National Accreditation Board for Testing and Calibration Laboratories (NABL)

Specific Criteria for Accreditation

of

Medical Laboratories

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AMENDMENT SHEET

S. No.	Amendment No.	Page No.	Cl. No.	Date of Amendment	Amendment	Reasons	Signature of QA Team	Signature of Competent Authority
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PREFACE

NABL documents are updated at regular intervals to keep pace with the latest technical developments and to synchronize with the International Standards. The updated issue of NABL 112A elaborates the requirements of International Standard ISO 15189:2022 as applicable to Indian setting. The document has been designed to make it user friendly for both NABL assessors and laboratories.

I extend my warmest thanks to all members of Technical Committee for their hard work and outstanding contributions in bringing out this issue of Specific Criteria. I sincerely appreciate the enthusiasm invested by members of NABL to ensure the success of updated document.

I further wish to thank immensely all the stakeholders for their valuable inputs which enabled us to go this extra mile.

My heartfelt thanks to the Chairman, NABL for his constant inspiration and able guidance during this entire endeavor.

CEO, NABL

CONTENTS

ABBREVIATIONS

ACTH	-	Adrenocorticotropic hormone
ADLM	-	Association for Diagnostics and Laboratory Medicine
AERB	-	Atomic Energy Regulatory Board
AF	-	Amniotic Fluid
AFB	-	Acid-Fast Bacillus
AFP	-	Alpha Feto Protein
ALL	-	Acute Lymphoblastic Leukemia
LOH	-	Loss of heterozygosity
APAC	-	Asia Pacific Accreditation Cooperation
APTT	-	Activated Partial Thromboplastin Time
ATCC	-	American Type Culture Collection
AV	-	Auto Verification
BAC	-	Bacterial Artificial Chromosome
BAL	-	Bronchoalveolar Lavage
Beta HCG	-	Beta Human Chorionic Gonadotropin
BLAT	-	Blast Like Alignment Tool
BLAST	-	Basic Local Alignment Search Tool
BMW	-	Bio Medical Waste
BOD	-	Biochemical Oxygen Demand
BRI	-	Biological Reference Interval
BSC	-	Bio-safety Cabinet
CAB	-	Conformity Assessment Body
CBC	-	Complete Blood Count
CD	-	Cluster of Differentiation
CBNAAT	-	Cartridge Based Nucleic Acid Amplification Test
CCMB	-	Centre for Cellular and Molecular Biology
CEPD	-	Continuing Education and Professional Development
CGH	-	Comparative Genomic Hybridization
CLIA	-	Chemiluminescent immunoassay
CLL	-	Chronic Lymphocytic Leukemia
CLSI	-	Clinical Laboratory Standards Institute
CMA	-	Chromosomal Microarray
CME	-	Continuing Medical Education
CMIA	-	Chemiluminescent Microparticle Immuno Assay
CPM	-	Confined Placental Mosaicism
CNV	-	Copy Number Variation
CPU	-	Central Processing Unit
CRO	-	Clinical Research Organization

CSF	-	Cerebrospinal Fluid
Ct	-	Cycle Threshold
CV	-	Coefficient of Variation
CVS	-	Chorionic Villus Sample
DGFASLI	-	Directorate of General Factory Advice Service & Labour Institutes
DNA	-	Deoxyribonucleic Acid
DP	-	Digital Pathology
ECLIA	-	Electrochemiluminescence Immunoassay
EDTA	-	Ethylene Diamine Tetra-acetic acid
EGFR	-	Epidermal Growth Factor Receptor
ELFA	-	Enzyme Linked Fluorescence Assay
ELISA	-	Enzyme Linked Immunosorbent Assay
EUCAST	-	European Committee on Antimicrobial Susceptibility Testing
EtBr	-	Ethidium Bromide
EQA	-	External Quality Assessment
ESR	-	Erythrocyte Sedimentation Rate
FCS	-	Flow Cytometry Standard
FFPE	-	Formalin Fixed Paraffin Embedded
FLAER	-	Fluorescent Aerolysin
FNA	-	Fine Needle Aspiration
FNAC	-	Fine Needle Aspiration Cytology
FISH	-	Fluorescence in situ hybridization
FTIR	-	Fourier Transform Infrared Spectroscopy
GOI	-	Government of India
G6PD	-	Glucose-6-phosphate dehydrogenase
GPI	-	Glycosyl Phosphatidyl Inositol
GPSs	-	Good Practice Statements
Hrs/hrs	-	Hour(s)
H & E Staining	-	Haematoxylin & Eosin Staining
HBV	-	Hepatitis B Virus
Her2	-	Human Epidermal Growth Factor Receptor 2
HGVS	-	Human Genome Variation Society
HIV	-	Human Immunodeficiency Virus
HLA	-	Human Leukocyte Antigen
HPLC	-	High Performance Liquid Chromatography
ICMR	-	Indian Council of Medical Research
ICT	-	Indirect Coombs Test
ICSH	-	International Council for Standardization in Haematology
IFCC	-	International Federation for Clinical Chemistry

IHC	-	Immunohistochemistry
ILAC	-	International Laboratory Accreditation Cooperation
ILC	-	Inter Laboratory Comparison
INR	-	International Normalized Ratio
IQC	-	Internal Quality Control
ISO	-	International Organization for Standardization
ISCN	-	International Society for Human Chromosome Nomenclature
ISHAGE	-	International Society of Hematotherapy and Graft Engineering
IT	-	Information Technology
IVD	-	In-vitro Diagnostics
KRAS	-	Kirsten rat sarcoma viral oncogene homology
LBC	-	Liquid Based Cytology
LCMS	-	Liquid Chromatography – Mass Spectrometry
LED	-	Light-emitting diode
LMWH	-	Low molecular weight heparin
LIS	-	Laboratory Information System
LJ Chart	-	Levey-Jennings Chart
LIMS	-	Laboratory Information Management System
LOD	-	Limit of Detection
LLOQ	-	Lower Limit of Quantification
MCC	-	Maternal Cell Contamination
MCH	-	Mean Corpuscular Haemoglobin
MCHC	-	Mean Corpuscular Haemoglobin Concentration
MCV	-	Mean Corpuscular Volume
MD	-	Doctor of Medicine
DNB	-	Diplomate of National Board
DCP	-	Diploma in Clinical Pathology
MNPT	-	Mean Normal Prothrombin Time
MOU	-	Memorandum of understanding
MRA	-	Mutual Recognition Arrangement
MRD	-	Minimal Residual Disease
NACO	-	National AIDS Control Organization
NAAT	-	Nucleic Acid Amplification Test
NBF	-	Neutral Buffered Formalin
NCDC	-	National Centre for Disease Control
NCBI	-	National Centre for Biotechnology Information
NCCLS	-	National Committee for Clinical Laboratory Standards
NGS	-	Next Generation Sequencing
NIH	-	National Institute of Health

NIPS	-	Non-Invasive Prenatal Screening
NMC	-	National Medical Commission
NPL	-	National Physical Laboratory
NTCC	-	National Type Culture Collection
NTEP	-	National TB Elimination Program
NIV	-	National Institute of Virology
OM	-	Optical Microscopy
PAPP-A	-	Pregnancy-Associated Plasma Protein A
PAP Staining	-	Papanicolaou staining
PBL	-	Peripheral Blood Lymphocytes
PCR	-	Polymerase Chain Reaction
PCPNDT	-	Preconception and Prenatal Diagnostic Testing
PIGF	-	Placental Growth Factor
POC	-	Products of Conception
Real Time RT - PCR	-	Real Time Reverse Transcriptase - Polymerase Chain Reaction
PND	-	Prenatal Diagnosis
PNH	-	Paroxysmal Nocturnal Hemoglobinuria
PT	-	Proficiency Testing
QBC	-	Quantitative Buffy Coat
QC	-	Quality Control
qPCR	-	Quantitative Polymerase Chain Reaction
QF-PCR	-	Quantitative Fluorescent PCR
RAM	-	Random-access memory
RBC	-	Red Blood Cell
RCF	-	Relative Centrifugal Force
RDW	-	Red Cell Distribution Width
RFLP	-	Restriction fragment length polymorphism
RNTCP	-	Revised National Tuberculosis Control Program
ROSE	-	Rapid Onsite Evaluation
RMPI	-	Rosewell Park Memorial Institute
RUO	-	Research Use Only
SARS-CoV-2	-	Severe Acute Respiratory Syndrome Coronavirus 2
SAM	-	Sequence Alignment Map
SBT	-	Sequencing based Typing
SCF	-	Sample Collection Centre/ Facility
SD	-	Standard Deviation
sRGB	-	Standard Red Green Blue
SNP	-	Single Nucleotide Polymorphism
SOP	-	Standard Operating Procedure

SSOP	-	Sequence-Specific Oligonucleotide Probes
SSP	-	Sequence Specific Primers
STR	-	Short Tandem Repeats
TAT	-	Turnaround Time
TB	-	Tuberculosis
TEM	-	Transmission Electron Microscopy
UV	-	Ultra Violet
uE3	-	Unconjugated Estriol
VAF	-	Variant Allele Frequency
VCF	-	Variant Call Format
VNTR	-	Variable Number Tandem Repeat
VTM/UTM	-	Viral Transport Medium/Universal Transport Medium
VRDL	-	Virus Research and Diagnostic Laboratory
WBC	-	White Blood Cells
WHO	-	World Health Organization
WSI	-	Whole Slide Imaging

i) INTRODUCTION

National Accreditation Board for Testing and Calibration Laboratories (NABL) is an accreditation body provides accreditation service to Conformity Assessment Bodies including medical testing laboratories. NABL is a signatory to Asia Pacific Accreditation Cooperation (APAC) and International Laboratory Accreditation Cooperation (ILAC) through Mutual Recognition Arrangements (MRA). These are based on mutual evaluation and acceptance of other MRA partners. Such international arrangements allow acceptance of test / calibration results between MRA partner countries.

This document specifies the criteria for the medical testing laboratories for obtaining NABL accreditation. Medical testing laboratory shall fulfill the requirements of this document in addition to the requirements of the ISO 15189:2022 - “Medical laboratories – Requirements for quality and competence”.

This specific criteria document shall be used in conjunction with ISO 15189:2022.. Wherever the standard is self-explanatory, this document has not addressed those clauses/subclauses. Further, the laboratory shall follow national, regional, local laws and regulations as applicable.

ii) DESCRIPTION AND TYPE OF LABORATORY

The requirements given in this document are applicable to all medical laboratories applying for NABL accreditation regardless of the level at which they function (micro/mini/small/medium/ large/very large) or the place in which they are located (village/town/district/city) or whether they are private/government/autonomous attached to a hospital/stand-alone.

The following classification of laboratories shall be used:

1. Micro : A laboratory receiving samples of up to 25 patients per day.
2. Mini : A laboratory receiving samples of 26-50 patients per day.
3. Small : A laboratory receiving samples of 51-100 patients per day.
4. Medium : A laboratory receiving samples of 101-400 patients per day.
5. Large : A laboratory receiving samples of more than 401-1000 patients per day.
6. Very Large: A laboratory receiving more than 1000 patients per day.

1. SCOPE

The requirements specified in this document are applicable for the following disciplines of the medical laboratory:

i. Clinical Biochemistry
ii. Clinical Pathology
iii. Haematology & Immunohaematology
iv. Microbiology & Infectious disease serology
v. Histopathology
vi. Cytopathology
vii. Flow Cytometry
viii. Molecular Diagnostics
ix. Histocompatibility & Immunogenetics
x. Cytogenetics

Note:

- *POCT, Immunological, serological tests and in situ hybridization tests (FISH, CISH etc.) are common to more than one discipline; therefore, these can be listed under respective discipline, irrespective of the section of the laboratory, the equipment/s are located.*
- *The tests of Nuclear Medicine can be applied under the discipline of Clinical Biochemistry.*
- *Molecular Diagnostics includes tests of Molecular genetics, Molecular Testing of infectious diseases, Hematopoietic diseases, solid tumors and prenatal genetic testing.*
- *Biochemical genetics tests can be applied under Molecular Diagnostics / Clinical Biochemistry.*
- *Karyotyping to be applied under Cytogenetics.*
- *Onsite demonstration of competence along with records of relevant training and experience in the respective discipline shall be considered for recommendations of personnel who review the results and authorize the release of reports in any discipline.*

Accreditation shall be considered only for those tests for which the laboratory itself is equipped and competent to carry out. The tests, for which quality cannot be ensured, shall not be included in the scope. The laboratory shall have samples (positive and negative) available for test witness (testing/retesting) at the time of assessment to demonstrate competence in the disciplines of the applied scope. Appropriate records to establish continuous ongoing work in the discipline shall be maintained.

The collection centre/facility for primary sample collection at sites other than its main laboratory shall also comply with the requirements of ISO 15189: 2022 and relevant NABL documents. {Document NABL 111-Procedure for recognition of sample collection centre/facility declared by medical laboratories (CABs)}

2. NORMATIVE REFERENCE:

ISO 15189:2022 - “Medical laboratories – Requirements for quality and competence”

3. TERMS & DEFINITIONS:

i.Biological reference interval / Reference interval:

Specified interval of the distribution of values taken from a biological reference population.

ii.Competence

Demonstrated ability to apply knowledge and skills to achieve intended result.

iii.Consultant

Person who provides expert advice professionally.

iv.Examination

Set of operations having the objective of determining the numerical value, text value or characteristics of a property.

v.Examination procedure

Specifically described set of operations used in the performance of an examination according to a given method.

vi.External quality assessment (EQA)

Evaluation of participant performance against pre-established criteria by means of interlaboratory comparisons.

vii.Interlaboratory comparison

Organization, performance and evaluation of measurements or examinations (3.8) on the same or similar materials by two or more independent laboratories in accordance with pre-determined conditions.

viii.Internal quality control (IQC)

Internal procedure which monitors the testing process to verify the system is working correctly and gives confidence that the results are reliable enough to be released.

ix.In vitro diagnostic (IVD) medical device

Device, whether used alone or in combination, intended by the manufacturer for the in vitro examination of specimens derived from the human body solely or principally to provide information for diagnostic, monitoring or compatibility purposes and including reagents, calibrators, control materials, specimen receptacles, software, and related instruments or apparatus or other articles.

x.Measurement accuracy

Closeness of agreement between a measured quantity value and a true quantity value of a measurand.

xi.Measurement uncertainty (MU)

Non-negative parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the information used.

xii.Medical laboratory

Entity for the examination of materials derived from the human body for the purpose of providing information for the diagnosis, monitoring, management, prevention and treatment of disease, or assessment of health.

xiii.Point-of-care testing (POCT)

Examination performed near or at the site of a patient.

xiv.Post-examination processes

Processes following the examination including review of results, formatting, releasing, reporting and retention of examination results, retention and storage of clinical material, sample and waste disposal.

xv.Pre-examination processes

Processes that start, in chronological order, from the user's request and include the examination request, preparation and identification of the patient, collection of the primary sample(s), transportation to and within the laboratory, ending when the examination begins.

xvi.Primary sample/Specimen

Discrete portion of a body fluid or tissue or other sample associated with the human body taken for examination, study or analysis of one or more quantities or characteristics to determine the character of the whole.

xvii.Quality indicator

Measure of the degree to which a large number of characteristics of an object fulfils requirements.

xviii.Referral laboratory

External laboratory to which a sample or data is submitted for examination.

xix.Sample

One or more parts taken from a primary sample.

xx.Turnaround time

Elapsed time between two specified points through pre-examination, examination, and post examination processes.

xxi.Validation

Confirmation of plausibility for a specific intended use or application through the provision of objective evidence that specified requirements have been fulfilled.

xxii.Verification

Confirmation of truthfulness, through the provision of objective evidence that specified requirements have been fulfilled.

4. GENERAL REQUIREMENTS:

a) Impartiality (Cl. 4.1 of ISO 15189:2022)

Laboratory activities shall be undertaken impartially and structured so as to safeguard impartiality: the organizational structure shall be such that there is no conflict of interest with other activities, defining the responsibilities: this may be seen where organization defines the structure particularly where the laboratories are the part of larger organization.

For being impartial, laboratory shall conduct its activities without any bias. Results of the laboratory should not be compromised due to being influenced by any relationships of the laboratory's personnel involved in the activities of the laboratory, with its customer.

To safeguard the impartiality in an organization shall clearly define the segregation of the activities in its organization which may be vulnerable to threat/risks to impartiality.

Threat/risks to impartiality may also arise within the laboratory itself by means of creating undue pressure on the analysts/ technicians to skip the test procedural steps for faster results delivery or to overlook the adverse results which will distress a Patient. Further undue pressure may also include offering monetary incentives to the employees for the number of tests conducted or the results of test. It is suggested that the identification of risks to impartiality should be carried out on an on-going basis or at a regular interval.

b) Confidentiality (Cl. 4.2 of ISO 15189:2022)

Legally enforceable commitments may be in the form of contract / agreement / work order between the laboratory and its patient.

5. STRUCTURAL AND GOVERNANCE REQUIREMENT

a) Legal entity (Cl. 5.1 of ISO 15189:2022)

For Legal entity requirements please refer NABL 153-Application Form for Medical Laboratories & 100A-General Brochure.
Laboratory shall also comply with local / regional / national requirements.

b) Laboratory director (Cl. 5.2 of ISO 15189:2022)

The qualifications of Laboratory Director (how so ever named) shall be appropriate for the disciplines & function of the laboratory, directed as per National/ Regional/ State/ Local regulatory requirements/ Acts/ Rules/ Legal orders/ Court Decisions/ Orders issued by Government/ Statutory Bodies as applicable and effective from time to time.

He/She shall have the overall responsibility of Technical / Advisory / Scientific operations of the laboratory. Laboratory Director shall be a full-time employee of the laboratory[#]. He/She shall be responsible for the implementation of the management system, including the application of risk management to all aspects of the laboratory operations so that risks to patient care and opportunities to improve are systematically identified and addressed. Duties and Responsibilities of the laboratory director shall be documented. He/She may delegate selected responsibilities to qualified and competent personnel and such delegation shall be documented. The Laboratory Director/designee shall also fulfill the other requirements of ISO 15189:2022.

[#]In a Mini/Micro/Small laboratory, the Laboratory Director can be a part time* employee. Other requirements/responsibilities remain the same.

*part time - minimum of four hours per day.

In a hospital setting or in a large or very large laboratory, each department/discipline may have a separate head. However, one of them if delegated as Laboratory Director, will be available for consultation and responsible for overall operations.

Note: *Checking of compliance to the regulatory requirements falls under the purview of respective applicable regulator.*

c) Laboratory activities (Cl. 5.3 of ISO 15189:2022)

Advisory activities (Cl. 5.3.3 of ISO 15189:2022)

Irrespective of the type of laboratory, i.e., Stand-alone or hospital-based, the laboratory shall have arrangements to communicate with its users for fulfilment of the requirements of the standard with regard to the choice of examinations and interpretations. Communication may be through direct contact, email etc. Hospital-attached laboratory personnel are encouraged to participate in clinical rounds and meetings.

d) Structure and authority (Cl. 5.4 of ISO 15189:2022)

Quality Management (Cl. 5.4.2 of ISO 15189:2022)

Laboratory shall have personnel for implementation, maintenance and improvement of management system for e.g., Quality officer/Quality Manager (how so ever named), either with dedicated or with other responsibilities.

He/She shall be a full-time employee.

e) Risk management (Cl. 5.6 of ISO 15189:2022)

i. The components of risk management are:
   - Risk identification - identification and listing of all risks across the entire testing processes covering pre-examination, examination, post-examination, manpower, equipment, facility and design, supplies, Quality control practices, policies etc.
   - Risk evaluation based on severity and likelihood of occurrence and detectability of occurrences, prioritization of risks.
   - Risk mitigation through preventive actions
   - Estimation of residual risk, through monitoring.

ii. The laboratory shall review its risk management at least once a year and whenever there is a change in process or design; records shall be kept that reflect the identified risks, their priority, actions taken to eliminate them and their effectiveness.

6. TECHNICAL & MANAGEMENT SYSTEM REQUIREMENTS FOR ALL DISCIPLINES (Cl. 6 to 8 of ISO 15189:2022)

The resource and process requirements have been sub-divided into two sections.
The first section includes the general requirements applicable to most of the disciplines and the second section supplements for discipline-wise requirements.

a) Personnel (Cl. 6.2 of ISO 15189:2022)

In all cases, it is the responsibility of laboratory to abide by the National/ Regional/ State/ Local regulatory requirements/ Acts/ Rules/ Legal orders/ Court Decisions/ Orders issued by Government/ Statutory Bodies as applicable and effective from time to time.

Note: *Implementation of criteria for automated selection and release of results, auto validation of results requires initial framing of clinical decision rules based on existing clinical guidelines/standards. This process requires significant input from qualified laboratory personnel, who are trained to understand the impact of laboratory results on management of the concerned diseases. Such criteria are specified, validated and approved before use by qualified, trained and experienced medical personnel as deemed appropriate by the laboratory management. The criteria shall be periodically verified.*

The staffing should correspond to the range, volume and complexity of the tests provided by each discipline. Large and very large laboratory shall include as a minimum, in every discipline MD/ DNB/DCP/PhD in discipline relevant and aligned with scope as per Cl. 1 of this document.

Medium sized laboratories shall include at least one full time MD/ DNB/DCP/PhD in any one of the discipline relevant and aligned with scope as per Cl. 1 of this document. Other disciplines shall have appropriately qualified, trained and experienced personnel of the relevant discipline as full/part time*.

Mini/Micro/Small laboratories shall have appropriately qualified, trained and experienced personnel in each discipline as full/part time*.

Records of attendance and activities done on-site by visiting MD/DNB/DCP/PhD and senior scientific staff members shall be kept.
* part time - minimum of four hours per day.

There may be specialized tests which are not specifically covered under academic curriculum because those tests are not done routinely or there are not enough samples in the laboratories of these academic institutions. Laboratories shall ensure competence of personnel for such tests by verifying and quantifying

the appropriateness of training the person has obtained beyond their qualification or even within the qualification wherever appropriate. Training for such tests obtained at any expert center (not limited to a national institute) or at laboratories examining sufficient number of samples for such tests is acceptable. Subsequently experience corresponding to the sample load in these tests and frequency of Continuing Education and Professional Development (CEPD) will be the criteria for continual improvement for competence.

In such situations, Competence assessments shall be conducted before assigning job responsibility. Competence assessments shall be documented with supporting data/records.

When personnel to review the results and authorize the release of reports are common resources shared by more than one discipline in the applied/accredited scope, the following to be ensured by the laboratory:

- Job responsibilities to be defined as deemed fit for each of the assigned disciplines. If involved in more than one discipline in a medium/large/very large laboratory, areas of primary and secondary responsibilities to be specified.
- Appropriate training and competence requirements to be established to ensure validity of examination results (includes IQC & EQA)
- Capable of trouble shooting or establish a process to handle the same
- Appropriate training on the regulatory requirements in the discipline as applicable e.g., BMW disposal.

b) Facilities and environmental conditions (Cl. 6.3 of ISO 15189:2022)

The laboratory shall have adequate space for efficient functioning and conditions to avoid cross contamination. Laboratory shall ensure that long-term adverse effects to staff are avoided by checking noise, chemical levels and ensuring ergonomics and avoiding physical injuries. Sections of the laboratory where laboratory processes involve handling of chemicals like formalin, acid/alcohol should have exhaust systems capable of removing fumes from the work areas without compromising the environmental requirements. It is desirable to have a fume hood with exhaust for this purpose.

The laboratory shall have effective separation for incompatible activities. The autoclave for sterile articles and for decontamination should be placed separately with proper exhaust.

The laboratory shall ensure that tests and activities performed in sections that are not under accreditation, do not adversely influence the safety of other areas or tests.

Note: *The laboratory shall ensure adequate space for patient reception, sample collection, workbenches, equipment and storage of volatile & inflammable reagents and bio-hazardous materials. Radioisotope related work shall be as per the requirements of the regulatory agency (AERB).*

The laboratory shall have adequate lighting, power supply arrangements and an uninterrupted power supply to ensure there is no compromise on laboratory activity and stored data. Extension boards without a fuse shall not be used for connecting equipment. Use of exposed cables should be kept to a minimum. Laboratory shall verify electrical safety of all points in use once in six months. All computers, peripherals, equipment and communication devices shall be supported in such a way that service is not likely to be interrupted. The laboratory shall have procedures in place to ensure the integrity of refrigerated and frozen samples / reagents / consumables in the event of a power failure. Wherever possible the sample segregation/ pre-examination processing area shall be separated from the testing area. Centrifuges should not occupy the same working bench as testing instruments where vibration may interfere with the results e.g., balances and semi-automated/ automated analytical analyzers.

Laboratories using carbon-dioxide (CO_2) cylinders should ensure that the cylinders are properly secured and do not pose any safety hazard.

Accommodation and environmental conditions are also applicable to primary sample collection facilities at sites other than the permanent laboratory facility as well as POCT.

c) Equipment (Cl. 6.4 of ISO 15189:2022)

All equipment including semi/ fully-automated analyzers, shall be verified for their performance prior to use. At the time of installation of new equipment / change of existing equipment / change in premises, the Installation Qualification (IQ), Operational Qualification (OQ) and Performance Qualification (PQ) shall be performed and documented. At a minimum, performance specification verification shall include accuracy, precision, linearity, carryover checks and method comparison wherever applicable.

i. Biosafety cabinets:

The laboratory shall connect appropriate exhaust systems for the BSCs and perform performance verification on installation and annually thereafter.

Burners shall not be used inside bio-safety cabinets. The use of disposable loops or loop sterilizer are desirable. Small centrifuges/shakers/vortex mixers may be used inside cabinets after assessing risks.

ii. Temperature-controlled equipment:

Equipment such as water baths, incubators, ovens, refrigerators and deep freezers are verified for accuracy / performance (with calibrated temperature-recording devices) for the intended temperature required. Daily temperature monitoring of the refrigerator and deep freezer shall be done. Temperature verification of various chambers/ shelves of refrigerator and deep freezer shall be recorded and the same shall be maintained till the next assessment.

Refrigerators and deep freezers requiring critical and continuous temperature control shall be fitted with 24X7 temperature recorders/data loggers. Auto-defrost function in freezers/ refrigerators shall not be used.

d) Equipment calibration and metrological traceability (Cl. 6.5 of ISO 15189:2022)

i. Analytical equipment:

- Calibration of equipment (Automated/Semi automated):

  All equipment such as cell counters, automated analyzers, automated coagulometers, POCT equipment, PCR machines, CLIA/ELISA readers, LCMS, FTIR and other such equipment shall be calibrated/ verified with respect to the stated performance specifications as provided by the manufacturer. The calibration /verification and criteria for acceptance of verification should be as per manufacturer's recommendations. Wherever the specifications are not provided by the manufacturer the calibration/ verification schedule shall be prepared by the laboratory based on the factors e.g., work load, frequency of usage of equipment, down time and taking risk into consideration. The calibration certificate shall contain/be supported by raw data. A calibration certificate with just a statement that the apparatus has been calibrated is not sufficient. All raw data or machine printout/screenshots shall be captured and documented for future use.

  During calibration of auto-analyzers, verification of power supply, photometer/ illuminometer/ fluorimeter/ LED that may/ may not be dye based, filter or emission light source lamps, pipettor assembly inclusive of metering pump and syringe, pressure checks wherever applicable, probe alignment and their carry over checks, temperature of temperature-controlled chambers, cuvette calibration (wherever

applicable) and system checks should be carried out. In addition to the verification, analyte calibration may be performed and appended. In case of semi-automated photometers, sipper calibration is also required wherever applicable.

Whenever, there is change in reagent formulation as required in many disciplines, changes to system's hardware/software to improve its performance, and introduction of an open channel / third party reagents on an analyzer, laboratory shall perform carry over checks in addition to demonstrating accuracy and precision.

- Electrophoresis apparatus:

  In fully automated electrophoresis apparatus, checks on power supply that include voltage input / output and its subsystems, migration voltage and current, temperature, pressure along with UV-sensitive charged couple device shall be verified for its performance within acceptable limits.

ii. Non- analytical equipment:

Policy on calibration and traceability of measurements shall be as per NABL 142 'Policy on Metrological Traceability of Measurement Results'. The equipment shall be calibrated from NPL, India or a calibration laboratory accredited by NABL or MRA partners, accredited for the specified scope.

For calibrations, the laboratory should include calibration points as per its use, in addition to the range of the equipment. For e.g., if the centrifuge has a range of 0 to 12,000 rpm (converted from g value), the lab uses 3500, 10,000 rpms for its work, both these points must be included in addition to lower, higher values across the range. The same is applicable for incubators, thermometers and pipettes.

The laboratory shall also determine its criteria for acceptance of measurement uncertainty values after calibration. If the uncertainty is too high and unsuitable for performing specific tests, calibration shall not be accepted, e.g., MU value of 0.5 µL at 3 µL, will not be suitable for pipettes in molecular testing. (Reference ISO 8655 Piston-operated volumetric apparatus)

All non-analytical equipment including thermometers, pipettes and centrifuges must be calibrated by a NABL accredited laboratory before being put into service for the first time. A manufacturer's calibration

certificate is not valid unless it contains an accepted procedure and traceable to SI unit (as per NABL 142).

The nominal maximum periods between successive calibration verification of general equipment are illustrated in Table 1.

Table 1

Item	Recommended maximum period between successive calibration by NABL accredited laboratory	Remarks
Autoclave	One year	Calibration of pressure gauge and temperature by thermal mapping. If an automated timer is present, it also shall be calibrated.
Balances and Scales	One year	Balances with in-built calibration facility must be verified using calibrated weights once a day before use.
Biological safety cabinet	One year	Verification of differential pressure, particle count, air flow velocities and HEPA filter integrity.
Laminar Flow	One year	Verification of differential pressure, particle count, air flow velocities and HEPA filter integrity.
Centrifuge	One year	Speed and timer to be calibrated. For refrigerated centrifuges, temperature measuring device to be calibrated.
Mass	Two years and can be extended up to three years if the mass is E1 Class (stainless steel)	OIML R111 Calibrated weight shall be kept in proper storage condition to avoid abnormal drift.
Piston-operated volumetric apparatus, pipettes and dispensers	One year	Variable volume pipettes shall be calibrated across the full range and at volumes that are most frequently used. For performance acceptability after calibration, refer to ISO 8655 for guidance.
Thermometers	One year	Calibration to include points of use

Item	Recommended maximum period between successive calibration by NABL accredited laboratory	Remarks
Thermocyclers	Once a year/as recommended by the manufacturer.	As per the Manufacturer recommendation
pH meter (Digital)	Once a year/as recommended by the manufacturer.	Verification each time before use with two standard buffer solutions appropriate to the expected pH of the sample being tested.

It must be stressed that the above calibration intervals depend upon ruggedness of the equipment, frequency of use, quality & periodicity of maintenance.

iii. Spectrophotometer and Colorimeter:

Calibration checks on all spectrophotometers or colorimeters shall be performed at six months/ one year interval preferably by manufacturer or authorized maintenance agency. These checks to include absorbance and wavelength accuracy, matching of cells in accordance with the manufacturer's instructions and/or appropriate procedures using traceable standard / reference materials. In colorimeter, blank and at least three points on the calibration curve must also be checked. These calibrations should be compared over time to detect any system deterioration.

e) Reagents and consumables (Cl. 6.6 of ISO 15189:2022)

All reagents, consumables, stains, media, kits, and antimicrobials shall be stored as recommended by the manufacturer. The label shall contain information like content and quantity, concentration or titer, date received / prepared, date of opening, storage requirements and expiry dates wherever applicable.

Similarly, reagents prepared in-house shall have the name & signature of individual who prepared the reagent, storage requirements, date of preparation & expiry.

Laboratory shall use Grade water-II for reconstitution of reagents, control and calibrator materials. Lab shall have records of water testing and the frequency of water testing shall be user defined to ensure that there is no impact on patient's report.

f) Service agreements (Cl. 6.7 of ISO 15189:2022)

The laboratory shall have a complete list of all users/ clinics/ hospitals/ laboratories/sample collection agencies from whom it receives samples. The users of laboratory shall be explicitly informed about the non-accredited status of tests requested while entering into a contract. This may be done by providing separate lists of accredited and non-accredited test parameters to users. A copy of accredited scope with method, TAT, shall also be made available for reference.

For POCT refer to the section for additional information.

g) Externally provided products and services (Cl. 6.8 of ISO 15189:2022)

NABL permits referral for second opinion for the tests of Histopathology, Cytopathology, Bone Marrow examination, Cytogenetics/ Molecular tests and for supplementary testing. Referral may also be required for confirmation of Biochemical, Microbiological and Haematological tests. The referral laboratory shall be NABL accredited for the tests under referral. The laboratory shall do risk assessment for transport of samples to the referral laboratory within acceptable time period to ensure sample integrity.

NABL also allows referral to experts of good professional standing, some central laboratories (NCDC New Delhi, NIV Pune, CCMB Hyderabad etc.) or other reputed institutions.

A test in any discipline may be referred to another accredited laboratory at the time of temporary incapacity of testing due to unforeseen circumstances such as breakdown of equipment, disasters, strikes etc.

Note: *NABL allows this relaxation only under exceptional situations and it is advised that the privilege provided to the laboratory is not misused.*

Referral laboratories and consultants shall be selected as per the criteria laid down by the laboratory.

Laboratory shall maintain records pertaining to lists of tests and the names & addresses of the referral laboratories from which services are obtained.

The referring laboratory shall give prior intimation to the users about the tests being referred.

The referring laboratory shall produce the original report of the referral laboratory or transcribe the report without alterations of clinical interpretation with additional remarks (if required) and specify the name of the referral laboratory, identify the tests performed and the results obtained by any such referral laboratory. Records pertaining to this shall also be made available.

A memorandum of understanding defining the responsibilities of the referring lab and the referral laboratories and consultants to be in place. Confidentiality shall be maintained.

Note: *The laboratory shall produce records of evaluation of the referral laboratories and consultants and a copy of the NABL certificate of referral laboratory along with the accredited scope for each laboratory.*

i. Telepathology

Telepathology services include interpretation of images from glass slides, whole slide imaging, electrophoretograms, gel images, fluorescence in situ hybridization, molecular results, flow cytometry dot plots, cell counter scatter plots/histograms etc. by referral laboratories and consultants.

Laboratory using telepathology services for referral laboratories and consultants who provide interpretations, advice and second opinions for these images/data, should communicate its requirement and specific guidelines for the consulting activities to be provided, after a thorough risk analysis for the same.

Guidelines shall be appropriate but not limited to the following:

- The laboratory should maintain the records of all referral laboratories and consultants with their areas of expertise.
- Laboratory should define the type of work being referred i.e., for review/second opinion, etc.
- Unequivocal traceability of the images/data to the patient they belong with the specific Lab ID/ biopsy number generated by the laboratory. These to be accompanied by the unique patient identifiers details of the laboratory to enable positive patient identification e.g., Name, gender, Lab id etc. which need to be documented in the report.
- For images, the pathologist from the referring laboratory will be responsible for ensuring that representative fields of the sections from glass slides are submitted for review/second opinion. List of number of images with details of stains used and magnification of the image capture for every image needs to be communicated to the referral laboratory/ Consultant. Details of

the material being sent, field selection and image quality of glass slides to reduce the errors in sampling, should be included.

- Quality indicators to monitor the performance of this activity to be defined and monitored based on risk assessment e.g., number discordant diagnosis between glass versus digital images, delays in turnaround times, and deferral rates (e.g., failure or inability to render a diagnosis etc.).

In addition to the above requirements, the requirement of clause 6.8 of ISO 15189:2022 would also apply to Telepathology.

h) Pre-examination processes (Cl. 7.2 of ISO 15189:2022)

Relevant clinical data is necessary for all tests. Request forms shall have provision to provide this information by the requesting physician.

For venipuncture, an evacuated tube system is preferred as the extracted blood comes directly in contact with the additives/anticoagulants. Syringes should be avoided for safety reasons. If it is necessary to use a syringe, a safety device for transferring blood to the tubes should be used so that opening the caps of evacuated tubes can be avoided. The vacuum ensures correct fills of sample draws. If such devices are not available or the laboratory is not using evacuated tubes, appropriate processes shall be established based on existing standards. Sample drawn through evacuated tube systems from central line devices/ IV catheters/ winged devices, suitable adapters should be used for direct sample drawn into the tubes.

All processes followed by the organization from routine venipuncture to capillary sample collections shall be part of the primary sample collection manual. If any deviation from routine has occurred in sample extraction it shall be documented in the request form. The laboratory shall assess the significance of deviation and the risk associated on interpretation of test results.

When using evacuated tubes, order of draw shall be followed.

Specific instructions for the proper collection and handling of primary samples shall be documented. The laboratory shall ensure that informed consent has been taken for HIV testing and invasive procedures e.g., FNA, lumbar puncture, body fluid aspiration, bone marrow aspiration etc.

The laboratory shall do risk assessment for transport of samples within specified or acceptable transport time to ensure that sample integrity is not compromised. The nature and type of measures required to maintain the samples in the

temperature range recommended for the specific parameter/ analyte will depend upon information gathered from such analysis. Accordingly, the laboratory should use appropriate packaging and cooling/ freezing material for transporting samples. Same storage temperatures within appropriate time periods recommended for parameters for re-analysis or when analysis is delayed due to transport shall be maintained during transportation. Many parameters/ analytes, except few, are stable at ambient temperature for up to 2 to 4 hours from collection time. For such parameters, if the test is carried out within this time frame, special packaging for transporting samples might not be necessary.

Laboratory shall use suitable, well insulated containers along with appropriate packaging and cooling materials which can maintain temperature from 4 to 8°C during transport. For extremely temperature sensitive analytes, laboratory shall use dry ice during transport. Laboratory shall take adequate measures to avoid temperature leakage during transport.

Note: *Depending on the nature of the analytes being tested the samples are either transported at 4 to 8°C or frozen e.g., ACTH, ammonia, Growth hormone, blood catecholamines, IGF-1, Vitamin C, Vitamin K etc. Some analytes need to be protected from exposure to light and hence require adequate wrapping of samples for e.g., 5 ALA, PBG, Vitamin A, Vitamin E, Vitamin K and Vitamin C.*

All persons involved in packaging and transport shall be trained on triple packaging of all specimens i.e., leakproof primary container, leakproof secondary container and rigid outer packaging.

The responsibility for the integrity of the primary sample or parts of the primary sample whatever be its source shall be with the laboratory. When the laboratory accepts samples from agencies or individuals which are not part of its organization, the laboratories shall demonstrate the assessment of their competency in maintaining sample integrity and the same to be documented. Adequate training provided to them to ensure competency or for understanding the processes of the laboratory or risk assessment should also be documented and frequently verified. If deviations are identified, appropriate corrective actions shall be undertaken and implemented, which shall also be reflected in the training. The competency assessment shall cover but not limited to patient preparation, sample collection procedure, packaging, storage, transportation, Biosafety, documentation, request forms and BMW management. All these shall be part of a contract/franchise agreement with such agencies or individuals and the same documented.

As and when the above event happens for the first time with any agencies or individuals with whom the laboratory does not have a formal agreement to accept samples extracted by them, then they shall have processes and checklists to verify if all the processes involved in maintaining the integrity of the sample have been appropriately done and documented. The laboratory shall not accept samples wherever it is not able to establish the integrity of the sample. If compromised samples are accepted by the laboratory, it is the primary responsibility of the laboratory to reflect the significance of the same in the test reports.

A log of sample collection time shall accompany the samples being transported to the laboratory. The above shall be applicable for collection facility at the main laboratory and sites other than the main laboratory viz., collection centers and sample collection agencies.

i) Examination processes (Cl. 7.3 of ISO 15189:2022)

In-vitro diagnostic (IVD) kits, where available, shall be used for reporting on clinical samples. The laboratory shall justify the rationale for the chosen alternative and provide evidence of its effectiveness.

i. Verification of examination methods (Cl. 7.3.2 of ISO 15189:2022)

Method verification shall be done when the test is introduced for the first time in the laboratory. The verification process includes accuracy, precision, linearity, analytical measurement range and inter instrument checks (either with previous existing equipment or with a similar measurement system by exchange of samples with another accredited lab). It is preferable to use patient samples to ensure matrix effect is kept to minimal. However, quality control material/ calibrator/ reference material may be used for verification process. When an open channel/ third party reagent is used on an automated system, verification process will include carry over studies in addition to those mentioned above.

Whenever there is a change in the premise/ replacement of a major part of equipment that affects the measuring system (e.g., pipettor motor and its sub assembly, photometer assembly), method verification will be done at a minimum to include accuracy, precision and inter instrument checks.

ii. Validation of examination methods (Cl. 7.3.3 of ISO 15189:2022)

When a laboratory modifies a regulatory-cleared and approved commercial test method by making changes, it is essentially considered as a new method. In such cases, the laboratory is considered as a test developer/ manufacturer as

well as the end-user and hence it shall establish acceptable performance criteria first and as end user, it shall validate the performance as part of implementation.

The following are examples of changes:

- different sample matrix and not stated in the kit insert (urine, body fluids)
- different collection method/container/transport media or conditions
- promoting different use (screening test vs diagnostic)
- type of analysis (quantitative vs qualitative)
- change in Incubation temperatures
- sample or reagent dilution
- using different calibration materials or set point
- changing or eliminating a procedural step

iii. Evaluation of measurement uncertainty (MU) (Cl. 7.3.4 of ISO 15189:2022)

- Measurement uncertainty of measured quantity values:

  One of the components of measurement uncertainty (MU) is precision. This is obtained from running stable controls. The laboratory shall run controls for each analyte where measurements are in quantitative values with frequency described under each discipline. The SD and %CV shall be derived from the laboratory mean and not from the control's target value assigned by the manufacturer. Actual %CV up to first place of decimal for each parameter shall be used for calculation.

  For practical purposes, imprecision data obtained from the routine application of internal quality control is recommended as the quantitative estimate of the uncertainty of measurement. With the caveat that quality control materials may not totally reflect the analytical behavior of patient specimens, this imprecision is most easily derived from long term Internal Quality Control (IQC) data, calculated as Standard Deviation (SD) or Coefficient of Variations (%CV).

  To record estimates of uncertainty of measurement imprecision should be documented as *k* X %CV. *k* is the coverage factor and at 95% confidence interval it equals to ±1.96 approximated to ±2, so the uncertainty of measurement could be set as:

  - Coefficient of Variation (%CV)
  - The uncertainty of measurement would be: ± 1.96 X %CV approximated to ± 2 X %CV.

It is recommended that a minimum of six months IQC data should be used to calculate routine imprecision. The value is to be updated annually where possible.

For qualitative set of tests, the laboratory shall enlist the factors which could contribute to the uncertainty of the results and ensure that they were given due attention while performing the test.

iv. Ensuring the validity of examination results (Cl. 7.3.7 of ISO 15189:2022)

- **Internal quality control (IQC) (Cl. 7.3.7.2 of ISO 15189:2022)**

Irrespective of the size of the laboratory, two levels of QC shall be included on the day of performing the test and subsequently one level every shift/8 hour. The frequency of run may also be based on risk assessment of harm to the patient due to an erroneous result, and the stability or robustness of the examination method. However, the controls shall be run at fixed timings during operational hours of the laboratory. Two levels of QC (obtained as a part of kit) for Newborn screening (based on Dried Blood spot samples) shall be analyzed with every batch of samples. The daily QC values shall be documented and LJ charts shall be plotted and reviewed daily. The laboratory shall derive its own mean and SD using a minimum of 20 data points to plot an LJ chart. Deviations for rare parameters shall be justified and recorded. The laboratory shall define the criteria for accepting or rejecting a run based on standard guidelines.

Controls for some analytes e.g., CBC have a short shelf life. Therefore, the laboratory mean cannot be calculated. In such situations, the laboratory can use the manufacturer's assigned mean and SD to detect out of control values. The laboratory shall, however, calculate their imprecision as %CV from the data obtained and shall ensure that the %CV continuously remains in the acceptable range.

The laboratory shall calculate the monthly mean, SD and %CV. The laboratory shall analyze QC outliers, trends, shifts and their causes and take immediate corrective action.

The laboratory shall analyze the 'out-of-control situation' by applying the following steps:

- search for recent events that could have caused changes
- examine environmental conditions.
- refer to the manufacturer's instructions for equipment/ reagents /calibrator and/or manufacturer's troubleshooting guide.

The laboratory shall maintain control charts to demonstrate the stability of the analytical measuring systems.

The laboratory shall employ suitable reference material traceable to SI units for calibration of measuring systems (Refer NABL 142). Traceability certificates for calibrators shall be obtained from kit suppliers and appropriately documented.

Alternate methods such as exchange of patient samples, patient sample retesting shall be employed for verifying accuracy of results of those tests for which calibration and control materials are not available. (For body fluids other than CSF and urine).

Additional approaches (but not alternative to use of controls) to maintain precision are performing duplicate tests on patient samples and use of daily moving averages or retained sample testing.

- **External quality assessment (EQA) / Proficiency testing (PT) (Cl. 7.3.7.3 of ISO 15189:2022)**

  The laboratory shall:

  - participate in EQA/ PT in each discipline prior to gaining accreditation
  - participate in an EQA program in case of change in test methodology, equipment changes and extension of scope.

The laboratory shall document corrective actions taken based on the EQA evaluation report.

For some tests, participation in PT program is not a feasible option for one or more of the following reasons:

- Non-availability of a formal National PT programme for analytes of interest.
- Only few laboratories performing the test.
- The analyte to be measured is unstable e.g., blood gases, ammonia, G6PD.

v. Alternative methodologies

For those tests where a formal EQA program is not available or is technically not considered suitable, the laboratory shall adopt any one of the alternative approaches enumerated below in order of preference to validate performance. Refer NABL 163 “Policy for participation in Proficiency Testing Activities’:

- Participation in sample exchanges with other laboratories.

- Inter-laboratory comparisons of the results of the examination of identical IQC materials, which evaluates individual laboratory IQC results against pooled results from participants using the same IQC material.
- Analysis of a different lot number of the manufacturer's end-user calibrator or the manufacturer's trueness control material.
- Analysis of samples using split/ blind testing of the same sample by at least two persons, or on at least two analyzers, or by at least two methods; e.g., microbiological organisms (Infectious molecular parameters).
- Analysis of reference materials considered to be commutable with patient samples;
- Analysis of patient samples from clinical correlation studies;
- Analysis of materials from cell and tissue repositories.
- Replicate testing
- Use of reference methods where available

vi. Comparability of results (Cl. 7.3.7.4 of ISO 15189:2022)

If the laboratory uses either two identical measuring systems, or more than one measuring system where the measurements are not traceable to the same reference material/ reference method, or the biological reference intervals are different, it is essential to perform a comparability study between the systems and prove that there is an agreement in performance throughout appropriate clinical intervals at least twice in a year using suitable statistical procedures such as Bland - Altman plot and/ or regression analysis. Such comparisons shall use 10-20 samples. A written procedure and complete record of all such data shall be retained till the next assessment. (e.g., automated vs. manual culture, identification and AST, two or more extraction or RTPCR systems for molecular testing under molecular diagnostics, etc.)

j) Post-examination processes (Cl. 7.4 of ISO 15189:2022)

i. Reporting of results (Cl. 7.4.1 of ISO 15189:2022)

Test reports shall be in accordance with Cl. 7.4.1 of ISO 15189:2022 and also include identification by name and/ or signatures of the person authorizing release.

ii. Result review and release (Cl. 7.4.1.2 of ISO 15189:2022)

The laboratory shall establish and display/make readily available critical limits for tests which require immediate attention for patient management. Test results within the critical limits shall be communicated to the user/authorized person after proper documentation.

iii. Preliminary report

Practically all hospital laboratories and a few stand-alone laboratories operate round the clock (24X7). After routine working hours, when there are no authorized personnel on duty, the laboratory shall have arrangements for releasing results in place which are required for immediate patient management. The laboratory shall ensure that:

- Daily IQC shows no violation of the documented policy and procedure.
- The technical personnel posted during this period shall be well trained and of proven competence to apply IQC rules
- The personnel authorizing the test reports shall go through the records on the next working day and issue a final report after verifying that the results of these tests showed no trend and that the IQC was valid. The records of these shall be available till the next assessment.

iv. Automated selection, review, release and reporting of results (Cl. 7.4.1.5 of ISO 15189:2022)

The authorized personnel have to establish the review interval for results using verified robust statistical and mathematical criteria. Test results on samples which do not require any further detailed interpretations, recommendations by authorized personnel may automatically qualify for auto verification and reporting. For establishing these criteria, the authorized person shall make use of available standards.

Algorithm based auto-verified (AV) reports should clearly be marked as “Auto verified”. The auto verification should be traceable to the authorized personnel via verification documents. All these rules (algorithms) reside in a computer system called the middleware (advanced LIS) which may be a standalone software or a cloud-based system and has specified hardware and network requirements.

The middleware manufacturer must provide a validation certificate for the AV process and this should be independently verified by the laboratory.

The auto-verification system (including standalone software, cloud-based systems, and hardware) shall be reviewed once in a year. If there is a significant change in the analytic principle or platform review criteria to be redefined.

All auto verification processes shall incorporate rapid suspension mechanisms when there may be a defect in the release process. A contingency plan shall be made available for reports released and awaiting release.

Note: *Implementation of automated selection and release of reports as per existing guidelines and standards (e.g., CLSI) or bodies (e.g., ADLM/IFCC/ICSH) requires robust software and middleware integrated with the LIS.*

v. Post-examination handling of samples (Cl. 7.4.2 of ISO 15189:2022)

The retention period for the samples of various disciplines is mentioned in the respective sections.

vi. Disposal of Bio Medical Waste

The laboratory shall follow the current Guidelines for Management of Healthcare Waste as per latest Biomedical Waste Management Rules and State and local guidelines as applicable.

k) Control of data and information management (Cl. 7.6 of ISO 15189:2022)

- **Information systems management (Cl. 7.6.3 of ISO 15189:2022)**

  - Results generated by manual tests or by an automated analyzer shall be communicated to the customers / users through a computerized or paper-based information system which manages workflow, quality and audit trail for the samples processed in the laboratory. The laboratory shall ensure that the confidentiality of patient information is maintained at all times.
  - When there is a comprehensive computerized information system, all functions from accession to reporting shall be verified after installation.
    - The general process should involve:
    - Input patient data and save demographics and clinical information
    - retrieve the same data
    - capture screen print
    - compare with data on paper form or in a paperless system
    - sign and file with date
  - There shall be a half-yearly review during which the above process is repeated for a minimum of 10 different types of samples / tests.
  - Interfaces: Interfaces between hardware (analyzer) and LIS or between software systems (LIS-HIS) shall be verified to ensure that the interface transmits data in the intended manner and that there is no misfiling of results in the database or in appropriate formatting of the report.
  - If there is major change in any of the components of the information system, the effect on the entire workflow for a selected sample shall be demonstrated to have no deleterious effect.
  - Security and confidentiality: There shall be role based authenticated access into the information system and there shall be procedures to inactivate users who are no longer authorized to access these systems. There shall be a facility to

demonstrate an audit trail to link the activity undertaken by a user with relation to patient data or software change.

l) Control of records (Cl. 8.4 of ISO 15189:2022)

The laboratory shall decide the retention time of records in accordance with national, regional and local regulations. However, NABL requires the following minimum retention period for ensuring quality service and patient care:

Table 2

Record	Retention period
TRFs/work sheets and reports in all disciplines	1 month
Molecular testing gel images, Real time PCR raw data	1 year (infectious diseases) or till the next onsite assessment whichever is later, 10 years (genetic diseases and cancer)
Flowcytometry/ Immunophenotyping data	5 years
Electrophoretogram / Immunofixation	1 year or till the next onsite assessment whichever is later
Haemoglobin HPLC data	2 years
Coagulation calibration/ standard graph	Lot changeover
Raw data & LJ chart of daily values of internal quality control/ raw data of EQA	1 year or till the next onsite assessment whichever is later
Histopathology Reports, Block & Slides	5 years
Cytopathology Reports, Slides	5 years
Cytogenetics, FISH images	5 years
Bone Marrow aspiration slides	5 years
IHC Control Slides	2 years

Note: *The records can be maintained as physical copies (instrument printouts or as photocopies) or electronically*

m) Evaluations (Cl. 8.8 of ISO 15189:2022)

i. Quality indicators (Cl. 8.8.2 of ISO 15189:2022)

The laboratory shall incorporate salient quality indicators for monitoring its performance. This shall describe the evaluation of various aspects of a laboratory's function such as but not limited to the following:

- sample collection and identification
- transportation and processing
- analysis and reporting of results
- turnaround time

- complaints
- downtime of processes
- uncertainty of measurements
- performance in PT / EQA scheme

ii. Internal audits (Cl. 8.8.3 of ISO 15189:2022)

The laboratory shall ensure that pre-examination, examination and post-examination processes are all covered during its internal audit along with the other processes including primary sample collection and POCT if applicable. Internal audit shall be conducted at least once in 12 months.

7. TECHNICAL REQUIREMENTS - DISCIPLINE WISE (Cl. 6 to 8 of ISO 15189: 2022)

7.1 CLINICAL BIOCHEMISTRY

a) Pre-examination processes (Cl. 7.2 of ISO 15189:2022)

Hormone stimulation tests – precautions:

Hormone stimulation tests are to be done under the supervision of a medical doctor in a hospital-based laboratory where emergency services are available for immediate handling of any adverse reactions if and when they occur.

b) Ensuring the validity of examination results (Cl. 7.3.7 of ISO 15189:2022)

- **Internal quality control (IQC) (Cl. 7.3.7.2 of 15189: 2022)**

  For blood gas measurements at least one level of control shall be assayed every eight hours. Appropriate controls for body fluid chemistry wherever available shall be used.

c) Post examination processes (Cl. 7.4 of ISO 15189:2022)

i. Reporting of results (Cl. 7.4.1 of ISO 15189:2022)

Wherever relevant, reports should have an interpretation.

ii. Storage period of examined specimen

The examined specimens shall be stored for re-examination and / or additional tests for a minimum period of 1 day at 2-8°C except for unstable parameters.

7.2 CLINICAL PATHOLOGY

a) Examination processes (Cl. 7.3 of ISO 15189:2022)

Criteria must be documented for identifying urine samples that may give erroneous results by dipstick reader and require manual evaluation. Intensely colored urine samples may result in false positive dipstick reactions with automated reflectance readers.

b) Ensuring the validity of examination results (Cl. 7.3.7 of ISO 15189:2022)

When there is available PT program the laboratory shall participate in the same. As an alternative approach to EQA participation, split sample testing of at least two samples, one normal and one abnormal shall be performed once in three months. It is not necessary to exchange samples with other laboratories.

i. Urine analysis:

Laboratory shall use controls to check dipstick quality every day. The controls may be prepared in-house provided there is a procedure or may use commercially available controls. It is necessary to check both positive and negative controls.

ii. Stool analysis routine & occult blood:

Laboratory shall check:

- The quality of occult blood kit with heated 2% RBC suspension each time a new box is opened.
- The quality of Lugol's iodine with starch when a new bottle is opened and once every week thereafter.
- For ova and cyst by salt floatation as well as Lugol's iodine split sample testing by different personnel shall be performed once every 3 months on at least 2 samples preferably one normal and one abnormal sample.

iii. Semen analysis:

Examination & reporting shall be as per the latest WHO guidelines. Laboratory shall do split sample testing once every 3 months on at least 2 samples (normal & abnormal samples) if the lab is not participating in a formal PT program.

iv. Body fluids:

Body fluids should be processed as soon as they are received by the laboratory. In case they are not processed immediately, the samples can be preserved for 12 hours at 4°C. For Body fluid analysis done by automation, 2 level QC shall be run on the day of analysis.

7.3 HAEMATOLOGY & IMMUNOHAEMATOLOGY

7.3.1 Haematology

a) Pre-examination processes (Cl. 7.2 of ISO 15189:2022)

For monitoring anticoagulant therapy, the request forms must indicate the purpose of the test e.g., monitoring heparin/ Low Molecular Weight Heparin (LMWH) and/ oral anticoagulant therapy as applicable. For Monitoring of Heparin either by APTT or Anti-Xa and for LMWH the time of sample collection shall be documented (ideally between 3-5 hours after the administration of the dose) since the processing of the sample and separation of the plasma shall be done within 1 hour of sample collection.

Blood specimens for coagulation tests should be collected in 3.2% sodium citrate. There must be guidelines for rejection of samples especially for under- or over- filled collection tubes for coagulation tests with training for the same evidenced. Reasons for rejection of these samples must be stated or communicated to the nursing staff, physicians or laboratory personnel responsible for sample collection.

b) Examination processes (Cl. 7.3 of ISO 15189:2022)

i. Complete Blood Count (CBC) specimens shall be checked for clots in samples where there is thrombocytopenia (visually, by applicator sticks, or by automated analyser histogram inspection or flags), before reporting results. CBC processing either automated or manual should be done within 8 hours but in no case later than 24 hours of sample collection as storage beyond 24 hours results in erroneous data on automated / semi-automated haematology analysers. If the laboratory is processing samples over 24 hours but within 48 hours adequate documentation of the stability study shall be available. Blood samples must be adequately mixed before analysis.

The laboratory should also report red cell distribution width (RDW) values. The scope should specify RDW in terms of SD or %CV.

Manual Verification of Platelet count on Peripheral Smear can be commented on as part of the complete blood count report or a comment along with platelet count if smear verification has been done. But it is not a parameter that can be included in the scope separately since there is a lack of proper quality control - accuracy and precision checks.

Packed Cell Volume Determination: The centrifuge shall be calibrated and capable of reaching at least 10000*g* for 5 minutes. The constant packing time (minimum

spin-time to reach maximum packing of cells) shall be determined and recorded for each instrument.

ii. ESR: Westergren or an equivalent method approved by ICSH or CLSI (Formerly NCCLS) shall be followed. ESR is to be performed within 6 hours of collection. Sample kept at 4°C can be processed up to 24 hours. Monitoring of %CV is not mandatory except for the automated method (due to algorithmic extrapolation). Split testing or exchange of samples between laboratories for ESR is also not required.

iii. White blood cell count: The haemocytometer shall be examined regularly to ensure that the lines are bright and free from scratch marks and dust particles. The correct standard thickness cover slips shall be used. The diluting fluid shall be filtered before use and checked periodically for background count. The fluid should be changed when required.

iv. Blood film examinations: The blood film shall exhibit satisfactory quality for staining properties, minimal debris, distribution and morphology of cells. Where appropriate an estimation of cell counts should be made from the blood film and correlated with abnormal counts reported.

v. Malarial parasites: Thick and/or thin films stained by Romanowsky is the method of choice preferably at a pH of 7.0-7.2. Quantitative Buffy Coat (QBC) used as a screening test must be followed up by thin film microscopy to identify the species. The parasite density in case of *Plasmodium falciparum* by currently recommended guidelines is to count the ring stages per WBC count in thick film and per RBC count in thin films.

vi. Manual haemoglobin (Cyanmethemoglobin Method): At least four concentrations must be used to construct a calibration curve.

vii. Coagulation tests: Specimens for coagulation tests must be checked for presence of clots. Coagulation tests must be performed within 4 hours of collection. If delay is expected plasma should be made platelet-free and kept frozen until test can be performed (at -20°C for up to 1 week and at -80°C for up to 1 year).

Platelet poor plasma shall be made in a centrifuge spun at 1500 to 2000g (Speed mentioned is in “g” & not rpm) for 15 minutes to achieve a platelet count of <10000 per µl. The counts must be verified on randomly selected specimens once in six months or following a major repair on the centrifuge which requires recalibration.

All reagents and test samples shall be incubated at 37°C immediately prior to testing, to ensure reaction temperature.

As better alternative diagnostic tests are available, Du test, LE phenomenon, Whole blood Clotting time are considered obsolete and shall not be included in the scope of accreditation. Bleeding time being an in-vivo test shall not be accredited.

c) Ensuring the quality of examination results (Cl. 7.3.7 of ISO 15189:2022)

Laboratory will ensure that in the frequency of running controls one shall follow the shut down- start up cycle.

i. Prothrombin time: The report shall contain the time taken by the test specimen to clot, Mean Normal Prothrombin Time (MNPT) and International Normalized Ratio (INR). MNPT (geometric mean of prothrombin time of 20 apparently normal healthy individuals) should be determined for every new lot of reagents, type of reagent and the instrument used and INR calculated accordingly. Biological Reference Intervals (BRI) show significant differences with each lot of reagents, type of reagent, technique and the instrument used and should be determined for each of the situations if the laboratory uses more than one system. The BRI stated in the literature is unsuitable for reporting the prothrombin time results.

ii. Automated reticulocyte counts**:** Automated reticulocyte counts shall be performed using only appropriate controls. Manual verification should be performed on at least one sample once in a week keeping in mind the bias that automated reticulocyte count is higher than manual reticulocyte count.

iii. Manual reticulocyte count: Recommended quality control by incorporating quality of retic stain, recording counts by two different technicians on same sample or using stored sample.

d) Post- examination processes (Cl. 7.4 of ISO 15189:2022)

- **Storage of examined specimen:**

  The examined specimens can be stored for re-examination and / or additional tests for the period and temperature as specified below:

  - Complete Blood Counts: Upto 24 hours at 2-8°C
  - Coagulation Tests: Upto 4 hours at room temperature

  The storage requirements for the samples which are retained for longer period are as follows:

  - Citrated blood or plasma for PT up to 24 hours shall be maintained between 18 - 24°C before testing.

- Plasma can be stored at or below -20°C for 1 week and -80°C for up to 1 year.
- Haemoglobin electrophoresis and HPLC: (Haemolyzate) 1 week at 2-8°C or longer below -20°C.

7.3.2 Immunohaematology

i. Blood grouping

- **ABO Grouping & Rh (D) type**

  The laboratory shall perform ABO Blood grouping by cell and serum testing using Tube, Microplate or Column agglutination technology by validated manual or automated methods. ABO grouping shall be done using 2 different techniques. One of the two methods, shall have serum testing (reverse grouping) using fresh cells for each group A, B and O cells in the event of not using commercially available cells. The cells should be prepared daily or RBC stabiliser if available and be free from haemolysis. Each batch of (A, B and O) cells should be confirmed for specificity. The slide or tile method for blood grouping shall be restricted only to the second method of verification, of blood grouping and not to validate the blood group.

  The Rh (D) type shall be determined using validated methods with anti-D reagents from 2 different sources preferably one IgM and the other blend. If negative it shall be tested to detect Weak D using the indirect antiglobulin method.

ii. Antibody screen

Antibody screen for detecting unexpected antibodies shall be done on serum /plasma with O pooled cells (which have been validated and fresh) or with commercially available screening panels. Antibody Identification shall be done only with the use of a validated cell panel. Protocol to be followed as per standard practice.

iii. Direct and Indirect Coombs Test

The laboratory shall follow standard accepted protocols for testing and shall include positive and negative controls. For Indirect Coombs Test (ICT) while using inhouse washed O cells the laboratory shall ensure that the reagent cells have a pool of three O group red cells. It will be of benefit if one of the O cells are Rh negative. While using in-house O pooled cells for ICT on a column agglutination platform each sample should be tested with a positive and negative if the O pooled cells are used beyond 2 days.

Every lot of blood group reagent needs to be checked for titre, affinity and avidity. Inter-lot comparison need not be performed.

iv. Crossmatch

This shall only be included in the scope of laboratories which are part of a blood bank/ transfusion services that issue Blood and Blood products as the reagents used for such tests are only available in blood banks/ centres. They shall follow a method for crossmatch that shall demonstrate ABO incompatibility and the presence of clinical unexpected or incomplete antibodies. Crossmatch shall be done using donor cells and patient serum/ plasma in different phases including up to the indirect antiglobulin phase if clinically indicated. These shall be documented.

7.4 MICROBIOLOGY & INFECTIOUS DISEASE SEROLOGY

a) Facilities and environmental conditions (Cl. 6.3 of ISO 15189:2022)

Microbiological testing shall be separated from other testing areas. Diagnostic and Health care laboratories performing infectious samples testing shall be designed for at least Bio-Safety Level 2 (BSL-2) (Ref. World Health Organization Laboratory Bio-safety Manual 4th Edition, 2020, p56; Table 4.1) or higher level depending upon the bio-risk of the pathogens being handled. Desirable equipment for a BSL-2 facility includes a Class II A biological safety cabinet (BSC) and two autoclaves, one for sterilization of the laboratory items and another one for decontamination. Appropriate practices include biohazard warning signs, "sharps" precautions and a bio-safety manual, defining any needed waste decontamination or medical surveillance policies (Ref. Biosafety in Microbiological and Biomedical Laboratories, 6th Edition, 2020, US Department of Health and Human Services Publication No. CDC 21-1112, p40), along with adequate precautions to prevent the generation of aerosols and splashes and the use of personal protective equipment (e.g., laboratory coats, gloves). A washbasin/ sink should preferably be near the exit of the section/laboratory available for hand washing. To prevent contamination of cleaned hands it is recommended to have sink equipped with elbow controls or foot pedals or sensors.

i. Media/reagent preparation room:

If media/ sterile reagents are prepared in-house, these laboratories shall have a dedicated area for media preparation. This area shall be free of moisture, well-ventilated with dust free environment to prevent contamination and have its own equipment. It is also recommended to have relevant dedicated reagents and storage facility within this room. Use of Laminar air-flow hood is recommended for preparing culture media.

ii. Mycobacteriology:

Tubercle bacilli may be present in clinical samples like sputum, gastric lavage fluids, CSF, urine, and tissues, hence sample processing may be carried out in a class II A2 Bio-safety cabinet. Laboratories that only manipulate specimens for direct smear microscopy or for the TB-CBNAAT assay, are considered "low-risk TB laboratories" and if class II BSC not available, the sample processing can be done on an open bench in an adequately ventilated area with 6-12 air changes per hour, with unidirectional airflow which is away from the technician.

iii. Laboratories that perform mycobacterial culture and sensitivity testing: Aerosol-generating activities like concentration method or culture shall be carried

out in a bio safety cabinet (BSC) class II A2 with all BSL3 practices including mechanical air ventilation with negative pressure, an ante-room with airlock. Laboratory shall use centrifuge cups with safety lids. Based on the type of work, these labs are classified into "Moderate or High risk" laboratories. Laboratories shall refer to the latest WHO Tuberculosis Laboratories Biosafety Manual, for air-exchanges, facility design, equipment, safety practices, specific risk assessment, emergency planning etc. BSL3 practices need to be followed for culture-based drug susceptibility testing and shall have a dedicated area for donning and doffing of personnel protective equipment. (Ref. latest WHO Biosafety Manual / Tuberculosis Laboratory Manual / Govt of India NTEP guidelines).

iv. Mycology:

For handling specimens for filamentous fungi, there shall be a dedicated area that shall be physically separated from the bacteriology and mycobacteriology section and shall not be used for any other culture work.

A laboratory performing fungus culture shall be equipped with a bio-safety cabinet class II A2 and BOD incubator. Other equipment may be chosen based on the risk level of fungi isolated and handled.

b) Storage Facilities: (Cl. 6.3.3 of ISO 15189:2022)

Laboratories shall have a dedicated area to store the segregated Biomedical waste. It is desirable that the biomedical waste area is located close to exit of the laboratory and the waste is not carried across the laboratory testing area.

c) Equipment (Cl. 6.4 of ISO 15189:2022)

i. Incubators:

The laboratory shall select incubators with appropriate performance specifications that comply with the temperature and growth requirements of organisms so that fastidious organisms are not missed.

CO_2 generating or anerobic gas packs shall be used in air-tight boxes of appropriate size as recommended by the manufacturer.

ii. Other equipment:

Autoclaves and hot air ovens shall be checked by both chemical and biological indicators. Chemical indicators shall be used with each batch, whereas a biological indicator shall be used at least once a week.

d) Reagents and consumables (Cl. 6.6 of ISO 15189:2022)

i. Materials used for collection and transport:

The laboratory shall ensure that all the material used for collection and transport are appropriate and verified for their usefulness and efficiency before being put in to use. Each lot/shipment shall be visually inspected and tested for sterility and performance depending on the purpose using appropriate strains (e.g., ATCC). Records shall be maintained.

ii. Media / Biochemical tests/ Serology tests:

The laboratory shall ensure that every lot/shipment of commercial or in-house prepared media are sterile, able to support growth and are appropriately reactive biochemically (Ref. CLSI document M22-A3-quality control for commercially prepared media). Growth shall be verified objectively using standardized inoculum of reference strains and measured semi-quantitatively. Acceptance criteria shall be documented. For this, the laboratory shall maintain stocks of characterized organisms which are traceable to ATCC/NTCC (with traceability and certificate of analysis).

e) Pre-examination processes (Cl. 7.2 of ISO 15189:2022)

- For Closed NAAT systems, clinical samples should be collected using manufacturer recommended containers and transport systems.
- Specimens for culture and sensitivity shall be processed immediately after collection. In case of delay in processing, the specimen is to be stored in refrigerator except for CSF, Urethral/ Endocervical swab for gonoccous culture and pus/ aspirate from suspected cases of amoebic liver abscess. In situations where the sample needs to be transported, it shall be collected in an appropriate transport medium. Avoidance of a delay in processing and the maintenance of a cold chain during transportation shall be ensured particularly for urine samples/ BAL/ endotracheal aspirates that are to be cultured since these tests are semi-quantitative. The laboratory may consider use of validated transport tubes with bacteriostatic agents for urine samples that are transported from long distances.
- For anaerobic/ fastidious organisms appropriate transport media and transport conditions shall be employed by the laboratory.

f) Examination processes (Cl. 7.3 of ISO 15189:2022)

The laboratory shall follow CLSI/EUCAST guidelines etc. for antimicrobial susceptibility testing. The laboratory shall define the level (e.g., Genus or Species) to which microbial identification is to be performed. This level should not compromise the quality

of results which can potentially affect patient care and infection control practices (e.g., *Candida auris*)

- **HIV testing**

NACO testing algorithms are to be complied with as applicable. NACO guidelines currently do not incorporate 4th generation (antibody plus antigen detection) kits for diagnosis of HIV infection. Therefore, laboratories using 4th generation tests, which have high sensitivity, as the screening test and using second/third generation test as the supplemental tests should follow NACO guidelines for reporting of indeterminate results; alternately, they can recommend confirmation by qualitative molecular tests.

g) Internal quality control (Cl. 7.3.7.2 of ISO 15189:2022)

i. Microbiology:

A suitable non-virulent strain shall be used for quality control wherever applicable. Standard reference strains of known susceptibility shall be tested along with clinical isolates while performing drug susceptibility testing, based on CLSI/ EUCAST guidelines. In case of conventional susceptibility testing against *Mycobacterium*, a standard strain of *M. tuberculosis* with known resistance pattern to different drugs shall be used with each batch of tests as a check on procedures.

ii. Serology:

For serological tests, the kit controls shall be put with every run as recommended by the manufacturer. MU or %CV shall be calculated for all quantitative serological parameters and qualitative serological parameters performed on quantitative platforms like ELISA, ELFA, CLIA, ECLIA and CMIA.

In-house external control sera shall be stored as multiple aliquots frozen at ≤ -20°C to prevent repeated freezing and thawing.

Table 3: Internal quality control guidelines

S. No.	Parameter	Frequency	Internal quality control Methodology	Acceptability Criteria
1.	Microscopy-stained preparation	Every time when staining performed	Appropriate standard strain of microorganism (e.g., ATCC)/ retained isolate/ EQA sample.	Satisfactory readings of staining on microscopic examination.
2.	Culture media & Biochemicals	Whenever a new lot	Appropriate standard strain of microorganism	The growth of the organism should be

	(other than for mycobacteria)	received / prepared (same as lot verification)	(e.g., ATCC).	supported. Use standardised inoculum; interpretation of growth done semi-quantitatively.
3.	Culture for mycobacteria	With each batch of testing	Appropriate standard strain of Mycobacteria	The growth of the organism should be supported. Use standardised inoculum; interpretation of growth done semi-quantitatively for solid media.
4.	Antimicrobial susceptibility testing	Weekly	Appropriate standard strain of microorganism (e.g., ATCC).	The zone sizes should be within acceptable limits (CLSI / EUCAST guidelines).
5.	Automated culture, identification and antimicrobial testing systems	As recommended by the manufacturer	Appropriate standard strain of microorganism (e.g., ATCC).	Growth, identification and/or antimicrobial susceptibility of standard strains should be satisfactory
6.	Serological assays-Qualitative result relying on test which produces qualitative output data (e.g., Immunochromatography, immuno concentration etc.)	After every 15-20 samples are tested or weekly whichever is earlier	Two prior tested patient samples/ EQA samples /third party controls, one negative and one positive (preferably low positive).	The results should be reproducible i.e. negative sample should give negative result and positive sample should give positive result.
7.	Serological assays-Semi-quantitative result (for e.g., VDRL test, WIDAL test)	Internal controls with every time the test is performed, external	Apart from internal controls (kit controls), external controls (prior tested patient samples/ EQA samples /third party controls) preferably one	The results should be reproducible i.e. negative sample should give negative result and positive sample should give positive result

		controls after every 15-20 samples are tested or weekly whichever is earlier	negative and one positive to be used.	within ± one dilution.
8.	Serological assays- Qualitative result relying on test which produces quantitative output data (e.g., ELISA, CLIA)	With each run	Apart from kit controls (internal controls), at least two prior tested patient samples, one negative and one positive (preferably low positive).	The results should be reproducible, i.e., negative sample should give negative result and positive sample should give a positive result. The test validation criteria should be met.
9.	Molecular assays- Qualitative (e.g., SARS-CoV-2 RT-PCR)	Every time the test is performed	Apart from kit controls (internal controls), at least two prior tested patient samples, one negative and one positive (preferably low positive, i.e., Ct value>25) at a frequency depending upon the risk. Sample should be run through both extraction and amplification	The results should be reproducible, i.e., negative sample should give negative result for the positive sample should be positive. The house keeping gene control (e.g., RNP, beta-globin etc) should be detected for ensuring sample quality.
10.	Molecular assays- Quantitative (e.g., HIV-1-qPCR, HBV-qPCR)	Every time the test is performed	Apart from kit controls (internal controls), at least two prior tested patient samples, one negative and one positive. Sample should be run through both extraction and amplification	The results should be reproducible, i.e., negative sample should give negative result and the difference in Log10 transformed values for the positive sample should be within ±0.5 or as per national guidelines. For closed systems the recommendations of the

				manufacturer shall be followed.
11.	Absolute CD4+T-Lymphocyte count & percentage by immunophenotyping (in the context of HIV)	Every run day	As per NACO guidelines	As per NACO guidelines

h) Post-examination processes (Cl. 7.4 of ISO 15189:2022)

Examined specimens shall be stored for re-examination and / or additional tests for a minimum period as specified below:

- Serum Samples: 3 days at 2 to 8°C (except for HIV, where NACO guidelines require these to be stored for 7 days)
- Samples for all types of culture**:** Until the final identification and antibiotic susceptibility report is issued. Sterile body fluids and other precious samples like aspirates shall be stored for a period of 10 days at 2 to 8°C.
- Molecular Microbiology: Sample aliquots should be stored at -80°C for 1 month or as per national guidelines.

i) Reporting of results (Cl. 7.4.1 of ISO 15189:2022)

- Microbiology results shall follow a uniform format and content, for each test under the scope. Wherever relevant, microscopy results shall include grading of cells/ organisms. Cultures shall be reported with organisms tested and their growth instead of mentioning “No growth” e.g., For Stool culture, report as “Culture negative for *Salmonella*, *Shigella*” instead of “No growth/comments as grown”, which could be misleading to the clinician.
- Whenever more than one test is performed on a sample and discordant results are reported, an attempt should be made by the laboratory to explain the reasons for such discrepancy. e.g., presence of fungi/ AFB in microscopy and absence of growth in culture.
- All tests of infectious diseases shall be reported along with comments on their interpretation and limitations.

7.5 HISTOPATHOLOGY

a) Accommodation and environmental conditions (Cl. 6.3 of ISO 15189:2022)

i. Safety of personnel from exposure to infectious & chemical hazards (such as formalin & xylene) must be ensured and an efficient exhaust & fume extraction system shall be in place.

ii. A separate room shall be allotted for gross examination of tissue, equipped with a fume hood, efficient exhaust, ensuring negative pressure.

iii. The record of formalin vapour levels covering activities like grossing, change of formalin and sample discarding shall be maintained.

iv. Permissible limits of Formaldehyde vapour and xylene as per Directorate of General Factory Advice Service & Labour Institutes (DGFASLI), Government of India.

Table 4

S. No	Chemical	Permissible Limits			
		Time - Weighted Average Concentration - 8 hours		Short-Term Exposure Limit - 15 minutes	
		ppm	mg/m3	ppm	mg/m3
1.	Formaldehyde	1.0	1.5	2.0	3.0
2	Xylene (o, m, p Isomers)	100	435	150	655

A separate well-ventilated place shall be dedicated for storage of wet specimens.

All chemicals shall be handled, stored and disposed according to local regulatory requirements.

b) Equipment (Cl. 6.4 of ISO 15189:2022)

i. Tissue Processors:

The laboratory shall verify the processing program for the type of tissues which it is likely to handle (small biopsies, large tissues, decalcified bones and brain/fatty tissues).

ii. Microtome:

- To be calibrated by the manufacturer once a year.
- Microtomes with non-disposable knives shall have a safety shield. Blade lock, blade guard and wheel locks should be used for all types of microtomes using disposable blades.

iii. Microscope:

Microscope shall have a scanning lens,10X and 40X objective. Spare bulbs and fuses shall be available in the laboratory. A field number 20 or above is desirable for

reporting in Histopathology and Cytopathology. An adequate field diameter for screening of histopathology and cytopathology slides. (even for clinical pathology and haematology) decreases the number of sweeps required to screen the histopathology sections and cytology smears (overlapping fields).

The laboratory should have an annual maintenance plan for all the microscopes to ensure optimal functioning optics.

c) Reagents and consumables (Cl. 6.6 of ISO 15189:2022)

The laboratory should use wax of optimum melting and crystallization temperature range, suited for the specimen, however for all routine purposes a good quality low melting point paraffin wax (<60°C) is desirable.

The laboratory shall ensure safe handling of paraffin wax to reduce the risk of molten wax at work place.

d) Pre-examination processes (Cl. 7.2 of ISO 15189:2022)

- The sample collection and transport are critical steps in ensuring optimum processing of the tissue and subsequent conclusive morphological examination, IHC and molecular testing.
- The cold ischemia time of <1 hour is recommended. Specimens should be cut and immersed in 10% Neutral Buffered Formalin (NBF) to allow for early penetration of fixative and optimum fixation.
- 10% NBF is the recommended fixative for histopathology, IHC and molecular diagnostics.
- Specimens collected from remote sites by laboratory collection teams should ensure transport to the referral laboratory at the earliest as over fixation may impact further molecular diagnostics results where needed.
- The diagnostic small biopsy should be fixed for minimum 6 hours in formalin and a resected specimen for minimum 24-72 hours (depending upon the type of tissue) pH of formalin should be regularly monitored and should remain close to neutral (pH 6.8–7.4) Formalin with pH<6.8 needs to be discarded as it can impact further molecular testing.
- Date of preparation of 10%NBF shall be recorded and should have a label mentioning expiry date.
- Sample receipt/ Sample acceptance exceptions: Histopathology specimens shall not be rejected on grounds of poor specimen integrity. They should be accessioned & remarks to be incorporated in the gross, microscopic descriptions and any impact on diagnostic interpretation as appropriate.

  In the case of specimen mislabeling or issues in specimen identification and traceability, the specimen shall not be accepted for testing without reconciling all

issues. In the intervening period, the specimen shall not be discarded. Appropriate temporary labelling and if necessary, processing of the specimen may also be undertaken.

e) Examination processes (Cl. 7.3 of ISO 15189:2022)

i. Fixation:

Fixation beyond 48 hours to be avoided, excepting for tissues with high fat content which may require 72 hours.

ii. Band saw or bone saw for bone cutting:

The laboratory shall take care to protect the operator from bone dust and possible injuries.

iii. Grossing:

The specimens shall be grossed, and the findings recorded by a pathologist or trainee pathologist deemed competent for the procedure.

iv. Tissue Processing:

- This shall be done in-house. It is not permissible to permanently outsource processing and slide preparation to another accredited laboratory.
- Depending on the workload the laboratory shall have a procedure to change the tissue processing reagents and maintain a record of it.
- A log recording of the 'time setting schedule' for an automated tissue processor shall be maintained.
- Temperature of the wax bath shall be checked and recorded daily and should not exceed 65°C.

v. Tissue embedding:

Care shall be taken not to use dyes that produce autofluorescence and interfere with fluorescent microscopy and molecular testing. If the small biopsies need to be stained for easy visualization during grossing and embedding natural food colors at low concentration are desirable. It is a good laboratory practice to conserve the diagnostic small biopsy material (Lung carcinoma, Guided biopsies for other tumors) for subsequent molecular and IHC studies.

vi. Microtome:

For molecular studies, adequate cleaning before and after cutting along with frequent changing of microtome blades should be practiced to prevent tissue carry over.

vii. Slide warming stage:

Temperature of the slide warming stage shall be checked daily and kept below the melting point of paraffin wax.

viii. Tissue flotation bath:

- The water in the flotation bath shall be changed at least once a day
- The surface of the water bath shall be skimmed regularly during section cutting to remove floaters.
- The temperature of the floatation bath with water needs to be monitored intermittently to ensure that it is just below the melting point of the wax.

ix. Staining:

- The frequency of changing the deparaffinization solutions (xylene / chloroform / alcohol) and stains should be recorded. This is based on workload.
- Special Stains: A positive control should be stained with each batch.

x. Frozen section/ squash smear:

- A specific area should be demarcated for performing frozen sections (if possible, near the OT complex).
- Fresh tissue received for frozen section shall be treated as infective and universal safety precautions shall be followed.
- Turnaround time (TAT) for frozen section / squash smears should not exceed 20 minutes. The laboratory shall identify & document situations where TAT may be exceeded.
- The decontamination and cleaning protocols for cryostat should be available and records maintained.

xi. Specimen suspected of prion disease (Creutzfeldt - Jakob disease):

In a suspected case of prion disease, facilities should be available for safe handling of specimens. The biopsy specimen shall be considered as bio-hazardous and transferred to concentrated formic acid (96%) for 48 hours, subsequently to 10% formalin for 24 hours and then processed. All instruments used for sectioning shall be left in 2M NaOH for 1 hour and washed in running water for 15 minutes before reuse. The microtome should be wiped clean with 2M NaOH and left for 1 hour. Subsequently, the instrument should be wiped clean with tap water followed by alcohol before reuse.

f) Ensuring the validity of examination results (Cl. 7.3.7 of ISO 15189:2022)

Record of daily quality checks shall be maintained (for processing Microtomy and routine stains).

g) Post-examination processes (Cl. 7.4 of ISO 15189:2022)

Result review and release:

i. The names of the person reporting the macroscopic and microscopic findings along with signatures shall be entered on the worksheet. There shall be adequate description of the macroscopic / microscopic findings.

ii. The histopathology examination requires clinical details, imaging correlation, and review of diagnostic previous material; the same should be recorded (wherever relevant) in the final reports.

iii. Frozen section results must be compared with the frozen section remains on final assessment and both results must be reflected in the final report.

iv. Reporting on Immunofluorescence studies on tissues the laboratory shall follow standard guidelines.

v. Report should be in accordance with recent terminology / classification, grading, scoring, nature of lesion and relevant information necessary for disease management as per latest international guidelines. Report shall also mention all additional tests performed such as special stains, immunohistochemistry etc.

vi. TAT for all biopsy specimens to be defined in accordance with specimen complexity and patient review/institutional practice. The laboratory to strive to achieve >75% within-TAT performance in a defined category of specimen. In cases where the TAT has exceeded the defined limits for ancillary testing and consultations the laboratory may consider giving out a provisional report, followed by a final report.

vii. Workflow systems: Use of workflow systems are encouraged to reduce labelling errors during all stages of specimen processing in. The software and hardware verification, monitoring and integration with LIS/HIS shall fulfil requirements of clause 7.6 of the ISO 15189:2022.

h) Post-examination handling of samples

i. Storage period of examined specimen: The examined specimens shall be stored for re-examination, issue and / or additional tests for a minimum period as specified below:

- Specimens: 30 days from the day of report authorization.
- Storage condition: Room temperature (18-27°C) and pest free archival site.

ii. Issue of tissues, slides/blocks: The laboratory should issue the gross specimen, representative slides and paraffin wax blocks to its patients on specific request for obtaining a second opinion, other testing or for treatment elsewhere. The laboratory shall have a procedure and maintain records of the same. However, attempts should be made to retain at least one representative slide on which the diagnosis was based for review.

Table 5

Mode of Disposal of BMW generated in Histo/Cytopathology: Laboratory to ensure that disposal of chemicals like formalin and Xylene are addressed as per Clause 6.8. of ISO 15189:2022 and this document.

S. No.	Types of Bio-Medical Waste	Mode of Disposal
1.	Anatomic bio waste (Human tissues, organs, body parts and fetuses below the viability, period) generated from the grossing room.	Yellow colored containers /non chlorinated bags to be handed over to an authorized agency for incineration or deep burial or plasma pyrolysis.
2.	The tissue trimmings from specimens (at the microtomy station) and old Paraffin Blocks.	All old blocks and scraps of paraffin including trimmings of sections should be collected on a disposable sheet and disposed in red non chlorinated plastic bags. The bags to be handed over to the authorized vendor for final disposal as per the current Government of India guidelines
3.	Disposal of Formalin used in laboratories. Decant the formalin from any tissue immersed in it. • Untreated formaldehyde containing solutions cannot be poured down the drain. • Neutralization with various commercially available reagents is the procedure of choice. • Formalin solutions at concentrations less than 0.1% (1000 ppm) are acceptable for sewer discharge	After recovery collect in a yellow container or non-chlorinated bags, the source of which should be labelled, and the bags sealed. Options: • Formalin collected in labelled containers can be discharged into the Effluent Treatment Plan (ETP) if the laboratory has one; after neutralizing the liquid waste with neutralizing agents available commercially. • The labelled and sealed containers to be handed over to the authorized agency with a documented MOU for the same for further disposal.
4.	Other Liquid waste like Isopropyl alcohol, DAB solution from IHC.	Hand it over in sealed labelled containers to the authorized agency with a documented MOU for the same for further disposal. Storage while awaiting disposal to be in a

		protected place with fire safety precautions.
5.	Disposal of Xylene used in laboratories	Xylene is a highly inflammable chemical with deep burial or controlled incineration as ideal procedure for disposal. Hand it over in sealed labelled containers to the authorized agency with a documented MOU for the same for further disposal. Storage while awaiting disposal in a protected place with fire safety precautions
6.	Disposal of Glass slides	Cardboard boxes with blue colored marking

7.5.1 Foetal autopsy:

i. Foetus is a product of conception irrespective of the duration of gestation. In clinical practice common terminology used for products of conception with respect to the duration of gestation.

ii. Upto end of 8 weeks: Products of Conception (embryo).

iii. 9 to 22 weeks: Early fetal death.

iv. 23 to 27 weeks: Intermediate fetal death.

v. 28 to term: late gestational fetal death (Still birth/Intra uterine death)

vi. It is preferable for a Pathologist trained in fetal autopsies to perform a foetal autopsy.

vii. The following pre-examinations requirements shall be provided with the foetal specimen:

- Request form from the Clinician with comprehensive maternal history which may include the relevant obstetric history, copies of imaging reports, maternal screening investigations.
- a death certificate for foetuses received after 28 weeks of gestation.
- Consent form duly signed by the parents /next of kin as declared in the records is desirable for intermediate gestation foetal death but MANDATORY for foetuses received after 27weeks of gestation.
- Autopsy procedure and reporting should follow the standard guidelines in literature.

viii. Preliminary report: to be provided to the referring clinician within three days of the post-mortem examination.

ix. Final report should be provided to the clinician within 8 weeks and incorporate all results of any special investigations like genetic study conducted on the foetal tissues.

x. Disposal of foetuses is as per local regulations beyond 27 weeks of gestation.

xi. If the remains after the autopsy are being handed over to the authorized BMW disposal agency the laboratory must provide copies of death certificate, request form of clinician and Consent form of parents/next of kin as stated above along with the foetus.

7.5.2 Transmission Electron Microscopy

a) Facilities & environmental conditions (Cl. 6.3 of ISO 15189:2022)

i. A separate room shall be allotted for tissue processing & staining with a fume hood for handling hazardous chemicals including osmium tetroxide, lead citrate etc.

ii. A separate room/enclosure to be provided for ultramicrotomy with adequate lighting and work space.

iii. A separate facility, with air-conditioning to be dedicated for preparation of specimen and performing transmission electron microscopy.

iv. The electron microscopy room shall have facilities in place for temperature control and chilled water supply.

b) Pre-examination processes (Cl. 7.2 of ISO 15189:2022)

- **Specimen:**
  - Paraffin embedded tissue may be reprocessed for TEM.
  - Autopsy tissue is not suitable for TEM.

c) Examination processes (Cl. 7.3 of ISO 15189:2022)

i. A procedure manual should be readily available with detailed procedure for the safe handling of epoxy resins.

ii. Sections of resin embedded tissue (one micron semi thin sections) are reviewed by Pathologists trained in the same to ensure selection of appropriate areas for TEM analysis.

d) Post-examination processes (Cl. 7.4 of ISO 15189:2022)

Post examination handling of samples**:** Disposal of samples of EM

Type of EM samples	Retention period
Wet tissues	1 week after final report
Resin block	5 years
Images and reports	5 years

7.5.3 Immunohistochemistry

Laboratory should specify type of specimens on which IHC assays would be performed which could be but not limited to the following:

- Formalin Fixed Paraffin embedded (FFPE) blocks.
- Air Dried Imprint smears
- Liquid based Cytology preparations
- Decalcified tissue
- Tissues fixed in any other fixative.

a) Process requirements (Cl. 7 of ISO 15189:2022)

i. Pre-examination processes (Cl. 7.2 of ISO 15189:2022)

Fixation:

The recommended optimal fixation time in NBF is 6 to 72 hours for IHC procedures. However, when this is not possible and beyond the control of the laboratory a disclaimer in the report to be included to cover the limitations of over fixation where relevant like Predictive markers.

ii. Verification of examination methods (Cl. 7.3.2 of ISO 15189:2022)

Verification of antibodies in use for their performance every 6 months is a good practice. However, the laboratory to develop verification protocols based on the frequency of usage of the antibodies.

Verification of IHC antibodies shall be performed for introduction of a new clone or change in the manufacturer for the same clone.

Laboratory director to give a rationale on the number of cases to be included in the verification process of Non-Predictive and Predictive markers at the time of introduction. For non-predictive markers a minimum number of 10 cases shall be verified and to include both positive and negative. For predictive markers/semi quantitative markers a minimum number of 20 cases shall be verified and these to include both positive and negative, Positive cases to includes the range of expected clinical results (expression levels).

The laboratory may choose to use Tissue Micro Array (TMA) /multiple sections on a single slide for verification procedures.

iii. Verification process for non-predictive markers

- Correlate the results using new antibody with the morphology and expected results using appropriate controls.
- Verify the assay for repeatability on the same tissue by the same person
- Verify the assay for reproducibility on the same tissue by a second person
- Verify comparability with another NABL accredited laboratory.

iv. Verification process for predictive markers

- Verification to include a-d as mentioned above.
- Verification to be robust and Positive cases in the verification to include the span of the expected range of clinical results (i.e., expression level), especially for those markers that are reported with a scoring grade or % of expression.

v. Ensuring the validity of examination results (Cl. 7.3.7 of ISO 15189: 2022)

- **Internal quality control (Cl. 7.3.7.2 of ISO 15189: 2022)**
  Laboratory to specify the list of antibodies with the tissue where an internal control is available, for which the routine use of an external control is not mandatory.
- **External controls:** One external tissue control per batch is mandatory as the preanalytical parameters may be different for the test and control blocks/tissue eg. quality of fixation, processing fluids etc. all of which influence antigen retrieval.
  For manual IHC staining one known positive and one known negative control for each antibody in a batch to achieve specificity. One negative reagent control for predictive markers to assess nonspecific aberrant staining in the tissue.
  For automated IHC staining on slide controls are preferred. It is desirable to include Positive controls with span of the expected range of clinical results (expression level), especially for those markers that are reported in gradation or % of expression and to select the tissue where an internal control is available makes the procedure robust.

- **Comparability of examination results (Cl. 7.3.7.4 of ISO 15189: 2022)**
  Automated IHC equipment are robust and precise in their performance when maintained as per the manufacturer`s recommendations. Records of positive controls are a good indication of their performance. When the laboratory has more than one equipment, processes shall be developed for usage of equipment for predictive and non-predictive diagnostic markers. Harmonization of

equipment shall be done if predictive /semiquantitative markers are run randomly when more than one equipment is used.

b) Post-examination processes (Cl. 7.4 of ISO 15189:2022)

Reporting of Results (Cl. 7.4.1 of ISO 15189:2022)

In addition to requirements of the Standard, reports of IHC shall include the following:

- Sample type - Tissue /FFPE block/Slide (any other as relevant)
- Fixation time - If available
- Antibody with details of the clone for predictive markers
- Comment on Controls eg: a statement such as “All Controls show appropriate activity” would be sufficient
- For Predictive Markers grading /scoring system followed with details of its interpretation.

7.5.4 Digital Pathology (DP)

Digital pathology is a dynamic, image-based environment that enables the acquisition, management, and interpretation of pathology information generated from digitized glass microscope slides. Whole Slide Imaging (WSI) allows the digitization of an entire glass slide by a scanning device to produce a high-resolution digital image that can be viewed, maneuvered and navigated on a computer screen akin to using conventional Optical Microscopy (OM).

This document focuses on the utility of DP for primary diagnosis, secondary consultations (telepathology consultation) and frozen sections diagnosis.

Primary diagnosis in DP is defined as reviewing digital slides in lieu of glass slides (e.g., review of digital images without the use of a microscope) and final reporting of the diagnosis to be used for patient care.

Telepathology involving static images are not included in this scope.

a) Scope of digital pathology:

Digital pathology is a product of complex multi-step processes, involving technical (scanners capabilities, software and hardware for viewing and archival of the slides), clinical (specimen type) and organizational (training and pathologist’s expertise in DP and institutional Information Technology (IT) capabilities.

Laboratories should specify the indication for which DP will be used.

Type of specimen to be scanned:

- Histopathology (H&E, IHC, Special Stains, Frozen sections)
- Cytology (FNAC & Effusion cytology, PAP smears – Conventional smears/ LBC slides)
- Haematology (Peripheral blood smears, Bone marrow aspirate slides)
- Molecular Pathology (FISH)

Volumes of workload /slides need to be scanned? (Scanning load)

- Laboratory to define whether all or selected work needs to be scanned and to have adequate number of scanners to meet the workload.

The scanner should be kept in the laboratory as close to the site of glass slide production.

b) Equipment (Cl. 6.4 of ISO 15189:2022)

i. Scanners:

- Technical details: The technical details of the digital scanner should be documented (as per the manufacturer) to include scanner model, slide capacity, scanning capability {Scanning of surgical pathology sections, frozen sections (including wet slide), immunohistochemistry slides, cytology smears}, scan magnification (as applicable) scanning mode (manual or automated), scanning time (for 15x15 mm area).
- Performance Capabilities to include the following:
  - Successful scanning rate (No of slides scanned on first attempt).
  - Digital artefacts (rate of artefacts, type of artefacts).
  - Quality of digital slides (on a scale of 1-3; 1- poor, 2- average, and 3 good).
  - If the scanning image quality is < 2, then necessary corrective action is to be taken.

ii. Workstation details:

Technical Specification of the operating computer unit (e.g., Processor, RAM). The minimum acceptable specification for the DP workstation computer should include:

- CPU: Intel Core 2 Duo (or newer) processor, running at 2 GHz (Intel i5 or above is preferred).
- RAM: 4 GB (8 GB or above is preferred).
- Hard Drive space: 50 GB (250 GB or above is preferred).
- Operating System: for e.g., Windows 7/8.1/10 (Win 10 Pro/11 is preferred).
- Graphic card: 2GB.

The acceptable specification for the DP workstation monitor (display) should be preferably as per the manufacturer`s recommendations, with an internet connection speed of 50 Mbps or above (100 Mbps is preferable).

Image management/ viewer system:

- Online/Offline
- Annotation tools available (Yes/ No)
- Integration with LIS (Yes/ no)

Storage server details:

- Onsite /cloud-based

- Technical details of the storage solution
- Legal documentation regarding patient data confidentiality and data security (especially in case of cloud-based storage)
- Backup plan for image database breakdown

Archival policies of the digital images - Digital images should be retained for 5 years, if original glass slides are not available.

c) Process requirements (Cl. 7 of ISO 15189:2022)

- **Proposed process/workflow:**

  The laboratory should document the proposed workflow for scanning as well as the reporting protocol for digital images.

d) Examination processes (Cl. 7.2 of ISO 15189:2022)

i. Verification records:

Validation of WSI is crucial to ensure the quality and consistency of diagnostic performance based on digitized slides. Verification of the DP solution should be performed to ensure that the system has been verified and is appropriate before routine use.

- The verification process should include a sample set of at least 100 cases for one application, or use case, (e.g., H&E-stained sections of fixed tissue, frozen sections, hematology) that reflect the spectrum and complexity of specimen types and diagnoses likely to be encountered during routine practice.
- The verification study should establish diagnostic concordance between digital and glass slides for the same observer (i.e., intra-observer variability). If concordance is less than 95%, laboratory should investigate and attempt to rectify the cause. All discordances should be reconciled with respect to types of problematic cases, scanner and/or histology issues and pathologist factors
- A washout period of at least two weeks is recommended between viewing digital and glass slides
- The verification study should encompass the entire WSI system. It is not necessary to separately validate each individual component (e.g., computer hardware, monitor, network, scanner) of the system or the individual steps of the digital imaging process.
- Pathologists adequately trained to use the WSI system should be involved in the validation process
- Documentation should be maintained recording the method, measurements, and final approval of verification for the WSI system to be used in the anatomic pathology laboratory.

ii. Good Practice Statements:

- Laboratories implementing WSI technology for clinical diagnostic purposes should carry out their independent validation studies
- Verification should be appropriate for and applicable to the clinical use
- The verification study should closely emulate the real-world clinical environment in which the technology is likely to be used
- Check list of challenging digital diagnosis and limitations with digital diagnosis to be made available.

iii. Records:

- Calibration logs of the scanner
- Log of failed scans
- Scanner breakdown logs
- Maintenance/ Service logs

e) Ensuring the validity of examination results (Cl. 7.3.7 of ISO 15189:2022)

Pre-scanning quality check of the glass slides is mandatory.

Factors to consider when assessing the images would be:

- Is the background clear?
- Is the image in focus?
- Is the staining crisp and clear?
- Are the images comparable across all scanners?
- Is there a significant difference in the interpretation of key diagnostic features in images obtained from different scanners?

Records of daily Quality control checks for the above image quality shall be maintained.

Laboratory to participate in an ILC by exchange of blocks with another accredited laboratory with DP. If ILC is not possible, an alternate methodology is to rescan random previously scanned slides and reported by a pathologist and compare the result.

7.6 CYTOPATHOLOGY

a) Facilities and environmental conditions (Cl. 6.3 of ISO 15189:2022)

The laboratory shall have a dedicated space for FNAC procedure.

b) Equipment (Cl. 6.4 of ISO 15189:2022)

The laboratory performing Cytopathology tests on CSF and Urine shall use cyto-centrifuge for processing the samples.

c) Pre-examination processes (Cl. 7.2 of ISO 15189:2022)

Where possible, all non-guided FNA shall be carried out by the Pathologist. Guided FNA shall be performed by a team composed of Radiologist & Pathologist. Onsite ROSE, where possible, should be done to establish sample adequacy and records maintained. In the absence of a Pathologist, a clinician / radiologist may perform FNA. Cytology samples intended for other ancillary testing (Molecular, Flow Cytometric, Microbiological) should be collected as per the guidelines defined in respective disciplines.

Cytology request form shall contain the following in addition to the requirements of the Standard:

i. **Non-Gynaecologic Cytology:**

- Relevant clinical history and clinical findings with provisional diagnosis
- Anatomical site of collected specimen
- Information regarding previous cytology report

ii. Gynaecologic Cytology:

- Details of menstrual phase and hormonal status
- Details of hormone therapy
- Details of contraception
- Details of previous surgery

iii. Intra-operative imprint/ aspiration cytology:

Detailed surgical information observed at the time of procedure.

d) Examination processes (Cl. 7.3 of ISO 15189:2022)

- All exfoliative cytology slides shall be stained by Papanicolaou technique.
- Non gynaecologic cytology slides FNAC, fluids, Sputum, urine, EBUS-TBNA, etc., shall be stained by May-Grunwald Giemsa /Giemsa with or without PAP / H & E.
- For all serous effusion fluids & FNA material, the laboratory is encouraged to do Cell Block and incorporate the results of it in its report.

e) Ensuring the validity of examination results (Cl. 7.3.7 of ISO 15189:2022)

- Daily check of the quality of staining/s shall be done and recorded with comments on cytoplasmic, nuclear & background staining.
- Frequency of change of reagents and stains shall be changed based on workload, and records shall be maintained.
- pH of the wash buffer to be maintained for May-Grunwald Giemsa or other stains where Giemsa is a part of the stain.
- Volume of workload for each cytoscreener per day shall be maintained.
- For gynaecologic screening program, appropriately trained cytotechnologists to be employed provided a pathologist review at least 10% of all negative smears. For Gynaecological screening program, ASCUS/SIL ratio as per Bethesda criteria shall be followed.

f) Post-examination of examination (Cl. 7.4 of ISO 15189:2022)

i. Reporting of results (Cl. 7.4.1 of ISO 15189:2022)

- In all cases where the aspiration has been done outside the lab, reference to this shall be mentioned in the report.
- Wherever relevant, reports should include a comment on sample adequacy with reference to standard guidelines.
- Cytoscreeners can be authorised to release all negative cervical cytology reports. All positive reports shall be reviewed and released by a pathologist.
- When fresh smears from the same patient are received; rescreening of previously reported slides to be done; discrepancies if any are to be reconciled.
- Recommended to follow current guidelines for reporting in cytopathology.
- For cervical, vaginal smears & thyroid FNAC the Bethesda System for reporting shall be followed.
- For intra-operative cytology, TAT shall not exceed 20 minutes.
- The turnaround time for all other cytology specimens shall not exceed 3 working days.

ii. Storage period of examined specimen

The examined specimens shall be stored for re-examination and/or additional tests for a minimum period as specified below:

- Fluids – 24 hours at 2-8°C.
- IQC slides should be stored for at least one week.

For issue of tissues, slides/blocks, refer to Histopathology discipline guidelines.

7.7 FLOW CYTOMETRY

a) Personnel (Cl. 6.2 of ISO 15189:2022)

The personnel responsible to report, review and authorize the results needs to have training in institutional or reputed flow cytometry laboratory.

b) Pre-examination processes (Cl. 7.2 of ISO 15189:2022)

All samples including bone marrow are precious and efforts shall be made to assay and do possible interpretations before completely rejecting a specimen.

Blood & bone marrow samples should be transported and stored at room temperature (20-24°C). They should reach the testing laboratory within 12-24 hours. Fluid samples, aspirates and suspected cases of Burkitt lymphoma should preferably be transported at 2-8°C. CSF samples should be processed within 1 hour of lumbar puncture; otherwise, they should be stabilized to avoid deterioration of cells due to the rapid in-vitro cytotoxic effects of CSF on leucocytes. They can be stabilized using transport media like RPMI with 5% fetal bovine sera or any other validated transport media. If acquisition is expected to be delayed, lysed & stained samples should be re-suspended in buffered-formaldehyde solution (fixative) and stored at 2 -8°C. Samples should not be frozen. For Paroxysmal nocturnal hemoglobinuria (PNH) studies, analysis of granulocytes should ideally be carried out within the first few hours of collection (maximum of up to 24 hrs.).

c) Examination processes (Cl. 7.3 of ISO 15189:2022)

i. Viability Testing:

The laboratory shall have a procedure for evaluation of viability. Viability testing may be done using dye exclusion methods (e.g., Trypan Blue) or by using DNA binding dyes (e.g., 7AAD). Laboratory should define the viability cut-off.

ii. Leukemia / Lymphoma Immunophenotyping:

Immunophenotyping is best done within 48 hours of sample collection (peripheral blood / bone marrow). Delayed processing may lead to degeneration. The laboratory shall establish procedures to ensure that viable cells are analyzed. It does not imply that all specimens with low viability must be rejected. If specimen viability is below the laboratory's established minimum criteria, test results may not be reliable, and this shall be noted in the test report. Routine viability testing may not be necessary. However, viability testing is recommended in specimens with a high risk of loss of viability, such as FNAC samples, stored fluid samples and disaggregated lymph node specimens.

iii. Antibody panels:

- Comprehensive panel of antibodies that covers common subtypes of hematolymphoid neoplasm shall be available based on the updated

guidelines and published literature. Laboratory can use either manufacturer's recommended volumes or in-house tittered volumes of antibody. It is recommended to determine in-house titrated volumes for antibodies to avoid suboptimal results. Antibody titration shall be done to calculate signal to noise (S/N) ratio and concentration with maximum S/N ratio should be used as staining volume for required number of cells in the assay. Clinical as well as other findings including morphology are very useful in deciding on a particular panel of antibody. Hence, it is recommended to examine morphology of the samples, if available, prior to the suggestion of antibody panel in a given sample. If antibody cocktails are used, verification of antibody cocktail should be performed using suitable control samples and it is advised to verify cocktail stability using expression level for each marker on internal positive control cell population. Reduction in number of samples requiring repeat processing and also reduction in repeat procedures can be monitored as quality improvement indicators.

- A minimum of “four color” immunophenotyping shall be used for immune cell subset analysis and diagnosis and subtyping of hematolymphoid neoplasms.
- For CD34+ stem cell enumeration, appropriately conjugated Class II or Class III anti-CD34 monoclonal antibodies to be used.
- PNH analysis has been divided into routine analysis (defined as identifying an abnormal population of 1% or more) and a high-sensitivity assay. A high sensitivity assay is recommended which can identify PNH clone size in red blood cells and white blood cells (WBCs, neutrophils and monocytes) down to a lower limit of quantification (LLOQ) of 0.01% for RBCs and 0.05-0.1% for neutrophils. Laboratory shall clearly mention in its scope whether it is doing routine analysis, high sensitivity assay or both. Detection of PNH clone size only based on RBC analysis is not recommended. Both neutrophils and monocytes lineages should be assessed for presence of Glycosyl Phosphatidyl Inositol (GPI) deficient phenotypes. Fluorescent Aerolysin (FLAER) based approaches are preferred for high sensitivity assays. PNH method validations must include both positive and negative samples. The LOD and LLOQ should be established by the lab doing high sensitivity assay for PNH.

iv. Cell cycle analysis and DNA ploidy:

- It is recommended to perform DNA-ploidy analysis simultaneously with immunophenotyping that helps in isolation of tumor cells from rest of the background cells.
- Minimum of “eight color” immunophenotyping shall be done for minimal/measurable residual disease in acute leukemia and multiple myeloma.

v. Cell concentration:

- It is important to define the cell concentration to be used per assay tube for a given assay.
- A recommended cell concentration of 0.5 - 1 million (0.1 -1 x 10^6) cells per assay tube should be used for diagnostic immunophenotyping of hematolymphoid neoplasm, of 2 - 5 million (2 - 5 x 10^6) cells per assay tube for minimal/measurable residual disease for acute leukaemia, and of 3 - 5 million (3 - 5 x 10^6) cells per assay tube for minimal/measurable residual disease in multiple myeloma. For DNA ploidy analysis, the cell concentration of 0.1 - 0.5 million (0.1 -0.5 x 10^6) cells per assay tube should be taken.
- It is important to note that as antibody staining is mainly volume dependent, the sample volume in the assay remains constant. For fluids, aspirates & specimens with low counts, lower cell concentration may be used and restricted panels may be applied as per the clinical scenario & morphology. The laboratory shall document cell concentration policy.

vi. Sample/Data acquisition:

In screening of peripheral blood/ bone marrow samples in a new case of hematolymphoid neoplasms, at least 10,000 total viable events should be acquired for each tube which should contain a minimum of 500 events of tumor cells/ blasts/ atypical lymphoid cells. More events may be acquired if there is marked degeneration of sample or when rare populations are being evaluated.

vii. CD4 counts:

For single platform measurements, manufacturer guidelines shall be followed.

viii. CD34+ stem cell enumeration:

A statistically valid number of CD34+ events are collected to ensure clinically relevant precision and accuracy. To achieve this precision, a minimum of 100 CD34+ events should be counted, as recommended by the ISHAGE guidelines.

Minimal/measurable residual disease: It is recommended to acquire adequate number of viable events for MRD studies to reach the requisite sensitivity of the MRD assay as per the LOD and LLOQ defined from published literature/ guidelines or established by the laboratory itself.

ix. DNA ploidy:

It is recommended to acquire a minimum of 500-1000 viable events of tumor cells and control cells (lymphocytes) to obtain adequate peak of DNA staining signal and optimum CV. The cell acquisition rate for DNA ploidy analysis should not be more than 200 cells per second.

x. Gating strategies:

Each laboratory shall define appropriate gating strategies for different lesions. CD45 versus light scatter gating is a must. For CD34+ stem cell enumeration sequential (Boolean) gating systems shall be followed. CD45/ CD3 gating is essential for CD4 subset enumeration.

In samples with the recent history of patients receiving monoclonal antibody therapy, a gating strategy using alternative gating markers should be included. For example, for anti-CD20 therapy, a gating strategy based on alternative gating markers such as CD19, CD22, etc. should be used.

xi. Quadrant markers:

Threshold determination for positive or negative population shall be based on the knowledge of the cell of interest. Controls such as unstained cells and/or isotype controls are suggested but have limited use and therefore, are not essential.

d) Ensuring the validity of examination results (Cl. 7.3.7 of ISO 15189:2022)

i. Instrument set-up & quality control:

The instrument shall be optimized for optical alignment, electronic standardization, sensitivity / linearity and compensation. Instrument function checks are ideally done by commercially available reference beads.

- The following parameters shall be monitored:
  - Laser current and laser power
  - PMT Voltages
  - Fluorochrome sensitivity
  - Laser delay (applicable on multi laser instruments)
  - Window extension (when applicable)
  - Area Scaling Factors (applicable on digital flow cytometers)
  - Fluidics
  - Percentage CV for detectors

- Fluorescence intensity: Percentage difference of median channel values for each fluorescence channel/detector.

- The values for PMT voltages and laser parameters shall be plotted on a LJ chart and monitored. Frequency of performance check: Laboratory shall have a policy on its instrument performance check based on its workload like daily or after every cold start i.e., after complete shutdown and restarting the instrument. If the instrument is not used regularly, it is recommended to do performance checks at least twice a week. It allows to plot a monthly LJ chart for adequate interpretation.

ii. Quality control for reagents and assays:

The frequency of running QC material depends on the type of test being performed.

- CD4 counts: For measurements of CD4+ T-cells, two levels of commercial controls are ideal. %CV should be less than 10%. CD34 stem cell enumeration: It is desirable to do commercial controls for CD34 + stem cell enumeration. %CV should be less than 10%. An assay has to be run in duplicates, so as to avoid random errors.
- Leukemia / lymphoma immunophenotyping: Internal positive controls can be used only for leukemia / lymphoma samples. Such internal control cells are the residual normal hematopoietic cells in the patient's own sample.
- Minimal/measurable residual disease:
  - It is suggested to define the sensitivity of the MRD assay using reference limit of detection (LOD) and lower limit of quantitation (LLOQ) from published literature/guidelines or establishing it by laboratory itself. It is not applicable to AML MRD as AML is a heterogeneous disease with highly variable immunophenotype which does not allow the application of LOD/LLOQ established for one immunophenotype to AML with different immunophenotypes.
  - If laboratory use fixed templates for analysis of control samples (BM samples) for each MRD assay at least every three months to update the MRD templates.
- DNA ploidy: The %CV for control cells e.g., lymphocytes should be less than 5%. A minimum of 70% of cells should be stained with DNA staining dye.

iii. Compensation controls:

The laboratory should have a procedure for multicolor compensation. This can be done manually or by automated methods. Compensation may be done using microbeads (spherobeads) or cells containing mutually exclusive populations of the

same fluorochrome. However, it is important to optimize the settings given by the beads with cells to be used in the actual experiment. Frequency of verifying and modification of compensation settings can be decided by the laboratory. However, it is essential to re-establish compensation values after any hardware change, laser realignment and change in filters, optics or any other such parameters which affect instrument performance. It is essential to note that compensation settings are stable for a given set of PMT voltages. Change in PMT voltages may lead to adverse effects on compensation values and shall be avoided. Laboratory shall recheck the applicability of the instrument generic compensation settings for each assay and if required, to modify these setting using label-specific or tube-specific or post-acquisition compensation.

e) Reporting of results (Cl. 7.4.1 of ISO 15189:2022)

The report shall include name / type of instrument & software used, cell preparation method, gating strategies, number of viable events acquired, and percentage of gated cells examined. It shall also include descriptive information about the immunophenotype of the abnormal cells, if identified and comments necessary to facilitate the interpretation. The details of the antibodies used may be given in a tabulated format along with the interpretation as positive or negative. Stress shall be laid on interpreting the intensity of positivity. While interpreting the intensity of positivity as bright, moderate, variable, subset, partial or dim, etc., the abnormal population shall be evaluated against known normal leukocyte populations. The final impression should be clearly stated along with a differential diagnosis, if required. Comments and suggestions regarding useful follow-up tests or other ancillary techniques should be added. The MRD reports should include the total number of viable cell or nucleated cell events from which MRD has been calculated and number of MRD events if positive. Reports should also include the LOD and LLOQ for each sample defined from published literature/guidelines or established by the laboratory itself. (LOD and LLOQ inclusion is not applicable to AML MRD).

The laboratory is recommended to follow recent WHO guidelines for the classification of hematolymphoid neoplasms.

f) Data Backup and Storage:

All FCS files, final flow cytometric data analysis in the PDF format, and raw files and final reports shall be stored for a minimum period of 5 years, as soft or hard copies, as applicable. The laboratory may consider giving the FCS files (particle data) to the patient on request for obtaining second opinion or for the treatment elsewhere. The laboratory shall have a procedure and maintain a record of the same.

7.8 MOLECULAR DIAGNOSTICS

A molecular diagnostics laboratory shall be designed to minimize the risk of cross contamination and ensure that pre and post amplification guidelines and safeguards are adhered.

7.8.1 PCR based Testing:

a) Facilities and environmental conditions (Cl. 6.3 of ISO 15189:2022)

- **PCR based molecular testing**:

  It is to be performed in physically separated areas, with the complete and effective segregation of post- PCR steps from all the prior steps (nucleic acid extraction, master mix preparation and PCR). The laboratory design shall ensure unidirectional workflow that prevents contamination of samples or reagents. This is more relevant for conventional PCR than for real-time PCR, since there is practically no risk of cross-contamination by the amplified product (which remains in a sealed tube).

b) Equipment (Cl. 6.4 of ISO 15189:2022)

A class II biosafety cabinet shall be available for sample preparation and nucleic acid extraction. All equipment in Molecular testing laboratory e.g., micropipettes, microfuge, vortex mixer, personal protective gear etc. should be dedicated only for the respective areas and should not be moved outside.

c) Pre-examination processes (Cl. 7.2 of ISO 15189:2022)

Appropriate clinical forms shall accompany foetal samples for prenatal diagnosis.

Nucleic Acid Preparation:

EDTA is the preferred anticoagulant for the collection of whole blood and the production of plasma for molecular test methods. Heparin is a PCR inhibitor and negative PCR results from a heparin tube may be interpreted with caution.

Blood for DNA analysis can be stored at room temperature for up to 24 hours or at 2 to 8°C for up to 72 hours prior to DNA extraction. For RNA studies from blood, extraction shall be performed within 6 hours. If extraction of RNA from blood is not possible immediately, sample shall be collected in a tube containing an RNA stabilizing additive. If cells are required to be used as the sample, it can be separated and stored in RNA stabilizing reagent like Trizol at (-)80°C or lower. For RNA studies from plasma, it shall be separated from EDTA blood within 6 hours and aliquoted. Plasma can be stored at 2 to 8°C for up to 5 days and for a longer period at (-)20°C (though (-)80°C is preferred).

Body fluids/ fresh tissue shall be chilled immediately, transported on wet ice to the laboratory and processed for DNA studies. Tissue can be stored at 2 to 8°C

for no longer than 24 hours prior to processing. Alternatively, tissue may be snap frozen at the collection site and kept frozen until further processing.

Long distance shipping of clinical samples for nucleic acid studies shall be in dry ice. If samples cannot be tested immediately, they shall be processed and aliquoted in a biosafety cabinet and stored at (-)80°C.

For cell-free nucleic acid based molecular assays (e.g., liquid biopsy for oncology and NIPT) samples shall be collected in special tubes which limit cellular degradation and resultant release of genetic material into the plasma, keeping the cell-free nucleic acid from contamination during storage, shipping, or batching. The tubes need to be transported as per manufacturer's instructions.

If RNA is to be extracted from a tissue sample, it shall be either snap frozen prior to storage at (-)80°C or lower, placed in a stabilizing solution, or processed for RNA extraction within 1 hour of collection.

RNA/ DNA extraction shall be performed by validated in-house methods or verified commercial kits. Repeated freezing and thawing of samples shall be avoided by prior aliquoting.

The laboratory shall maintain a record of histological assessment of neoplastic cell content for paraffin-embedded tumour specimens from which DNA or RNA is extracted for examination (e.g., EGFR, KRAS or KIT analysis). Acceptance and rejection criteria for tumor content need to be defined based on the assay type and the technology used.

Tris/EDTA (pH 7.4) is considered the preferred buffer for DNA storage, because buffering limits pH variations. Distilled water can be used if DNA is to be used for PCR and/ or endonuclease digestion within a few days after its isolation. To store purified RNA, use sterile hydrophobic, RNAase- free plastic tubes that have not been handled with ungloved hands.

The quality of the sample and nucleic acid extraction should be documented by amplification of a housekeeping gene. As RNA degrades rapidly, the laboratory shall have a policy about the receipt of transported samples for RNA based assays. However, for precious but delayed samples, negative results shall be interpreted with caution. A comment shall be added in the final report highlighting this issue.

d) Examination processes (Cl. 7.3 of ISO 15189:2022)

i. PCR Design and Optimisation

For in-house developed tests, those loci shall be used for analysis which are documented in public databases (NCBI/ Ensembl) or by publication in peer-reviewed scientific literature. Primer/ Probe sequences shall be subjected to a BLAST/ BLAT search to identify other homologous genomic sequences which could interfere with hybridization of the probe to the target sequence and documented. In the data sheet information on primers/ probes, specific sequences, PCR conditions and the size of the expected amplicons shall be included.

For in house assays, all reaction conditions (reagents and thermocycling parameters) shall be established for each molecular assay and documented. Amplicons designed for use in multiplex PCR reactions shall be thoroughly assessed for compatibility prior to use. Optimization shall demonstrate that all amplicons have suitable specificity.

All in-house assays shall be validated prior to use in a diagnostic testing. The validation shall show the amplification of desired amplicon, the sensitivity and specificity, reproducibility of the assay and limitations of the test to meet the requirements of the intended use. Records of validation performed shall be maintained. Peer-reviewed publications by the laboratory, that include the required information, can be considered adequate for validation. However, the verification of the same to be done prior to clinical reporting. Restriction digests shall include control(s) with a documented genotype at the locus tested.

For all qualitative tests that use a quantitative cut-off value to discriminate between positive and negative results; the cut-off value shall be confirmed using all the standards provided in the kit with each lot change or at least every six months whichever is earlier. Internal controls are used in all nucleic acid amplification techniques to detect false negative reactions caused by extraction failure or the presence of an inhibitor, as applicable.

ii. cDNA synthesis and reverse-transcription PCR

When RNA is the starting material, cDNA is first synthesized from the RNA using oligo (dT), random hexamer primers, or mRNA-specific primers. The usual safeguards against contamination by PCR products shall be used.

Reverse-transcription PCR controls shall include controls for positive, normal, and negative (no DNA) reaction controls. A normal control for the specific region of the gene to be analysed shall be included in each assay.

For RNA based assays to detect efficiency of cDNA synthesis, amplification of ubiquitously expressed endogenous “housekeeping” genes as recommended in literature shall be monitored.

iii. Real-time PCR (RT-PCR)

For quantitative methods like real-time PCR, a standard curve shall be generated initially using the complete set of standards, after which a minimum of 3 standards which cover the range of testing (lower limit, upper limit, and an in-between value), shall be included with each run. Data from repetitive runs shall be used to monitor the %CV of the assay (monthly LJ chart).

iv. Post-PCR

A variety of detection systems can be employed post-PCR for molecular testing. These include gel and capillary electrophoresis, membrane hybridization, microarrays, and real-time amplification. These systems shall be validated and well documented for each assay and appropriate controls be used with each run.

The laboratory shall demonstrate that a level of specificity characteristic of the selected detection system has been attained internally and that the level of specificity is adequate for detecting the expected products.

v. Storage of samples

Unlabeled PCR products can be stored for a maximum of 72 hrs. at 2-8°C; fluorescent labeled PCR products/ cycle sequencing reaction products shall be stored at -20°C for a maximum of 48 hrs., if not immediately processed.

Laboratories where-in post PCR steps utilizes intercalating dyes like ethidium bromide (EtBr a carcinogen) to visualize the PCR product (amplicon), care shall be taken while handling the dye with all personnel protective equipment. The laboratory also shall have a system for the appropriate disposal and shall follow the BMW guidelines for cytotoxic materials. Buffers and liquids containing EtBr shall be collected in a dedicated EtBr waste container and stored in the chemical waste accumulation area and shall be treated with activated charcoal prior to the disposal in the drainage. Electrophoresis gels and other solids containing EtBr shall be placed in an appropriate colour coded bag marked with cytotoxic waste symbol. Dyes such as SYBR Safe, GelRed, GelGreen, and EvaGreen, that are safer alternatives to EtBr, should preferably be used instead of EtBr.

7.8.2 Molecular Genetics

a) DNA Sequencing Analysis

Each laboratory shall validate this technique for each gene to be analyzed. Validation with known mutations as well as normal samples is required. Results of validation studies for each gene analysed shall be available for review.

Verification of sequence data using data obtained from sequencing the opposite strand and/ or a second sequencing reaction is required. Some mutations may be missed if sequencing is performed in only one direction. For direct sequencing, a second PCR amplicon shall be used for repeat sequence analysis.

Base differences are correlated with the known gene structure and other relevant data, and the likely effect of the base change on the gene is predicted. The laboratory must follow the HGVS nomenclature (http://www.hgvs.org/rec.html) for reporting genetic variations. Standard databases and software can be used for any interpretation.

During assay validation, testing is undertaken to determine the estimated lower limit of detection for sequencing performed on mixed populations of cells (for example, in tumour samples), and the limit of detection should be available. Generally, 20% Variant Allele Frequency (VAF) is the LOD for sequencing assays and hence care should be taken to ensure appropriate tumor cell percentage in cells, tissues, or the area of the slide from which the DNA is extracted. Sequencing assays are optimized to minimize background noise and achieve high signal to noise ratios to ensure a readable signal throughout the length of the target region. The ACMG guidelines should be utilised for germline variant classification and interpretation in hereditary diseases. Similarly, AMP recommendations should be followed for the categorization and interpretation of somatic variations.

b) Prenatal Genetic Testing

Prenatal testing shall be carried out in centres with valid license from Ministry of Health and Family Welfare as per Pre-Natal Diagnostic Techniques (Regulation and Prevention of Misuse) Act, Government of India.

For each prenatal genetic test, the laboratory shall determine the appropriate prenatal specimen and specify the amount of material required for testing. If there is sufficient material and whenever possible, prenatal testing can be performed in duplicate using DNA extracted from two separate specimens.

It is recommended that the mutation status of one or both parents, as appropriate, be tested prior to testing of foetal specimens, preferably within the same laboratory.

MCC (Maternal Cell Contamination) represents a potential source of error in prenatal diagnosis. The laboratory shall have procedures in place to assess the presence and level of MCC. A combination of several polymorphic STR (short tandem repeats) or VNTR (Variable number tandem repeat) loci is recommended for ruling out MCC. However, some of these cases require a paternal sample to complete the testing for MCC. The validation of MCC assays shall include sensitivity studies to determine if the appropriate levels of MCC can be detected.

7.8.3 Quantitative Fluorescent PCR (QF-PCR) for pre-natal testing

a) Pre-examination processes (Cl. 7.2 of ISO 15189:2022)

Informed consent from the patient is required.

A laboratory performing pre-natal testing must abide by the PCPNDT act and pertinent amendments. For samples from twins, twin identifiers should be used, to the extent possible.

b) Examination processes (Cl. 7.3 of ISO 15189:2022)

- Commercial assays as well as published primer sets shall undergo verification on positive and negative samples in the laboratory.
- Noncommercial markers that have not been previously reported shall be validated on samples from 50 unrelated individuals.
- Both minimum and maximum peak heights for interpretation shall be established.

i. Assay design

- All prenatal samples shall be tested using markers for chromosomes 13, 18, and 21. Sex chromosomal markers are optional but shall be included where there is a suspicion of sex chromosomal anomaly.
- Markers for chromosomes 15, 16, and 22 are desirable when the test is done on products of conception and foetal tissue.
- At least 4 markers must be used per chromosome.
- For sex chromosomal testing, there must be at least 4 X chromosomal markers and 2 Y specific markers. A non-polymorphic marker such as amelogenin should be used to assess the X:Y ratio as well as at least one X counting marker.
- Markers should, as far as possible, not be clustered but be dispersed across the length of the chromosome.

- For in-house assays, basis principles of multiplex design are to be followed. Markers should be chosen to avoid homology to repetitive elements, CNVs and SNPs as well as primer-dimer interactions.
- The assay should be optimized for the detection of mosaicism. Markers that show a high degree of homozygosity in the population are to be avoided as they produce uninformative results.
- Di-nucloetide markers should be avoided as they tend to produce stutter peaks.

ii. Processing

- Procedures that minimize the risk of maternal cell contamination should be followed when collecting and processing the sample.
- For chorionic villus samples (CVS), multiple villi from different sections of the specimen should be sampled. This will minimize misinterpretation due to confined placental mosaicism. It is also recommended that the same pool of villi be used for culture back-up.
- For Amniotic Fluid (AF) blood staining should be noted and documented.
- Processing should be done initially on the uncultured sample. However, it is desirable to have a culture backup. This will enable further investigation of aneuploidies for structural rearrangements that may be inherited. It will also provide a back-up sample in case the DNA is mishandled or insufficient for further testing following a negative report. Also, where there is a high risk or evidence of significant MCC in AF, it may be better to process the cultured sample which is less likely to carry cells of maternal origin.
- For cord blood, the fetal origin of the sample shall be confirmed. The Keilhauer test or fetal genotyping may be used for this.
- An H2O blank is to be included in every run. Positive controls need not be included in every run but it is desirable to monitor assay performance by running positive samples repeatedly on an intermittent basis.
- Ratio ranges for normal, trisomic and inconclusive results are to be verified or established.
- It is desirable to establish or verify ratios for different sex chromosomal aneuploidies if they are part of the assay.
- To interpret a result as normal, at least two informative markers consistent with a normal biallelic pattern must be present on each chromosome. However, a single informative marker with other alleles being uninformative may be reported as ‘consistent with a normal biallelic pattern’; however, this should be qualified and ideally confirmed by another modality.

- To interpret a result as representing a trisomy, at least two informative markers should be consistent with trisomy, with all the other markers uninformative.
- When reporting an abnormal result, the identity of the sample should be verified through genotyping against the mother's genotype or by another method.

c) Reporting of results (Cl. 7.4.1 of ISO 15189:2022)

- When reporting abnormal results, the name and cytogenetic location of the informative markers must be mentioned.
- QF-PCR is a standalone test and further testing is not required for confirmation. However, further testing by karyotype may be recommended to detect structural variation, or rule out Confined Placental Mosaicism (CPM). Parental testing may be advised to exclude inherited abnormalities that carry a risk of recurrence.
- In the event of non-concordance with Non-Invasive Prenatal Screening (NIPS), a statement that the QF-PCR diagnostic result supersedes the NIPS screening result should be included.
- Limitations of the QF-PCR should be included in the report. These include being a targeted assay, inability to detect balanced rearrangements, deletions and monosomy, structural abnormalities, and low levels of mosaicism.
- It is recommended that 90% of samples are reported within 3 working days.

7.8.4 Chromosomal Microarray

a) Equipment (Cl. 6.4 of ISO 15189:2022)

i. The following information regarding the equipment /platform shall be available with the laboratory:

- list of probes with their physical co-ordinates as per a specified genomic build and cytogenomic locations.
- region-wise coverage, spacing and density of probes.
- resolution of the instrument.
- all QC undertaken by the manufacturer to assess performance and reproducibility (see validation below).
- parameters specific to a platform (number of consecutive probes, log2 ratio, SNP allele ratios, quality control metrics, etc.) that are necessary to conclude that a copy number call represents true copy number variation.
- confidence intervals at boundaries of copy number variants.
- software algorithm recommended by the manufacturer to maximize performance.

ii. Comparative genomic hybridization (CGH) and single nucleotide polymorphism (SNP) array platforms may be used for constitutional indications. However, arrays using SNP markers have certain advantages including being able to detect homozygosity that may be relevant to uniparental disomy or autosomal recessive conditions. Therefore, it is desirable that the platform used incorporates SNP probes.

iii. The resolution shall be at least 400 kb for both deletions and duplications across the genome. BAC array platforms do not meet this resolution and are therefore not to be used.

iv. The laboratory may establish size restriction for CNV calls which may vary depending on whether it is a deletion or a duplication, or a region with an associated syndrome versus others. The size restrictions applied shall be documented in the procedure manual and mentioned on reports.

b) Examination processes (Cl. 7.3 of ISO 15189:2022)

i. It is preferable to perform the assay on uncultured cells as a routine so as to avoid cultural artefact.

ii. Back-up cultures must be retained for all pre-natal samples undergoing CMA analysis.

iii. It is desirable that prenatal samples are routinely tested for MCC by a rapid method prior to running the assay. If MCC is detected in AF, a cultured sample may be used for the assay.

iv. Mosaicism detected by CMA should be investigated to confirm its presence and level. It may represent a culture artifact (pseudomosaicism), true fetal mosaicism or, for CVS, CPM.

c) Validation of examination methods (Cl. 7.3.3 of ISO 15189:2022)

Validation is specific to each sample e.g., constitutional, neoplastic, prenatal, formalin fixed tissue, etc. For prenatal samples, validation should be performed separately for cultured and uncultured amniotic fluid and CVS.

Software settings should be optimized for aberration detection and then established parameters should be used consistently throughout verification/validation and subsequent testing.

d) Biological reference intervals and clinical decision limits (Cl. 7.3.5 of ISO 15189:2022)

The laboratory may use its own reference set or one provided by the manufacturer. The laboratory should document the source of reference DNA and how it is used. Assay

conditions for the reference set should closely match that of the test samples. Databases referenced for annotation must be updated regularly.

e) Reporting of results (Cl. 7.4.1 of ISO 15189:2022)

- CNVs should be denoted by the latest ISCN terminology.
- The categorization of copy number variants should follow the rules laid down by the American College of Medical Geneticists.
- In case of detection of a pathogenic variant, it is desirable to provide additional information such as name of the syndrome, relevant genes and any useful references.
- Loss of heterozygosity (LOH) should only be mentioned in the report if significantly increased from normal. Cut-offs for this, are to be decided by the laboratory and specified in the report.
- The laboratory should have a defined policy for reporting of benign variants or variants of undetermined significance. The reporting policy for these shall be specified in the report.
- All Clinical samples/extracted nucleic acid for infectious disease molecular diagnostics are to be retained for a minimum period as mentioned below (National / State guidelines or legal requirements would take precedence for duration):

Sample for infectious disease molecular diagnostics	1 year at (-)80°C (aliquoted)
Clinical samples for other molecular diagnostics	5 years at (-)80°C

f) Control of records (Cl. 8.4 of ISO 15189:2022)

It is recommended that the laboratory consider a minimum of 2-year storage of a file type that would allow regeneration of the primary results as well as future reanalysis with improved analytic pipelines.

7.8.5 Next Generation Sequencing:

a) Recommendations for NGS analytical wet bench process:

The overall NGS wet laboratory consists of processes which includes specimen handling, NGS library preparation and sequence generation. The following are the necessary recommendation which may be followed for effective and successful wet laboratory experiments:

i. Specimen type, nucleic acid isolation and quality control:

Laboratories shall identify the relevant sample types needed for the test (e.g., blood, buccal cells, cultured cells, or formalin-fixed, paraffin-embedded (FFPE) or

fresh tissue), the acceptance and rejection criteria for different types of specimens shall be documented and validation should take into account issues relevant to all sample types and nucleic acid isolation methodologies used.

ii. **Target Information**:

The laboratory shall have detailed description of the analytical target regions (for example, genes or organisms in a panel, exome, genome, or other targeted regions such as introns or promoter sites), as well as whether the technique employs a metagenomics approach. The region of the genome that does not fulfil assay quality parameters must be evaluated using a different method or eliminated from the reported target zone.

iii. **Library construction and enrichment:**

The step-by-step library construction procedure shall be defined during the initial validation/verification of the assay and the details of the same should be included in the technical SOP. Protocols for enrichment of the target regions either by amplification based or hybridization-based capture shall be defined and If appropriate, methods and reagents for depletion of host or undesired nucleic acids (e.g., oligonucleotide-based depletion) should be included.

iv. **Assay multiplexing:**

In case of assay multiplexing, detailed information about the use of the molecular barcodes/index and its protocol for sample pooling shall be made available.

v. **Use of control sample**:

The laboratory shall put emphasis of use of appropriate controls during the analytical wet bench process, as applicable (for example, controls that show detection limits, controls to ensure adequate nucleic acid extraction and detection of specific taxonomic classes, such as viruses, bacteria, mycobacteria, and fungi, or a control(s) with known variant(s).

vi. **Sequencing platform and reagent versions:**

The laboratory shall have processes to keep track on the manufacturing versions of the sequencing reagents and disposables (e.g., Chips, flow cells, reagents etc.). The instrument software versions used to generate on-instrument (primary) data and output format (e.g. FASTQ files) need to be updated and documented periodically.

vii. **Acceptance and rejection criteria:**

The acceptance and rejection criteria for the sequencing data which is generated after the sequencing run is completed shall be defined and validated using metrics and quality control parameters established during test optimization.

viii. Criteria for identifying when the analytical wet bench method fails, and the specimen is not processed further, shall be included. These may include, but are not limited to:

- post-fragmentation nucleic acid size distribution
- pre-capture library concentration and size distribution
- post-capture library concentration and size distribution
- final library quantification
- flow cell cluster density
- overall chip loading and live ISPs percentage
- number of Test fragments
- sequence read base quality scores
- sequence reads passing instrument quality filters
- total numbers of sequence reads per target (on-target)
- uniformity of the sequencing
- error rate

b) Recommendation for NGS analytical dry bench (Bioinformatics) process:

The laboratory shall document the procedure that describes the steps involved in the bioinformatics process (also known as the bioinformatics pipeline) used to analyze, interpret, and communicate NGS test data.

i. Sequence alignment

To evaluate the acceptability of a sequence alignment for variant calling, key quality measures for alignment (e.g., mapping quality scores or % alignment (mapping fraction)) should be defined and thresholds specified. Assessment should be made to reduce the likelihood of inaccurate variant calls by investigating genomic areas with known homology, such as pseudogenes or segmental duplications.

ii. Variant calling

A bioinformatics pipeline should include algorithms for detecting various forms of clinically relevant variants from NGS data (SNVs, indels, CNVs, and so on). Laboratories should define the largest and smallest size limits of INDEL/CNVs that may be successfully detected. HGVS terminology should be utilized for validation and reporting purposes, with the most 3′ position of the reference sequence taken into account for INDEL representation. During validation, the laboratory should determine the detection reliability for each expected variation type, as well as associated limitations, if any.

iii. Analysis of variant allele frequency

Laboratories shall define the variant allele frequency range corresponding to the heterozygous and homozygous states for hereditary diseases. However, in the case of acquired cancer or mitochondrial disorders, laboratories shall identify the lower limit of variant allele frequency detection as well as the estimated lower limit of variant allele frequency detectable by the assay. The laboratory shall create an internal guideline for dealing with potential somatic mosaicism in inherited disease testing.

Variants in acquired cancers and mitochondrial genome diseases can be found at variant allele frequencies ranging from 1% to 100%. Thus, laboratory should define condition in which additional verification is required to distinguish the low frequency variants from instrument errors.

iv. Data storage

The decisions regarding retention of files shall consider the patient's context and legal obligations. The final significant file format which needs to be stored is VCF file containing variant information. The latter should be retained for a minimum of 5 years, however in the case of testing of minor, it should be stored for longer time i.e., at least for 5-10 years. In addition, some form of raw data (e.g., fastq/SAM/BAM files or compressed versions thereof) should be stored for at least 5 years. These files retention period can be further increased based on annual turnover and the capacity of the laboratory to handle big data.

c) Recommendation for interpretation and reporting of NGS results

The following are the necessary recommendations, which may be followed for effective interpretation and reporting of NGS results:

i. Variant interpretation and reporting:

The interpretation and reporting of sequence variations adheres to the recommendations and guidelines of professional organizations. Human sequence variants must be reported using HGVS nomenclature as well as a standard versioned reference identifier to the transcript/protein (e.g., Ref Seq Accession Number, Ensemble Transcript) that enables unambiguous mapping of the variant. It is necessary to indicate the reference genome assembly and version number used for alignment and variant calling. The genomic coordinate of a variant chromosomal location should be supplied. The ACMG guidelines should be utilized for germline variant classification and interpretation in hereditary illnesses. Similarly, AMP/ASCO recommendations have been developed for the categorization and interpretation of somatic variations.

ii. Reporting of Incidental or Secondary Findings:

The laboratory shall have a written policy for reporting findings that are unrelated to the clinical purpose of the testing (for example, incidental or secondary findings). Laboratories may follow updated ACMG recommendations as applicable from time to time, for reporting a specific number of genes or design their own reporting policy with availability of informed consent from the patient.

iii. NGS report:

The laboratory shall report the findings of NGS in the form of a diagnostic report that should have information such as patient demographics, details of the test ordered, number of genes covered in the panel, details of the variant identified and its interpretation including variant classification, test methodology and the limitation of the test.

The person analyzing and interpreting NGS data prior to reporting shall have the necessary competence, with evidence of training in this activity.

d) Recommendation for verification/validation of NGS test

Laboratory director to give a rationale on the number of cases to be included for verification/validation of NGS test when there is a deviation from the standard guidelines. However, for validation and verification of the NGS and other molecular assays, the laboratory shall include a minimum of twenty specimens (mix of positive negative and reference standard), addressing the accuracy, precision, sensitivity, specificity and limit of detection of the assay. For validation and verification of the Oncology panels, the laboratory may refer to “A Joint Consensus Recommendation of the Association for Molecular Pathology and College of American Pathologists; PMID: 28341590”.

7.9 HISTOCOMPATIBILITY AND IMMUNOGENETICS

a) Personnel (Cl. 6.2 of ISO 15189:2022)

The person who is responsible for the HLA activities and signing out reports needs to have had training in a Histocompatibility laboratory with reporting in parallel with an expert of at least 50 HLA crossmatches, 50 molecular typing (SSOP, SSP, SBT) and at least 10 NGS runs depending on which area the training is focused on for authorization of reports.

The laboratory shall ensure participation of the laboratory personnel in CEPD programmes.

The desirable requirements for CEPD per year are as follows:

Laboratory personnel	Training duration
Person to report, review and authorize the results/ Technical Manager	48 hours
Technical supervisor/scientist	24 hours
Technical personnel	12 hours

b) Pre-examination processes (Cl. 7.2 of ISO 15189:2022)

i. In addition to the standard requirements for request form the following information should be included for HLA:
   - Patient and donor information including relationship.
   - Sensitization history such as previous blood transfusion, pregnancy or transplant and the last dialysis date.

ii. Any other information as required by the latest Organ Transplant Act & State and National regulations to be followed.

c) Examination processes (Cl. 7.3 of ISO 15189:2022)

HLA typing:

This covers requirement for serological, flowcytometry & molecular typing relevant to HLA.

i. HLA typing by serological methods

HLA antigen typing by serological methods is no longer an acceptable method as per the provisions of the Transplantation of Human Organs and Tissues Rules, 2014 (http://notto.nic.in/act-end-rules-of-thoa.htm). However, it may still be used for diagnosis of disease association and other non-transplant related needs (e.g., HLA-B27 testing).

For cytotoxicity-based HLA testing the specific reactions must be recorded to indicate percentage of cell death and interpreted according. Background reactions with Negative control sera shall not exceed 20% and use of negative

and positive control antisera is essential for each test. The laboratory has to ensure that each new batch of rabbit complement used is evaluated to determine that it can mediate cytotoxicity with commercially available or previously validated complement along with Negative and Positive control antisera. Results of such validation are to be recorded.

ii. Molecular testing for HLA alleles

- DNA-based HLA typing is mandatory for donor selection for patients requiring organ or hematopoietic stem cell transplant.
- Suitable methods of DNA storage shall be available to preserve integrity of the material if not used immediately or kept for future use.
- The laboratory may select one or more of the following techniques based on the clinical need;
  - Specific oligonucleotide probe hybridization (SSOP/SSO)
  - Sequence-specific primer amplification (SSP)
  - Sequencing-based typing (SBT)
  - Next Generation Sequencing (NGS)
- High accuracy and high-resolution commercial HLA typing kits utilizing these techniques are available with several manufacturers. Standard protocols are to be followed for all molecular methods mentioned above and minimal requirements for test validation need to be performed prior to use and records of validation maintained. The laboratory shall use internal quality control (Negative, Positive or Disease specific) in each run.
- The method of allele assignment must be designated and the Database of HLA sequences updated to the most current version of the IPD-IMGT/HLA database for reporting.
- Laboratories using NGS technology for HLA should be aware of the extent of the sequencing capacity of the kit being used – specific exon /entire genome which will determine the resolution of the HLA typing. Validation to cover representative alleles for patient and donor population for at least 20 samples though 50 samples is ideal. Validation must be performed on well characterized samples from any repository maintaining high resolution HLA genotyping data. Follow the SOP based on the kit being used and document the minimum quality control required for validating each run prior to interpretation.
- Manual or electronic data must be stored for a period of 10 years or as per regulatory requirements.

- For more details refer NGS under molecular discipline but keep in that mind that the HLA kit comes with clearly defined protocols which have to be adhered to.

iii. HLA cross matching

The HLA cross match test for determination of the antibody status requires an acceptable donor cell population. Depending on the test needs, the laboratory shall use donor peripheral blood lymphocytes, separated peripheral B lymphocytes, separated peripheral T lymphocytes, chronic lymphocytic leukemia (CLL) cells, splenic lymphocytes, lymph node lymphocytes or lymphoblastoid cell line.

iv. CDC cross match test

- HLA cross-matching for solid organ transplantation shall use either the standard NIH (CDC) micro lymphocytotoxicity method or its variants utilizing the anti-human lymphocyte globulin. For most cases the serological cross-match test may be performed with Peripheral Blood Lymphocytes (PBL) of the donor or purified donor T and / or B cell populations, such that each cell preparation shall have 80% or higher cell purity.
- Laboratory cross-matching policy shall define the recipient serum used (with date) in the cross-match, the dilutions if any done to the recipient's serum, the nature of serum (fresh, stored at 4°C or previously frozen sera) and the donor cellular targets which must include donor T-cells, and may or may not include donor B-cells.
- Use of sulphydryl reducing agents like Dithiothreitol (DTT) with the recipient serum is desirable to rule out Positive reactions caused by IgM antibodies.
- The protocol for final interpretation of the CDC Crossmatch as per standard acceptable practice shall be documented in the procedure manual.

v. Flowcytometry based cross matching

- In flowcytometry based cross-match, a set of fluorochrome labeled antibodies is used to identify the target cell type (T or B) and the isotype of the anti-HLA antibody bound to the target cell.
- The laboratory must ensure the use of proper isotype controls for the secondary antibodies used in each test. Each batch of secondary antibody along with Positive and Negative Control antisera should be validated against an existing batch of such reagents and the results documented.
- The specific parameters necessary to call a test valid should be specified.

- The cut offs for positive T cell and B cell crossmatch must be documented along with the records of validation. The objective criteria for accepting controls should be defined.
- Negative and positive controls shall be run with every individual test and the calculations for the final result should be clearly elucidated. Techniques to increase specificity /decrease background can be used in specific cases when needed based on a validated protocol.

vi. Luminex or Solid phase assays for antibody testing:

- The following tests come under this category Luminex anti HLA antibody screen, Luminex phenotype for panel reactive antibody testing, single antigen-based bead detection & the Luminex crossmatch using donor lysate.
- HLA antigens or antigenic epitope determinants coupled to fluorescent microspheres can be used to evaluate presence or absence of anti-HLA antibodies in serum samples.
- Calibration and verification of the instrument to be performed as per manufacturer's recommendation, once a week or on the day of use whichever is more frequent.
- Cut offs for interpretation needs to be documented.
- Ideally a combination of different platforms -serological, solid phase crossmatch, flowcytometry should be used to assess the patient's anti HLA antibody profile prior to transplant. Assays such as the Luminex Donor specific antibody crossmatch using Donor lysate has its own limitations such as lack of reproducibility and specificity and is not the most easily standardized but is sometimes used in resource constrained settings. It is therefore not a test to be used in isolation to detect the presence or absence of antibodies but could be used as a complimentary test and not a substitute for more sensitive tests such as the Single antigen bead assay.
- An in-depth understanding of the sources of error of all tests should be documented and taken into consideration at the time of interpretation and release of reports.

vii. Interpretation of Data (HLA)

- All results shall be checked by two individuals (identified in records) independently, one of whom must be the person responsible to review, report and authorize the results.
- Interpretation in each area should be as per standard guidelines and nomenclature such as ASHI/ EFI/ Any other approved guidelines. All

questionable or inconsistent data shall be resolved by either repeating the assay or using an alternative method.

- It is recommended that the immunological risk be assessed and categorized prior to transplant (Refer British Transplantation Society Guidelines, July 2015 with the latest review in 2020; Guidelines for the detection and characterization of clinically relevant antibodies in allotransplantation).

d) Ensuring the validity of examination results (Cl. 7.3.7.3 of ISO 15189:2022)

Negative and Positive controls are to be used for cross matches, antibody testing and HLA disease risk testing and where appropriate. Laboratory needs to document and specify the internal control practices as per the test method used. Performance of controls used in each kits /assay (in-built or additional) should be documented including acceptable cut off values, interpretation and troubleshooting.

e) Post-examination processes (Cl. 7.4 of ISO 15189:2022)

i. Reporting of results

- In addition to the requirements of the standard, the report shall include the following:
  - Methodology: Summary of the methods used, Loci/Disease locus tested, test performed.
  - Limitations of the assay (if any) which can impact on clinical care.
  - Interpretation of the results within the clinical context indicating the level of resolution that the HLA typing has been done.
  - To conform to the current World Health Organisation Nomenclature for Histocompatibility antigens.
- The HLA laboratory should define critical reporting such as an unexpected positive crossmatch or a de-novo donor specific antibody.

ii. Post examination handling of samples

Retention of samples

CDC cell preparation	24 hours provided viability is within acceptable limits
DNA	5 years at (-)20°C to (-)80°C in aliquots
Patient /Recipient sera	5 years at (-)20° C to (-)80° C in aliquots

(National / State guidelines or legal requirements would take precedence for duration)

7.10 CYTOGENETICS

a) Conformance with requirements (Cl. 5.3.2 of ISO 15189:2022)

A laboratory offering pre-natal genetic diagnosis (PND) or pre-implantation genetic diagnosis (PGD) of chromosomal/ metabolic/ mono and polygenic disorders shall be licensed by the appropriate authority (e.g., Municipal Corporation/ Health Department of Taluka or Zilla, as applicable) for handling fetal and embryonic samples. For PGD, the laboratory shall not perform sex selection before implantation of embryonic cells.

The laboratory shall follow all the rules and regulatory issues of the PCPNDT Act. Form E (form for maintenance of records by genetic laboratory) should be maintained by the laboratory for all samples received for prenatal testing.

b) Personnel (Cl. 6.2 of ISO 15189:2022)

i. The personnel to report, review and authorize the results in addition to the minimum credentials specified in the Clinical Establishment (Registration and Regulation) Act, 2010 and its pertinent amendments, should be able to demonstrate competency in Cytogenetic reporting.

ii. Competency in reporting Cytogenetics may be supported by one or more of the following:

- **Certification:** A person who has completed a certified formal training program in cytogenetics including cytogenetic reporting that is conducted by and within a recognized academic institution, or is recognized by a university or a standard setting organization or regulatory body.
- **Training:** A person who has undergone a period of training in chromosome analysis and reporting with exposure to at least 500 samples that includes sufficient representation of abnormal samples including numerical and structural abnormalities, normal variations, mosaicism, sex chromosomal abnormalities/DSDs, and/or malignancy associated abnormalities. Log books and training portfolios may be evidence for this.
- **Experience:** A person who has already been reporting in the field of cytogenetics for a duration of at least 3 years and has independently reported at least 1000 samples. The competency for reporting cancer cytogenetics and constitutional cytogenetics should be considered distinct and individually attained through exposure/training in the specific areas.

iii. Quality of reporting shall be used as determinant of competence. The following may be evidentiary in this regard:

- Accurate usage of ISCN terminology in reports.
- Delivery of appropriate recommendations where required eg. requirements for further testing
- Discretionary application of protocols that are appropriate for certain common contexts, and reporting such results where required with appropriate information on their significance eg. determining level of mosaicism in prenatal samples.
- Abidance with common established standards and regulations with regard to reporting e.g., means of avoiding disclosure of sex in prenatal samples, reporting only clonal abnormalities, etc.
- Demonstration of familiarity with limitations of the test that he/she is reporting.

In addition to the above, it is recommended that personnel to report, review and authorize the results actively seek to update their knowledge in their field through CEPD programmes. This may take the form of training programs, workshops, CMEs, academic programs, or on-line courses.

c) Facilities and environmental conditions (Cl. 6.3 of ISO 15189:2022)

It is desirable to have a biosafety cabinet (Class II A) rather than laminar air-flow in the culture room for setting up the cultures.

The maintenance schedule and protocol for the Cytogenetics cell culture laboratory shall be documented. Regular cleaning and mopping of the floor are mandatory.

d) Pre-examination processes (Cl. 7.2 of ISO 15189:2022)

A consent form shall accompany all samples that are to be tested for constitutional disorders, including prenatal samples.

The laboratory should ensure prior to processing that clinical details as stated on the request form correspond to the type and quantity of sample received and other pre-examination conditions, and verify that the test requested is the most appropriate for the sample received. Any deviation and its potential impact on testing should be conveyed to the clinician without delay. As cytogenetic laboratories process samples that may be difficult to collect again it may not be possible to avoid processing of samples that do not meet recommended conditions. For example, an amniotic fluid sample that is insufficient for duplicate cultures may be processed as a single culture; however, the implications must be discussed with the requisitioning clinician prior to processing. The intimation of such information is moreover to be maintained as a record by the laboratory.

Analysis of buccal smears for Barr bodies is not recommended for the diagnosis of DSD (Disorders of Sex Development).

In prenatal diagnosis, FISH should not be a stand-alone test but should be offered only as an adjunct test to another testing modality such as karyotyping or QF-PCR.

Aneuploidy FISH is not to be included in the test menu of an infertility work-up.

Recommended conditions for sample collection, transport and storage for conventional cytogenetic analysis are tabulated below. These conditions should be specified in the sample collection manual made available to clients. The process for dealing with deviant samples shall be specified in procedure manuals for sample reception.

Table 6

Tissue	Sample volume	Container	Mode of collection	Transport / Storage
Whole Blood/ Cord Blood/ Bone Marrow	Minimum 2- 3 ml	Sterile green top sodium heparin vacutainer	Sterility must be maintained	Sample to be transported at room temperature and should be processed as soon as possible. In case of delay, sample to be stored either in an air-conditioned room (22-25°C) or on the door shelf of the refrigerator
Chorionic Villus	10-15mg	Sterile 15 ml centrifuge tube or 1.5 ml micro centrifuge tube containing sterile transport medium	Sterility must be maintained	Sample to be transported at room temperature and should be processed as soon as possible. In case of delay in processing, the villi samples to be cleaned and placed along with culture medium (in a sterile petri dish) inside a carbon dioxide incubator. When immediate cleaning is not possible and storage period is longer,

Tissue	Sample volume	Container	Mode of collection	Transport / Storage
				sample should be placed on the door shelf of the refrigerator
Products of conception (POC)	20-30mg	Sterile 50 ml centrifuge tube with sterile saline with few drops of antibiotic or transport medium	Sterility must be maintained	Sample to be transported at room temperature and should be processed as soon as possible. If immediate processing is not possible the sample to be stored on the door shelf of the refrigerator
Other Solid Tissue including Tumors and Skin	4 - 5 pieces, 2 - 4 mm^2 Punch biopsy of skin must include dermis. Lesional and non-lesional skin shall be kept in separately marked containers	Sterile 50 ml centrifuge tube or 1.5 ml micro centrifuge tube containing sterile saline with few drops of antibiotic or transport medium	Sterility must be maintained	Sample to be transported at room temperature and should be processed as soon as possible. In case of delay in processing, the samples to be cleaned and placed along with culture medium (in a sterile petri dish) inside a carbon dioxide incubator. When immediate cleaning is not possible and storage period is longer, sample should be placed either in an air-conditioned room (22-25^{0}C) or on the door shelf of the refrigerator
Amniotic Fluid	10-15ml	Two sterile 15 or 50 ml centrifuge tubes	Sterility must be maintained	Sample to be transported at room temperature
Fine needle aspirates /	5-10 ml	Sterile 50 ml centrifuge	Sterility must be maintained	Sample to be transported at room temperature and

Tissue	Sample volume	Container	Mode of collection	Transport / Storage
Pleural or other fluids		tube		should be processed as soon as possible. In case of delay, sample to be stored either in an air conditioned room (22-25°C) or on the door shelf of the refrigerator

Samples must reach the lab within 48-72 hours of their collection.

The samples for chromosomal breakage syndromes should preferably be collected prior to any blood transfusion. However, this may not always be possible. For stress cytogenetics analysis the sample should be collected with adequate gap post-transfusion and same should be mentioned in the report. Cases tested post-transfusion that in spite of high clinical suspicion, do not demonstrate sensitivity to Mitomycin C/Diepoxy butane, should be subjected to molecular testing.

For metaphase FISH analysis, sample criteria remain as mentioned in Table 6. In addition, stored cell pellets from samples, cultured and harvested for karyotyping, may also be used for metaphase FISH, provided there are sufficient cells.

For interphase FISH blood and bone marrow samples collected in EDTA may be used. Other examples of samples that may be used for interphase FISH include cells that are harvested, fixed in acid alcohol or formalin, frozen, flow processed, or cytospun, paraffin embedded tissue, touch preparations, buccal smears, urine sediment, biopsies and archival fixed tissue. The processing of each type of sample needs to be standardized and validated/verified by the laboratory.

If maternal cell contamination is discovered this information is to be promptly conveyed to the clinician and its possible impact on results is to be discussed. The presence of maternal cell contamination may necessitate the modification of routine procedures. Such modifications should be recorded and their impact on turn-around time should be discussed with the clinician.

If bone marrow aspirate received for cytogenetic analysis is suboptimal (e.g., partially or completely clotted, viscous, haemolyzed, inadequate, etc.,) the referring clinician should be informed immediately. A cell count may be done to check for the availability of sufficient cells. All attempts should be made to salvage a poor sample and obtain relevant information, particularly if it is a diagnostic or pre-

treatment sample. Therefore, additional tests such as FISH analysis may be done as required.

e) Examination processes (Cl. 7.3 of ISO 15189:2022)

i. Conventional cytogenetic analysis:

The recommended number of metaphases for a cytogenetic study to be deemed complete is as follows:

- Constitutional abnormalities, post-natal and pre-natal: At least 20 metaphases are to be counted. A minimum of five metaphases shall be analysed and 2 karyogrammed.
- Disorders of sexual differentiation: Standard 20 cell assessment. If no abnormality is established at least 10 additional metaphases are to be scored for sex chromosomal abnormalities.
- Where mosaicism is strongly suspected, an attempt should be made to score at least 50 metaphases before FISH analysis is recommended.
- For establishing mosaicism in prenatal cases, depending upon the chromosomes involved, more cells shall be scored as per standard published guidelines. The guidelines to be followed in such cases shall be specified in the laboratory manual and shall be made available in the laboratory for easy reference.
- For chromosome breakage studies, the number of cells scored to assess spontaneous chromosome breakage shall be as per the recommendations of the protocol followed and is to be stated in the laboratory manual. Scoring has to be done both in the patient as well as a normal control. Normal control used should preferably be matched for age and gender. The laboratory should have well established ranges for positive and negative samples.
- Definition of a clonal abnormality shall be as follows: presence of at least two cells containing the same extra chromosome(s) or structural chromosome abnormality or by the presence of at least three cells that have lost the same chromosome. Non-clonal abnormalities are generally not to be included in a report unless there is a strong reason for doing so eg., suspicion of mosaicism. If included, they must be qualified as abnormalities for which clonality could not be established.
- For karyotyping in haematological malignancies at least 20 cells should be counted and analysed. When any abnormal clone is identified, at least two metaphases of the abnormal clones (related and unrelated) as well as any sideline clones that may be present should be karyogrammed along with one metaphase from a normal cell. In case no abnormality is detected, a minimum of two metaphases should be karyogrammed. More than 20 metaphases may

sometimes be required to be analysed to establish clonality for a single cell abnormality which is associated with the stated / suspected disease, or if the metaphases are of poor quality.

Note: *For standardization, the terms mentioned above are defined as follows:*

- *Counting: Establishing the number of centric chromosomes per metaphase.*
- *Analysing: Evaluating each chromosome in a banded metaphase in its entirety under the microscope, through photos or using digital imagery.*
- *Karyogram: Arrangement of paired chromosomes according to the ISCN format.*
- *Scoring: Evaluation of cells for a specific cytogenetic feature.*

- Cultures set-up for prenatal samples should be performed in duplicate or independently, where possible.
- For chorionic villus samples (CVSs) it is recommended to establish and analyse long-term cultures, even if short-term cultures are in place as long-term CVS cultures are more likely to represent the foetal karyotype than short-term cells/cultures.

ii. Fluorescent in Situ Hybridization (FISH)

- Slides of interphase and metaphase FISH analysis shall be scored by two readers familiar with the expected signal pattern of the probe used.
- Discrepancies may be resolved by a third reader.
- The minimum number of cells to be counted for interphase FISH analysis is as follows:

  - Constitutional abnormalities except mosaicism studies - 50 cells.
  - Hematological -malignancies - 200 cells.
  - Mosaicism studies - 200 cells.
  - Detection of chimerism following sex-mismatched bone marrow transplants – 500 cells.

- If small numbers of cells with an abnormality are detected, the images of these cells should be saved and their coordinates noted for subsequent identification and verification.
- In Multiple Myeloma, FISH testing should be performed on enriched plasma cells. If the laboratory prefers to perform Myeloma FISH testing based on percentage of plasma cells in the sample submitted, the cut-off criteria for such decision making should be documented along with the method of choice for enrichment.

- For metaphase FISH analysis, clonality is defined according to ISCN guidelines. To demonstrate that an abnormality is clonal, it must be present in a minimum number of metaphases, as described below:
  - Trisomy / structural abnormality - two metaphases
  - Monosomy - three metaphases

- Use of expired probes on patient samples should be strongly discouraged.

f) Ensuring the validity of examination results (Cl. 7.3.7.3 of ISO 15189:2022)

i. Karyotyping

- The laboratory shall include culture failure rates among its quality indicators.
- For all samples, more than one culture shall be routinely set up and samples from more than one culture shall be used in reporting.
- The laboratory shall periodically review the ratio of normal versus abnormal results generated, particularly in leukemia or other hematological malignancies.
- The interlaboratory comparison if done, should preferably be performed on samples or at least processed cell pellet. Sharing of stained slides or images for interlaboratory comparison is strongly discouraged.

ii. Fluorescence in situ hybridization (FISH)

- Specificity of the FISH probes should be verified by using on normal metaphases obtained from healthy male individuals to ensure that the probes are binding to the regions as intended, as well as on known positive controls as and where available.
- Known negative samples should be used to obtain cut off for various clinically relevant signal patterns. The cut-offs need to be defined and documented by the laboratory. Cut off criteria for reporting should be calculated using standard statistical methods, which should be documented in the procedure manual. Records of such calculations should be maintained.
- Use of home-brew probes is not recommended for routine diagnostic application or if therapy is based on the results of FISH analysis.

g) Reporting of results (Cl. 7.4.1 of ISO 15189:2022)

- For interpretation of a cytogenetic study analysis, clinical and family history shall be considered.
- The latest ISCN nomenclature shall always be followed in reporting of karyotyping results.

- The karyotyping report shall specify the number of metaphases counted, analysed and karyotyped.
- For FISH results every attempt should be made to define findings in the ISCN nomenclature. For complex FISH results, it may be permissible to instead represent the results in an unambiguous tabular format.
- In addition to the description of the karyotype / result of FISH or other analysis following the most current recommendations of the ISCN, a clear explanation of the report in words must be included.
- An appropriate comment on the significance of any findings must be included in the report.
- 'Normal variable chromosomal features are not to be mentioned in the karyotype nomenclature routinely, but may be mentioned in the text, notes or comments. The laboratory should have a defined protocol as to whether and where these are reported in the text. If mentioned, an appropriate statement qualifying such findings as polymorphisms/normal variants must be included. The comments should be articulated clearly to mitigate any potential misunderstanding by both the clinician and the patient. Customers must be informed of the laboratory's reporting policy for findings that are considered normal variations.
- Additional tests, if already performed, should be cross-referenced in reports where relevant e.g., for family studies.
- The FISH report shall unambiguously specify the probe used with its target(s) and fluorochrome(s).
- The karyogram and if possible, the designated metaphase may be included in the report.

h) Control of records (Cl. 8.4 of ISO 15189:2022)

The recommended minimum period for retention of records is as follows:

Table 7

Type of Record	Retention Period
Cytogenetic / FISH images / Photographs	5 years
Test reports	10 years or more
Banded Slides	3 years
Log books and other records	3 years
Cell pellets from cancer studies	3 years at 2-4°C or lower (-20°C or lower is recommended if the pellet is to be stored for longer periods of time)
Cell pellets from post-natal constitutional	2 months at 2-4°C or lower. Positive samples

studies	may be retained for longer at the discretion of the laboratory
Cell pellets from pre-natal constitutional studies	One year. Positive samples may be retained for longer as per the policy of the laboratory.
Primary sample comprising blood/Bone Marrow	5 days at 2 to 4°C
Residual cultures	Till the report is finalized

There should be at least 2 image records to document each abnormality and 2 image records for a normal cell line in constitutional cases as well as in oncology testing. Normal cells may not be captured in case of the presence of normal as well as abnormal cells.

The laboratory shall have a policy and protocol for providing original slides or images to patients for obtaining a second opinion or for treatment elsewhere. For cytogenetic studies of leukemia, especially with a low mitotic index, images shall be the first choice since the other laboratory may not be able to locate the metaphases analyzed and considered for reporting.

FISH on Formalin Fixed Paraffin Embedded (FFPE) tissues

- FISH probes that are to be used for testing in solid tumors should be subjected to verification.
- Verification should also include known positive samples wherever available, known negative samples and FISH on metaphase preparation for verifying the specificity of the FISH probe.
- FISH in solid tumors where national or international guidelines are available, the same should be followed for reporting purposes e.g., Reporting of Her2Neu FISH. Where such guidelines are not available, laboratories may use well published cutoff and reporting criteria from reputed medical journals, however the cutoff should be verified in-house as well as documented. The reporting criteria for all the solid tumor FISH testing should be documented.
- 2 to 4µm thick sections should be used for FISH testing on FFPE tissues.
- The H & E section from the FFPE block should be analyzed by a histopathologist for the presence of tumor cells and its suitability of FISH testing. Caution should be exercised in tissue biopsies with limited tissue. In such situations after confirming the adequacy of FISH testing, adequate number of sections should be taken on coated slides. Thereafter the final section should be stained again with H & E to verify that the tumor is not lost while sectioning. If the tumor has been lost due to deeper sections, the immediately preceding section should thus be stained and

examined to ensure that unstained slides that will be used for FISH test ing definitely have tumor, and therefore reportable.

- FISH results should be scored by two independent observers. A third observer may score the FISH slide in case of discrepancy. Minimal number of cells as defined in guidelines as well as by the laboratory should be scored and documented.
- A minimum of two representative images from the FISH testing should be captured and stored.
- The laboratory should participate in EQA wherever available or interlaboratory comparison for all types of solid FISH tumors probes under scope. FFPE blocks or sections on coated slides may be shared for inter-laboratory comparison purposes. Sharing stained slides or images is not acceptable. The results of ILC program should be reviewed by competent personnel and documented.

7.11 POINT OF CARE TESTING (POCT)

Point of Care Testing (POCT) refers to testing performed nearer to the patient and patient bedside. This includes POCT devices used in hospital settings irrespective of its location that excludes devices used for patient self-testing and those in the central testing laboratory.

For molecular POCT, refer to requirements under the section on Molecular Testing.

Based on the complexity of POCT device, categorization is as follows:

Table 8

Category based on complexity	Device	Type of results	Example
Low	Cassettes or single use strips or card test	Qualitative or semi-quantitative; Manual reading of results	Pregnancy card test Fecal occult blood test urinalysis
Moderate	Moderately complex instrumentation	Qualitative or semi-quantitative or quantitative; Device display of results	Automated urinalysis instrument, electrolyte analyzer, HbA1c analyser Glucometer
High	Multiple analytes, multiple cartridges with multitude of internal parts and interface capabilities	Quantitative results; Device display of results	Blood gas analyser Complete blood counter (CBC), Cardiac marker analyser (such as troponins and BNP)

a) Structural and governance requirements (Cl. 5.0 of ISO 15189:2022)

Laboratory Director shall have the overall responsibility of Technical / Advisory / Scientific operations of the POCT coordinating committee. Such a committee should include representatives of those who use the services (physicians, physician assistants), those who deliver the services (nurses, nurse practitioners, health care providers, technical assistants) along with the representative of organization's management team. Committee is responsible for the implementation of the management system, including the application of risk management to all aspects of the laboratory operations so that risks to patient care and opportunities to improve are systematically identified and addressed.

b) Resource requirements (Cl. 6.0 of ISO 15189:2022)

i. Personnel

Testing may be performed by non-laboratory personnel such as physicians, physician assistants, nurses and technical assistants.

ii. Competence requirement

The POCT results depend heavily on robustness of device and competence of personnel. Training strategy for POCT shall include understanding the context of the test (Clinical requirement, action taken on the result provided, nature & method of the test), patient preparation (e.g., diurnal variation, drugs), sample requirement & its collection, preparation of analytical device, performance of Quality control and test along with reporting, interpretation, documentation and health and safety issues. Competency assessment of the operators shall be periodically evaluated for assessing skills and further training requirements.

iii. Training of users

All POCT users shall receive training for the POCT device prior to authorizing the personnel to operate the POCT. Training shall be hands-on approach and must be deemed competent. Operator training is vital for quality POCT programs.

c) Equipment (Cl. 6.4 of ISO 15189:2022)

i. POCT Device

All devices (handheld and bench top) shall have the following features documented:

- Specimen type, sample preparation requirements, test menu and performance characteristics such as accuracy, precision, specificity for the analyte, turnaround time, calibration frequency, potential interferents, calibrators and reagent stability, lot-to-lot variation for reagents and calibrators, and QC requirements, Quality control and operator lockout management and data / software connectivity. It is ideal and preferable to choose a POCT device with analyte principles to be the same as the central clinical laboratory testing facility**.**
- Device verification for analytical performance at the Initial stage of its introduction and ongoing verification shall be done. The minimum guidelines are as follows:

Table 8

	Type of device	**Precision**	**Comparison**	**Linearity**
Initial Device verification	Low	Both negative and positive QC to be run for 5 days	5 abnormal and 5 normal patient samples. Compare to central /clinical lab method	NA

	Moderate & High	Within run (two levels of QC run a total of 5 times in one run)	20 patient samples to ensure it covers the entire range of assay measurement. Compare to central/ clinical lab method	Vendor supplied linearity material to be analyzed in duplicate or series of dilution from a high patient sample and measure 3 levels in duplicate
Ongoing Device verification for additional devices/ backup within an existing POCT program	Low	Do not require further evaluation		
	Moderate	Within run (two levels of QC run a total of 5 times in one run)	5 to 10 patient samples or EQA /PT specimens comparison with central clinical lab method	Required only if linearity performance was marginal during initial verification
	High	Within run (two levels of QC run a total of 5 times in one run)	10 patient samples or QC sample (that span assay measurement range) comparison with central clinical lab method	Vendor supplied linearity material to be analyzed in duplicate or series of dilution from a high patient sample and measure 3 levels in duplicate

- Intra- Instrument comparison for all types of devices should be done using PT sample or split-sample testing once per year with a minimum of two specimens including normal and abnormal specimens. Split-sample testing shall be with another accredited laboratory for POCT.
- Interlaboratory comparison of non-labile parameters shall be done with another NABL accredited laboratory for POCT.

ii. Intra-instrument comparison:

- Within the same organization, the devices located in different areas are compared for the same analytes. Eg. device 1 glucometer located in PICU and device 2 glucometer located in MICU.
- For comparison either a whole blood sample collected from one patient can be aliquoted for testing or finger prick specimen from the same patient to be tested for glucose on both devices.
- For moderate and high complexity devices, Inter-Instrument comparison between POCT and central clinical laboratory shall be performed twice per

year with a minimum of three specimens with low, medium and high concentration (six specimens in total per year).

d) Reagents and consumables (Cl. 6.6 of ISO 15189:2022)

- New lots of reagents and change in spares / consumables should be verified for precision and compared to central clinical laboratory.
- New reagent lot evaluation: Devices with low complexity shall be verified once with one QC material (each of positive and negative QC). Devices with moderate & high complexity shall be verified three times for each level of QC material.

e) Documentation of examination procedure (Cl. 7.3.6 of ISO 15189:2022)

Policies and procedures for all POCT devices shall be documented and a copy made available at all locations using the device.

f) Ensuring the validity of examination results (Cl. 7.3.7 of ISO 15189:2022)

i. Internal quality control (Cl. 7.3.7.2 of ISO 15189:2022)

One level should be performed per shift and same to be documented after verifying for acceptance. Wherever possible, for quantitative parameters, quality control ranges should be established, and variations within ± 3 SD are acceptable. Root cause analysis should be done for all QC results outside of acceptable limits and documented.

New Quality control material lot-to-lot evaluation for low complexity device shall compare one measurement with previous QC material. In the case of moderate and high complexity devices, quality control material lot-to-lot evaluation shall be done by comparing three times for each quality control level.

An infectious disease POCT for antibody shall include anti-human immunoglobulin control.

ii. External quality assessment (EQA) (Cl. 7.3.7.3 of ISO 15189:2022)

POCT devices shall be subject to minimum of two verification/comparison using EQA samples per year or with split samples when EQA is not available (with another NABL accredited laboratory in a different organization).

g) Post-examination processes (Cl. 7.4 of ISO 15189:2022)

i. Reporting of results (Cl. 7.4.1 of ISO 15189:2022)

The result output from POCT device shall be released directly and same shall be documented. Report should include all necessary components such as patient

identification, reference intervals, unit of measurement, date, time, traceable to operator performing test and release of report.

ii. Critical result reports (Cl. 7.4.1.3 of ISO 15189:2022)

POCT program shall have a procedure to notify the concerned authority of results that fall outside of critical decision limits.

h) Evaluations (Cl. 8.8 of ISO 15189:2022)

i. Quality indicators (Cl. 8.8.2 of ISO 15189:2022)

Quality of the POCT process depends on regular monitoring of quality indicators that should include but not limited to positive patient ID procedures, specimen and reagent labelling, performance of QC testing according to the procedure for the device, EQA performance and compliance with policies related to follow-up of results, such as critical results, or results above or below the assay measurement range of the POCT device.

ii. Internal audit (Cl. 8.8.3 of ISO 15189:2022)

Internal Audit shall be performed once a year per POCT program although regular audits are crucial to identify non-conformances and compliance with POCT policies and procedures. Audit should include but not limited to procedure compliance with positive patient identification, performance of QC at defined frequency, documentation of results, evidence of follow-up on results, compliance with POCT ordering procedures and documentation process, labeling and storage of reagents as required and detailed in the procedure.

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