of infection to workers who come into continuous contact with pathogenic organisms. Even experienced workers are at risk and there is no microbiological laboratory where the potential hazard of acquiring 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 procedures and 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 skills necessary for controlling these hazards and the continual reinforcement of good laboratory practices are essential to sustain a safe and healthy microbiology laboratory.

The environment/infrastructure of any testing facility must be designed in such a manner that the safety of personnel & quality of work is not compromised. There must be enough space in the laboratory to permit specimen collection, storage, perform tests in unhindered fashion and adequately dispose waste, besides provision of space for non-laboratory activities. Specimen movement and workflow through the laboratory must be such that it prevents specimen loss, specimen mix-up, and minimises exposure of laboratory personnel to laboratory hazards. Sample flow should be unidirectional as far as possible. Organized, efficient workflow ensures that patients and patient samples do not have common pathways.

11.1 Practice of laboratory safety

In order to maintain effective operations, a laboratory should have adequate space in relation to:

(1) Patient reception

(2) Sample collection

(3) Work bench

(4) Equipment use

(5) Specimen/reagents/general storage facility including cold storage where applicable

(6) Washing

(7) Record room/area

(8) Waste disposal facility including biomedical wastes

(9) Non-laboratory activities such as eating and access to safe drinking water

- In laboratory facilities where samples are collected from patients, consideration should be given to optimum collection conditions, patient comfort and privacy. Provision should also be made for comforts of physically disabled patients.
- Workflow patterns should be carefully considered so that laboratory accidents can be prevented. Working spaces should be comfortable, with appropriate chairs of appropriate height. Safe routes to enter and to leave the laboratory should be planned for everyday use, as well as for emergency exits.
- The laboratory facility must also provide enough space for non-laboratory activities, such as eating and drinking, to avoid bringing of food or drink into the laboratory area. Appropriate toilet facilities must be provided outside the laboratory and there should be access to safe drinking water.
- Controlled access to laboratory areas must be ensured and measures instituted to safeguard samples and resources.
- The laboratory should have adequate lighting, power plugs and uninterrupted power supply. The use of exposed cables should be kept to a minimum.

- Laboratory windows should be covered by fly screens if opened during working hours.
- The laboratory should have adequate communication systems (telephone, email, fax etc.) for the efficient transfer of messages.
- All laboratory and storage spaces should be as uncluttered as possible. Unnecessary, obsolete or unused equipment should be decontaminated and removed to prevent accidental tripping, while easy, unrestricted access to laboratory benches and equipment must be ensured. Storage areas should be large enough to avoid clutter. Procedures should be in place to ensure the integrity of refrigerated and/or frozen samples/reagents/consumables in the event of an electrical failure.
- Ventilation should be good enough to allow adequate air circulation so as to help control the build-up of hazardous vapors. Fume cupboards need to be used whenever hazardous materials (e.g; concentrated acids, formalin etc) are being used.
- Ideally, a laboratory should maintain a constant temperature of 22 ± 2°C, but this also depends on what procedures are being performed and what kind of recommendations are in order. The terms "room temperature" and "ambient temperature" should be avoided when defining operating temperature. It is best to list the range of temperature (eg 18-25 degrees C) that must be followed for optimal results. In fact, it is important to know the temperature of the exact laboratory location where procedures are performed. All laboratory procedures should be protected from direct sunlight, which may affect performance of assays or temperatures at which they are operating.
- Ensure the implementation of local/regional guidelines for storage and disposal of hazardous materials.
- All floors, walls, ceilings, and bench tops of the laboratory must be clean and well maintained with a pre-defined/pre-planned protocol for cleaning & maintenance.

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 employees. Some of the important preventive measures are described below :

- Laboratory floor plan with directions towards the emergency exits and stairwell.
- Fire drills, some of which may be without warning
- Distinct marking of fire exits
- Fire exits should be kept unblocked
- Fire extinguishers must be available, functional and staff trained in their use. They must be checked annuallys to ensure they function effectively
- Installation of fire alarms
- Declaration of the laboratory as a no smoking zone and with restricted access.
- 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.

In case of fire follow the principle of RACE : **Rescue** (anyone in immediate danger), **Alert** (call for help), **Confine** (close all windows and doors) and **Extinguish** (use fire extinguisher).

Chemical hazards

A chemical is hazardous if it meets any of the following conditions: It is cancer-causing, toxic, corrosive, an irritant, a strong sensitizer, flammable, or reactive, and thus poses a threat. They are classified in the following way

Class 1 – Explosives; Class 2 – Gases (compressed, dissolved or liquefied); Class 3 – Flammable & Combustible liquids; Class 4 – Solids (flammable, spontaneously flammable or dangerous when wet); Class 5 – Oxidizing Substances; Class 6 – Poisons; Class 7 – Corrosive Substances .

Chemicals of different classes must be segregated, properly labeled and stored in their respective designated cabinets. Hazardous and combustible materials should be kept to the minimum. All containers must be capped & sealed when not in use so as to reduce the possibility of a spill and the accompanying release of fumes into the laboratory.

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
- Use fume cupboards for handling hazardous chemicals
- Provide emergency shower
- Provide an eye washer
- Provide safety goggles or full-face respirators

Electrical hazards

Some electrical units can cause electric shock and fire hazards. Explosions in eletrophoretic units causing fires have occurred frequently. The microwave oven has become a common piece of equipment, but its use has introduced a significant new hazard to the laboratory. Careless operating procedures have resulted in the 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 equipment. That is why all electrical equipment should be earthed (grounded); information regarding the location

of the master switch should be prominently posted and uninterrupted source of power provided.. All electrical equipment must be inspected periodically by qualified personnel. Equipment should be disconnected from the power source when not in use (e.g; centrifuge) except critical ones (e.g. refrigerators, freezers, incubators etc.)

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 micro-organisms 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 non-pathogenic microorganisms before higher-risk micro-organisms are introduced into the laboratory routine. The salient preventive measures (Table 11.1) must be practiced 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 off properly
- Promote regular hand washing 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.

Material safety data sheets (MSDS)

MSDS are technical bulletins that contain detailed information about physical and chemical hazards, handling procedures and emergency response procedures for hazardous chemicals as well as storage and handling precautions. MSDS information includes : Nomenclature, including chemical family and formula; Hazardous ingredients; Physical data, recommended PPE; Storage recommendations; Fire and explosion hazard, toxicology; Health hazard, Spill and leak with recommended actions; Waste disposal information; First aid and Special protection information.

Businesses are required to provide to all their customers the MSDS for all chemicals they manufacture and distribute. Laboratories need to heed precautions listed in the MSDS in order to ensure the chemicals they use are handled and stored safely.

Chemical spills

A chemical spill is considered to be minor only if the person who spilled it is familiar with the chemical, knows the associated hazards and knows how to clean up the spill safely. The recommended steps for dealing with a minor spill include:

- Alert coworkers, then clean up spill;
- Follow procedures for disposal of materials used to clean up spill;
- Absorb free liquids with appropriate absorbents, such as for caustic liquids use polypropylene pads or diatomaceous earth, for oxidizing acids use diatomaceous earth; for mineral acids use baking soda or polypropylene pads and for flammable liquids use polypropylene pads. Then neutralize residues and decontaminate the area.

Anything beyond a minor spill must be handled by experts, not necessarily from within the laboratory. Steps to deal with major spills include alerting co-workers, moving to a safe location and calling authorities to report the situation.

11.2 Biohazard levels

Safety begins with the collection of the specimen. The right 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 along with 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 both 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 characterized strains of viable microorganisms not known to cause disease in healthy adult humans. Emphasis is placed on:

- the use of mechanical pipetting aids
- hand washing
- 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 immuno-deficient or immuno-suppressed 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 micro-organisms assigned to this containment level.

Primary hazards to personnel working with these agents are accidental auto-inoculation 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 administrator's point of view the 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 personnel should enter a laboratory displaying the universal biohazard sign. Doors displaying a biohazard symbol should not be propped open, but 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 undertaken to reduce the potential hazards of aerosol generating processes such as centrifugation, and grinding for both low and moderate risk materials.
- Pathogenic micro-organisms should be handled in safety cabinets/ hoods only.

Transportation of infectious material within the 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 which need 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 the materials leave the laboratory area, unless they 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 in charge of all safety procedures and equipment. He/she should carry out all educational and training services so that all 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 are a result of inadequate standards of practice, ignorance and inexperience as well as thoughtlessness. Overcrowding, badly maintained equipment and poorly designed premises are key contributors. Despite the prominence given in the literature to the risk of transmission of hepatitis B virus and human immuno-deficiency 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

Micro-organisms 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 (e.g. 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

Hemorrhagic 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 ten 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 that are 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 metal or plastic to prevent it from being damaged by pests. A medical officer should be consulted regarding the contents of the box. A first aid chart stating immediate treatment for cuts, burns, poisoning, shock and collapse should be prepared and displayed in the laboratory.

Biological spills

When surfaces are contaminated by biological spills, the suitable actions to take are:

- Define/isolate the contaminated area.
- Alert co-workers.
- Put on appropriate PPE.
- Remove glass/lumps with forceps or scoop.
- Apply absorbent towel(s) to the spill; remove bulk and reapply if needed.
- Apply disinfectant to towel surface.
- Allow adequate contact time (20 minutes).
- Remove towel, mop up, and clean the surface with alcohol or soap and water.
- Properly dispose of materials.
- Notify the supervisor, safety officer, and other appropriate authorities.

Disinfectant: For most spills, use a 1:50 solution (1 g/l chlorine) of household bleach (sodium hypochlorite solution containing 50 g/l chlorine). For spills containing large amounts of organic material, use a 1:10 solution (5 g/l chlorine) of household bleach, or an approved mycobactericidal.

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 neighbors with culture material. It is also important to prevent contamination of the work place itself with micro-organisms 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/flowers 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 micro-organisms present in specimens or cultures to the vessels in which they are contained and,
- Prevent environmental micro-organisms (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

Safety training

Training staff about safety procedures is key in achieving satisfactory results. Training should include information about universal precautions, infection control, chemical and radiation safety, how to use personal protective equipment (PPE), how to dispose of hazardous waste, and what to do in case of emergencies; Laboratory staff must receive safety training. At a minimum, the safety training must include:

- Blood-borne pathogens : Includes information on standard precautions, risks and types of infectious diseases contracted through exposure, proper safeguards, and methods of handling potential contaminants
- Personal protective equipment (PPE): All laboratory personnel must be trained on the proper use of PPE prior to starting work in the laboratory.
- Chemical hygiene/hazard communications
- Use of safety equipment: eyewash, emergency shower, fire extinguisher, first aid.
- Packaging: Labelling of shipped materials and transportation of potentially infectious material-IATA.

- Waste management/biohazard containment (segregation and appropriate disposal of biohazards).
- General safety/local laws related to safety.

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. Cardinal principles of waste management include:

- Segregation of waste (eg. infectious vs non- infectious, solid vs liquid and sharps vs non-sharps)
- Appropriate treatment prior to disposal
- Proper 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 waste 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, policies and procedures should be formalized and documented 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. The color coding for various classes of waste is given below. However the laboratories must use the color codes as per local practices.

Sl No.	Waste class	Type of container	Colour coding	Treatment /Disposal option
1	Human anatomical waste, blood & body fluids	Single-use containers/ Plastic holding bags	Red	Incineration / Deep Burial
2	Microbiology & bio-technology waste	Single-use containers/ Plastic holding bags	Yellow	Autoclaving/ Microwaving/ Incineration
3	Waste sharps	Re-usable/ single-use sturdy containers of plastic, glass or metal	Blue	Shredding & Deep Burial
4	Discarded medicines	Re-usable/ sturdy cardboard/ glass/ plastic holding bags	Blue	Shredding & Deep Burial
5	Soiled wastes (linen)	Plastic bags/ sacs	Yellow/ Black	Disinfection and machine cleaning
6	Disposables (other than sharps)	Re-usable/ sturdy containers/ plastic holding bags	Yellow/ Black	Disinfection- Chemical / Autoclaving, Shredding, Burial
7	Chemical wastes	Sturdy containers/ plastic holding bags	Yellow/ Black	-

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 appropriate heat-resistant containers.

If a primary waste container has become damaged or its exterior is 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 non-infectious 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 non-infectious 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 if it is 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 land-filled. 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.

Important points to remember

- Training of staff, provision of safety equipment and compliance with all regulations are crucial
- Safety in the responsibility of both the management and the workers
- Safety is the most important aspect of quality assurance
- It is effective to have one person (safety manager) to co-ordinate and oversee all safety activities
- Benchmarks must be established for safety standards
- "Common-sense" practices for safety are most important
- Safety manuals must be written and used for each laboratory
- Universal precautions should be practiced with all specimens at all times

Records must be kept of all incidents or occurrences of injuries and follow up documented

12

Practice of quality assurance in clinical laboratory

Quality assurance in clinical microbiology should be comprehensive and must cover all aspects, right 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 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, ensuring the right transcription, timely delivery of the results to the right user 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

Parameter	Guidelines
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 or as per local requirement
Referral specimens	Use only authorized referral laboratory Include name of reference laboratory on patient's reports
EQAS	Participate in appropriate external quality assessment schemes Consider adopting internal quality assessment programmes
Equipment performance	Document function checks of equipment Perform as frequently as recommended by manufacturer 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

Parameter	Guidelines
User prepared media	Record amount prepared, source, lot numbers, sterilization method, preparation date, pH, expiration date. Check medium for color, consistency, depth or slant, smoothness, haemolysis, contamination, bubbles Test media with QC micro-organisms 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 programmes is to ensure the generation of reliable laboratory results. 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 so as to 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

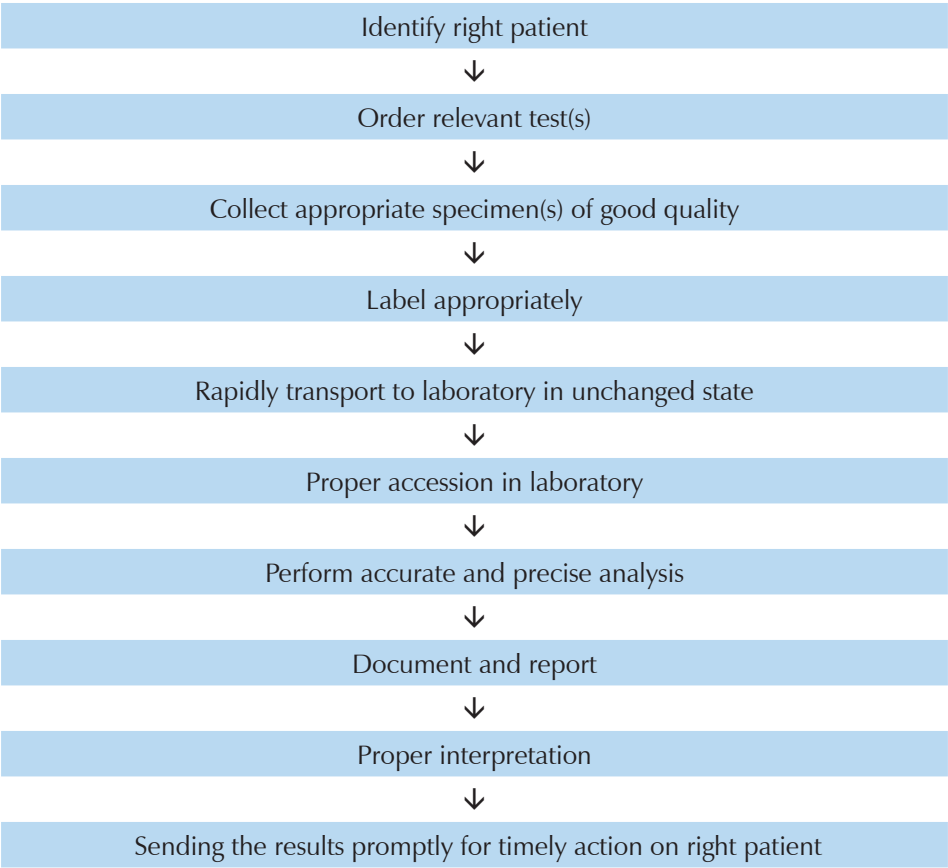
- Routine bacterial culture and parasite examination on patients hospitalized for more than three days
- CSF cultures for mycobacterium of specimens with normal cell counts and glucose and protein levels
- Urine cultures for asymptomatic patients receiving antibiotics
- More than one specimen from same 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 Figure12.1.

12.3 Laboratory efficiency

Laboratory efficiency is an outcome of a good quality system. It is the responsibility of the manager of the laboratory to ensure that the best possible results are delivered most efficiently and in a reasonable period of time. This can be achieved with the active involvement of all staff members. Cost effectiveness is part of the efficiency as are turnaround times and the efficient use of personnel and materials.

Figure 12.1: Algorithm of quality assurance in clinical microbiology



12.4 Statistical challenges in quality assurance programme

There are a wide range of statistical methods which can be used as part of the QA programme in the laboratory. Not all tools need to be applied each time a test is put up or a new technique is introduced. 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.

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.

Table 12.3: Interpretation of Westgard Rules

Westgard Rule	Observation	Interpretation
Warning Rules	$1_{2\ SD}$ is violated	If control value exceeds mean value by +/-2 SD
	$2_{2\ SD}$ is violated	If two consecutive control values exceed mean value on the same side of mean by +/-2 SD. This indicates systematic error.
	$4_{1\ SD}$ 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	$1_{3\ SD}$ is violated	If control value exceeds mean value by +/- 3SD. The results based on this assay are rejected.
	$R_{4\ SD}$ 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 the same side of the mean. The results based on this assay are rejected.

Cumulative sum

Cumulative sum (CUSUM) is the method for identifying shifts or trends occurring in an assay. It indicates systematic errors which may include the 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 materials used. Even the best technologist cannot produce good results with poor quality materials.

In the vast majority of cases, bad analysis is the result of 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 – all these can cause erroneous analytical results. They may all be categorized as poor quality material.

13.1 Pipettes

Pipettes, the most frequently used volumetric glassware, play a crucial role in determining the degree of accuracy and precision in several laboratory analyses. The greater the number of manual test methods used in the laboratory, the greater the role played by the pipette. That is why they should be selected properly and used with care. Completely automated laboratories do little or no pipetting at all.

Volumetric or transfer pipettes, often marked "TD", meaning "to deliver", are used for the transfer of a specified volume of a non-viscous, diluted, aqueous solution. Viscous solutions do not drain properly; therefore these pipettes cannot be used in the quantitative transfer of viscous solutions. Since pipettes and burettes are calibrated for water, an error is introduced whenever they are used to handle solutions with viscosity and surface tension significantly differing from those of water.

Every laboratory should have a written procedure for pipetting so that there are no personal variations in the standard procedure. The precision of the analysis can be hugely improved if all technicians adhere to uniform and careful procedures. For example, the practice of mouth pipetting must be universally 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 into 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 eye level.

(e) Hold the mouthpiece tightly.

Wipe the lower outside portion 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 must be labeled, dated and stored properly and not used beyond their expiry date, and if these show signs of deterioration, such as abnormal turbidity and/or discolouration.

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 organization 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

The 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 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 centers and should be used in calibrating national and 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 precautions must be taken in the storage and preservation of reagents and standards:

- Store all aqueous solutions in plastic bottles that can be tightly closed
- The concentration of standards through evaporation and air oxidation of reagents are sometimes the cause of bad analyses. These can be minimized if bottles are tightly shut. Since colored aqueous solutions

absorb light in the visible region and may undergo photochemical reactions if stored in white plastic bottles, they should be stored in amber-coloured 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.

  All such bottles should be labeled "REFRIGERATE" so that even an inexperienced technician will not leave the bottle out at room temperature. The labeling is easily 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 know. Such contamination could result in small or gross errors in the analyses, which reduce precision and can also result in the test being repeated again. To avoid these difficulties, it is best to first transfer a small amount of the material into a suitable beaker. The remainder solution in the beaker should not be poured back into the original container but 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. That is why 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. Some are used to support the growth of micro-organisms showing typical colonial and morphological appearance. Others are 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

Sources of media

Some years ago culture media used to be prepared from basic chemical ingredients, but laboratories are no longer required to do this. Laboratories should ensure that media prepared in-house are sterile, able to support growth and are appropriately reactive biochemically. Therefore, the laboratory must maintain the stock of reference organisms. These should be used to test the media. Blood-based media should be prepared using appropriate animal blood procured from an authorized source.

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 quality, after preparation, because of changes that can be brought about by the process of reconstitution and sterilization.

Dehydration with additives

For isolation of fastidious organisms, certain additives need to be used when media are prepared in the laboratory. Common additives 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 behavior.

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.

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 an 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 dehydration and makes it unfit for use. Dehydration of the medium can be reduced by preparing only the required number of plates of media and sealing the 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 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 in specimens.

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 this is expensive, media which are known 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, a new supplier or new product is 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 laboratory experience. To meet certification requirements, laboratories need to perform quality control procedures according to a prescribed pattern. Careful records of quality control procedures should be maintained and periodically reviewed to determine the stability of media so that corrective measures can be taken in the right 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 micro-organisms 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 along with their desired frequencies of testing so that they have continuous reliable results, are 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*	2517725922	Pink red bacilli, Blue bacilli
Acridine orange	*Esch. Coli,* *Staph.aurues*	2592225923	Fluorescent bacilli/ cocci
Giemsa	Thin film blood smear		Distinct staining of WBCs and RBCs
Gram	*Esch. coli,* *Staph.aureus*	2592225923	Gram -ve bacilli Gram +ve cocci
Iodine solution	Formalin treated stool specimen with cysts		Visible cyst nuclei
Spores	*Bacillus* species		Spores stain one color and bacillus stains with counter stain
Romanowsky's stain	Thin film blood smear		Distinct staining of WBCs and RBCs

Quality control of stains should be performed on a weekly basis as well as 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 isolates obtained from clinical specimens. 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	*Staphylococcus aureus* *Streptococcus species*	+ –	Bubbling reaction No bubbling
Coagulase	*Staphylococcus aureus* *Staphylococcus epidermidis*	+ –	Clot formation in 4 hours No clot
Indole	*Escherichia coli* *Enterobacter aerogenes*	+ –	Red ring at surface Yellow ring at surface
Methyl red	*Escherichia coli* *Enterobacter aerogenes*	+ –	Instant red colour No colour change
Oxidase	*Pseudomonas aeruginosa* *Escherichia coli*	+ –	Purple colour in 20 seconds No colour in 20 seconds
Voges Proskauer	*Enterobacter aerogenes* *Escherichia coli*	+ –	Red colour No colour change
Bacitracin disc	*Streptococcus group A* *Enterobacter faecalis*	+ –	Zone of inhibition No zone of inhibition
Optochin disc	*Streptococcus pneumoniae* *Viridans Streptococcus*	+ –	Zone of inhibition No zone of inhibition
ONPG disc	*Escherichia coli* *Proteus vulgaris*	+ –	Yellow colour No change in colour
Oxidase disc	*Pseudomonas aeruginosa* *Escherichia 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 catalase, oxidase, and coagulase
- Weekly for bacitracin, Optochin and ONPG

A test procedure not giving anticipated results with control organisms should not be used till 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 micro-organisms. 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 micro-organisms
- Ensure stability of characters
- Prevent contamination
- Ease of supply and transport of preserved micro-organisms
- 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 preservation of microorganisms

Method	Survival period	Stability of characters	Chances of contami-nation	Cost	Ease of Supply and transport	Duration
Subculture	+	+	++++	+	+	+
Gelatin discs	++	++	+	+	++	++
Lyophili-zation	+++	+++	+	+++	+++	+++
Liquid nitrogen	++++	++++	+	++	+	+++

16.1 Preservation of stock cultures

There are many means of preserving living micro-organisms, 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 sub- culturing 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 summarized below:

Enterobacteriaceae

Escherichia coli (ATCC 25922) is one of the required 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 Escherichia 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 *Pseudomonas 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 one of the required 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. *Staphylococcus epidermidis* is used for a negative control in the coagulase and DNase tests.

A strain of *Staphylococcus* that produces β-haemolysin and is suitable for the CAMP test for identifying group B β-hemolytic streptococcus 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, *Enterococcus 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. *Streptococcus salivarius* is needed as a representative of the β-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 an 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 micro-organisms 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 commensal 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 micro-organism 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 micro-organisms 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 aminoglycosides and cephalosporins.

17.4 Choice of drugs

The choice of drugs used in a routine antibiogram is governed by considerations of the anti-bacterial 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

Microorganisms	Basic set	Additional set	Supplemental set for Urine
Staphylococcus spp.	Oxacillin (for *Staph. aureus*) Cefoxitin (for *Staph. aureus* and coagulase negative staphylococci) Penicillin G Erythromycin Clindamycin Gentamicin Trimethoprim Sulfamethoxazole	Levofloxacin or Ciprofloxacin MIC of vancomycin	Norfloxacin Nitrofurantoin
Enterobacteriaceae	Ampicillin Amoxicillin-clavulinic acid or Ampicillin-sulbactam Cefazolin Cefoxitin Gentamicin Trimethoprim-sulfamethoxazole Ciprofloxacin	Cefuroxime Cefotaxime or Ceftriaxone Cefoperazone Cefepime or Cefpirome Amikacin Imipenem Ertapenem Meropenem	Norfloxacin or Ofloxacin Nitrofurantoin

Microorganisms	Basic set	Additional set	Supplemental set for Urine
Shigella spp. and *Salmonella* spp.	Ampicillin Trimethoprim-sulfamethoxazole Ciprofloxacin or Norfloxacin	Levofloxacin	
Vibrio cholerae	Ampicillin Tetracycline Trimethoprim-sulfamethoxazole		
Pseudomonas aeruginosa	Ceftazidime Gentamicin Ciprofloxacin	Piperacillin-tazobactam Cefoperazone Cefepime or Cefpirome Imipenem Meropenem Amikacin	Norfloxacin or Ofloxacin

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.

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.

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 Clinical and Laboratory Standards Institute (CLSI), 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 45-50°C 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 10-30 minutes at 36°C 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 33186) 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 one 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 100 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 degree 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 sulphonamides 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 micro-organism 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 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 benzyl penicillin, 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
Amino glycoside zone too small	pH of medium too high
Amino glycoside 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 〈 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 (lactamase negative strains of *Staph.aureus*)
Enterococcus appears sensitive to amino glycoside 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 17.4. 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	–
Cefepime	30 µg	23-29	31-37	24-30
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	–	–
Colistin	10 µg	-	11-17	11-17
Erythromicin	15 µg	22-30	–	–
Gentamicin	10 µg	19-27	19-26	16-21

Antibiotic	Disc potency	Diameter of zone of inhibition (mm)		
		Staph. aureus (ATCC 25923)	*Esch.coli* (ATCC 25922)	*P.aeruginosa* (ATCC 27853)
Meropenem	10 μg	29-37	28-34	27-33
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	–
Tigecycline	15 μg	20-25	20-27	9-13
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	–

*CLSI: 2009

17.9 Standard procedure for quality control

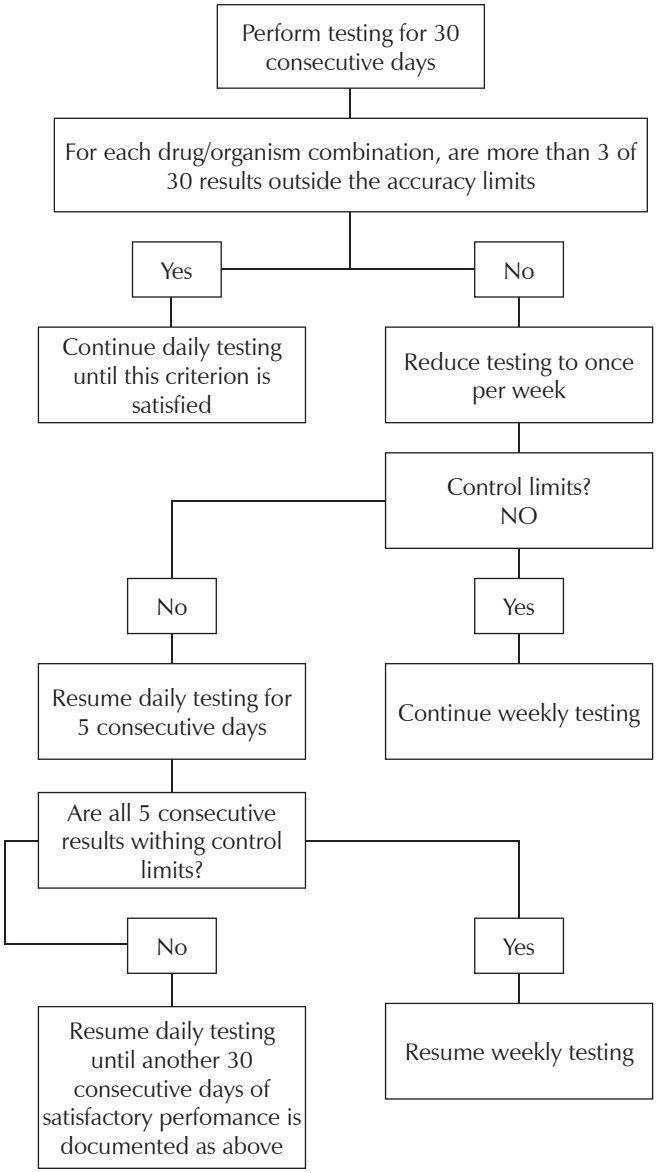
The quality control procedure should use standard reference strains of bacteria that are tested in parallel with the clinical culture. They should preferably be run every week (Figure 17.1), or with every fifth batch of tests, and in addition, every time that a new batch of Mueller Hinton agar or a new batch of discs is used.

Standard strains

These are:

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

Figure 17.1: Frequency of QC Testing in Antimicrobial Susceptibility Testing



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 sub-cultured onto fresh slants every 2 weeks.

17.10 Frequency of quality control testing

Salient features of quality control in antibiotic susceptibility testing are summarized 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 infectious agents to establish its contact with the immune system. Their diagnostic importance stems from the demonstration of a rising titre of antibodies to the agent which inter alia indicates a progressive infection. In rare instances the presence of antibody in a single sample is 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 cl assification and sub classification of infectious agents (e.g. *Salmonella, Shigella, Streptococcus,* etc.).

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

- Antigen only
- Antibody only
- Antigen and/or antibody concurrently

The advantages of serological methods include :

- Rapid identification of agent where pathogens are difficult to culture
- High specificity of detection of antigen
- Simplicity of performance

- Safe procedure
- Diagnostic aids
- Epidemiological tool
- Retrospective confirmation of exposure/ diagnosis
- To differentiate between recent and past infections

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 although some may be expensive, all require certain reagents (sera or antigens etc.) which have limited shelf life and standardized techniques which must be documented in the SOPM.

18.1 Procedure manual

An important element in maintaining day-to-day uniformity in laboratory results is an established procedure (SOP) which details all phases of the laboratory's operation (including safety precautions), and is used by all laboratory personnel. It should include the following instructions:

- The principle of the procedure used for examinations;
- Collecting, transporting, and storing specimens
- Performance specifications (e.g. linearity, precision, accuracy
- Detection limit analytical sensitivity and specificity;
- Required equipment and reagents;
- Procedural steps;
- Quality control procedures;
- Interferences (e.g. lipaemia, haemolysis, bilirubinemia) and cross reactions;
- Biological reference intervals;
- Reportable interval of examination results;
- Alert/critical values, where appropriate;
- Laboratory interpretation;

- Safety precautions;
- Potential sources of variability.

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. Often, 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 inter-laboratory 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 semi-quantitative 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, diagnosis should be undertaken with the utmost seriousness 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 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 specimens

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 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 with a particular batch of kit and should not be used in a different batch of kit.

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 aliquots 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. Records should be kept on a regular basis by individuals who are trained and 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. Serum panels for antigens or 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)	Non-reactive serum control	No clumping
	Weakly reactive serum control	Clumping of graded activity
	Reactive serum control	Clumping of graded activity
Latex agglutination test (ASO)	Negative control serum	No clumping
	Positive control serum	Clumping

Antibody test	Control procedures required	Expected results
Direct agglutination (Widal test, STA for Brucellosis)	Antigen control	No clumping
	Negative control serum	No clumping
	Positive control serum	Clumping
Passive haemagglutination (ASO)	Streptolysin control	Haemolysis
	Red cell control	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 as part of quality assurance. 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	Capsular swelling
	Haemolytic streptococci	No reaction
	H.influenzae type b	Capsular swelling
	Acinetobacter	No reaction
Co agglutination test (β-Haemolytic streptococci, N. meningitides antibody)	Group A,B, β-haemolytic streptococci, *N.meningitidis*	Agglutination with corresponding microorganism

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 and disinfection

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:

Decontamination of equipment and the environment

Decontamination is a process which removes or destroys microorganisms to render an object *safe* for use. It includes cleaning, disinfection and sterilization.

Cleaning

Cleaning is a process that removes foreign material (e.g. soil, organic material, micro-organisms) from an object.

Disinfection

Disinfection is a process that reduces the number of pathogenic microorganisms, but not necessarily bacterial spores, from inanimate objects or skin, to a level which is not harmful to health.

Antisepsis

Antisepsis is a process whereby microorganisms that exist on living tissue are destroyed or inhibited.

Sterilization

Sterilization is a process whereby all forms of microorganisms, including bacterial spores, are killed.

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 (e.g. 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. Among the various indicators, 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 and pressure 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 *Bacillus 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 knowing 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 Bacillus stearothermophilus are used, and with dry heat sterilizers, spores of Bacillus 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, 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 Bacillus subtilis var niger are employed, whereas with formaldehyde Bacillus stearothermophilus is used.

In the case of liquid sterilant, spores of Bacillus 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:

Thermo chemical 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.

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

Table 19.2: Preferred methods of sterilization forcommon 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	Glycerin	Knives	
Stopper	Needles	Scalpels	
Tubing	Oils	Scissors	
Glass		Paper	
Slides		Cups	
Syringe and needles		Plates	
Test tubes		Plastics	
Enamel metal trays		Flasks	
Wire baskets		petri dish	
Wood		Tubes	
Tongue depressors		Rubber tubing	
applicator		Catheters	
Steel tumbler		Drains	

Disinfection

Disinfection is generally a less lethal process than sterilization. It eliminates nearly all recognized pathogenic micro-organisms but not necessarily all microbial forms (e.g., bacterial spores) on inanimate objects. The effectiveness of a disinfection procedure is controlled significantly by a number of factors. Among these are:

- The nature and number of contaminating micro-organisms (especially the presence of bacterial spores); the amount of organic matter present (e.g., soil, feces, and blood)
- The type and condition of instruments, devices, and materials to be disinfected
- The temperature
- The presence of any organic matter necessitates longer contact time with a decontamination method if the item or area is not pre-cleaned. For example, a steam cycle used to sterilize pre-cleaned items is 20 minutes at 121°C. When steam sterilization is used to decontaminate items that have a high bio-burden and there is no pre-cleaning (i.e., infectious waste) the cycle is longer. Decontamination in laboratory settings often requires longer exposure times because pathogenic micro-organisms may be protected from contact with the decontaminating agents.

The sources of laboratory contamination are:

- Fomites
  - Surfaces – laboratory benches, floor, door handles.
  - Instruments – glassware, seating, computers
- Aerosols
- Animals

Decontamination in the microbiology laboratory may require disinfection of work surfaces, decontamination of equipment so it is safe to handle, or may require sterilization. Regardless of the method, the purpose of decontamination is to protect the laboratory worker, the environment, and anyone who enters the laboratory or handles laboratory products. Reduction of cross-contamination in the laboratory is an added benefit. Routine decontamination

of laboratory benches, furniture, equipment, etc, requires the use of chemical disinfectants.

Choosing a disinfectant

Microorganisms present a range of resistances to chemical disinfectants; no single disinfectant is effective in all situations. The following points must be considered when selecting a disinfectant :

(a) Type of micro-organisms, numbers and presence of spores

(b) Physical situation (surface type, suspension, etc.)

(c) Contact available between disinfectant and microorganisms

(d) Possible interaction between disinfectant and materials

(e) Duration of contact

(f) The concentration of the chemical in the disinfectant solution

Most disinfectant solutions need to be regularly prepared as fresh solutions to avoid growth of micro-organisms in the solution and to ensure optimum activity of the disinfectant chemical. In order to prepare working dilutions one must follow the manufacturer's recommendations for dilution of concentrated disinfectant and prepare fresh dilutions daily using clean, dry containers and using clean water for dilutions.

Chemical germicides used for decontamination range in activity from high-level disinfectants (i.e., high concentrations of sodium hypochlorite [chlorine bleach]), which might be used to decontaminate spills of cultured or concentrated infectious agents, to low-level disinfectants or sanitizers for general housekeeping purposes in laboratories. A list of chemical germicides and their activity levels is given below:

Activity of Different Types of Disinfectants						
Toxicity Against	Disinfectant					
	Phenolic Com-pounds	Hypo-chlorites	Alcohols	Formal-dehyde	Glutaral-dehyde	Iodophors
Fungi	good	slight	nil	good	good	good
Bacteria (gram +/-)	good	good	good	good	good	good

Activity of Different Types of Disinfectants						
Toxicity Against	Disinfectant					
	Phenolic Com-pounds	Hypo-chlorites	Alcohols	Formal-dehyde	Glutaral-dehyde	Iodophors
Mycobacteria	fair	fair	good	good	good	good
Spores	nil	fair	nil	good (<40°c)	good (<20°c)	slight
Lipid Viruses	slight	slight	slight	slight	slight	slight
Non-Lipid Viruses	variable	slight	variable	slight	slight	slight
Skin	slight	slight	nil	slight	slight	slight
Eyes	slight	slight	slight	slight	slight	slight
Lungs	nil	slight	nil	slight	slight	nil

The most common chemicals that can be used are :

Chlorine – in the form of sodium hypochlorite (available in concentrated solutions as household bleach –as 4%, 5% or 6% sodium hypochlorite) or other chlorine releasing compounds – is active against vegetative forms of bacteria and viruses and is the preferred disinfectant for HIV and hepatitis viruses. It is less effective against spores. For effective disinfection, a pH range of 6-8 is optimum. The effective strength of chlorine solutions decreases on storage so working solutions should be freshly prepared daily. Chlorine combines rapidly with proteins, so, in the presence of organic materials, the concentration of chlorine needs to be increased. For example, an equal volume of 5000-10000 ppm (0.5%-1% sodium hypochlorite) available chlorine is required for the inactivation of HIV and hepatitis viruses in blood or serum or in areas that have been grossly soiled with blood or body fluids. Benches, floors, walls and other inanimate objects likely to be contaminated, but not visibly soiled, should be cleaned with 500-1000 ppm (0.05% – 0.1% sodium hypochlorite) available chlorine. The hypochlorite solution should be in contact for at least 10 minutes. Hypochlorite solutions are corrosive to stainless steel and other metal surfaces and tend to bleach and damage fabrics.

Alcohols – 80% v/v ethyl alcohol or 60-70% v/v isopropyl alcohol solutions – are useful for cleaning surfaces and disinfecting skin. They are also active against vegetative bacteria and lipid containing viruses, but are inactive against spores. They are ineffective against Mycobacterium species and HIV dried

on surfaces in the presence of sputum or serum. Alcohols are unsuitable for application to proteinaceous material as they tend to coagulate and precipitate surface proteins which may then result in protection of the microorganisms present. Alcohols are flammable, so they should not be used near flames or sparks. Because of their volatility, alcohol disinfectants should be used sparingly in biological safety cabinets and not with equipment that is likely to cause sparks. Alcohol disinfectants may be used from a dispensing bottle, but should never be sprayed as a mist. Both ethyl and isopropyl alcohol are toxic, with isopropyl being the more toxic of the two.

A spill control plan must be available in the laboratory. The steps to be followed for laboratory disinfection after spills (spill cleanup) are:

- Use a chemical disinfectant that is known to inactivate the spilled agent
- Bleach is typically a very good choice
- Place absorbent material on the spill (cotton, gauze, newspaper)
- Pour the solution around the spill to contain it and then towards the middle
- Allow sufficient contact time to inactivate all of the spilled agent – 15 minutes is a good starting point ; 30 minutes for spores
- Wearing proper PPE (lab coat, gloves, eye protection), clean up the spill and place materials in biohazard waste.
- Wipe the area with water or ethanol to remove residue

20

Quality control for equipment

Many kinds of equipment are used in the laboratory. Equipment plays a critical role in assuring quality of results. They can be classified as, Instruments (units that measure), and Machines (units that perform a specific function). Each piece of equipment must function properly to ensure quality of laboratory service. Choosing the right equipment, installing it correctly, ensuring that new equipment works properly, and having a system for calibration and maintenance are all part of the equipment management program in the quality management system. The following factors influence optimal utilization of equipment :

- Proper installation
- Periodic calibration
- Validation
- Regular maintenance
- Training to operator

20.1 Purchase of equipment

Various parameters should be considered before finalizing the purchase of equipment. For example :

- Instrument specifications should fit the intended purpose with comparative cost-effectiveness
- 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 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 that is not possible, the temperature must be recorded every day and before opening the incubator.

pH Meter

A pH meter needs to be standardized 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 micro-organisms.

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, semi-automatic 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.

Thermometers

A calibrated thermometer is a very critical piece of equipment in a microbiology laboratory for recording temperatures. Mercury-filled thermometers may be used for measuring temperatures between 40^0C to 200^0C, while alcohol-filled thermometers are required for measuring sub-zero temperatures in freezers. 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 for all laboratory personnel to ensure that all equipment is in good working condition everyday and to document the same. 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 to do. Some of the suggested acceptable temperature ranges, as recommended in the national standards of some 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°C	+/-5°C	Storage of specimens, sera, reagents and stock cultures
Freezer at -70°C	+/-10°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. Here are some suggestions for appropriate documentation :

- Name and serial number of instrument
- Elements to be checked and data 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 cost of inspection, lubrication and adjustment of instruments is insignificant when compared with the cost of emergency repairs, rebuilding or overhauling equipment, as well as additional man hours and materials involved in producing test results when the 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

Equipment management programme

A good equipment management program is necessary to ensure accurate, reliable and timely results by helping to maintain a high level of laboratory performance, reducing variations in test result and improving the technologist's confidence and knowledge, lengthening instrument life, reducing interruption of services due to breakdowns and failures, lowering repair costs, preventing delays in testing and improving customer satisfaction.

Equipment management includes:

- Equipment selection & acquisition.
- Equipment installation.
- Equipment validation.
- Equipment calibration and performance evaluation.
- Equipment maintenance, service and repair.
- Equipment inventory, documentation and record maintenance.
- Equipment troubleshooting.
- Equipment retirement and disposal (condemnation).

The essential features of equipment management are summarized in Table 20.2

Table 20.2: Components of equipment management

Component	Essential features
Equipment selection and acquisition.	Should fit the purpose Should meet performance characteristics Should be as per available physical infrastructure Safe and easy to operate
Equipment installation.	Should be suitably located in the laboratory so as to allow accessibility, smooth path of workflow and sequential utilization
Equipment validation.	Validate the performance of newly received equipment prior to use to confirm its suitability,
Equipment calibration and performance evaluation.	Calibrate equipment at regular intervals Evaluate performance as calibration only checks the quality and does not upgrade it.

Component	Essential features
Equipment maintenance, service and repair.	Internal preventative maintenance activities as well as vendor provided maintenance/repair for laboratory equipment is important Develop SOP for maintenance and repair
Equipment inventory, documentation and record maintenance.	An equipment inventory system assists in the control of equipment. Records should be maintained for each item of equipment contributing to the performance of examinations.
Equipment troubleshooting.	Develop a flow chart on troubleshooting of common anticipated problems Train operators in the use of trouble shooting flowchart
Equipment retirement and disposal (condemnation).	Develop policy and procedure for retiring older laboratory equipment when it is beyond economic repair or has become obsolete.

All laboratories should have a well-organized equipment management programme. The programme should address equipment selection, preventive maintenance, and procedures for troubleshooting and repair. It is essential that all documentation and records pertaining to the equipment be maintained. These will include a complete and accurate inventory of all laboratory equipment, documents provided by the manufacturer on operation, maintenance and troubleshooting and records of all preventive maintenance, calibration service and repair activities.

21

Suggested further reading

August MJ et al. Eds. Quality control and quality assurance practices in clinical microbiology. *Cumitech*. 1990; 3A: 1-14.

Balows A, Hausler WJ, Herrman KL et al. *Manual of clinical microbiology*. 5th edn. Washington: American Society for Microbiology, 1991.

Bhatia, Rajesh and Ichhpujani RL. *Quality assurance in microbiology*. New Delhi: CBS Publishers & Distributors, 1995.

Medical laboratories: requirements for safety. BS-ISO 15190. 12 November 2003. http://shop.bsigroup.com/ProductDetail/?pid=000000000030098497 – accessed 16 May 2012.

Cheesbrough M. Ed. Medical laboratory manual for developing countries. (Butterworth & Co., Sevenoaks) 1989.

El-Nageh, Heuck CC, Appel W, Vandepitte J, Engbaek K, Gibbs WN. *Basics of quality assurance for intermediate and peripheral laboratories*. WHO Regional Publications, Eastern Mediterranean, Series No. 2. Alexandria: WHO Eastern Mediterranean Regional Office, 1992.

Howanitz PJ, Kowanitz JH. *Laboratory quality assurance*. New York: McGraw Hill Book Company, 1987.

Medical laboratories: particular requirements for quality and competence. ISO15189. 2007: http://www.iso.org/iso/catalogue_detail?csnumber=42641 – accessed 16 May 2012.

Last JM: In A Dictionary of Epidemiology (Oxford University Press), 1995
Miller JM. Quality control in Microbiology.
(CDC, Atlanta Ga), 1987

Specific criteria for accreditation of medical laboratories. NABL 112. http://ahllnablresources.blogspot.in/2005/11/specific-criteria-for-accreditation-of.html - accessed 16 May 2012.

Prier JE, Bartold JT, Friedman H. Eds. *Quality control in microbiology*. Baltimore: University Park Press, 1975.

Report. ISO/DIS: 3524-1, 1990, 3.14

Snell JJS, Farell ID, Roberts C. Eds *Quality control: principles and practice in the microbiology laboratory*. 2nd edn. London: Public Health Laboratory Services, Colindale, 1999.

Taylor RN, Huong AY, Fulford KM, Przybyszewski VA, Hearn TL. *Quality control for immunologic tests*. Atlanta Ga: CDC, 1985.

Vandepitte J. Et al. *Basic laboratory procedures in clinical bacteriology*. Geneva: WHO, 1991.

World Health Organization. *Basic laboratory procedures in clinical bacteriology*. Geneva: WHO, 1991

World Health Organization. *Manual on basic techniques for a health laboratory*. Geneva: WHO, 1980.

World Health Organization. *Safety in health care laboratories*. Geneva: WHO, 2003.

Annex 1: Contributors

Dr Sudarshan Kumari
Former Regional Adviser
Department of Communicable Diseases
World Health Organization
Regional Office for South-East Asia,
New Delhi-110002
Kumaris1941@gmail.com

Prof V. Ravi
Head of Neurovirology
National Institute of Mental Health and Neurosciences (NIMHANS)
P. O. Box No. 2900
Hosur Road-560029, Bangalore
vravi@nimhans.kar.nic.in

Dr Rekha Jain
Technical Specialist for Laboratory Strengthening towards Accreditation and GCLP
Unit-F, Gemini Park CHS,
V N Purav Marg, Mankhurd (E)
Mumbai 400088, India
drrekhaj@gmail.com

Mrs Suwanna Charunut
Bureau of Laboratory Quality Standards,
WHO Collaborating Centre on Strengthening Quality in Health laboratories
Department of Medical Sciences,
Building 9, 7th Floor, Tiwanond Road, Amphur Muang, Nonthaburi
Thailand 11000
suwanna.j@dmsc.mail.go.th

Mrs.Partoompit Wimonwattrawatee
(Head of External Quality Assessment Section,BLQS),
Bureau of Laboratory Quality Standards,
WHO Collaborating Centre on Strengthening Quality in Health laboratories
Department of Medical Sciences,
Building 9, 7th Floor, Tiwanond Road, Amphur Muang, Nonthaburi
Thailand 11000

Mr.Suthon Vongsheree (Head of Laboratory Accreditation Section1)
Bureau of Laboratory Quality Standards,
WHO Collaborating Centre on Strengthening Quality in Health laboratories
Department of Medical Sciences, Building 9, 7th Floor, Tiwanond Road, Amphur Muang, Nonthaburi
Thailand 11000

Dr Anuj Sharma
National Professional Officer
WHO Country Office for India
New Delhi 110001
Sharmaan@searo.who.int

Ms Jyoti Malhotra
D-35, Press Enclave, Saket
New Delhi
jomalhotra@gmail.com

Dr Aparna Singh Shah
Lab Scientist
Department of Communicable Diseases
World Health Organization
Regional Office for South-East Asia
New Delhi-110002
shahap@searo.who.int

Dr Rajesh Bhatia
Regional Adviser, BLT
Department of Communicable Diseases
World Health Organization
Regional Office for South-East Asia
New Delhi-110002
bhatiaraj@searo.who.int

Index

Accession list, 40
Accidents in the laboratory, 88
Accreditation, 71
Analytical factors, 14
Antisepsis, 144
Benefits of quality, 2
Biohazard levels, 84
Calibration, 156
Categories of pathogens, 89
Collection of specimen, 164
Cumulative sum, 103
Deming cycle, 3
Quality system,
Development, 24
Disinfection, 149
Document
    Control, 36
    Layers of, 34
    Value, 33
    Types, 34
External quality assessment scheme, 57
Good laboratory practices, 6
Incident reports, 45
Intermediate laboratory services, 27

Internal quality assessment, 65
International organization for standards
    ISO, 1, 8
Laboratory-acquired infections, 89
Objectives of quality assurance, 16
    Total quality management, 16
Peripheral laboratory services, 25
Practice of laboratory safety, 78
Preservation of stock cultures, 119
Preventive maintenance, 157
Quality
    Assessment of, 54
    Factors affecting analytical, 14
    Pre-analytical factors, 10
    Post-analytical factors, 14
Quality assurance, 15
Quality assurance programme, 16
    Components 17
    Computers 18
Quality assurance in antibiotic susceptibility testing, 123
    Factors influencing zone size in, 131
    Troubleshooting guide for disc diffusion, 132
Quality assurance in clinical laboratory, 107
Quality management system, 19
Quality audit, 67
    Equipment, 142, 153
    Serology, 137
    Sterilization, 144
    Bacteriological media, 111
    Stains, 116
Quality system, 21
    Key elements, 21
Rapid diagnostic tests, 31
Referential (quality) standards, 22
Requisition form, 41
Safety in the laboratory, 82

Safety records, 45
Standard deviation index, 103
Standard operating procedures, 47
    Essential components, 49
    Structure of SOP, 50
Sterilization, 170
Test Report, 42
Traceability, 9
Validation, 52
Waste Management, 94
Westgard Rules, 103
Work Card, 41

There is an increasing dependence on clinical and public health laboratories for better patient management and also for preventing the spread of emerging pathogens. With rapid and significant growth of laboratories at all levels of health care, it has become mandatory to check results to make them reliable and cost-effective, as well as comparable with those obtained by international laboratories. The International Standards Organization (ISO) has provided several guidelines and standards for achieving quality in laboratory results.

These guidelines dwell upon the basic concepts of quality assurance in microbiology and also describe essential practices and steps of ensuring quality in various activities that a microbiology laboratory is expected to undertake in its support to primary health care system in a biosafe environment and in accordance with ISO. Following these guidelines will help in delivery of reliable, cost-effective and timely laboratory results and support clinical and public health actions.

World Health Organization
Regional Office for South-East Asia
World Health House
Indraprastha Estate
Mahatma Gandhi Marg
New Delhi-110002, India

ISBN 978 92 9022 415 0
9 789290 224150