National Guidelines on Quality Management Systems In HIV Testing Laboratories

National AIDS Control Organisaiton
Ministry of Health & Family Welfare, Government of India

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सत्यमेव जयते

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Message

Laboratory diagnosis must be accurate and reliable so that person who is HIV positive should not be labeled as HIV negative and vice versa. Therefore importance of maintaining quality and continuously improving the quality is of utmost importance.

Over the past few years, in collaboration with Centres for Disease Control and Prevention (CDC) and Project Concern International (PCI) our HIV testing reference laboratories, both national and state, have been systematically assessed for compliance with internationally recognized quality standards and have been provided technical assistance to attain these standards. This has enabled almost all National Reference Laboratories (NRLs), half of State Reference laboratories (SRLs) and some integrated Counseling and Testing Centers (ICTC) and CD4 enumeration laboratories to achieve NABL (National Accreditation Board for Testing and Calibration Laboratories) accreditation conforming ISO 15189 standards.

I congratulate Lab Services Division and thank all experts for formulation of new national guidelines in HIV testing laboratories for further mass quality across all ICTCs and other places where this testing is happening.

27.4.15

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Preface

With around 2.1 million cases of HIV in the country, HIV/AIDS remains one of the major health concerns in India. Government of India has successfully responded to the HIV epidemic from time to time. One of the cornerstones of containing HIV infections is timely and accurate diagnosis of HIV infection among various population groups. Annually more than 2.2 crore HIV tests are performed in the country. Maintaining quality of this huge number of tests is of utmost importance for accurate diagnosis.

Medical laboratory services are critical crosscutting support services and are essential to patient care, however, attaining, maintaining and improving accuracy, reliability and timeliness of test results are major challenges for health laboratories. This guideline is intended to provide a comprehensive reference on Laboratory Quality Management System in HIV diagnostic laboratories in the public and private sector. The guideline is meant for all stakeholders involved in health laboratory processes, from senior management, to middle level administration, to finally bench-work laboratorians.

The first version of guidelines published in 2007 viz. "Manual on Quality Standards for HIV Testing Laboratories" for improving quality of laboratory services was in use until recently. This mission has been taken further, towards every laboratory meeting both technical competence requirements and quality management system requirements that are necessary for it to consistently deliver technically valid results and seeking accreditation. NACO has revised the guidelines based on the international standards particular to medical laboratories' requirements for quality and competence.

Leading national experts and international agencies have contributed to the development of these guidelines and I acknowledge contributions of CDC, PCI and all contributors immensely.

(Dr. Naresh Goel)

एड्स का ज्ञान : बचाए जान
TALK AIDS : STOP AIDS

Table of Contents

Abbreviations

AIDS	Acquired Immunodeficiency Syndrome
AMR	Analytical Measurement Range
BMW	Biomedical Waste Management
CAPA	Corrective Action Preventive Action
CDC	Centers for Disease Control and Prevention
CLSI	Clinical Laboratory Standard Institute
COV	Cut off value
CQI	Continual Quality Improvement
CV	Coefficient of Variation
DCGI	Drug Controller General of India
ELISA	Enzyme Linked Immuno-Sorbent Assay
EQAS	External Quality Assessment Scheme
FDA	Food and Drug Administration
FEFO	First Expiry First Out
GCLP	Good Clinical Laboratory Practice
HBV	Hepatitis B Virus
HBsAg	Hepatitis B surface Antigen
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
IATA	International Air Transport Association
ICTC:	Integrated Counseling and Testing Center
IP	In Patient
IQ	Installation Qualification
ISO	International Organization for Standardization
LIMS	Laboratory Information Management System
MRA	Mutual Recognition Agreement
MSDS	Material Safety Data Sheet
NABL	National Accreditation Board for testing and Calibration Laboratories
NACO	National AIDS Control Organisation
NACP	National AIDS Control Program
NaOCl	Sodium Hypochlorite
NARI	National AIDS Research Institute
NC	Non-Conformance
NCDC	National Center for Disease Control
NCE	Non-Conforming Event
NICED	National Institute for Cholera and Enteric Disease

Abbreviations

NIMHANS	National Institute of Mental Health and Neurosciences
NML	National Metrology Laboratory
NRL	National Reference Laboratory
NRL on Q	National Reference Laboratory on Quality
OD	Optical Density
OFI	Opportunity for Improvement
OIML	International organization of legal metrology
OM	Occurrence Management
OQ	Operational Qualification
PCI	Project Concern International
PEP	Post Exposure Prophylaxis
PID	Patient Identification
PPE	Personal Protective Equipment
PQ	Performance Qualification
PT	Proficiency Testing
QA	Quality Assurance
QC	Quality Control
QMS	Quality Management System
QSE	Quality System Essential
QSP	Quality System Procedure
RCA	Root Cause Analysis
RPM	Revolutions per Minute
SACS	State AIDS Control Society
SD	Standard Deviation
SI units	International System of Units
SIMS	Strategic Information Management System
SOP	Standard Operating Procedure
SRL	State Reference Laboratory
TAT	Turn Around Time
TMU	Temperature Monitoring Unit
TTI	Transfusion Transmitted Infection
UM	Uncertainty of Measurement
VIM	International Vocabulary of Metrology
WI	Work Instructions

Glossary

Accident: An undesirable or unfortunate happening that occurs unintentionally

Accreditation: The process by which an independent and authorized body gives formal recognition that an organization is competent to carry out specific tasks

Amended Report: The revised version of an original report created, based on the receipt of additional information or analysis.

Assessment: A systematic process of collecting and analyzing data to determine the current, historical or projected status of an organization

Audit: A systematic, independent and documented process for obtaining audit evidence and evaluating it objectively to determine the extent to which audit criteria are fulfilled (ISO 9000)

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.

Biosafety: Laboratory biosafety describes the containment principles, technologies and practices that are implemented to prevent unintentional exposure to, or accidental release of, pathogens and toxins.

Biosecurity: Institutional and personal security measures designed to prevent the loss, theft, misuse, diversion or intentional release of pathogens and toxins.

Calibration: The process of comparing of a measurement instrument, kit or test system of unverified accuracy to that of a reference standard of known accuracy to detect any variation from the required performance specification.

Coefficient of Variation: The standard deviation (SD) expressed as a percentage of the mean.

Confidentiality: Confidentiality has been defined by the International Organization for Standardization (ISO) in ISO-17799 as "ensuring that information is accessible only to those authorized to have access" and by the International Code of Ethics as “Except when obligated by the law of the country concerned, a doctor shall not disclose without the consent of the patient, information which he has obtained in the course of his professional relationship with the patient”

Competence: Demonstrated personal attributes and demonstrated ability to apply knowledge and skills (ISO 9000)

Complaint: A concern lodged by any customer or client, including a patient, family member, physician, other health care staff, other laboratories, etc.

Continual Quality Improvement: A part of the quality management system focused on increasing the ability to fulfil quality requirements. It is possible to achieve continual improvement through small, incremental changes using scientific methods.

Corrective Action: An action to eliminate the cause of a detected nonconformity or other undesirable situation (ISO 9000)

Glossary

Document: Information and its supporting medium. This may be paper-based or electronic.

Document Control: A system to establish and maintain the proper use of time or version sensitive documents.

Engineering controls: Well-designed work areas and equipment that minimize or eliminate exposure to hazards

Error: A deviation from truth, accuracy, or correctness; a mistake.

External Audit: Second-and third-party audits.

External Controls: Controls not included in the test kits and are used in addition to the internal controls.

External Quality Assessment: is a system of objectively assessing the laboratory performance by a designated external 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.

Feedback: Communication from customers about how delivered products or services compare with customer expectations.

Flowchart: A graphical representation of the steps in a process. Flowcharts are drawn to better understand processes.

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

Gap Analysis: The comparison of a current condition to the desired state.

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

Inter-laboratory Comparison: The organization, performance and evaluation of tests for the same analyte by two or more laboratories in accordance with predetermined conditions.

Management Review: A periodic meeting of management at which it reviews the status and effectiveness of the quality management system of the laboratory.

Mean: The arithmetic average of a group of values. This is determined by summing the values and dividing by the number of values.

Medical Ethics: A system of moral principles that apply values and judgments to the practice of medicine.

Measurand: A quantity intended to be measured.

Measurement Uncertainty: A non-negative parameter characterising the dispersion of quantity values being attributed to a measurand, based on the information used.

Non-conformity: Failure to meet a requirement of the quality management system or the relevant standard. Non fulfillment of a requirement.

Occurrence: Something that happens; an event, incident, complaint, non-conformance, or accident.

Occurrence Management: A central part of continual improvement; it is the process by which errors are identified and handled.

Organizational Chart: Defines the working structure for the organization; Organizes jobs along lines of authority; defines the reporting, decision making and results accountability hierarchy and span of control; works in combination with job descriptions to define the working structure of the organization.

Path of Workflow: laboratory- sequential processes in a laboratory's activities that transform a request for examination into the laboratory information that is captured in the report of results. ISO standards group laboratory processes into pre-examination, examination and post-examination categories. Comparable terms used currently include: pre-analytic, analytic and post-analytic processes; or pre-test, test and post-test processes.

Performance Review: A periodic review and evaluation of an individual's job performance.

Personal Protective Equipment: Specialized clothing or equipment worn by an employee to protect against health and safety hazards.

Policy: a documented statement of overall intentions and directions defined by those in the organization and endorsed by management.

Post-examination Phase: Processes following an examination including the systematic review, formatting and interpretation, authorization for release, reporting and transmission of the results, and storage of samples for the examinations.

Pre-examination Phase: Chronological steps beginning with the clinician's request including the examination requisition, followed by the preparation of the patient, the collection of the primary sample, the transportation to and within the laboratory, and ending when the examination phase/process begins.

Precision: A measurement of the scatter or random error between repeated measurements expressed statistically as the standard deviation. The less variation a set of measurements has the more precise it is.

Preventive action: Proactive action/s taken to eliminate the cause of a potential non-conformity or any other potentially undesirable situation or actions taken to 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.

Glossary

Procedure: Specified way of carrying out an activity of a process defining in detail the work that should be done, how it should be done, who should do it, and under what circumstances.

Process: Set of interrelated or interacting activities that transforms inputs into outputs.

Proficiency Testing: The evaluation of participant performance against pre-established criteria by means of inter laboratory comparisons (ISO 17043)

Quality: Degree to which a set of inherent characteristics fulfills requirements. (ISO 9000)

Quality Assurance: A planned and systematic set of quality activities focused on providing confidence that quality requirements will be fulfilled. Quality assurance calls on laboratories to be "fit for purpose" and to "do it right the first time".

Quality Control: A set of procedures undertaken by the laboratory staff for continuously assessing laboratory work and emergent results, in order to decide whether they are reliable enough to be released and to ensure day-to-day consistency.

Quality Indicators: Established measures (Observations, statistics, or data) defined by a laboratory to determine how well it is meeting its quality intentions , customers' needs as well as other operational and financial performance expectations.

Quality Manager: An individual with delegated responsibility and authority to oversee compliance with the requirements of the quality management system, who reports directly to the level of laboratory management at which decisions are made on laboratory policy and resources.

Quality Manual: The primary document (level 1) specifying the quality management system and quality policy of an organization.

Quality Management System: A management system of coordinated activities to direct and control an organization with regard to quality (ISO 9000); note: Systematic and process-oriented efforts are essential to meet quality objectives.

Quality Policy: The overall intentions and direction of an organization related to quality as formally expressed by executive management.

Quality System Essentials: Set of coordinated building blocks for quality management. The 12 quality system essentials are : Documents and Records; Organization; Personnel; Equipment; Purchasing and Inventory; Process Control; Information Management; Occurrence Management; Assessment/Audit both External and Internal; Process Improvement; Customer Service; and Facilities and Safety.

Random Error: the dispersion of independent test results obtained under specified conditions. It is expressed as the maximum allowable coefficient of variation (CV %) of the results in a set of replicate measurements.

Record: An evidence of results achieved or activities performed (ISO 9000).Records demonstrate traceability and provide evidence of verification, preventive action and corrective action.

Reference Laboratory: A laboratory that provides specialized expertise to other laboratories. This expertise may be in the performance of additional or specific examinations, or as consultation on submitted cases.

Regulation: A principle, rule or law designed to control or govern. A governmental order having the force of law.

Root Cause Analysis (RCA): A process for identifying the basic or causal factor(s) that underlies variations in performance, including the occurrence or possible occurrence of a nonconforming event.

Scope of Accreditation: The scope of accreditation of a testing laboratory is the formal and precise statement of activities which the laboratory is accredited for.

Sensitivity: The probability that a test will detect an analyte when it is present in a specimen. The ability of a test to correctly identify individuals, who have a given disease or condition. The sensitivity of a test is the probability of a positive test in people infected with HIV, expressed as a percentage.

Sharps: Any object that can penetrate the skin, including, but not limited to, needles, scalpels, and broken capillary tubes.

Shifts: in the mean occur when an abrupt change is followed by six or more consecutive QC results that fall on one side of the mean but typically within 95% range as if clustered around a new mean. On the sixth occasion this is called a shift and results are rejected.

Source Documents: The paper form onto which data are written.

Specification: Any requirement with which a process, equipment or other activity must conform.

Specificity: The probability that a test will be negative when an analyte is absent from a specimen. The ability of a test to correctly exclude those individuals who do not have a given disease or condition. The specificity of a test is the probability of testing negative in people not infected with HIV, expressed as a percentage.

Specimen (sample): Any sample material taken from a biological entity for testing, diagnostic, propagation, treatment or research purposes.

Standard Deviation: It shows how much variation or dispersion, there is from the average (mean or expected value). A statistical measure of variation used to describe a frequency distribution; the square root of the average of the squared deviations from the mean; to calculate a SD, the data points are first averaged, then this mean value is subtracted from each data point, giving the “difference score”; these difference scores are then each squared, and the squared difference scores are added together; the sum of the squared difference scores is then divided by the number of original data points less one, or “n-1”; a square root of the resulting quotient is the SD.

Glossary

Standard Operating Procedure: Detailed, written instructions to achieve uniformity of the performance of a specific function.

Standard Precautions: An approach of infection control in which all specimens containing or contaminated with human blood and body fluids are treated as if infectious.

Supplier: Organization or person that provides a product or service.

Systematic Error: the expressed difference between the average result obtained by a procedure under specified conditions and an accepted reference value or the deviation of the mean from the target value. Bias is expressed as the maximum allowable difference (Delta diff) of an average result in a set of replicate measurements and its expected reference value.

Traceability: Ability to trace the history, application, or location of that which is under consideration.

Traceability of measurement: The property of 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.

Trends: occur when values gradually, but continually, move in one direction over six or more analytical runs. Trends may display values across the mean, or they may occur only on one side of the mean. On the sixth occasion, this is determined to be a trend and results are rejected.

Turn Around Time: Length of time from when a sample arrives in the laboratory and when the final result is issued.

Uncertainty of Measurement: A parameter, associated with the result of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the measurand.

Validation: Confirmation through the provision of objective evidence that the requirements for a specific intended use or application have been fulfilled (ISO 9000).

Verification: The act of determining whether products and services conform to specific requirements. Confirmation through the provision of objective evidence that specified requirements have been fulfilled (ISO 9000).

Work Instructions: A set of detailed, sequential, stepwise instructions for performing a task.

Introduction

Laboratory services are an essential component in the diagnosis and management of patients infected with the Human Immunodeficiency Virus (HIV). Universal availability and routine access to quality assured HIV related laboratory services is a key objective of the National AIDS Control Program (NACP). Good Laboratory systems are required for HIV prevention, care support and treatment, therapeutic monitoring and surveillance. National AIDS Control Organization (NACO) has established a three-tiered pyramidal HIV laboratory system to support the quality management, mentoring and EQAS, with an Apex Laboratory and National Reference Laboratories (NRLs) at the national level through the State Reference Laboratories (SRLs) down to the point of testing sites at the Integrated Counselling and Testing Centres (ICTC).

An estimated seventy percent of medical decisions are made on the basis of laboratory test results. Health outcomes depend on the accuracy of testing and reporting. If inaccurate results are delivered, the consequences can be significant and include unnecessary treatment; treatment complications; failure to provide proper treatment; delay in correct diagnosis and additional and unnecessary diagnostic testing. These consequences result in increased resource utilization costs, time and personnel effort, and often in poor patient outcomes. In an HIV testing laboratory, the results have social, medical, ethical, and legal implications.

In order to achieve the highest level of accuracy and reliability, it is essential to perform all processes and procedures in the laboratory in the best possible way. The laboratory is a complex system, involving many steps of activity and many people. The complexity of the system requires that all processes and procedures be performed properly. Therefore, the quality management system model, which looks at the entire system is very important for achieving good laboratory performance consistently.

Laboratory management needs to be firmly committed to quality assurance and adequate resource allocation. Quality is the responsibility of all staff members of the organization supported by relevant trainings, standards, procedures and documentation.

Quality standards are an integral part of the Quality system. This guideline is in alignment with the international standard by the International Organization for Standardization, “Medical laboratories- Requirements for quality and competence” ISO 15189 and the specific criteria of the National Board of Testing and Calibration Laboratories- NABL 112.

A laboratory quality system is only as good as the staff that works with it. No matter how good a quality system is, if it is not carried out consistently in daily practice, high quality cannot be achieved. Trained laboratory personnel must perform, supervise, interpret and validate

laboratory analysis at all times.
A good documentation system is necessary for the smooth functioning, good clinical laboratory practices (GCLP) and accreditation of a laboratory. Document control is an essential part of document management and involves creating, regularly reviewing and updating, distributing and maintaining all documents and information.

Health laboratories should be free from recognized hazards that may cause serious harm to their employees, the general public or the environment. Staff is expected to follow safe work practices, keep their work and material secure and follow an ethical code of conduct.

Appropriate building space, design, utilities and equipment are essential to delivering safe and effective services. Laboratory management must establish and implement an equipment management program that regularly monitors and demonstrates the proper calibration and function of instruments, reagents and analytical systems. It should also have a documented and recorded program of preventive maintenance and validation.

Assuring the quality of laboratory results is the core objective of any health laboratory and is a continuous on-going process. Participation in Proficiency Testing (PT), inter-laboratory comparison and External Quality Assessment (EQA) will ensure accurate and reliable results thereby increasing the credibility and acceptance of the laboratory. Quality Control (QC) measures should be practiced daily. The goal of QC is to detect errors and correct them before patients' results are reported.

Laboratory management should develop relevant quality indicators to systematically monitor and evaluate the laboratory's performance and contribute to the overall health services and health outcomes. Quality should be assessed through periodically scheduled audits (internal or external) the results of which should guide management in further improving the quality of laboratory services.

All these aspects have been covered in the present document which will help the laboratories to strengthen their Quality Management System (QMS) and facilitate seeking accreditation.

NACO is committed to working continually towards sustaining and improving the quality of HIV related laboratory practices and positioning the laboratory for accreditation.

Quality Assurance in the HIV Testing Laboratory

Quality is an absolute requirement for any testing laboratory. The ISO 9000:2007 defines quality as the degree to which a set of inherent characteristics fulfills requirements. Laboratory quality can be defined as the accuracy, reliability and timeliness of reported test results. To be useful and contribute in patient care appropriately, laboratory results must be as accurate as possible, all aspects of the laboratory operations must be reliable, and reporting must be timely.

For a HIV testing laboratory, the generation of false positive and false negative results is associated with social, medical, ethical and legal implications; hence it is extremely important to avoid the occurrence of inaccurate results.

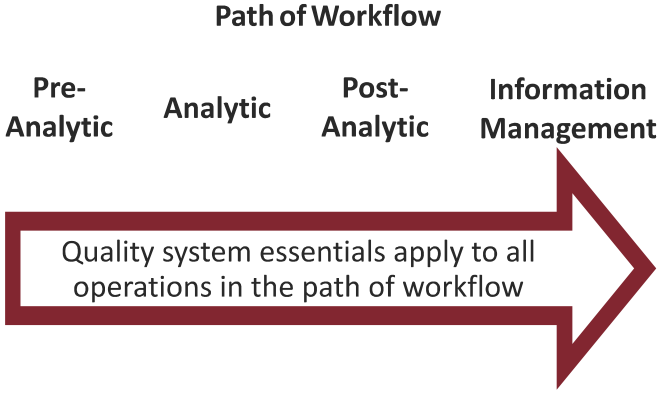
To attain quality, a laboratory must have a Quality Management System (QMS) in place. QMS can be described as a set of essential building blocks for a laboratory's work operation to fulfil stated quality objectives. Such a system provides the means to direct and control the laboratory with regard to quality. Quality Management System is not limited to only testing activities in the laboratory, but extends much beyond the testing process.

The Clinical Laboratory Standard Institute (CLSI) through a process of voluntary consensus has identified twelve Quality System Essentials (QSEs) as the foundational building blocks that function effectively to support the laboratory's path of workflow. The path of workflow comprises of sequential processes in a laboratory's activities that transform a request for examination into laboratory information that is required to report the results. It is divided into three phases (processes): pre-analytic (pre-examination), analytic (examination) and post-analytic (post-examination) phases or processes of laboratory testing.

Structure for a Quality System:

Quality System Essentials

1. Organisation
2. Customer focus
3. Facilities and Safety
4. Personnel
5. Purchasing and Inventory
6. Equipment
7. Process management
8. Documents and Records
9. Information Management
10. Nonconforming Event Management
11. Assessments
12. Continual Improvement

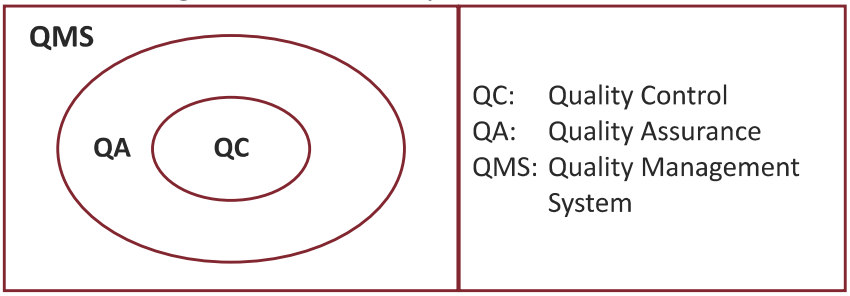


The same are defined in the international standard ISO 15189:2012 (Medical laboratories- Requirements for quality and competence) as Management requirements with 15 sub clauses (4.1 to 4.15) and Technical requirements with 10 sub clauses (5.1 to 5.10).The HIV laboratory's fulfillment of these requirements means that it meets both the technical competence requirements and the management system requirements that are essential for it to consistently deliver technically valid results.

Errors can potentially occur during the many processes that take place from the time a test is requested to the time the test results are interpreted and released. In order to mitigate or reduce the risk of errors in the laboratory, the QMS developed includes Quality Control (QC), Quality Assurance (QA) and Continual Quality Improvement (CQI).

A diagrammatic representation (Figure 2.1) explains the relationship between QC, QA and QMS.

Figure 2.1: Relationship between QC, QA and QMS



Quality Control (QC) is defined as the activities used to monitor the testing process and ensure that the test run is valid. It refers to processes used to minimize errors during the analytical phase of testing activities.

Quality Assurance (QA) is a planned and systematic set of quality activities focused on providing confidence that quality requirements are fulfilled. It is a process whereby the overall quality of the laboratory test report is assured. It is a dynamic and ongoing process of monitoring a system for reliability and reproducibility of results that provides an opportunity for corrective action when established criteria are not met. It includes minimizing errors during pre-analytical, analytical and post- analytical phases of testing.

Quality assurance is an all-encompassing activity dealing with pre-analytical, analytical and post- analytical phases, whereas quality control is specific to the analytical phase only.

The pre-analytical phase comprises of those steps involved before the actual performance of the test. This is one of the most important steps during which majority of errors can occur. The analytical phase includes various steps involved in the testing process. The post- analytical phase includes those steps involved in the interpretation and reporting of test results. The various activities occurring in each phase are listed in Table 2. 1

Table 2.1: Activities affecting the quality of test results

Pre-analytical	Analytical	Post-analytical
▸ Test requisition ▸ Preparation and identification of patient ▸ Specimen selection, collection, labelling ▸ Specimen transport ▸ Specimen accession (acceptance/ rejection criteria) ▸ Specimen storage ▸ Selection and storage of test kits ▸ Performance, calibration & maintenance of equipment ▸ Trained and competent personnel	▸ Specimen processing ▸ Reagent preparation and use ▸ Test performance : adhering to test algorithms & SOPs ▸ Use of QC procedures : inclusion of internal and external controls ▸ Determine measurement uncertainty for each measurement procedure ▸ Participation in EQAS/PT ▸ Results interpretation	▸ Transcribing results from worksheet to report forms ▸ Review of results ▸ Authorized release of results ▸ Communicating the result to the appropriate person within the turnaround time ▸ Retention and storage (archiving) of specimen ▸ Retention of examination results ▸ Waste disposal

Activities like proper documentation, maintenance of quality and technical records, safety and training with competency assessments are essential in all the three phases of testing.

Benefits of a quality management system:

- Generates accurate and precise results
- Avoids inappropriate test selection, unnecessary investigations and incorrect results that can have serious health implications, and may lead to adverse events including financial and emotional burden.
- Helps the physician in quickly establishing the proper diagnosis , thus generating confidence and better health care for the patient
- Creates a good reputation for the laboratory
- Ensures inter laboratory comparability and stimulates improved performance
- Motivates staff to work better
- Ensures support in the event of legal challenge or other complications
- Saves money by getting it right at the first time
- Mandatory requirement for accreditation

The HIV network of testing laboratories at all levels (National Reference Laboratories-NRLs, State Reference Laboratories-SRLs, Integrated Counseling and Testing Centers-ICTCs) should develop, implement and maintain a QMS. Every laboratory should routinely monitor and assess the quality in the pre-analytical, analytical, and post-analytical phases/processes.

Ensuring Quality in the pre analytical phase

Test Requisition Form: Each specimen must be accompanied with a test requisition form which should include

- Name, age and gender of the patient
- Registration number: Patient Identification number (PID) and/or hospital In-Patient/Out-Patient number
- Type of specimen
- Identity of the requester
- Date and time of collection
- Identity of the person who collected the specimen
- Brief clinical information

Pre-test counseling and informed consent must precede sample collection

Labeling of specimen: The label should include following information:

- Name, Age and Gender of the patient
- Date of collection
- Patient's identification number

The requisition form (Annexure 2.1) and the label on the specimen should be compared and verified before acceptance

Accession of specimen received: All specimens must be inspected at the time of receiving and before testing to ensure that they are suitable. The quantity of the specimen should be adequate to perform the test (e.g. 2 - 5 ml of whole blood for HIV serology) to perform the test. The accepted samples must be entered into the Specimen Acceptance Register (Annexure 2.2)

- **Rejection criteria:**
  - Insufficient quantity
  - Lipaemic sera (serum is milky white from its high fat content)
  - Haemolysed sera (pink to red tinged serum)
  - Visually turbid serum due to contamination
  - Inappropriate container
  - Leaking / soiled container
  - Specimen not accompanied by the requisition form
  - Specimen not transported at recommended temperature
  - Specimen container label absent, incomplete or illegible

A fresh specimen should be requested for testing when specimen is rejected. A note must be made in the Specimen Accession Register as to the reason for rejection. The requester/collection site must be informed of the specimen rejection and a record of this communication must be maintained.

If the specimen is irreplaceable or critical, the requesting physician takes responsibility for identifying and providing proper information. A note must be made in the accession register and in the report, for reasons of acceptance of a compromised specimen. The report should also mention that the result of the test might not be valid because of the condition of the specimen

Appropriate testing specimen: The specimen/sample used for testing with a particular test kit should meet the requirements of the kit literature and the laboratory's Standard Operating Procedure (SOP).

The laboratory must have information available for patients and users of the laboratory services. The information needs to provide the following guidance and instructions for those who order laboratory examinations : the location of the laboratory, details of which laboratory tests are available and when with the turnaround times, which test requires documentation of patient consent for HIV testing, which laboratory examination require special instructions/preparation such as pre-test and post - test counseling for HIV testing, appropriate information concerning sample required, collection containers, preservatives or anticoagulants, primary sample volumes, special precautions, turnaround time, biological reference intervals, and clinical decision values; instructions for completion of the request form, method/procedure of blood collection, labeling of samples, sample acceptance / rejection criteria, instructions for transportation and storage of samples; proper disposal of materials used in the collection; availability of clinical advice on ordering of examinations and on interpretation of examination results; the laboratory's policy on protection of personal information; the laboratory's complaint procedure.

Test equipment and environment: All equipment (including refrigerators, centrifuges, ELISA readers/ washers, pipettes) required for testing should be calibrated and checked for performance. Calibration, maintenance and equipment operation have been described in the chapter on equipment.

Specimen transport: Specimen should be packaged and transported from point of collection to the laboratory in a leak proof container using standard precautions along with the requisition form. Specimen should be transported within the required time interval, and temperature range, ensuring the integrity of the sample and safety of the person transporting the specimen and the environment in accordance with all applicable transport and safety requirements and regulatory guidelines. Details of specimen packaging and transportation is described in the Guidelines of HIV Testing

Specimen storage: Serum/ plasma to be stored at 2-8^{o}C in the refrigerator for up to 1 week. For longer storage serum/plasma should be stored at -20^{o} C or below.

Storage of test kits: Kits should be stored in the refrigerator or as per manufacturer's recommendations.

Selection of test kits: The National HIV Testing Strategy and algorithm should be strictly adhered to for the selection of test kits and sequence of testing.

Availability of trained and competent personnel: Trained technical personnel who have undergone regular competency evaluations are authorized to perform tests.

Ensuring quality in the analytical phase of testing:

Quality Control

Quality Control (QC) refers to the procedures undertaken for continuous and immediate monitoring of laboratory work in order to decide whether results are reliable enough to be released. Quality control procedures are a tool to detect problems that could invalidate patient results. It consists of examining "control" materials of known substances along with patient samples to monitor the accuracy and precision of the complete analytic process. The goal is to detect, evaluate and correct errors due to test system failure, environmental conditions or operator performance before patient results are reported. Different QC processes are applied to monitor quantitative, qualitative and semi-quantitative tests.

The laboratory must plan for and document its quality control plan, including the levels of quality control materials to be used, frequency of performing QC, types of QC materials and the QC acceptance/rejection criteria including possible corrective action customized for each examination procedure based on that procedure's capabilities. QC data must be reviewed periodically and all staff trained.

Internal quality control (Internal to the test Kit): These are the controls included in every test kit that is supplied by the manufacturer. These include both positive and negative controls. They are intended to be used with the same lot number of the kit with which it has been supplied in the same pack and should not be interchanged between kits. Internal controls are generally adjusted by the manufacturer so that expected results are obtained with each lot of kit.

Internal Controls in HIV ELISA are used to calculate the cut off value (COV). Even with day to day use of ELISA test kits from the same manufacturer and with identical batch numbers, some degree of variations in the internal controls (supplied with the kits) are encountered that in turn result in the variation of the calculated cut off value that is calculated on the basis of OD values of the internal controls. This is due to variation in factors like preparation of the reagents, plate to plate and well to well variation in the amount of coated antigens, incubation conditions etc. Such factors influencing the OD values of the controls would also expectedly influence the OD values of the test samples in a similar direction. But the relative reactivity of a given test sample

in relation to cut off value would not change much and standardizes the data. This relative reactivity of a test sample in relation to cut off value in a particular run is expressed and termed E ratio. This is the ratio between the sample OD and cut off OD (OD/COV).

Rapid tests also contain built-in controls integrated into the design of a test kit device automatically run with each test performed. These controls may also be referred to as procedural controls. Most built-in controls monitor only a portion of the analytical phase, and they vary from one test to another as to what is being monitored. For example, built-in controls for some kits may indicate that all the reagents impregnated into the device are active and working properly, whereas built-in controls for other kits may only indicate that a sample was added and solutions flowed through the device correctly.

Internal controls have limitations as these can be used only with the kits from which they originate and are prepared artificially in a manner that minor deterioration of the kit may not be detected by the results of the internal controls. Hence, in addition to the Internal QCs, the use of external quality controls is also necessary.

External quality control (External to the test kit): These are a set of controls not supplied with the kit. They are applied for each run/ assay to monitor the entire test system, the suitability of the physical testing environment (temperature, humidity, level workspace), and whether the person conducting the test performs it correctly.

It is important to select the appropriate control materials. When choosing controls for a particular method, values that cover medical decision points must be selected, such as one with a normal value, and one that is either high or low, but in the medically significant range. For HIV testing this would be at a minimum, positive and negative controls and additional borderline positive is recommended. The borderline positive/reactive control is capable of detecting any minor error in assay performance. This is especially important in HIV ELISA tests in order not to generate false results among test samples having an OD near the cut off value. Controls should have the same matrix as patient samples; this usually means that the controls are serum or plasma-based. Because it is more efficient to have controls that last for some months, it is best to obtain control materials in large quantity and store as small aliquots.

Control materials are available in a variety of forms. They may be frozen, freeze-dried, or chemically preserved. Control materials may be purchased, obtained from a central or reference laboratory, or prepared in-house. Purchased controls may be either assayed or unassayed. Assayed controls have a pre-determined target value, established by the manufacturer. When using assayed controls the laboratory must verify the value using its own methods. When using either unassayed or “in-house” controls, the laboratory must establish the target value and control limits of the analyte.

Steps in preparing a borderline reactive external quality control sample are:

- Selection of a high titer HIV positive plasma/serum. Source could be either an HIV infected individual or a transfusion unit of blood found to be positive for HIV.
- Serial dilution of the high titer HIV positive specimen using normal human serum. This is to keep the antibodies in natural serum protein environment. Adequate quantity of serum for use as diluent can be obtained from a transfusion blood/plasma unit negative for HIV, HBV and HCV.
- Perform two fold serial dilutions (doubling dilutions) wherein a fixed volume of HIV positive plasma/serum is mixed and transferred into successive tubes containing an identical volume of diluent (i.e. normal human serum).
- Selection of a suitable sample dilution to achieve the desired titer for use as external control with borderline reactivity. Results usually show a sigmoidal curve.
  The dilution suitable for using as low positive controls are generally selected at 'E' ratio of around 2.0 times the cut off.
- Batch production: Preparation of bulk external (low positive) control sample. Volume requirement will depend on the period for which the external control of the current batch needs to be used (e.g. for one year or 6 months); the sample volume required for each assay; how often the assay is to be performed
- Batch validation to check that the batch has been sufficiently mixed and is homogenous so as to minimize the inter-aliquot as well as inter run variation
- Aliquoting and storage. Aliquots stored at -20 degree Celsius with volume per aliquot sufficient to last for one week in routine testing. One aliquot is thawed at the beginning of the week for use for that week only, following which it is discarded. It is stored at 20^{o}C to 80 ^{o}C in between during the week.
- Determination of acceptable ranges of quality control to validate each ELISA test run. This is done by employing statistical parameters like mean, standard deviation and coefficient of variation. Subsequently, the external controls, in conjunction with the internal kit controls, can be used to validate all test runs. The external QC sample is tested in at least 20 runs (e.g. in 20 consecutive days) to make statistically significant observations. The mean and standard deviation of 'E' ratio of the external controls are calculated from the set of 20 data points. The coefficient of variation (CV) calculated on different dates is minimal (i.e. <15%).

For HIV rapid tests: If the positive quality control is not positive or negative quality control is not negative, then the test should be repeated. If further investigation is needed, there are several possible causes for an incorrect control in a qualitative test. First, the reagents may be added in wrong order. Second, the test procedure may be done correctly, but the results might be read too soon or too late. Also, the reagents could be outdated or possibly have deteriorated from inadequate storage. Document that there is a problem and list the QC code and expiration date. If everything seems to be accurate and the problems are still evident, then the kit manufacturer should be informed about the problem.

Laboratories must use at least two levels of quality control material for HIV ELISA testing which is negative, positive and it is recommended to use a borderline positive/reactive (Annexure 2.4), if possible. The laboratory can prepare them in-house. To validate each ELISA test run, acceptable control limits of external quality control (positive, borderline reactive) must be determined. This is done by employing statistical parameters like arithmetic mean, standard deviation and coefficient of variation. Subsequently, the external controls, in conjunction with the internal kit controls, can be used to validate all test runs.

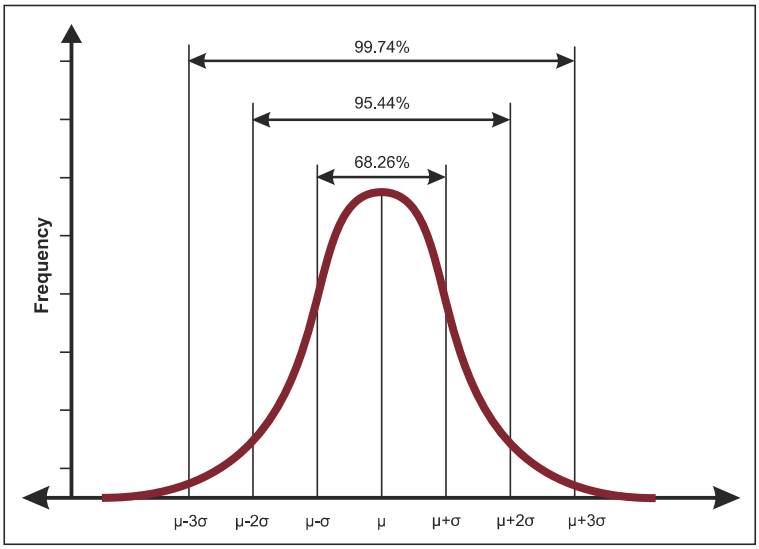
For HIV ELISA tests, statistical analysis is used for the quality control monitoring process, and Levey-Jennings charts plotted to provide a useful visual tool for this monitoring. To make statistically significant observations, at least 20 data points should be collected through intermediate precision repetitive testing over a period of 20 to 30 days for each lot number. When collecting this data, procedural variation that occurs in daily runs must be included; for example, different operators, different times of day, different reagent lot, maintenance and calibration of equipment etc. The mean and standard deviation of 'E' ratio (OD/COV) of the external controls are calculated from this set of 20 data points. Appropriate control limits are then established (+/- 1SD, 2SD and 3 SD from the mean) to evaluate if the test run is "in control" or if the control values are not reading properly – "out of control". When only one control is used, an examination run is considered to be "in control" if a value is within 2 SD of the mean unless a shift or trend is seen. If 2 or more levels of controls are used, Westgard multirule system is applied to evaluate the test run. The monthly coefficient of variation (CV) must also be calculated and compared to see that variation is minimal (i.e. <20%).

A scientific calculator, an electronic spreadsheet, or a statistics program, all of which have functions for calculating the standard deviation of a group of measurements can be used for the statistics. The **Coefficient of Variation** (C.V.) describes the standard deviation as a percent of the mean. C.V. is used to compare the precision of a variety of determinations that have different normal values and even different units as well. Calculate the coefficient of variation (C.V.) on a monthly basis for comparison and analysis.

The Levey-Jennings control chart is used to graph successive (run-to-run or day-to-day) quality control values. A chart is created for each test and level of control. They are constructed with control values (E ratio) plotted on the y axis versus time (run date) on the x axis and lines are drawn from point to point to accent any trends, shifts, or random excursions. Control/decision limits are drawn as horizontal lines at distance from the mean measured in +/- 1, 2 and 3 standard deviations (SD). The charts are labeled to indicate name of test and control material, analytical system, lot number of control material, current mean and standard deviation, and the time period covered by the chart. Appropriate statistical QC rules are used to detect systematic (trends or shifts) and random errors.

When an analytical process is within control, since the QC data values are expected to have a normal Gaussian distribution (Figure 2.2), approximately 68% of all QC values fall within ±1 standard deviation (1s/1σ). Likewise 95.4% of all QC values fall within ±2 standard deviations (2s/2σ) of the mean. About 4.5% of all data will be outside the ±2s limits when the analytical process is in control. Approximately 99.7% of all QC values are found to be within ±3 standard deviations (3s/3σ) of the mean. As only 0.3%, or 3 out of 1000 points, will fall outside the ±3s limits, any value outside of ±3s is considered to be associated with a significant error condition and patient results should not be reported.

Figure 2.2: Normal Frequency Distribution (Gaussian Curve)



When the quality control sample that is used in a test run is out of the acceptable range, the run is considered to be "out of control". When this happens, the testing process should be stopped, and the technician must immediately undertake a root cause analysis (RCA) to identify and correct problems. Possible problems to consider are degradation of reagents or kits, control material degradation, operator error, failure to follow manufacturer's instructions, an outdated procedure manual, equipment failure, and calibration error.

Once possible sources of error have been identified, and corrections have been made, the control material should be rechecked. If they read correctly, then patient samples, along with another quality control specimen should be repeated. Do not simply repeat the testing without looking for sources of error and taking corrective action. Patient results **must not be** reported until the problem is resolved and the controls indicate proper performance.

It is important that calibrators and control materials not be confused. Calibrators are solutions with a specified defined concentration that are used to set or calibrate an instrument, kit, or system before testing is begun. Calibrators are often provided by the manufacturer of an instrument. They should not be used as controls since they are used to set the instrument. They usually do not have the same matrix as patients' samples.

Frequency of use of quality control material: At a minimum

- For HIV Rapid tests-Minimum once a week (beginning of the week) and for HIV ELISA- with