

**NABL 112**



**National Accreditation Board for  
Testing and Calibration Laboratories  
(NABL)**



# **Specific Criteria for Accreditation of Medical Laboratories**

**ISSUE NO. : 04**

**ISSUE DATE : 11-Feb-2019**

**AMENDMENT NO. : 01**

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## AMENDMENT SHEET

Sl	Page No.	Clause No.	Date of Amendment	Amendment	Reasons	Signature QM	Signature CEO
1.	25-28	---	26.04.2019	Alignment of clause no. with ISO 15189:2012	Typographical error	-Sd-	-Sd-
2.							
3.							
4.							
5.							
6.							
7.							
8.							
9.							
10.							

## 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 112 elaborates the International Standard ISO 15189:2012 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**

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## ABBREVIATIONS

AERB	- Atomic Energy Regulatory Board
APAC	- Asia Pacific Accreditation Cooperation
CBC	- Complete Blood Count
CLSI	- Clinical Laboratory Standards Institute
CRO	Clinical Research Organization
CSF	- Cerebrospinal Fluid
CV	- Coefficient of Variation
DNA	- Deoxyribonucleic Acid
EDTA	- Ethylenediaminetetraacetic acid
ELISA	- Enzyme Linked Immunosorbent Assay
EQA	- External Quality Assessment
ESR	- Erythrocyte Sedimentation Rate
FNA	- Fine Needle Aspiration
FNAC	- Fine Needle Aspiration Cytology
FISH	- Fluorescence in situ hybridization
H/h	- Hour(s)
H & E Staining	- Haematoxylin & Eosin Staining
HBV	- Hepatitis B Virus
HGVS	Human Genome Variation Society
HIV	- Human Immunodeficiency Virus
HLA	- Human Leukocyte Antigen
HPLC	- High Performance Liquid Chromatography
ICSH	- International Council for Standardization in Haematology
ILAC	- International Laboratory Accreditation Cooperation
INR	- International Normalized Ratio
ISO	- International Organization for Standardization
ISCN	International Society for Human Chromosome Nomenclature
IVD	In-vitro diagnostics
LJ Chart	- Levey-Jennings Chart
MCH	- Mean Corpuscular Haemoglobin
MCI	Medical Council of India
MCHC	- Mean Corpuscular Haemoglobin concentration
MCV	Mean Corpuscular volume
MNPT	- Mean Normal Prothrombin Time
MRA	- Mutual Recognition Arrangement
NACO	- National AIDS Control Organization
NCBI	National Centre For Biotechnology Information
NCCLS	National Committee for Clinical Laboratory Standards
NIH	- National Institute of Health
PAP staining	- Papanicolaou staining

PCR	- Polymerase chain reaction
PND	- Prenatal diagnosis
PT	Proficiency Testing
QBC	- Quantitative Buffy Coat
QC	- Quality Control
RBC	- Red Blood Cell
RFLP	Restriction fragment length polymorphism
RNTCP	Revised National Tuberculosis Control Program
SCF	Sample Collection Centre/ Facility
SD	- Standard Deviation
SOP	Standard Operating Procedure
TAT	- Turnaround Time
UV	- Ultra Violet
WBC	- White Blood Cells
WHO	- World Health Organization

## 1. INTRODUCTION

Laboratory accreditation activities are administered under the direction of the National Accreditation Board for Testing and Calibration Laboratories (NABL), involving assessment team and accreditation committee as recommending authorities. 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.

The requirements in this document on specific criteria are based on the International Standard, ISO 15189:2012 - "Medical laboratories – Requirements for quality and competence". It specifies requirements for competence and quality that are particular to medical laboratories. The laboratory's compliance to requirements of the standard and its technical competence are assessed by NABL for accreditation.

The specific criteria document must be used in conjunction with ISO 15189:2012. It provides an interpretation of the latter document and describes specific requirements. Further, the laboratory shall follow national, regional, local laws and regulations as applicable.

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## 2. SCOPE

The scope of accreditation is applicable to the following disciplines of medical laboratory:

- i. Clinical Biochemistry
- ii. Haematology
- iii. Clinical Pathology
- iv. Microbiology & Infectious disease serology
- v. Histopathology
- vi. Cytopathology
- vii. Flow Cytometry
- viii. Cytogenetics
- ix. Molecular Testing

**Note:**

- i. *Immunological and serological tests are common to many disciplines; therefore, these can be listed under respective disciplines.*
- ii. *For guidance on preparation of scope of accreditation, refer sample scope given as Annexure-I(a) Sample scope has been detailed for Histopathology, Cytopathology, Microbiology & Infectious disease serology and Cl. Biochemistry. For other disciplines the same format and guidelines shall be followed.*
- iii. *The tests of Nuclear Medicine can be applied for accreditation under the discipline of Cl. Biochemistry.*

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 assured, shall not be included in the scope.

To be eligible for accreditation for disciplines of Histopathology and Cytopathology, a laboratory should receive at least 300 specimens every year in the respective discipline. The latter does not apply to specialized areas viz.: Nephropathology, Neuropathology, where the number of samples received may be fewer. Similarly, in the discipline of Haematology the laboratory should receive at least 100 bone marrow aspiration samples per year for the test to be accredited.

The collection centre / facility for primary sample collection at sites other than its main laboratory shall also comply with the relevant requirements of ISO 15189: 2012.

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### 3. 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 (small / medium / large / very large / laboratory with multiple locations) or the place in which they are located (village / town / district / city) or whether they are private / government / quasi-government attached to a hospital / stand-alone.

The following classification of laboratories shall be used:

- a) Small sized: A laboratory receiving samples of up to 100 subjects per day
- b) Medium sized: A laboratory receiving samples of up to 101- 400 subjects per day
- c) Large sized: A laboratory receiving samples of more than 401-1000 subjects per day
- d) Very large sized: A laboratory receiving more than 1000 subjects per day
- e) Multiple location: A laboratory with more than one location in the same district with same legal identity

## 4. MANAGEMENT REQUIREMENTS

### Organization and management responsibility

A Medical laboratory must produce relevant evidence of legal identification which can be any of the following: Registration under the Indian Companies Act, Limited Liability Act, Partnership Act, Registration of Business as Sole Proprietor, Indian Trust Act, Societies Registration Act, Any Government notification in support of establishment of institution / laboratory or any approval from local or regulatory bodies. Laboratory shall also comply with local / regional / national requirements.

A laboratory operating at more than one location within a district having the same legal identity will be considered as a single laboratory, provided the analytes measured at different sites do not overlap and the laboratory will be issued a single certificate. For example, facility for cytogenetics could be at another site. However, if a laboratory requires separate certificates for individual location, separate application for accreditation should be submitted for each location.

For Multiple location laboratory, a single certificate will be issued with main laboratory's address on the accreditation certificate and details of all locations in the annexure (i.e. scope of accreditation).

Laboratories operating at more than one location having separate legal identities will be treated as independent laboratories even though they may be part of same organization.

Laboratories having one legal identity but operating in different districts will be treated as independent laboratories even though they are part of the same organization.

The accreditation certificate for a laboratory cannot be transferred and shall be valid only for premises for which it is issued.

### Qualification norms for Laboratory Director / Chairman / Head (howsoever named)

The qualifications of Laboratory Director / Chairman / Head (howsoever named) shall be same as given in 5.1. She / He shall have the overall responsibility of Technical / Advisory / Scientific operations of the laboratory.

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The Laboratory Director / designee shall also fulfill the other requirements of ISO 15189:2012. In the case of a laboratory where there are more than one person designated as Laboratory Director, one of them should be available to ensure that she / he is responsible for overall operations.

In a hospital setting or in a large or very large laboratory, each department / discipline may have a separate head. However, one of them, represented as Laboratory Director shall be available at all times for consultation.

#### Requirements for Quality Manager

Quality Manager / designee shall be trained in 4-days Quality Management as per ISO 15189. She / He should be a full time employee, and can be delegated with additional responsibilities.

#### **Service agreements**

The users of laboratory shall be explicitly informed about the non accredited status of tests requested while entering into contract. This may be done by providing separate lists of accredited and non accredited test parameters to users. A copy of accredited scope shall also be made available for reference.

#### **Examination by referral laboratories**

Referral laboratory is an external laboratory to which the laboratory management chooses to submit a sample or subsample for examination or when routine examinations cannot be carried out. This differs from a laboratory that may include public health, forensics, tumor registry, or a central (parent) facility to which submission of samples is required by structure or regulation.

NABL allows referral for second opinion for the tests of Histopathology, Cytopathology, Bone Marrow examination, Genetic tests and also for supplementary tests. Referral may also be required for confirmation of Biochemical, Microbiological and Haematological tests. The referral laboratory has to be NABL accredited.

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.

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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 information is kept both in the 'referral' file and the patient file.

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.

*Note: The laboratory shall produce:*

- 1. Records of evaluation of the referral laboratories and a copy of the NABL certificate along with the scope of each laboratory.*
- 2. MOU which may be maintained in a simple form.*

### **Advisory services**

Stand-alone laboratory shall communicate with their clients (patients / clinicians) with regard to the choice of tests under different clinical conditions, whenever required or sought. Communication may be through direct contact, email and / or documentation. Hospital-attached laboratory personnel are encouraged to participate in clinical rounds and meetings. The records of the above shall be maintained.

## Control of records

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 1**

Haematology (CBC) / Clinical Biochemistry	1 week
Microbiology and Infectious disease serology	1 year
Molecular testing gel images, Real time PCR raw data	2 years (infectious diseases), 10 years (genetic diseases and cancer)
Flowcytometry / Immunophenotyping data	10 years
Electrophoretogram / Immunofixation	10 years
Haemoglobin HPLC data	10 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 assessment whichever is later
Histopathology Reports, Block & Slides	10 years
Cytopathology Reports, Blocks & Slides	5 years
Cytogenetics, FISH images	10 years

### Note:

1. The records can be maintained as physical copies (instrument printouts or as photocopies) or electronically
2. Every effort should be made to retain blocks and slides in Histopathology and Cytopathology. If, however, they are returned to the patient, this must be documented and records kept

## Evaluation and audits

The laboratory shall ensure that pre-analytical and post-analytical processes are also covered during its internal audit along with the other processes.

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
- equipment downtime
- uncertainty of measurements
- performance in PT / EQA scheme

.....

## 5. TECHNICAL REQUIREMENTS

The technical 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 includes discipline wise requirements.

### PART 1 – GENERAL

#### 5.1. Personnel

As per the latest Notification issued by Ministry of Health & Family Welfare (MoHFW) dated 18<sup>th</sup> May, 2018 notifying Clinical Establishment (Central Government) Amendment Rules, 2018, all laboratories are required to comply with it as applicable.

*Note 1: If the above notification is not applicable, laboratory may give valid justification by producing evidence of alternate applicable rules and regulations.*

**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.**

Laboratory Director/Head of Laboratory/Technical Head (Howsoever named) shall have the overall responsibility of operations of the laboratory. For review, evaluation and release of results, he may delegate selected duties/ responsibilities to qualified personnel.

*Note 2: NABL is a voluntary accreditation body and has no statutory powers. Checking of compliance to the regulatory requirements falls under the purview of respective/applicable regulator.*

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## 5.2. Accommodation and environmental conditions

The laboratory shall have adequate space for efficient functioning, a pleasant ambience and conditions to avoid cross contamination.

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

*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 requirements of the regulatory agency (AERB).*

The laboratory shall have adequate lighting, power plugs and uninterrupted power supply. Use of exposed cables should be kept to a minimum. The laboratory shall ensure that adequate uninterrupted power supply is available so that there is no compromise of stored data. 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 processing area should be segregated / separated from the testing area. In particular, centrifuges should not occupy the same working bench as testing instruments where vibration may interfere with the results e.g. centrifuges & balances / analytical instruments like automated analyzers.

Gas cylinders shall be kept secure to prevent unintended movement (ISO 15190: 2003; Cl. 17.1).

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

## 5.3. Laboratory equipment, reagents and consumables

Verification of all automated / semi-automated systems is also required and includes precision, accuracy, carryover and wherever applicable, linearity.

The laboratory shall check each lot of control and reagent against an earlier tested in-use control / reagent lot. The laboratory can follow guidelines mentioned in Annexure III.

Records of comparative data shall be maintained.

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## Storage & Labelling

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.

## Calibration:

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

The nominal maximum periods between successive calibrations of general equipment are illustrated in Table 3 below.

**Table 3**  
**Calibration requirements**

<b>Item</b>	<b>Recommended maximum period between successive calibration by accredited laboratory</b>	<b>Procedure and comments for calibration verification</b>
Autoclaves	One year	Calibration of pressure gauge and temperature by thermal mapping.
Balances and Scales	One year	In addition balances with in-built calibration facility must be verified using calibrated weights for calibration once a day before use.
Biological safety cabinet	One year	Calibration of manometer, particle count and air flow also to be checked once in a year. For checking the functioning of UV light colony count to be performed once in 15 days.
Laminar Flow	One year	For horizontal laminar flow where sterile work is being done like media preparation, at least one blood agar media plate shall be used once in 15 days and there should be no growth.
Centrifuge	One year	Tachometer (contact / non-contact type) or Stroboscope. Calibrate temperature using PRT (Platinum resistance thermometer) with digital indicator and timing devices (by a stop watch) in

		specialized high speed (> 10000 rpm) centrifuges.
Mass	Two years and can be extended up to five 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	Initial manufacturer's certificate at the time of purchase and calibration checks every one year thereafter	i) In-house gravimetric checks: Repeat at least 10 times volume delivery and weighing. (The laboratory may refer ISO 8655-6:2002 for guidance). ii) Adjustable devices: take readings at several settings of volume. iii) In-house verification of pipette calibration: Volumes less than 100µl should be checked with balances of appropriate sensitivity / resolution.
Thermometers	One year	Calibration of master thermometer at required temperature for other thermometers in laboratory verification by using calibrated thermometer at 2-3 points covering entire range.

All equipment including thermometers, pipettes and centrifuges must be calibrated by an 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 traceability (as per NABL 142).

It must be stressed that these calibration intervals depend upon ruggedness of the equipment, frequency of use, quality & periodicity of maintenance and life of the equipment. Laboratory may seek a compliance report for the equipment from the accredited calibration laboratory.

All equipment must be calibrated following preventive maintenance, breakdown and repairs or more frequently as recommended by the manufacturers.

At the time of installation of new equipment, the Installation Qualification (IQ), Operational Qualification (OQ) and Performance Qualification (PQ) should be performed and documented.

Periodic calibration verification should be carried out to ensure continued stable performance of the equipment.

### Calibration of Automated Instruments:

The laboratory shall have a calibration schedule for automated instruments. All automated instruments such as cell counters, Clinical Biochemistry automated analysers, automated coagulometers and ELISA readers shall be calibrated by manufacturer at least once a year and be verified for calibration after preventive maintenance. The calibration certificate shall contain raw data; just a certificate that the apparatus has been calibrated is not sufficient. All raw data or machine printout / screen shots should be captured and documented for future use. The calibration should be as per manufacturer's recommendations and criteria for acceptance of verification.

During calibration of autoanalysers 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.

### In-house calibration of equipment

Certain items of equipment (listed below) may be calibrated by the laboratory itself without the service of an external calibration body, provided the laboratory has necessary reference standards and materials (with traceability) and such calibration procedures do not demand special techniques which are outside the capability and experience of the laboratory staff. The calibration status, date of calibration and due date shall be exhibited on the equipment.

### pH Meter:

pH meter shall be calibrated each time before use. This is done by using two standard buffer solutions appropriate to the expected pH of the sample being tested and the records of the same to be maintained.

### ISE analyzers & ABG analyzers

Calibration verification is in the form of self calibration. However, the electrode slope should be recorded to ensure that there is no deviation. Thereby requirement for change of electrodes is not missed.

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### Spectrophotometer and Colorimeter

Calibration checks on all spectrophotometers or colorimeters shall be performed at six months / one year interval preferably by manufacturer or AMC agency. These include checks on absorbance, linearity, matching of cells in accordance with the manufacturer's instructions and / or appropriate procedures using traceable standard / reference materials. A 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.

### Electrophoresis Apparatus

Instrument performance shall be routinely monitored during use with appropriate controls. System components (e.g. electrodes, tank and power supply), must be checked periodically.

### 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. The temperature shall be monitored daily, especially where the deterioration of material (sample or reagents) affects the test results. An attempt should be made to cover various chambers of refrigerator and deep freezer. The temperature is monitored with calibrated recording devices at multiple points with the doors shut. Records 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. Deep freezers shall not have an automated defrosting system without a manual override.

### Maintenance of Equipment:

Microscopes:

Microscopes used for screening shall have 10 X and 40 X objectives. Spare bulbs and fuses shall be available in the laboratory.

Calibration is not required for microscopes. However, regular cleaning and maintenance is essential for satisfactory operation. The stage and lenses shall be cleaned after use. Servicing shall be carried out by competent personnel.

#### 5.4. Pre-examination processes

Relevant clinical data are necessary for most specialized tests. Request forms should be designed so that the requesting physician provides this information.

For venipuncture, an evacuated tube system is preferred. 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 viz. Luer Adapter.

The guidelines for order of draw as mentioned in Annexure IV may be followed.

Specific instructions for the proper collection and handling of primary samples shall be documented in a primary sample collection manual. 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.

The above shall be applicable for collection facility at the main laboratory and sites other than the main laboratory viz., collection centers. Additional requirements related to collection centers are mentioned in "Guidelines for Operating Collection Centre(s) of the Medical laboratories", of this document.

The laboratory as a policy shall not accept samples, with labile analytes such as ammonia, acid phosphatase, blood gases and lactate that are not collected in-house.

## 5.5. Examination processes

The kit insert shall not replace an SOP.

### Measurement uncertainty of measured quantity values:

One of the components of measurement uncertainty (MU) is precision / imprecision. This is obtained from running stable controls. The laboratory shall run controls for each analyte where measurements are in metric 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).

For the purpose of recording estimates of uncertainty of measurement imprecision should be documented as  $k \times \%CV$ .  $k$  is the coverage factor and at 95% confidence interval it equals to  $\pm 1.96$  approximated to  $\pm 2$ , so the uncertainty of measurement could be set as:

- a. Coefficient of Variation (%CV)
- b. The uncertainty of measurement would be:  $\pm 1.96 \times \%CV$  approximated to  $\pm 2 \times \%CV$

It is recommended that a minimum of six months internal QC data should be used to calculate routine imprecision, to be updated annually where possible.

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

## 5.6. Ensuring quality of examination results

The laboratory shall design the internal quality control procedure appropriate to its size & scope. The results will be plotted on LJ charts.

Controls for some analytes such as CBC and blood gases 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 SDs 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 perform root cause analysis and take corrective actions as when out of control situations are detected.

### Inter-laboratory comparisons

#### External Quality Assessment (EQA) / Proficiency Testing (PT):

The laboratory shall:

1. participate in EQA / PT in each discipline prior to gaining accreditation
2. have a plan to cover critical tests
3. participate in an EQA program in case of change in test methodology, equipment change and extension of scope

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

Testing of EQA / PT samples must be integrated within the routine laboratory workload and analyzed in rotation by personnel who routinely test patient samples. In case of slides received as EQA samples, they are to be reported by appropriate levels of staff involved in the department without sharing of data. They should then collectively discuss the results before they are dispatched.

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

1. Non-availability of a formal national PT programme for analytes of interest
2. Only few laboratories performing the test
3. The analyte to be measured is unstable e.g. blood gases, ammonia, G6PD
4. Control material of the same matrix is not available
5. The sample is completely consumed during performance of the test (e.g. ESR)

**Note:** *It is unethical to take an extra sample of blood for repeat testing without informed consent*

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For utilizing Proficiency Testing reports to improve quality of a laboratory, the guidelines given as Annexure V can be followed.

#### Alternative approaches

For those tests where a formal EQA is not available, the laboratory shall adopt alternative approaches to validate performance. Such alternative approaches are:

- Replicate testing
- Examination of split samples (within the laboratory)
- Use of reference methods & materials, where available
- Exchange of samples with other accredited laboratories

When the laboratory exchanges samples with other laboratories as an alternative approach to EQA participation, following needs to be addressed:

- In the case of comparison between 2 laboratories, one will function as the “reference laboratory” against which the other will be compared. This is to be documented as an MoU
- When there are several laboratories, compare the result against the “reference laboratory”-

The results obtained shall be compared statistically and for guidance, the laboratory may refer to the most current edition of CLSI document EP9 - Measurement Procedure Comparison and Bias Estimation Using Patient Samples.

Other quality assurance procedures which are useful in addition to IQC but do not replace split sample analysis are:

- Review of daily mean
- Delta check
- Clinical correlation
- Correlation with other laboratory results

#### Comparability of results

If the laboratory uses more than one measuring system where the measurements are not traceable to the same reference material / reference method, or the biological reference interval are different, it is essential to perform a comparability study between the systems and prove that there is 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. A written procedure and complete record of all such data shall be retained till the next assessment.

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## 5.7. Post-examination processes

The retention period for the samples of various disciplines is mentioned in the respective sections either under clause 5.4 or 5.7.

### Disposal of Bio Medical Waste (BMW):

The laboratory shall follow national, state and local guidelines for BMW handling and disposal.

- a) Treatment outside laboratory premises
- b) Treatment inside laboratory

#### a) Treatment outside laboratory premises:

The laboratory shall have a documented contract with a licensed BMW Management Contractor or common waste management facility as per the local, regional or national guidelines. The laboratory shall follow the protocols laid by the contractor regarding segregation of waste. This shall also be displayed in colored posters at different sites of BMW generation for ready reference of the staff. Evacuated tubes may be treated as per the contractor's requirement.

#### b) Treatment inside laboratory

- i) Infectious microbiology waste – All infectious waste shall be autoclaved before disposal.  
The laboratory shall have a separate autoclave for waste treatment
- ii) Other samples shall be treated with 1% fresh hypochlorite solution for 30 mins. This can then be poured down the drain in running water
- iii) An effluent treatment plant may be placed as per local requirements

## 5.8. Reporting of results

Identification by name and / or signatures of the person authorizing release shall be included in the test report in accordance with Cl.5.8.3 of ISO 15189:2012.

Biological Reference Interval should be established by the laboratory for the method used only where required e.g.: Activated Partial Thromboplastin Time & Prothrombin Time (PT & APTT) should be reported as time in seconds with a reference interval. This is also the case for PT and INR. The results of coagulation tests vary with the analyzer & the reagent therefore it is necessary to establish biological reference intervals for these tests. If it is not practical to establish the biological reference interval for a particular analyte the laboratory should carefully evaluate the published data for its own reference intervals, and retain documentation of this evaluation.

## 5.9. Release of results

The laboratory shall establish and display critical limits for tests which require immediate attention for patient management. Test results within the critical limits shall be communicated to the concerned clinician after proper documentation.

### Interim report

Practically all hospital laboratories and a few stand-alone laboratories operate round the clock (24X7). After routine working hours the laboratory may not have an authorised personnel on duty. The laboratory shall have the following arrangements in place. If feasible an authorised personnel should be posted to supervise and authorise release of urgent tests in the disciplines of Clinical Biochemistry, Clinical Pathology, Haematology and Microbiology that are required for immediate management of the patient. If there is no authorized personnel on duty, the report will be "Interim".

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 regular authorised personnel shall go through the records on the next working day and issues 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.

### Automated selection and reporting of results

Many results on test samples may not require any further interpretations, recommendations or further review by authorized personnel. These results would automatically qualify to be released as such. Further, these could qualify as having been verified by an authorized person if he or she has established the criteria for results to qualify as not requiring further review. For establishing these criteria the authorized person shall make use of available standards (e.g., CLSI Auto 10) or Guidelines (e.g., ICSH for haematology - Laboratory Hematology 2005; 11:83-90. The International Consensus Group for Hematology Review: Suggested Criteria for Action Following Automated CBC and WBC Differential Analysis).

Besides rule based verification, this could bring in the concept of delta check where a patient's result is compared with an earlier one within a predetermined interval making some of the results eligible for automated release. After these criteria are selected, the laboratory shall validate the impact of these rules / criteria and its ability to automatically select the results for release. Once validated all the staff will be made aware of the same and this process will be introduced under supervision. All these criteria and validation of rules are to be part of an SOP. The review of criteria will be required if analytic principle or platform undergoes a significant change.

(Refer Guideline algorithm for automated selection and reporting of results; mentioned in Annexure VI)

### Revised reports

If a report is revised, a hard or soft copy of the original and revised reports together with the reason for revision shall be recorded and maintained.

## 5.10. Laboratory Information Management

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 have a documented procedure to ensure that the confidentiality of patient information is maintained at all times.

### Information system management

- a. When there is a comprehensive computerized information system, all functions from accession to reporting shall be verified after installation.
- b. The general process should involve:
  - i. Input patient data and save demographics and clinical information.
  - ii. retrieve the same data
  - iii. capture screen print
  - iv. compare with data on paper form or in a paperless system
  - v. sign and file with date
- c. There shall be a half-yearly review during which the above process is repeated for a minimum of 10 different types of samples / tests.
- d. 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.
- e. Rule based systems for automated selection and reporting of results, shall be verified.
- f. 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.
- g. 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.
- h. Redundancy (Back-up), manual procedures for down times and disaster recovery systems along with SOPs should be in place and certified by appropriate (local) information technology authority.

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## PART 2 – DISCIPLINE WISE

### Clinical Biochemistry

#### 5.3 Laboratory equipment, reagents and consumables

A microbial count is not required as long as Grade water I/II is used for reconstitution of reference material and reagents.

#### 5.4 Pre-examination processes

##### 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<sup>0</sup>C except for unstable parameters.

##### Hormone stimulation tests – precautions

Hormone stimulation for these tests is 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.

#### 5.6 Ensuring quality of examination results

Two levels of QC shall be included at least once on the day of performing the test irrespective of the size of the laboratory. If the laboratory is operational 24X7, two level controls shall be run in the peak hour subsequently one level every 8 hours. The daily QC values shall be documented and LJ charts (Levey-Jennings chart) shall be plotted on a daily basis. The laboratory shall derive its own mean and standard deviation (SD) using a minimum of 20 data points to plot an LJ chart. The laboratory shall define its own criteria for accepting or rejecting the run and be able to justify the application.

The laboratory shall analyze QC outliers, their causes and take immediate corrective action.

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

- i. Search for recent events that could have caused changes
- ii. Examine environmental conditions.
- iii. Follow manufacturer's troubleshooting guide
- iv. Refer to instructions of manufacturers of equipment, reagents or QC / calibrator

The laboratory shall calculate the monthly mean, SD and %CV. The laboratory shall maintain control charts to demonstrate the stability of the analytical measuring systems.

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The laboratory shall employ suitable reference material traceable to International Standards for calibration of measuring systems and methods. Traceability certificates for calibrators shall be obtained from kit suppliers and appropriately documented.

Alternate methods shall be employed for verifying accuracy of results of those tests for which calibration and control materials are not available.

Note: The use of third-party controls is advised, though not mandatory.

For blood gas measurements at least one control shall be assayed every eight hours. In addition, one control should be run with each patient sample unless the instrument automatically calibrates itself at predefined intervals.

### **5.8 Reporting of results**

Wherever relevant, reports should have an interpretation.

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## Haematology

### 5.3 Laboratory equipment, reagents and consumables

Automated Haematology analyzers shall be calibrated using 'calibrators' that have traceability to standard reference material or methods. Controls often lack absolute accuracy and are not recommended to be used as calibrators.

Coagulometer shall be calibrated once in a year and as per manufacturer's instructions.

### 5.4 Pre-examination processes

For monitoring anticoagulant therapy the request forms must have a column for the physician to indicate the purpose of the test e.g. monitoring heparin / low molecular weight heparin and / or oral anticoagulant therapy as applicable.

#### Indwelling Lines or Catheters:

Phlebotomists drawing blood from indwelling (arterial, central venous) or umbilical lines should have thorough training. While drawing blood from indwelling lines or catheters errors due to dilution and / or contamination from flushing solutions should be avoided.

When an intravenous solution is being administered in a patient's arm, blood should be drawn from the opposite arm. If an intravenous infusion is running in both arms, samples may be drawn after the intravenous infusion is turned off for at least two minutes before venipuncture and applying the tourniquet below the intravenous infusion site.

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. Reasons for rejection of these samples must be stated or communicated in writing to the nursing staff, physicians or laboratory personnel responsible for sample collection.

#### 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: <24 hours at 2-8<sup>0</sup>C

Coagulation test: <4 hours at room temperature

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

- i) Plasma can be stored at or below -20<sup>0</sup>C for 1 week and -80<sup>0</sup>C for up to 1 year.
- ii) For PT up to 24 hours if samples are maintained between 18 - 24<sup>0</sup>C and for heparin monitoring it shall be within an hour.



Haemoglobin electrophoresis and HPLC: (Haemolyzate) 1 week at 2-8<sup>0</sup>C or longer below -20<sup>0</sup>C

Bone Marrow aspiration slides: 5 years

Bone Marrow biopsy: 10 years

### 5.5 Examination processes

CBC specimens must be checked for clots (visually, by applicator sticks, or by automated analyzer histogram inspection or flags), significant in-vitro haemolysis and interfering lipaemia 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 analyzers. Blood samples must be adequately mixed before analysis.

A laboratory using a five part differential counter should preferably report absolute counts. The %CV for each differential shall be included in the scope of accreditation.

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

Packed Cell Volume Determination: The centrifuge shall be calibrated and capable of reaching at least 10000g for 5 minutes. The constant packing time (minimum spin-time to reach maximum packing of cells) shall be determined and recorded for each instrument.

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<sup>0</sup>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.

White 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.

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

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Bone marrow examination: The bone marrow film should exhibit satisfactory quality for staining properties, cell morphology and their distribution.

Reticulocyte count: Manual or automated must be performed within 24 hours of collection. Stain should be filtered before use. The reticulocyte percentage should be based on the count of at least 1000 red blood cells.

Malarial parasites: Thick and thin films stained by Romanowsky is the method of choice. Quantitative Buffy Coat (QBC) used as a screening test must be followed up by thin film microscopy to identify the species. The laboratory shall ensure that the buffer for the Romanowsky dye is at pH 6.8. At least 100 oil immersion fields should be screened before reporting negative. All the samples received by laboratories in the endemic area shall be double checked for malarial parasite by an experienced staff. Positive *P. falciparum* should be reported with parasite index on at least 100 red cells and parasitaemia reported immediately.

Manual Haemoglobin (Cyanmethaemoglobin method): At least four concentrations must be used to construct a calibration curve.

Direct Antiglobulin Test (Direct Coombs test): All positive results shall be verified by manual method performed by using gel method.

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<sup>0</sup>C for up to 1 week and at -80<sup>0</sup>C for up to 1 year).

Platelet poor plasma shall be made (using a calibrated centrifuge) spun at 1500 to 2000g 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<sup>0</sup>C immediately prior to testing, to ensure reaction temperature. Water-baths must be temperature controlled. Timers shall be checked for accuracy at least annually.

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

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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.

### 5.6 Ensuring quality of examination results

Internal quality control is necessary to ensure precision and repeatability. For this the laboratory shall use stable controls procured from commercial sources. The data should be plotted on control charts (L.J. charts). Laboratory shall use 2 levels of controls at least once a day. The 24X7 laboratory shall use these controls every 12 hours interval.

Additional approaches (but not alternative to use of controls) to maintain precision are performing duplicate tests on patient samples and use of daily means. In these situations, precision of routine work can be monitored by performing duplicate tests on patient samples. SD of differences between results on 20 duplicate samples is determined and  $\pm 2SD$  limits specified. Subsequent duplicate values should be within these defined limits. Patient data can also be used to monitor precision in a laboratory performing  $\geq 100$  samples a day. Day-to-day variation in MCV, MCH and MCHC should be analyzed using Bull's algorithm. This facility is available in the software of many auto analyzers.

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 reagent, type of reagent and the instrument used and INR calculated accordingly. Biological Reference Intervals (BRI) show significant differences with each lot of reagent, 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.

Automated reticulocyte counts: Automated reticulocyte counts shall be performed using only appropriate controls (2 levels controls are preferred). Manual verification should be performed on at least one sample once in a week.

Bone marrow smears: They should preferably be both Bone marrow touch smears as well as smears spread as peripheral smears. Iron staining shall also be done as a part of bone marrow examination workups.

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## Clinical Pathology

### 5.5 Examination processes

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

### 5.6 Ensuring quality of examination results

It is not necessary to exchange samples with other laboratories. As an alternative approach to EQA participation, split sample testing by different person can be performed once in three months.

#### Urine Analysis:

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

Laboratory shall cross check dipstick with manual methods every 6 months and records to be kept.

#### Stool analysis routine & occult blood:

Laboratory must check

- i. The quality of occult blood kit with heated 2% RBC suspension each time a new box is opened.
- ii. The quality of Lugol's iodine with starch when a new bottle is opened and once every week thereafter.
- iii. For ova and cyst by salt floatation as well as Lugol's iodine.

Split sample testing by different person must be performed once every 3 months.

#### Semen Analysis:

Laboratory must do split sample testing once every 3 months.

Facility shall be there to perform fructose test.

Examination & reporting shall be as per the latest WHO guidelines. Due attention to cellular morphology should be paid while examining the sample.

#### 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 biochemistry the quality control protocol should be identical to Clinical Biochemistry analytes. Appropriate control for body fluid chemistry shall be used.

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## Microbiology & Infectious disease serology

### 5.2 Accommodation and environmental conditions

Microbiological testing has to be separated from other testing areas. Incompatible activities shall be performed in separate areas, e.g., the area for media preparation shall be different from that of sample processing in Microbiology.

Diagnostic and health care laboratories must all be designed for at least biosafety level 2 (BSL-2). Desirable equipment for a BSL-2 facility includes a biological safety cabinet and an autoclave (World Health Organization Laboratory Biosafety Manual 3<sup>rd</sup> Edition, 2004, p9; p2, Table 2 and p3, Table 3). Appropriate practices include biohazard warning signs, “sharps” precautions and a biosafety manual, defining any needed waste decontamination or medical surveillance policies (Biosafety in Microbiological and Biomedical Laboratories, 5<sup>th</sup> Edition, 2009, US Department of Health and Human Services Publication No. CDC 21-1112, p59), along with adequate precautions to prevent the generation of aerosols and splashes and the use of personal protective equipment (e.g. laboratory coats, gloves).

#### Mycobacteriology and Mycology

Laboratories that only manipulate specimens for direct smear microscopy, or for the Xpert MTB / RIF assay, are considered “low-risk TB laboratories”. In such laboratories, sample processing may be carried out on an open bench in an adequately ventilated area (with 6-12 air changes per hour, with directional airflow, away from the technician, across the work area with potentially infectious materials, away from occupied areas of the room and outside). The bench used to process specimens shall be separate from areas used to receive specimens and from administrative areas used for paperwork and telephones. Ventilated workstations / Class II biological safety cabinets can be an optional solution when adequate natural or mechanical ventilation are not practical (Tuberculosis Laboratory Biosafety Manual, 2012, World Health Organization, p20).

However, when a concentration method precedes staining, or if mycobacterial culture is included in the scope, the work should be carried out in a Class II biological safety cabinet.

For mycological culture, only where fungal media that permit the growth of filamentous fungi are used a separate class II biological safety cabinet is necessary, which shall not be shared for other culture work.

All biological safety cabinets shall be certified at least annually to ensure that filters are functioning properly and that air flow rates meet specifications.

A laboratory performing fungus culture shall be equipped with a biological oxygen demand (BOD, heating and cooling) incubator to meet the environmental conditions for the isolation of dimorphic fungi that require incubation at 25°C.

Otherwise, no back-up alternative incubator is necessary, especially in a small laboratory.

### **5.3 Laboratory equipment, reagents and consumables**

In the field of Microbiology, clinical samples, standard strains and media shall all be segregated from each other during storage.

Autoclaves, hot air ovens and anaerobic jars shall be checked by both chemical and biological controls. Chemical controls must be used with each batch, whereas a biological control should be used at least once a week. Records of physical parameters (*viz.*, temperature, pressure) are to be maintained.

#### Media / Biochemical Tests

The laboratory shall ensure that commercial or in-house prepared media are sterile, able to support growth and are appropriately reactive biochemically. For this, the laboratory must maintain stocks of characterized organisms (procured from a standard source / PT provider / NABL accredited laboratory). These are to be used to test the media and biochemical tests. Blood agar should contain 5-10% sheep blood.

#### Stains / Reagents / Kits

Stains, reagents & kits must be labeled, dated and stored properly. They shall not be used beyond their expiry date or if they show signs of deterioration such as abnormal turbidity and / or discoloration. Appropriate controls shall be used, as per standard references, for stains and reagents. Control smears should be stained whenever a new batch of a stain is prepared.

#### Standard strains for antibiotic susceptibility testing

Each lot of antibiotic sensitivity discs must be checked for activity and potency using standard reference strains before being placed in service and at least weekly thereafter.

## 5.4 Pre-examination processes

### Skin antisepsis for blood culture

Cleansing with 70% isopropyl alcohol initially followed by 1 - 2% iodine tincture or an iodophor (aqueous iodine solution) is recommended standard practice. Iodophor require 1.5 to 2 minutes contact time while iodine tincture needs 30 seconds for its effect. Alternatively, a combination of 2% chlorhexidine with 70% isopropyl alcohol can be used [Cumitech1C: Blood Cultures IV. Baron EJ, Ed. 2005. American Society for Microbiology (ASM) Press, USA, p5]. Ethyl alcohol may be used instead of isopropyl alcohol.

Specimens for culture and sensitivity must be processed immediately after collection. In case of delay in processing, the specimen is to be stored in refrigerator except for CSF and pus / aspirate from liver abscess for suspected *Entamoeba trophozoites*. In situations where the sample has to be transported, it must be collected in an appropriate transport medium. Avoidance of a delay in processing and the maintenance of a cold chain during transportation must be ensured particularly for urine samples / BAL / endotracheal aspirates that are to be cultured since this test is semi-quantitative.

## 5.5 Examination processes

### Culture

Enrichment and selective media should be used for isolation of organisms from stool, sputum, throat, urethral & cervical swabs, etc., wherever indicated. For urine samples the laboratory shall perform, report semi-quantitative cultures and use media and procedures that permit isolation of Gram positive cocci, Gram negative bacilli & Candida.

### Antibiotic susceptibility testing

The laboratory shall follow CLSI (formerly NCCLS) / EUCAST / BSAC guidelines for antibiotic susceptibility testing.

The number of antibiotic discs applied on the Petri dish to test antibiotic sensitivity shall be as per these recommendations.

To prevent the misuse of antibiotics, the list of antibiotics for which susceptibility tests to be reported shall be decided based on standard guidelines, consultation meetings with referring clinicians, antibiotic policy (in hospital settings) and clinical & therapeutic status of the patient.

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### Tuberculosis serology

Based on the recommendation of WHO urging member countries to ban the inaccurate and unapproved blood tests that claim to diagnose active tuberculosis through antigen and antibody detection (news release dated 20<sup>th</sup> July 2011) and the Government of India's subsequent advisory to all states, serological tests for tuberculosis are no longer accepted in the scope of accreditation. The Quantiferon test may be included in the scope but it should be reported with the disclaimer that it cannot differentiate between latent infection and active tuberculosis.

The mantoux test, being an in-vivo test, is not considered in the scope.

### Ziehl Neelsen staining

Staining for acid fast bacilli (AFB) should follow the guidelines of the Revised National Tuberculosis Control Programme (RNTCP) of the Government of India, including staining of direct (unconcentrated) specimens with grading of smears that are AFB positive. Subsequent concentration & decontamination procedures should follow standard references and appropriate biosafety guidelines.

### HIV testing

Laboratory performing HIV testing shall follow National AIDS Control Organization (NACO) guidelines which mandate pre- and post- test counseling by a counselor. In case the laboratory does not have its own counseling facility, the referring clinician shall certify that the individual has been counseled before the test and will be counseled after the test. Written informed consent of the individual must be taken before the blood sample is collected. Evidence of counseling and consent shall be retained by the laboratory. Besides English, the patient information sheet and consent form is to be printed in the commonly used vernacular language of the region.

The report shall be sent to the referring clinician or the counselor who informs the individual tested about the HIV report and ensure post-test counseling. The results of the HIV test should be kept strictly confidential.

NACO guidelines do not incorporate 4th generation (antibody plus antigen detection) kits for HIV testing and therefore cannot apply to laboratories that are using these tests.

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## Denque serology

As dengue is reportable to the health authorities, the guidelines / advisories of the National Vector Borne Disease Control Programme (NVBDCP), Government of India, shall be followed. Dengue rapid test (e.g. ICT) reports should contain a disclaimer stating that the report is based on a screening test and is provisional and that the diagnosis of acute dengue infection should be confirmed by an IgM capture ELISA.

### **5.6 Ensuring quality of examination results**

#### Internal and External Quality Controls for ELISA / ELFA

- i. Negative and positive (internal) controls are available with the kit and shall be used with each run.
- ii. In addition, laboratory shall use a commercial control or an (external) control with a value close to the cut-off for the test. The CV% calculated for inter-assay variation using this control is to be submitted to NABL along with the application.

In-house external control sera should be stored as multiple aliquots frozen at  $\leq 20^{\circ}\text{C}$  to prevent repeated freezing and thawing.

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.

Sensitivity for those antibiotics e.g. vancomycin for *S. aureus* and colistin that cannot be reported on the basis of disc diffusion method shall be reported on the basis of MIC testing based on broth / agar dilution or automated systems or an E- test or equivalent. All antibiotic sensitivity interpretation shall be done using the latest national / international guidelines.

Stains for acid fast bacilli should be checked with the known positive and negative control organisms and the results recorded for each new batch. Control smears for acid fast stain should include smears with few to moderate number of acid fast bacilli. Positive and negative control smears should be included daily.

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### Quality indicators

The quality indicators to be monitored may include specimen rejection rate, transit time, blood culture contamination rate and turn-around time (TAT) etc.

### **5.7 Post-examination processes**

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

Serum samples: 3 days at 2- 8<sup>0</sup>C (except for HIV, where NACO guidelines require these to be stored for 7 days)

Samples for culture: Until the final identification and antibiotic susceptibility report is issued

### **5.8 Reporting of results**

All serological tests for infectious diseases should be reported along with comments on their interpretation and limitations.

### **5.9 Release of results**

Automatic generation and release of reports in Microbiology may be considered only for samples of blood and sterile body fluids which are negative by automated culture systems (e.g. Bactec). These can be reported as “No growth after \_\_\_\_\_ hours. Final report to follow at \_\_\_\_\_ days” as per the defined policy of the laboratory. All requirements must be met for the automatic generation of such reports, as described in PART 1 General, section on "Technical Requirements" in this document.

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## Histopathology

### 5.2 Accommodation and environmental conditions

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

A separate room shall be allotted for gross examination of tissue, equipped with a fume hood, efficient exhaust and formalin vapour monitoring.

All chemicals should be handled with care and disposed according to local regulatory requirements.

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

### 5.3 Laboratory equipment, reagents and consumables

#### Tissue Processing

- a. Depending on the workload the laboratory shall have a procedure to change the tissue processing fluids and maintain a record of it
- b. A log recording of the 'time setting schedule' for an automated tissue processor shall be maintained
- c. Temperature of the wax bath shall be checked and recorded daily

#### Microtome

- a. To be calibrated by the manufacturer once a year. However, traceability certificate for the same may not be insisted upon
- b. The setting of the microtome indicating the thickness of sections shall be checked before use
- c. Microtomes with non-disposable knives shall have a safety shield

#### Slide warming stage, if used.

- a. Temperature of slide warming stage shall be checked daily

#### Flotation bath

- a. The water in the flotation bath shall be changed at least once a day
- b. The surface of the water bath shall be skimmed regularly during section cutting to remove floaters

## 5.4 Pre-examination processes

### Acceptance and rejection criteria

Histopathology specimens should not be rejected on grounds of poor specimen integrity. They should be accessioned & remarks be incorporated in the gross, microscopic descriptions and 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 labeling and if necessary, processing of the specimen may also be undertaken.

### 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:

Specimens – 30 days

Slides & Blocks – 10 years

## 5.5 Examination processes

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

### Processing:

This shall be done in-house. It is not permissible to outsource processing and slide preparation to another accredited laboratory

### Staining:

- a. The frequency of changing the de-parafinizing solutions (xylene / chloroform / alcohol) and stains should be recorded. This is based on workload
- b. Special Stains: A positive control should be stained with each batch. The control slides shall be filed and retained for the same time period as the test slides

### Frozen section / squash smear:

- a. A specific area should be demarcated for performing frozen sections
- b. Fresh tissue received for frozen section should be treated as infective and universal precautions should be taken
- c. Frozen sections / squash smears should be recorded as other specimens in the request form. Left over tissue must be processed for permanent section
- d. The turnaround time for frozen section / squash smears should not exceed 30 minutes

- e. Frozen section / squash smears shall be retained and filed along with the permanent sections for stipulated time period

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. The blocks shall be labeled as bio-hazardous. The trimmings of the block shall be disposed off by incineration. All instruments used for sectioning shall be left in 2 Molar NaOH for 1 hour and washed in running water for 15 minutes before reuse. The microtome should be wiped clean with 2 Molar NaOH and left for 1 hour. Subsequently, the instrument should be wiped clean with tap water followed by alcohol before reuse.

Electron Microscopy:

- a. A separate room shall be allotted for tissue processing with a fume hood for handling osmium tetroxide
- b. A separate dust-free facility, with air-conditioning shall be available for preparation of specimen and performing electron microscopy
- c. The electron microscopy room shall have:
  - i. facilities in place for temperature control and chilled water supply
  - ii. insulated cabling kept away from the work areas
  - iii. proper seating available to allow for optimal ergonomic positioning of the person using the microscope
  - iv. Dark room with adequate ventilation
  - v. Warning light on the door of the dark room indicating usage

Processing of specimens shall be done by a trained technician under supervision / authorization of the officer-in-charge of electron microscope laboratory.

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

**5.6 Ensuring quality of examination results**

When a repeat specimen for Histopathology from a patient is received, all previous slides must be reviewed if possible and reflected in the final report.

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

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## Immunohistochemistry

Verification of antibodies for IHC every 6 months is a good practice. However, the laboratory is advised to have a daily record of positive controls when the antibodies are used. This is a better method of verification and a way of checking when the dilution needs to be adjusted.

For those antibodies where an internal control is available, the routine use of external controls is not mandatory.

### Proficiency testing:

- National / International EQAS where available
- When not available, introduce
- Inter laboratory comparison
- Intra-laboratory review of masked slides
- Review by supervisory staff

## **5.7 Post-examination processes**

The laboratory shall dispose the reagent waste as per Bio-medical waste (management & handling) rules, 1998. The laboratory can also refer to BIS, Indian standard code of safety for benzene, toluene and xylene IS : 4644-1968 (Reaffirmed 2002).

The laboratory may consider giving the original slides to its patients on specific request for obtaining second opinion or for treatment elsewhere. The laboratory shall have a documented procedure and maintain records of the same. However, attempts should be made to retain at least one representative primary slide on which the diagnosis was based for review.

## **5.8 Reporting of results**

It is not mandatory to have the signatures of two Pathologists on a histopathology report.

1. 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.
2. Report should be in accordance with recent terminology / classification, grading, scoring, nature of lesion and relevant information necessary for disease management. Report shall also mention all additional tests performed such as special stains, immunohistochemistry etc.
3. All reports shall be checked for accuracy by a Pathologist before authorizing and issuing printed or electronic reports.
4. The turnaround time for issue of reports should not exceed 4 days. In case any special procedures are carried out to further characterize the pathology, an interim report should

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be issued to facilitate immediate management of the patient wherever required. A final report should be issued after carrying out the special procedures in a reasonable amount of time depending upon the degree of specialization and consultancy needed.

5. When the examination of a permanent section is preceded by frozen section and / or followed by other diagnostic modalities like immunohistochemistry, in-situ hybridization the final report shall also include these results with interpretation.

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## **Cytopathology**

### **5.1 Personnel**

The cytotechnologists / cytoscreeners shall have a workload of not more than 100 slides per 8 hours a day (as per CLSI guidelines) so that quality of screening is not compromised.

### **5.2 Accommodation and environmental conditions**

The laboratory shall have a dedicated space for FNAC procedure. It is not necessary to sterilize the environment by using UV light.

### **5.3 Laboratory equipment, reagents and consumables**

All equipment such as centrifuges capable of creating bio-hazardous aerosols should be used in extractor cabinets or rooms fitted with extractor facilities.

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

### **5.4 Pre-examination processes**

- i) The procedure describing the sampling requirement for each specimen shall be readily available at all submitting locations (laboratory / clinic / hospital) and shall contain the following information:
  - a. Preparation of patient for sampling
  - b. Consent form for Fine-Needle Aspiration (FNA)
  - c. Collection techniques
  - d. Specimen identification and labeling
  - e. Fixation requirement e.g. anticoagulant used, fixative (wet fixed and / or air dried) and storage requirements
  - f. Transportation instructions
  - g. Safety precaution for all of the above (with special reference to HIV and Hepatitis)
  - h. All laboratory staff handling infectious material shall be vaccinated against HBV
    - a. Where possible, FNA shall be carried out by the Pathologist. In the absence of a Pathologist, a clinician / radiologist may perform FNA following documented procedures as provided by the laboratory and sign the requisition form



- b. A request form should accompany every specimen and contain the following information:
  - i. Full demographic data
  - ii. Relevant clinical history and clinical findings with provisional diagnosis
  - iii. Anatomical site of collected specimen
  - iv. Date and time of specimen collection
  - v. Information regarding previous cytology report
  
- ii) For gynecological cytology the request form shall also contain:
  - a. Details of menstrual phase and hormonal status
  - b. Details of hormone therapy
  - c. Details of contraception
  - d. Details of previous surgery
  
- iii) For intra-operative imprint / aspiration cytology, the request form shall also contain detailed surgical information observed at the time of procedure

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<sup>0</sup>C

Slides – 5 years

The laboratory may consider giving the original slides to its patients on specific request for obtaining second opinion or for treatment elsewhere. The laboratory shall have a documented procedure and maintain records of the same. However, attempt should be made to retain at least one representative primary slide on which the diagnosis was based for review during the follow up.

**5.5 Examination processes**

All exfoliative cytology slides shall be stained by Papanicolaou technique. FNAC slides shall be stained with May-Grunwald Giemsa with or without PAP / H & E staining for interpretation.

## 5.6 Ensuring quality of examination results

- i) For gynecologic screening program, appropriately trained cytotechnologists are permitted to sign out negative results, provided Cytopathologist review at least 10% of all negative smears
- ii) Re-screening of previously reported slides on receiving fresh smears from the same patient, during follow up
- iii) Records of checking the quality of staining and volume of workload for each screener shall be maintained. The laboratory shall avoid overloading the screener
- iv) Procedures and records for follow up shall comply with:
  - a) Reviewing all previous slides for an individual patient
  - b) Matching previously reported abnormal smears with histopathology sections submitted for examination from the same patient
  - c) Comparison of all abnormal cytological findings with results of colposcopy or biopsy
- v) The laboratory shall have procedures for following up discrepancies identified between biopsy result and cytology report
- vi) For gynaecological cytology the ASCUS : SIL ratio shall comply with the latest Bethesda recommendations.

**Note:** *The above criteria / procedures shall be applied to fluids cytology / FNAC where applicable.*

## 5.8 Reporting of results

A Pathologist shall review and sign all reports screened by a Cyto-technologist. Explanatory notes shall accompany any unsatisfactory or equivocal report. The turnaround time shall not exceed 3 working days. All malignancies or suspected malignancies shall be reported immediately in writing. For intra-operative cytology, the smears will be stained and interpreted within 30 minutes and the result immediately communicated to the surgeon. In case of reports with abnormal cytology findings, the Pathologist should make recommendations regarding further clinical / histological evaluation, where relevant.

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## Flow Cytometry

### 5.3 Laboratory equipment, reagents and consumables

All instruments and equipment should be properly installed, operated, maintained, serviced and monitored to ensure that malfunctions of these instruments and equipment do not adversely affect the analytical results. The maintenance procedures should be followed as per manufacturer's guidelines. A log detailing the daily, weekly, monthly, semi-annual and annual maintenance procedures shall be maintained. Any unscheduled maintenance procedures must be documented.

The laboratory shall have a policy for confirming and documenting the reactivity of the reagents used. This may be achieved by direct analysis with reference materials, verification testing of old vs. new reagents and checking against routine controls. All reagents should be stored as per manufacturer's guidelines.

The laboratory must assign an expiration date to any reagents that do not have a manufacturer provided expiration date. The assigned expiration date should be based on known stability, frequency of use, storage conditions and risk of deterioration. In addition, for various reagents / antibodies, laboratory shall keep the documentation of receipt, storage conditions, in-use state, antibody cocktails and assay development / optimization.

### 5.4 Pre-examination processes

The laboratory shall have a documented policy for sample acceptance and rejection criteria, anticoagulant used, time of collection, transport temperature, processing and storage (24-72 hrs based on specimen type and anticoagulant used). Laboratory may define its own rejection criteria. However sub-optimal and / or unacceptable samples include presence of clot, hemolysis, improper container, inappropriately labeled & samples received beyond 48 hours after collection. In case of unacceptable samples, the same shall be informed to the treating physician and recorded. 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 may be transported and stored at room temperature (20-24<sup>0</sup>C). They should reach the testing laboratory within 12-24 hours. Fluid samples, aspirates and suspected cases of Burkitts lymphoma may be transported at 2-8<sup>0</sup>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

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transport media. The examined specimens shall be stored for re-examination and / or additional tests for a minimum defined period. 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 upto 24 hrs) and of RBCs upto 7 days.

### 5.5 Examination processes

Laboratory shall monitor various pre analytical, analytical and post analytical variables.

#### Assay Procedures

##### a. Viability Testing:

The laboratory shall have a documented policy 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).

- i. 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.
  - ii. Viability testing in lymphocyte subset enumeration may not be necessary on specimens of whole blood which are analyzed within 24 hours of drawing. ISHAGE (International Society for Hematotherapy and Graft Engineering) recommends assessment of viable CD34 stem cell counts (post thaw stem cell product).
- b. Antibody Panels:
- i. Comprehensive panel of antibodies that covers common subtypes of hematolymphoid neoplasm shall be available. Clinical as well as other findings including morphology are very useful in deciding on a particular panel of antibody. Laboratory shall demonstrate documented panels and also the procedures as well as records of adding additional reagents / antibodies, if no opinion could be made based on primary panels. It is a good laboratory practice to prepare antibody cocktails for panels for routine immunophenotyping of hematolymphoid neoplasm. Reduction in number of samples

requiring repeat processing and also reduction in repeat procedures can be monitored as quality improvement indicators.

- ii. Minimum of three color immunophenotyping shall be done for immunophenotyping of hematolymphoid neoplasms and for CD4 cell counts.
- iii. For CD34+ stem cell enumeration appropriately conjugated Class II or Class III anti-CD34 monoclonal antibodies are used. For performing IVD tests (using kits / materials) the manufacturer's instructions shall be followed. If procedures are altered, documented proof shall be provided that the modification has not altered the results.
- iv. PNH analysis has been divided into routine analysis (defined as identifying an abnormal population of 1% or more) and high-sensitivity analysis (in which as few as 0.01% PNH cells are detected). Laboratory shall clearly mention in its scope whether it is doing routine analysis, high sensitivity analysis or both. For routine analysis, PNH testing on neutrophils with or without monocytes shall be performed. PNH diagnosis only based on RBC analysis is not recommended. When standardizing the PNH testing in the laboratory, it should be performed on a known PNH positive sample with both Type I & Type III cells rather than a normal sample. Standard guidelines must be followed for use of reagents / antibody for different types of cells.
- v. Flow Cytometry assessment of HLA-B27 shall be done with at least two different antibody clones of defined specificity for HLA-B27.

c. Cell Concentration:

It is important to define the cell concentration to be used per assay tube for a given assay. This may be based as per the manufacturer guidelines or on existing clinical practices. A recommended cell concentration to be used for immunophenotyping of hematolymphoid neoplasm is 0.1 -1 million (0.1 -1 x 10<sup>6</sup>) cells per assay tube. It is important to note that as antibody staining is mainly volume dependent, the sample volume in the assay remains constant. The cell number can be adjusted by spinning down the cells and re-suspending them in a desired volume. 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. Laboratory shall document cell concentration policy.

d. Sample / Data acquisition:

In screening of peripheral blood / bone marrow samples in a new case of hematolymphoid neoplasms, at least 10,000 total 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.

- i. CD4 counts: For single platform measurements, manufacturer guidelines shall be followed.
- ii. CD34+ stem cell enumeration: A statistically valid number of CD34+ events are collected to ensure clinically relevant precision and accuracy. The allowable coefficient of variation for CD34+ cell counts should be 10%. To achieve this precision, a minimum of 100 CD34+ events should be counted, as recommended by the ISHAGE guidelines.

e. Gating Strategies:

Each laboratory shall have documented policies of appropriate gating strategies for different lesions. CD45 versus light scatter gating is a must. For evaluation of lymphoma / myeloma, the concept is to track the abnormal population using an antibody / antibodies as tracking markers. For CD34+ stem cell enumeration sequential (Boolean) gating systems shall be followed. ISHAGE protocol is ideal for CD34 stem cell enumeration. CD45 / CD3 gating is essential for CD4 subset enumeration.

f. 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 / isotype controls are suggested but have limited use and therefore not essential.

## 5.6 Ensuring quality of examination results

Instrument set-up & quality control: The instrument shall be optimised for optical alignment, electronic standardization, sensitivity / linearity and compensation. The laboratory shall have a documented policy defining the procedure for appropriate instrument function checks to be performed for all instruments prior to a daily run. Instrument function checks are ideally done by commercially available reference beads. These may differ depending upon the type of instrument. 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)
- Fluids

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The values for PMT voltages and laser parameters shall be plotted on a LJ chart and monitored. Though there are many protocols available in the literature to monitor the instrument, it is prudent to follow manufacturer's instructions and specifications. The laboratory shall use vendor supplied beads and respective automated software systems for monitoring the instrument.

Frequency of performance check: Laboratory shall have a policy on its instrument performance check based on its work load like daily or after every cold start. If instrument is not used regularly, it is recommended to do performance check at least once a week.

Cross-instrument performance: If more than one instrument is used in the laboratory for the same test, verify that results are concordant / equivalent and this shall be done at least twice in a year.

Quality control for reagents and assays :

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

- i. CD4 counts: For measurements of CD4+ T-cells, two levels of commercial controls are ideal. CV should be less than 10%
- ii. CD34 stem cell enumeration: It is desirable to do commercial controls for CD34 + stem cell enumeration. CV should be less than 10%. Assay has to be run in duplicates, so as to avoid random errors. The difference between replicates should not be more than 10%
- iii. 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

Like external controls, there must be objective criteria for acceptable performance of internal controls. Laboratory shall document its quality control policy for each test and maintain records. It is recommended to do single platform analysis for absolute counts.

Compensation Controls:

The laboratory needs to document the procedure of how the color compensation is going to be setup. 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

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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 affect on compensation values and shall be avoided.

National as well as International published guidelines shall be followed.

### 5.8 Reporting of results

The report shall include name / type of instrument & software used, cell preparation method, gating strategies 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 and not on percentages. While interpreting the intensity of positivity as normal, bright or dim, the abnormal population shall be evaluated against known normal leukocyte populations. Final impression should be clearly stated along with a differential diagnosis, if required. Comments and suggestions regarding useful follow up test or other ancillary techniques should be added. For laboratory doing other ancillary techniques like cytogenetics and molecular diagnostics, it is desirable that the final report has a mention of these. The laboratory is recommended to follow recent WHO guidelines for the classification of hematolymphoid neoplasms.

#### Data Backup and Storage:

Analysis plots on which final diagnosis is made shall be stored either in PDF format or as a hard copy. All list mode files and final reports shall be stored for a minimum period of 10 years. The laboratory may consider giving the list mode files (particle data) to the patient on request for obtaining second opinion or for treatment elsewhere. The laboratory shall have a documented procedure and maintain record of the same.

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## Cytogenetics

### 4.1 Organization and management responsibility

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. The license / certificate shall be prominently displayed in the patient waiting area / laboratory reception and laboratory operational area. The laboratory shall also prominently ascertain that it does not disclose or report the sex of the fetus for PND. 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 PND Act. The patient's consent must be collected and maintained along with PND test reports. The laboratory shall be able to produce such documents at the time of assessment.

#### 4.13 Control of records

The recommended minimum period for retention records is as follows:

Cytogenetic / FISH images / Photographs: at least 10 years

Test reports: at least 10 years or more, as for the images

G-banded slides: at least 5 years

Log books and other records: 5 years

### 5.2 Accommodation and environmental conditions

The cytogenetics laboratory shall have dedicated areas for cell culture, harvesting, slide preparation and staining, with an exhaust system capable of removing acid / alcohol fumes. The cytogenetics laboratory shall also have designated areas for receiving specimens, FISH analysis (including probe-labelling in a laboratory with an in-house probe preparation facility), reagent preparation, microscopy, washing & sterilization, specimen aliquoting (for archival storage) and storage of consumables.

The maintenance policy for the cytogenetics cell culture laboratory shall include infection control of the laminar flow hoods by checking bacterial colonies on agar culture at regular intervals and checking of CO<sub>2</sub> incubator temperature & humidity. Regular cleaning and mopping of the floor is mandatory.

The laboratory in which cultures are set up shall have a facility for storage of sterile containers and other accessories required for tissue culture in order to avoid repeated movement of staff.

Every staff member working with tissue culture shall be familiar with sterile technique, preparation, handling of culture media and safe laboratory practices.

Refrigerators shall be used solely for laboratory purposes. Cell culture reagents shall have dedicated areas for storage in the refrigerator. The compartment for temporary storage of sterile specimens should be isolated from media.

If working with DNA, a separate laminar airflow chamber shall be used for PCR master mixing. The chamber used for culture / DNA mixing shall not be used for master mixing. There must be effective separation between DNA extraction / mixing area and the master mixing area.

#### 5.4 Pre-examination processes

Clinical details and relevant family history shall accompany the specimen. A consent form shall accompany a fetal sample for prenatal diagnosis.

Recommended conditions for sample collection, transport and storage for conventional cytogenetic analysis are tabulated below:

**Table 4**

<b>Tissue</b>	<b>Sample volume</b>	<b>Container</b>	<b>Mode of collection</b>	<b>Transport / Storage</b>
Whole blood / Cord blood / Bone marrow	Minimum 2- 3 ml	Sterile green top sodium heparin vacutainer	When using a vacutainer use an evacuated tube system to collect the blood. If using an ordinary syringe and a vacutainer, use a transfer device following the same order of draw in case of multiple tubes. 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 eppendorf tube containing sterile	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

		transport medium		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 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 tumours and skin	4 - 5 pieces, 2 - 4 mm <sup>2</sup> 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 eppendorf 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°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	Sterile condition must be maintained	Sample to be transported at room temperature and

Pleural or other fluids		tube 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
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Fluorescence in-situ hybridization (FISH) analysis:

Sample collection: as mentioned in Table 4

Fixed cell suspensions and stained or unstained slides or formalin fixed paraffin embedded tissue sections may also be considered for FISH. The laboratory shall provide proper guidelines for preparing such specimens.

In prenatal diagnosis, FISH technique is considered to be a rapid test; however, it provides very specific information based on the probe used. Therefore, for prenatal diagnosis of chromosomal disorders the laboratory shall have a policy of offering FISH only as an adjunct to karyotyping. FISH alone shall not be considered for offering prenatal diagnostic service.

Other pre-examination requirements are based on the choice of test to be done depending upon the request received from the referring clinician. However, it is important to review the clinical details as stated on the request form, the type & quantity of sample received and other pre-examination conditions and verify that the test requested is the most appropriate for the sample received.

Karyotyping shall be performed for determination of the sex chromosome constitution in suspected disorders of sexual development. Analysis of buccal smears for Barr bodies is not recommended as the sole test for the diagnosis of these conditions, as this test may not detect structural abnormalities involving the sex chromosomes.

Cytogenetic analysis shall be the first test of choice for the primary diagnosis of acute leukemia. However, for monitoring response to treatment when a specific abnormality and in the events of culture failure or low mitotic index; FISH alone may be considered.

In cases of relapse, cytogenetic study shall also be carried out to look for additional chromosomal changes which are indicators of clonal evolution.

Note:

*Aneuploidy - FISH shall not be included in the test menu of an infertility work up and not be considered as a stand-alone prenatal genetic test.*

Recording details of sample:

For interphase FISH analysis the type of tissue used for the test shall be stated clearly on the test requisition form.

Storage period of examined specimens:

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

Blood samples for karyotyping – 6 days at 2-8<sup>0</sup>C

Fixed cell suspensions – 5 years at -20<sup>0</sup>C

### **5.5 Examination processes**

The laboratory shall ensure that all measures are implemented to minimize failure rate of cell / tissue culture. These results shall be reviewed periodically.

The laboratory shall check the sterility of medium before use. For in-house preparation of cell culture medium the protocol given by the manufacturer shall be followed.

For samples of bone marrow / peripheral blood / solid tissues / amniotic or other fluids, more than one culture shall be set up (overnight / 24 / 48 / 72 hours / direct / long term culture) with proper labeling of culture flasks / tubes.

The competency of laboratory personnel for harvesting to obtain a good metaphase index should be periodically evaluated by splitting the specimen and harvesting by two persons independently.

Special attention shall be paid while slide preparation, to control overspreading and mixing of chromosomes of different cells.

Conventional cytogenetic analysis:

When performing cytogenetic analysis, the coordinates of each metaphase, and the microscope used shall be recorded, so that the metaphases may be located easily for checking. For the same reason, slides should be oriented in a consistent manner (i.e. label on right or left) for all cases. Should there be a change in orientation due to breakage or any other cause; the changed orientation should be recorded.

The laboratory shall follow the recommendations of the most recent edition of the International System for Cytogenetic Nomenclature (ISCN), for conventional cytogenetic analysis.

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

- i. Constitutional abnormalities, post-natal and pre-natal: At least 20 metaphases, a minimum of five metaphases shall be karyotyped and analysed (band for band).
- ii. Disorders of sexual differentiation: At least 25 metaphases.
- iii. For mosaicism studies in post-natal samples, an attempt should be made to analyse at least 50 metaphases before FISH analysis is recommended.
- iv. For mosaicism studies in prenatal diagnosis, depending upon the chromosomes involved, upto 30 more cells from both the normal as well as a third primary culture shall be counted as per standard guidelines.
- v. 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 (a minimum of 40-50 cells in most cases). Scoring has to be done both in the patient and as well as a normal control.

For leukemia and other tumours, at least 20 cells.

More than 20 metaphases may need 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.

General recommendations for cancer cytogenetics:

- If the bone marrow aspirate received is suboptimal (partially or completely clotted / viscous / haemolysed / volume is not adequate), 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.
- If the mitotic index is low, efforts should be made to obtain as many cells as possible, especially if the karyotype is normal. More slides may be made to increase the yield of metaphases. An attempt should be made to determine the reason for the low mitotic index (low sample volume / leucopenia / leucocytosis / marrow fibrosis / clotted sample / technical issues).
- In haematological malignancies, efforts should be made to detect abnormal cells which may often have poor morphology. Therefore, if cytogenetic analysis is performed solely on metaphases with good chromosome morphology, the abnormal cells would not be detected.

- Correlation with peripheral blood and bone marrow and other laboratory findings would help to refine the search for associated abnormalities and better interpret the cytogenetic changes.
- In follow-up studies on specimens from hematological malignancies, review of the previous cytogenetic analysis is essential.

Fluorescence in situ hybridization (FISH):

Probes shall be stored as per the manufacturer’s recommendations in the dark. Probes should be validated before use on normal or known positive controls, if available.

Probes specific for common conditions are available commercially. These can be used for diagnostic or follow-up purposes.

Use of home-brew probes is not recommended for routine diagnostic application or if therapy is based on the results of FISH analysis. In case home-brew probes are used for FISH, the validation record on normal and positive control shall be documented prior to use.

Slides of interphase and metaphase FISH analysis should be scored by two readers familiar with the expected signal pattern of the probe used.

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.

The minimum number of cells to be counted for interphase FISH analysis is as follows:

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

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

FISH can be used as an additional or supplementary test for confirmation of locus of chromosome break point in an abnormal karyotype.

## 5.6 Ensuring quality of examination results

### Karyotyping:

The laboratory shall offer cytogenetic services in an ethical manner especially with regard to prenatal diagnosis. Patient confidentiality shall be strictly maintained and reports must be given only to the referring physician or the patient, in line with the policies of the laboratory.

The laboratory shall periodically review the success rate of cell / tissue cultures and ratio of normal versus abnormal results generated, particularly in leukemia or other malignancies.

## 5.7 Post-examination processes

Examined unfixed specimens of bone marrow aspirates / peripheral blood / FNAs / other samples shall be stored: at least 3-5 days at 2-8°C.

Slides and pellets: at least five years

Fixed archival specimens and cell pellets shall be stored: five years at -20°C

All reports and images of conventional cytogenetic analysis and FISH analysis shall be retained by the laboratory: at least 10 years

## 5.8 Reporting of results

For interpretation of a cytogenetic study analysis, clinical and family history shall be considered. ISCN nomenclature shall always be followed in reporting of cytogenetic results. 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. This is particularly important when there is an abnormal finding or a finding which could be a normal variation. An appropriate comment on the significance of such a finding must be included in the report. The karyogram, and if possible, the designated metaphase may be included in the report. The system of reporting shall be documented.

Counseling shall be done by trained personnel only.

The laboratory may consider giving the original slides to its patients on specific request for obtaining a second opinion or for treatment elsewhere. The laboratory shall have a documented procedure for this purpose and maintain records of the same. However, an attempt should be made to retain at least one representative primary slide on which the diagnosis was based for subsequent review, if required.



In case the slides do not present metaphases of an analyzable standard due to long period of preservation, images (soft or hard copy) shall be considered as an alternative to fulfill the requirement. 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 analysed and considered for reporting.

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## **Molecular Testing**

A Molecular testing laboratory shall ensure that pre and post PCR guidelines and safeguards are adhered to.

### **5.2 Accommodation and environmental conditions**

PCR based molecular testing 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 must ensure that contamination of samples or master mix by amplicon does not occur.

### **5.3 Laboratory equipment, reagents and consumables**

A class II biosafety cabinet shall be available for sample preparation and nucleic acid extraction. All equipment in the post-PCR area, e.g., micropipettes, microfuge, vortex mixer, personal protective gear etc. should be dedicated only for this area and should not be moved outside. Calibration for equipment shall be done as per manufacturer's requirements.

### **5.4 Pre-examination processes**

Samples arriving in the laboratory shall be accompanied by requisition forms with the patient's name, hospital number (if applicable), laboratory number or other unique identifier. Details about the date & time of sample collection and date, time & temperature at which it was received shall be documented.

Requisition form shall have sufficient clinical information to ensure appropriate & accurate testing and interpretation of results. A judgment about specimen quality shall be made at the time of sample receiving. Any problems related to specimen collection (tubes, anticoagulants, transport solutions, labelling, etc.) or quality (lysis, clotting, etc.) shall be noted. Appropriate individuals from the referring facility shall be contacted regarding any unacceptable samples or incomplete information. Records shall be maintained for such communication (email, phone call etc.).

Appropriately designed clinical forms shall accompany fetal samples for prenatal diagnosis.

Nucleic Acid Preparation:

If commercial methods are used for nucleic acid extraction then manufacturers' recommendations shall be followed.

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, extraction shall begin within 4 hours. If extraction is not possible immediately, sample shall be collected in a tube containing an RNA stabilizing additive. Alternatively, the buffy coat can be removed and stored in Trizol reagent at -70°C or lower.

Serum shall be shipped frozen on dry ice for either DNA or RNA studies. Plasma shall be shipped at 2 to 8°C and stored at -20°C. Any body fluid / fresh tissue shall be chilled immediately and transported on wet ice to the laboratory for DNA studies. If CSF specimens cannot be processed immediately, the specimens being tested for viruses should be placed at -70°C. 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.

Any body fluid for RNA studies shall be chilled immediately on wet ice and the RNA to be extracted within 1 to 4 hours of collection. If RNA is to be extracted from a tissue sample, it shall be either snap frozen prior to storage at -70°C or lower, placed in a stabilizing solution, or processed for RNA extraction within 1 hour of collection.

Owing to the labile nature of RNA and the ubiquitous presence of RNase, it requires specialized handling with instruments and reagents which are intended specifically for RNA work (e.g. DEPC treated / RNase-free). These shall be serviced, cleaned and rendered RNase free with special care and records maintained. Care shall be taken to avoid contamination of reagents, laboratory equipment and disposables with RNase. Gloved hands, sterile plastic-ware, barrier tips, and DEPC-treated glassware shall be used to minimize contamination with RNase.

RNA / DNA extraction shall be performed by validated protocols. Complete references shall be included in standard operating procedure. The quality of the starting material affects the quality and yield of the isolated nucleic acids. In addition, repeated freezing and thawing of samples shall be avoided, as this leads to DNA / RNA of reduced size or reduced yields of pathogen DNA (e.g. viral DNA).

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, RNase- free plastic tubes that have not been handled with ungloved hands. Alkaline pH (7.1 to 7.5) is much more effective than acidic or neutral conditions for preservation of RNA samples.

The quality of RNA shall be documented by amplification of a housekeeping gene & gel analysis to ensure that it is an appropriate starting template. The quality / quantity of DNA should be checked using a spectrophotometer / fluorometer.

As RNA degrades rapidly, the laboratory shall have a policy about the receipt of transported samples for RNA based assays. If there is delay due to transportation of samples, the laboratory shall have validated extraction process and test for use in stored / delayed samples. However, in the latter samples, negative results shall be interpreted with caution. A comment shall be added in the final report highlighting this issue.

## **5.5 Examination processes**

### PCR Design and Optimisation

Prevention of contamination of specimens by other nucleic acid targets is a significant challenge. The major sources of contaminants are amplified targets such as PCR products, plasmids or phage. Specific work practices shall be in place to prevent the contamination of specimens since it has the potential to alter a patient's results.

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 for primers / probes, specific sequences, PCR conditions and the size of the expected amplicons should be included.

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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. Restriction digests shall include control(s) with a documented genotype at the locus tested. Laboratory personnel must know how to recognize a partial digest and a degraded specimen. All analytical agarose gels shall include molecular size standards.

#### cDNA Synthesis and RT-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.

A second round of PCR (nested-PCR) using a nested primer pair is required to amplify low abundance mRNA transcripts. This method offers additional sensitivity as well as the added specificity. However it brings the potential for serious contamination problems. In the second round of PCR, tubes containing first-round PCR product shall be opened one at a time to prevent potential tube-to-tube contamination. PCR controls including a negative (no DNA) control shall also be re-amplified to permit detection of low-level contamination.

RT-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 analyzed 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.

For quantitative PCR, a low and high level positive control, preferably close to the levels of clinical decision making shall be used. Data from repetitive runs shall be used to monitor the %CV of the assay.

### Post-PCR

All materials (and equipment: see 5.3 above) including stationery, worksheets, SOPs, etc. in the post-PCR area, shall be dedicated only for this area and there shall be no movement of these to the outside.

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.

Unlabelled 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.

### 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 analyzed 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 shall be used for any interpretation.

### Prenatal Genetic Testing

Prenatal testing shall be carried out in centers 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.

Genetic testing shall meet the requirements of the Ethical Guidelines on Biomedical Research for Human Participants of the Indian Council of Medical Research (ICMR) ([http://icmr.nic.in/ethical\\_guidelines.pdf](http://icmr.nic.in/ethical_guidelines.pdf)).

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Prenatal genetic analysis can be performed using both direct and cultured cells from amniotic fluid and chorionic villi. 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 important that excess samples, if any, are stored until the molecular analysis is completed and reported.

It is recommended that the mutation status of one or both parents, as appropriate, be tested prior to testing of fetal specimens, preferably within the same laboratory. To the extent possible, laboratories should have a follow-up program in place to monitor the accuracy of their prenatal testing.

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 required 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.

### **HLA typing**

#### **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).

Serological or cytotoxicity based HLA antigen assignment is to be carried using the microlymphocytotoxicity method only (popularly known as the Terasaki test).

Determination of HLA class I antigens shall be performed on peripheral blood lymphocytes (PBL) purified on a density gradient and shall be defined by at least 2 antisera, that are certified as operationally monospecific (most commercial serological HLA typing kits available can guarantee this).

Determination of HLA class II antigens shall be performed on B-cell preparations with a documented B-cell purity of 80% or more. Class II antigen assignment by the use of operationally monoclonal antibodies to each DR and DQ antigen shall be determined by (a) 2 antibodies directed to private epitope specificity, or (b) 1 antibody having private epitope specificity and 2 antibodies with public epitope specificity, or (c) 3 antibodies with partially non-overlapping antibodies directed at public epitope determinants.

For cytotoxicity based HLA testing, there shall be established limits for defining positive and negative results by approximate percentage of cell death. Only the specific reactions giving a cytotoxicity score of 80 to 100% cell death may be scored as Positive. 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.

#### Molecular Testing for HLA alleles

DNA-based HLA typing is mandatory for donor selection for patients requiring organ or hematopoietic stem cell transplant. Laboratory must ensure good quality DNA by recording the test sample absorbance ratio at 260/280 nm in a spectrophotometer. The acceptable ratio range is 1.6 – 2.0, with a minimum DNA concentration of 50ng/uL. Use of poor quality DNA may lead to false results.

The laboratory shall take into consideration the following while interpreting results: a) absence of control bands with no specific amplification indicates failed reactions. b) If there is an apparent homozygous result, or if a failed reaction could change an allele assignment, then the test must be repeated. c) Occasionally, the control band may not amplify efficiently when a positive product is seen. This is due to substrate competition and laboratory should not invalidate the test. d) Weakly amplified bands of obvious wrong product size may sometimes be present and may be disregarded if the strength and clarity of the overall amplification is good and interpretation is clear.



The laboratory may select one or more of the following techniques:

- i. Sequence-specific oligonucleotide probe hybridization (SSOP/SSO): Fluorochrome or dye labelled sequence specific probes are hybridized to PCR amplified target DNA and then detected. It allows for handling of large number of samples and its variant. The Reverse-SSO (RSSO) is currently the most popular. In this case, the probes are attached to a bead or membrane, and labeled amplified DNA is added for hybridization. RSSO also allows for semi-automated HLA typing systems to be developed and several such systems are being offered commercially. SSO/RSSO is of much interest in situations where large volume testing is required as in HLA donor registries or low-resolution screening of several possible donors.
- ii. Sequence-specific primer amplification (SSP): Fastest of all methods, it relies on detection of target alleles in PCR amplified DNA using sequence specific primers. The primers have a specificity-dependent nucleotide on the 3' end for accuracy. SSP is highly accurate and high-resolution SSP kits are also available.
- iii. Sequencing-based typing (SBT): PCR amplified target alleles are purified and sequenced. It is the most reliable and accurate method allowing for detection of rare and unknown HLA alleles. Since it provides the highest resolution, SBT is to be used when high resolution testing is desirable as in donors- recipient matching for stem cell transplants and in examining disease associations.

High accuracy and high-resolution commercial HLA typing kits utilizing these techniques are available with several manufacturers.

#### 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.

## CDC cross match test

HLA cross-matching for solid organ transplantation shall use either the standard NIH (CDC) microlymphocytotoxicity 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 has 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 sulphhydryl reducing agents like Dithiothreitol (DTT) with the recipient serum is desirable to rule out Positive reactions caused by IgM antibodies.

Laboratory shall ensure that the following criteria are strictly followed for serological testing:

1. Freshly isolated or previously frozen lymphocytes should have a viability of at least 80% and should contain less than 1% platelets or granulocytes.
2. B lymphocytes isolated for the identification of class II antibodies should contain less than 10% of non-B cells.
3. The background incidence or spontaneous cell death, as assessed by a negative control antiserum, should be less than 20%.
4. All reagents, kits and other biological used testing are to be stored as per manufacturer guidelines and any deviations in storage temperature are to be recorded.

## Flowcytometry based Cross-Matching

In flowcytometry based cross-match, instead of rabbit complement, a set of fluorochrome labeled secondary 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. The flowcytometer shall be calibrated as per manufacturers' instructions. 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.

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### Luminex based cross-matching

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.

The test must be performed exactly as per manufacturer specifications and in the event of any deviation, the test results should be interpreted with caution. The laboratory must ensure calibration and validation of the system on a daily basis or prior to every test. Storage of test material as per manufacturer instructions must be ensured and documented.

### Interpretation of Data

All results shall be checked by two individuals (identified in records) independently, one of whom must be the authorized signatory.

All file materials relating to individual and/or family studies should be cross-referenced for accessibility.

All questionable or inconsistent data must be resolved by either repeating the assay or using an alternative method. The use of positive controls for specific mutations can be helpful in certain situations. The possibility of mistaken paternity, maternal cell contamination, sample mix-up, co-mingling of specimens and allele drop-out should be considered when results are not consistent with the family history or phenotype.

## **5.6 Ensuring quality of examination results**

### Internal Quality Control

A no template control shall be included in each run to detect contamination (as the last sample in the series). The solution replacing the DNA in the PCR reaction shall be a reagent used in sample preparation such as buffer used to rehydrate DNA. This control shall be run right through the extraction, amplification and detection steps.

Controls (negative, normal, positive standards and sensitivity) shall be used as necessary in molecular assays (GAP PCR, long PCRs, restriction enzyme based or RFLP assays, dot blot and reverse dot blot, allele specific oligonucleotide PCR, multiplex PCR and real-time PCR), as necessary. 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

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each run. A low intensity positive control with known level of positivity (e.g. 5% of mutant DNA in normal DNA) shall be used as a sensitivity control for tests detecting mutations/ SNPs (Single Nucleotide Polymorphism). Size markers to check for size of amplicon or DNA digests shall be used. For heteroduplex based mutation screening, that uses conformation sensitive gel electrophoresis, a known heteroduplex positive control shall be used as a control.

### External Quality Assessment

The laboratory should participate in EQA / PT programmes if available.

If these are not available, alternative approaches should be used, as mentioned in the ISO standard and elsewhere in this document.

### **5.7 Post-examination processes**

Molecular testing samples are to be retained for a minimum period as mentioned below (National / State guidelines or legal requirements would take precedence for duration):

- i. DNA - 5 years at -20<sup>0</sup>C (2 years for infectious disease molecular testing)
- ii. Extracted RNA - 5 years at -70<sup>0</sup>C (2 years for infectious disease molecular testing)

Alternatively, the clinical samples should be stored at -70<sup>0</sup>C.

### **5.8 Reporting of results**

In general, the report shall include the following:

- i. Patient / Sample information: collection date, date (and time, if applicable) of receipt in the laboratory, specimen type, name of individual, gender, date of birth, ethnicity / race where appropriate, laboratory identification number, date of report and reason for testing.
- ii. Methodology: Disease locus tested, test performed, methodological details if possible with relevant references, NCBI, UCSC or Ensembl gene ID, mutations tested, notation of any deviation from the laboratory's standard practice, limitations of the assay, the genotype and / or haplotype established for the individual.
- iii. Results and interpretation: a statement interpreting the data (interpretation should be understandable to a non-geneticist professional), including clinical implications, follow-up test recommendations, genetic counseling indications and recommendations, documentation if a preliminary report has been issued and signature of the authorized personnel.

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To describe sequence variants the HUGO (Human Genome Organization) nomenclature is highly recommended. If legacy nomenclature is used relevant references shall be added. In case of novel mutations, comments may be included on reasons why this variant is being thought to cause the observed phenotype. To report somatic mutations in cancer, the COSMIC (Catalogue of Somatic Mutations in Cancer) database can be used as a guide.

### **5.9 Release of results**

Reporting of genetic results needs individualized and tailored interpretation depending on the clinical problem and genetic results, and hence, electronic signature shall not be considered.

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## 6. Guidelines for Operating Sample Collection Centre/Facility (SCF) of the Medical Laboratory

Maintaining the integrity of the test sample at all stages of collection, handling, transportation to the main laboratory and processing plays a vital role to ensure reliability of test results. Therefore, it is important to ensure quality at the collection centres. The detailed procedure for recognition of sample collection centres/ facilities (SCF) declared by medical laboratory is mentioned in NABL 111.

Collection centres are defined as follows:

- (a) Ownership: Collection centres owned by the laboratory or its parent organization and personnel are employees of the laboratory
- (b) Management: Laboratory or its parent organization does not own the collection centre but is entirely responsible for day to day operations and its employees
- (c) Franchisee: Laboratory or its parent company does not own the collection centre but has an arrangement for sample collection under an agreement e.g. hospitals, Nursing home.

Apart from the above, laboratory shall declare details of all other source(s) of sample collection other than the medical laboratory or sample collection centre/ facility. Laboratory shall ensure integrity of samples from these sources. They shall be assessed on a random basis by NABL, however, claim of recognition under Recognized sample collection centre/ facility can not be made by them laboratory/ sources.

The collection centres/facilities shall meet the following guidelines:

All issues related to the operation of collection centres and maintenance of quality shall be addressed by the laboratory in the quality system of the laboratory. Specific instructions for proper collection and handling of primary samples at the collection centre and transportation of these samples to the laboratory shall be documented in a primary sample collection manual, which shall be a part of the quality system of the laboratory and the collection centre.

Laboratory shall document policies and procedures to ensure maintenance of proper hygiene, lighting, environmental conditions and privacy in its collection centres. Collection centres should have adequate space to avoid any cross contamination. During the sample collection in collection centres, laboratory shall ensure the safety, comfort and privacy of the patients.

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The laboratory shall have policies and procedures that integrity of the samples is not affected during collection, storage and transportation. Collection centres shall ensure maintenance of required temperature during transport as mentioned below:

#### Temperature monitoring

Integrity of temperature sensitive parameters / analytes during transport of samples is a major concern in a distant testing scenario. Use of appropriate packaging material, of suitable and well insulated containers, of coolants (4-8°C) and dry ice (for ultra cold temperature) are measures that help in maintaining stability of such samples. However, ensuring a constant and desirable temperature in transit i.e. during the period from collection of the sample to its testing is a major challenge.

The following guidelines will be helpful in this direction:

1. The laboratory may run pilot studies to determine the time taken for samples to reach the laboratory by the route and mode of transport that it plans to use to transport patients' samples for testing. The nature and type of measures required to maintain the samples in the temperature range recommended for the specific parameter / analyte will depend on information gathered from such trial runs. Accordingly, the laboratory should use appropriate packaging and cooling / freezing material for transporting samples. Most parameters / analytes, except some, are stable at ambient temperature for up to 2 - 4 hours from collection. Hence, if the test is carried out within this time frame, special packaging for transporting of samples might not be necessary.
2. It is the laboratory's responsibility to ensure that samples are continuously maintained at the temperature recommended for preservation and transport of samples for the tests to be performed. Monitoring of the temperature of samples during transit using electronic data loggers is encouraged to achieve this objective. These devices are inexpensive and are reusable. The laboratory can include such a device inside the package containing the samples, download and examine the data at the time of receiving the samples in the laboratory. Appropriate corrective measures should be taken by the laboratory if temperature inside the package goes above or below that recommended for the tests to be performed. Samples not maintained at the desirable temperature during transit shall not be accepted for testing.

3. All acceptable samples that are not going to be processed immediately after accessioning shall be transferred to and preserved immediately at appropriate temperatures till testing. This is important for ensuring integrity of samples

Laboratory / collection centre (wherever samples are collected) shall have access to hygienically maintained toilets.

Laboratory shall ensure that its collection centres dispose waste as per the national laws (eg. Biomedical Waste Act) and the local regulations on waste disposal (e.g. the State Pollution Control Board)

For some tests the sample has to be separated & stored (e.g. platelet poor plasma for lupus inhibitors or separation of serum / plasma to be sent in frozen condition); the laboratory shall ensure that adequate training is imparted to the staff for this. Transport of microbiological specimens shall be as per the guidelines of Manual of Clinical Microbiology 10<sup>th</sup> edition 2011, ASM Press.

The staff employed in collection centres shall be adequately trained. The training shall include but not be restricted to issues as:

- i. Policies, procedures and guidelines
- ii. Maintenance of proper hygiene and environmental conditions
- iii. Methodology for collection of sample and the amount required
- iv. Processing of collected samples
- v. Packaging of samples
- vi. Proper transportation of the samples / specimen
- vii. First aid measures to be taken, in case of abnormal events
- viii. Safety and waste disposal

Spillage: Treatment of spills - Any spill should be covered with a blotting paper / paper towel to reduce the volume of spill. Pour 1% hypochlorite over it and leave it for 30 mins. Discard this in the yellow / red bags as per the waste segregation guidelines.

Occupational safety: Needle stick injury and the action taken to be recorded.

Laboratory shall ensure the evaluation of the training imparted to staff in collection centres and maintain records.

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Laboratory shall have a plan to conduct internal audit of its collection centres so that they meet NABL guidelines. Laboratory shall conduct internal audit of each of its collection centre at least once a year. Management review of the laboratory shall also discuss the internal audit of its collection centres.

Only those collection centres which are declared to NABL shall be claimed by the laboratory as a part its laboratory system. The laboratory shall include the name and address of its collection centre in the test reports. The sample collection centre can claim recognition in line with NABL 133.

Collection centre(s) of the laboratories will be assessed by NABL These may or may not be assessed by the same assessor who has conducted assessment of the laboratory. Their assessment may be conducted separately by another assessor at a different time. Assessors shall assess the records maintained by the collection centres, including the internal audit records of collection centres. Competence of the staff especially the phlebotomist shall also be assessed.

If major non-conformities or a total system failure is observed during the assessment of a collection centre, the collection centre/facility will not get any recognition or will be derecognized, if it already holds recognition. In case the laboratory fails to take corrective actions or there is a consistent system failure, an appropriate and proportionate action against the laboratory will be taken.

Only those collection centres/facilities which are declared to NABL shall be claimed as recognized sample collection centres/facilities of that laboratory during its valid accreditation cycle.

The following pages present a checklist for assessing the collection centres, which form the additional requirements for accreditation of Medical laboratories operating collection centres.

Records mentioned in the checklist 3 shall be available at the collection centre during assessment.

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## 7. Checklist for Assessment of Sample Collection Centre/ Facility (SCF) of Medical Laboratory

Collection Centre / Facility: \_\_\_\_\_

### Premises

			Remarks
1.	Type of the Collection Centre / source of sample	Owned / Managed / Franchise/Any other source of sample collection which is not categorized above	
2.	Size of premises	Adequate / Inadequate	
3.	Average Number of patients per day		
4.	Does it meet the requirement of the workload	Yes / No	
5.	Reception and waiting area separate from collection area	Yes / No	
6.	Hand washing facility	Yes / No	
7.	Access to hygienically maintained toilet facility	Yes / No	
8.	Provision of privacy during collections	Yes / No	
9.	Hours of operation have been displayed	Yes / No	

### Accommodation and Environmental Conditions

1.	Is it adequately lit and clean	Yes / No	
2.	Is the humidity and temperature suitable	Yes / No	
3.	Are cleaning policies available	Yes / No	
4.	Is it adequately ventilated and prevented from dust	Yes / No	
5.	Does it have adequate space & separation to avoid cross contamination	Yes / No	
6.	Is the house keeping adequate	Yes / No	

### Equipment

1.	Refrigerator (temp. record; calibrated temp. recording device)	Yes / No	
2.	Centrifuge (Calibration records)	Yes / No	
3.	Proper storage of supplies	Yes / No	
4.	Suitable chair and/ or couch for collection of blood, etc.	Yes / No	
5.	Basic first-aid material	Yes / No	
6.	Telephone	Yes / No	
7.	Air conditioning, if applicable	Yes / No	
8.	Power backup for equipment	Yes / No	

## Material

1.	Material required for specimen collection e.g. evacuated blood collection tubes, syringes, tubes, swabs etc.	Yes / No	
2.	Presence of expired supplies	Yes / No	

## Staffing

1.	Staff members	_____ nos.	
2.	Number of phlebotomists		
3.	Is manpower appropriate to the workload?	Yes / No	
4.	Training records	Yes / No	
5.	Does the staff possess knowledge of first-aid measures to deal with situations they are likely to encounter in the course of specimen collection?	Yes / No	

## Documentation

1.	List of services provided	Yes / No	
2.	Sample collection manual available	Yes / No	
3.	Records of Internal audit	Yes / No	

## Health and Safety

1.	Collection staff to observe universal precautions (to wear gloves, lab coat & protective mask)	Yes / No	
2.	Vaccinated against Hepatitis B	Yes / No	

## Safety and Waste Disposal

1.	Approved receptacles for sharps and for contaminated waste available	Yes / No	
2.	Transport and disposal of waste is in accordance with applicable regulatory requirements	Yes / No	

## Transport of Pathology Specimens

1.	Does the collection centre follow national / international regulations for the transport of infectious and other diagnostic specimens by air and by surface so that in the event of an accident, courier staff and the general public may not be exposed to blood and body fluids	Yes / No	
2.	Has the specimen collection staff participated in training in specimen collection, transport, handling of emergencies etc?	Yes / No	
3.	Has the staff participated in retraining within two years interval?	Yes / No	
4.	Is the parcel of infectious substances attached with a plastic envelope containing 'Bio-hazard' label	Yes / No	

## Packaging

1.	Is the primary container leak proof?	Yes / No	
2.	Does the secondary container possess sufficient absorbent material to absorb the contents if the primary container leaks?	Yes / No	
3.	Are both the above containers properly labeled?	Yes / No	
4.	Is the secondary container packed into appropriate outer packing and labeled appropriately?	Yes / No	
5.	Is cooling agent included in the outer package if cold chain is to be maintained?	Yes / No	
6.	Monitoring the transport condition by electronic data loggers (wherever applicable)	Yes / No	
7.	Is the outer package labeled, addressed and taped securely	Yes / No	
8.	Are slides mailed in rigid slide container to prevent breakage	Yes / No	

## Complaints / Feedbacks

1.	Does the collection centre have provision for receiving of complaints / feedbacks	Yes / No	
2.	Are the complaints / feedbacks reviewed and resolved by the laboratory	Yes / No	

.....

## Guidelines for Scope Preparation

HISTOPATHOLOGY

Sl.No.	Materials or items examined / tested	Specific tests / examination performed	Specification, standard (method) or technique used	Range of testing/ Limit of detection	%CV / MU
<b>A.</b>	<b>Biopsy</b>				
1.	Small, Medium & Large tissues	Grossing , Decalcification, Processing. Paraffin embedding Microtomy H& E Staining (Conventional)	Light Microscopy with Interpretation	Descriptive	NA
2	Biopsy with Immuno- fluorescence Renal / Skin etc	Grossing – Processing, Paraffin embedding Microtomy H& E Staining (Conventional) + Cryosections Staining with FITC for IF	Light Microscopy + Flourescent Microscopy with Interpretation	Descriptive	NA
3	Slides for second opinion	Stained / Unstained slides	Light Microscopy with Interpretation	Descriptive	NA
4	Paraffin Blocks for second Opinion	Microtomy H & E Staining (Conventional)	Light Microscopy, Interpretation	Descriptive	NA
5.	Cell block preparations for fluids / aspirates	Processing Microtomy H & E Staining (Conventional)	Light Microscopy with Interpretation	Descriptive	NA
<b>B</b>	<b>Frozen Section</b>				
	Biopsy : As only representative samples are sent for frozen	Grossing, Processing, Microtomy Staining (H & E, Toluidine blue, PAP) (Conventional)	Light Microscopy with Interpretation	Descriptive	NA

<b>C</b>	<b>Histo-chemistry (All special stains performed to be listed)</b>				
1	Tissue / Paraffin block / Unstained slide	PAS stain Mcmanus method	Light Microscopy with Interpretation	Descriptive	NA
2	Tissue / Paraffin block / Unstained slide	Reticulin stain	Light Microscopy with Interpretation	Descriptive	NA
3	Tissue / Paraffin block / Unstained slide	Grocott Methanamine silver stain for fungi	Light Microscopy with Interpretation	Positive / Negative	NA
4	Tissue / Paraffin block / Unstained slide	Congo red stain	Light Microscopy with Interpretation	Positive / Negative	NA
5	Tissue / Paraffin block / Unstained slide	ZN stain for AFB	Light Microscopy with Interpretation	Descriptive	NA
6	Tissue / Paraffin block / Unstained slide	Perl's Stain, for Iron	Light Microscopy with Interpretation	Descriptive	NA
7	Tissue / Paraffin block / Unstained slide	Warthin starry stain	Light Microscopy with Interpretation	Descriptive	NA
8	Tissue / Paraffin block / Unstained slide	Alcian Blue stain	Light Microscopy with Interpretation	Descriptive	NA
9	Tissue / Paraffin block / Unstained slide	Elastic Verhoeff's Van Gieson (EVG) Stain	Light Microscopy with Interpretation	Descriptive	NA
10	Tissue / Paraffin block / Unstained slide	Mucicarmine stain	Light Microscopy with Interpretation	Descriptive	NA
11	Tissue / Paraffin block / Unstained slide	Toluidine blue stain	Light Microscopy with Interpretation	Descriptive	NA
12	Tissue / Paraffin block / Unstained slide	Giemsa stain	Light Microscopy with Interpretation	Descriptive	NA
<b>D</b>	<b>Immunohistochemistry / Immunocytochemistry</b>				
1	Tissue / Paraffin block / Cytology slides / Unstained slide on APES / Polylysine coated / charged slides	Conventional + Antigen Retrieval & IHC staining (to mention manual / automated & the name of the marker)	Light Microscopy with Interpretation	Descriptive	NA

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<b>E</b>	<b>In-situ hybridization</b>				
1	Tissue / Paraffin block / Cytology slides / Unstained slide on APES / Polylysine coated / charged slides	Manual / Automated	Light Microscopy with Interpretation	Descriptive	NA

### **CYTOPATHOLOGY**

<b>A</b>	<b>Cytopathology: Non Gynaec</b>				
1.	Palpable or non palpable lesion involving any organ	FNA, smear preparation, MGG staining with or without PAP / H & E	Light Microscopy with Interpretation	Descriptive	NA
2.	Body fluids (Ascitic, Pleural, CSF, Synovial, Pus, BAL fluid, ET secretions, nipple discharge)	Cytospin/ Centrifugation, smear preparation, Pap, H & E and MGG staining (To include CSF in the scope the laboratory needs to have a cytospin)	Light Microscopy with Interpretation	Descriptive	NA
3.	Unstained smears	MGG staining with or without PAP / H & E	Light Microscopy with Interpretation	Descriptive	NA
4.	Scrapings / brushings (GIT, bronchial, oral)	MGG staining with or without PAP / H & E	with Interpretation	Descriptive	NA
5.	Scraping from vesiculobullous lesions of skin	Tzanck Smear MGG Stain	Light Microscopy with Interpretation	Descriptive	NA
<b>B</b>	<b>Cytopathology: Gynaec</b>				
1.	Cervical and vaginal smears (Conventional / Liquid based, to be mentioned)	PAP stain	Light Microscopy with Interpretation	Descriptive	NA

## MICROBIOLOGY & INFECTIOUS DISEASE SEROLOGY

<b>A BACTERIOLOGY</b>					
1.	Sputum, Pus, Body Fluids except Blood, swabs	Identification of bacteria by Gram's Staining	Light Microscopy	Qualitative	NA
2.	Throat & nasopharyngeal swabs	Staining of metachromatic granules	Albert's Staining / Light microscopy	Qualitative	NA
3.	Stool	Examination for Cryptosporidium, cyclospora	Modified Acid fast (Ziehl Neelsen) staining	Qualitative	NA
4.	Stool	Hanging drop for cholera	Microscopy	Qualitative	NA
5.	Respiratory, skin, pus, discharges	AFB staining for Nocardia, actinomyces	Modified Kinyoun's method & light microscopy	Qualitative	NA
6.	Blood, bone marrow, sterile body fluids	Aerobic Culture	Culture – Aerobic by Automated method	Qualitative	NA
7.	Body fluids, Aspirate fluid, Pleural fluid, Ascitic fluid, Peritoneal dialysate and Bile	Aerobic Culture Identification & Antibiotic sensitivity	Routine Culture – Aerobic, ID by automated/manual methods AST by Disk Diffusion / MIC / E test	Qualitative	NA
8.	Stool, Bronchial secretions	Adenovirus antigen Detection	Rapid Immuno-chromatography	Qualitative	NA
9.	Throat Swab	Gr A Streptococcus antigen detection	Latex Agglutination	Qualitative	NA
<b>B MYCOBACTERIOLOGY</b>					
10.	Sputum, Pus, Body Fluids except Blood, swabs	Detection of Acid Fast Bacilli	Direct and / or Concentration method / Ziehl Neelsen / Kinyoun's stain and Microscopy	Qualitative	NA
11.	Sputum, Pus, Body Fluids except Blood, swabs	Detection of Acid Fast Bacilli	Fluorescent Microscopy by Auramine Staining	Qualitative	NA
<b>C INFECTIOUS DISEASE SEROLOGY</b>					
12.	Serum	AntiStreptolysin-O Antibody	Latex Agglutination	Qualitative	NA
13.	Serum	Leptospira IgM	Immuno-chromatography	Qualitative	NA

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14.	Serum	ANA( Anti Nuclear antibodies)	Immunoflorescence	Qualitative	NA
15.	Serum	Anti HBs Ag	ELISA	Semi – Quantitative (Reactive / Non-reactive)	7.3

### **CL. BIOCHEMISTRY**

1.	Urine	5 HIAA	Column Chromatography	0.9-200 mg/l	10.7
2.	Serum	ALP - Isoenzymes	Gel Electrophoresis	Qualitative	NA
3.	Serum / plasma	Apolipoprotein A1	Immunoturbidimetry	20-400 mg/dl	4.5
4.	Serum / plasma	Apolipoprotein A1	Turbidimetry Dry Chemistry	30 – 240 mg/dl	4.5
5.	Serum	Apolipoprotein B	Immunoturbidimetry	20-400 mg/dl	7.1
6.	Serum	Apolipoprotein B	Turbidity Dry Chemistry	35 – 300 mg/dl	7.0
7.	Serum	B U N / Urea	U V Kinetic GLDH	3 – 240 mg/dl	3.5
8.	Serum	B U N / Urea	Urease	2.0 – 120 mg/dl	3.0
9.	Urine	Urea / Urea Nitrogen	U V Kinetic GLDH	150 - 12000 mg/dl	1.1
10.	Urine	Urea / Urea Nitrogen	Urease	67 – 2520 mg/dl	2.7
11.	Urine	Bence Jones Proteins	Capillary Electrophoresis	Qualitative	NA
12.	Serum	Bilirubin Direct	Diazotized Sulphanilic Acid	0.1 - 25.2 mg/dl	5.7
13.	Serum	Bilirubin Total	Diazotized Sulphanilic Acid	0.1 - 35.1 mg/dl	4.5
14.	Serum	C Reactive Protein (CRP) Ultrasensitive	Immunoturbidimetry	0.15 – 20mg/l	6.4
15.	Serum	High Sensitivity CRP	Turbidimetry	0.10 – 15 mg/L	4.8
16.	Urine	Calcium	Spectrophotometry (o-CPC)	0.6-30 mg/dl	5.4

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17.	Urine	Calcium	Arsenazo III	1.0 – 17.80 mg/dl	1.9
18.	Serum	Calcium – Ionized	Ion Selective Electrode	0.2-5 mmol/L	5.7
19.	Serum	Calcium Total	Spectrophotometry(o- CPC)	0.4 – 20 mg/dl	2.9
20.	Serum	Calcium Total	Arsenazo III	1.0 – 14.0 mg/dl	1.9
21.	Serum	Magnesium	Chelation Method	0.20 – 10.0 mg/dl	1.7
22.	Urine	Magnesium	Colorimetric CPZ III	1.2-30.4 mg/dl	3.2
23.	Urine	Magnesium	Chelation Method	1.2 – 60 mg/dl	4.9
24.	Urine	Microalbumin	Immunoturbidimetry	3-400 mg / l	7.9
25.	Urine	Microalbumin	Turbidimetry	6.0 – 190 mg/l	1.4
26.	Serum	P S A (Free Hormone) (Prostate Specific Antigen)	Chemiluminiscence	0.01-50 ng/ml	7.7
27.	Serum	P S A (Total) (Prostate Specific Antigen)	Chemiluminiscence	0.003 – 100 ng/ml	3.9
28.	Serum	P S A (Total) (Prostate Specific Antigen)	Electro Chemiluminiscence	0.064 – 100 ng/ml	2.5

## Guidelines for Lot Verification

### Lot verification or parallel testing of controls

1. **CI. Biochemistry:** New lot of controls should ideally be run in parallel with old lot of controls
2. **CBC and coagulation controls:** The new QC lots may be verified against the old lot by running them parallel.

Acceptance criteria – Review the data to ensure that there are no trends. Outlier should be eliminated before calculating the laboratory mean and ranges. The ranges are acceptable when it is within manufacturer's recommendations (available in the instrument or method documentation). Calculate the new mean and standard deviation for each analyte (as applicable)

### Lot verification or parallel testing of reagents

#### **HIV EIA and other EIA Assays:**

1. A minimum of 2 patient samples (negative, low positive and high positive if available) or an entire strip from a previous run are tested in parallel with QC on both the old and the new lot numbers.
2. The QC and patient results should be reproducible between the two lots (reproducibility includes both the OD readings and the interpretations).

#### **HIV RNA PCR Quantitative Assay:**

1. A minimum of 2 patient samples (not detected, low positive and high positive, if available) or an entire strip from a previous run are tested in parallel with the QC on both the old and the new kit or reagent lot number.
2. The QC and patient results should be reproducible between two lots. Typical criteria for the acceptability of quantitative PCR assays would be that any variation should not be greater than three-fold or they should be within 2 fold.

### **Chlamydia, HIV PCR Qualitative Assays:**

1. A minimum of 2 patient samples should be run in parallel on both the old and the new lots.
2. The QC and patient results should be reproducible between the two lots. The results should confirm that there is agreement between the results for the two kits (i.e. negative results are negative on the new kit and positive results are positive).

### **CD4 / CD8 Assay:**

1. A minimum of 2 patient samples (One normal and one abnormal sample) or QC should be run in parallel when antibody lot numbers, reagent lot numbers or reagent kits, such as true count, lot numbers are changed.
2. The QC and patient results should be reproducible between the two lots. Acceptability limits for parallel is difference of <10% for both percent and absolute counts.

### **Chemistry Assays:**

1. A minimum of 2 patient samples or QC should be run on the old and new lot number.
2. Acceptability limits are within +/- 1SD or within +/-10%.

### **CBC Analyzer Reagents**

1. Material of known value may include patient samples or controls – minimum of 2 patient samples.
2. Background checks must be performed on inert materials such as diluent to ensure that new lots do not interfere with patient results.

### **Coagulation Reagents:**

#### **PT Reagent**

1. Parallel testing of new lots of PT reagents also includes verifying the reference range, geometric mean and programming the correct ISI (international Sensitivity Index) into the coagulation analyzer.
2. To verify the reference range and geometric mean it is necessary to collect specimens from 20 “normal” subjects and to run a PT with the new lot of thromboplastin reagent. 90% of the samples must fall within the current range in order to verify the range and geometric mean. If they do not, a new reference range study must be conducted to determine them. Microsoft Excel or other appropriate clinical reference range software must be used to calculate the new range and geometric mean.

3. Perform comparison studies between the old and new lot number to verify the consistency of patient results and controls. The R value for the correlation study should be  $\geq 0.97$ .
4. Validate the PT reference range with 20 specimens. If the reference range does not validate perform a new reference range study using at least 60 specimens.
5. Finally, perform a manual check of the INR and compare with the instrument generated INR result.

**PTT (APTT) Reagents:**

1. Parallel testing of PTT reagents should be conducted well in advance of the expiration of the old reagent.
2. Perform comparison studies between the old and new lot number using patient samples and controls. The R value for the correlation study should be  $R \geq 0.97$ .
3. Please note that if patient on heparin therapy are being monitored, the laboratory should perform a new heparin curve with each change of reagent lot.

**Semi quantitative Tests Urine analyser strips:**

1. A minimum of 2 patient samples are run in parallel on both the old and the new lots (The samples should demonstrate varying results across the range for different strip analytes).
2. The QC and patient results should be reproducible between the two lots. Generally negative results should remain negative, positive results should give the same results or be one level up or down from the original result).

**Qualitative Testing (Rapid HIV test kits, urine / serum qualitative hCG etc.)**

1. A minimum of 2 patient samples are run in parallel on both the old and the new lots.
2. The QC and patient results should be reproducible between the two lots i.e. negative results are negative on the new lot and positive results are positive.

**Guidelines for following Order of Draw**

The following order of draw is recommended when drawing multiple specimens for clinical laboratory testing during a single venipuncture. Its purpose is to avoid possible test result error due to cross contamination from tube additives.

- (1) Blood culture tube
- (2) Coagulation tube (e.g. blue closure) – To be the 1st tube when blood culture is not being collected
- (3) Serum tube with or without clot activator, with or without gel (e.g. red closure)
- (4) Heparin tube with or without gel plasma separator (e.g. green closure)
- (5) EDTA (e.g. lavender closure)
- (6) Tube with glycolysis inhibitor

## Guidelines for utilizing Proficiency testing reports to improve quality of a laboratory

To achieve specified levels of quality, there are two aspects that must be addressed:

1. Internal quality control and quality procedures
2. Use of a proficiency testing service for external quality assessment

EQAS (External Quality Assessment Scheme) is designed to provide the external proficiency testing services so as to enable laboratories to compare themselves with peer groups and thus be able to assess themselves primarily on the issue of accuracy.

For using EQAS to achieve acceptable quality; the pre-analytical, analytical and post-analytical factors should be taken into account.

### A. Pre-analytical:

1. Try to perform the tests as early as possible from the date of receipt of EQA samples. The lyophilized / stabilized samples should be stored in a refrigerator (2-8°C) until testing. Do not freeze the lyophilized samples.
2. All EQAS samples must be treated in the same manner as a routine patient specimen.
3. Most programs provide lyophilized / stabilized samples. Follow instructions carefully about the reconstitution and preparation of sample. If exact volume of water is not used for reconstitution, the resulting sample may be more dilute or concentrated. Use of a calibrated volumetric pipette is recommended.
4. Mix gently to avoid frothing. After reconstitution, the sample should be kept at room temperature for the prescribed period of time.
5. The testing should be performed within the recommended period of time after reconstitution. If samples have been reconstituted and left for long periods of time at room temperature, refrigerated or frozen and thawed, the results will become unreliable.

### B. Analytical:

1. The procedure for testing must be identical to that for all the patient samples. No additional precautions should be performed prior to testing EQA materials except at the preparatory stage. This is because the test is being done to determine the quality of routine procedures being followed in the laboratory (as being followed for any random patient sample). For instance if the laboratory repeats the test on the EQA sample

whereas routine patients are tested only once then the purpose of participation in EQAS is defeated.

2. The laboratory using any form of automation should not perform non-routine quality / maintenance procedures on the analyzer prior to testing the EQA material.
3. For laboratory that performs manual testing, it is preferable that the same analyst who carries out the routine testing also performs the proficiency test. If this is not done, then the laboratory will fail to get a true reflection of its practices related to patient specimens.

**C. Post-analytical:**

1. The reporting of EQA results should be in the same manner as used for reporting patient results. Some of the routine laboratory methods of reporting such as reviewing patient's prior results may not apply in these situations.
2. Precautions must be taken to ensure that there are no transcription errors.
3. Reconstituted EQA samples should not be stored for future testing as they tend to deteriorate and will not provide accurate results.
4. Lyophilized samples if available may be stored in a refrigerator for up to one year. Prior to use check for the presence of liquid in the vial or discoloration of the pellet. These are indicators of deterioration. Do not freeze lyophilized specimens.

Handling unsatisfactory scores:

The laboratory will at some time receive unsatisfactory scores. A single poor score should not cause for excessive concern. That being stated, each unsatisfactory results should be investigated carefully. This should not be a reason to precipitate procedural change until a careful analysis has been performed. As a part of analysis the laboratory can use the following protocol:

1. Perform a root cause analysis as to why the unsatisfactory result was obtained.
2. Begin from sample handling and address all aspects mentioned in pre-analytical factors.
3. Some errors may result from the non-standard sample preparation process required for EQA samples. However, all other sources of error should be ruled out before labeling the error as being such.
4. EQA results can be used to suggest preventable measures. Careful assessment of acceptable results and comparison with unsatisfactory ones will be helpful in this regard.
5. Besides individual results, trends should be examined as they could signal potential problems. Even if current results are satisfactory, timely action will help prevent future poor results. Example: when several of the results lie on one side of the mean.



6. Accreditation processes require root cause analysis, corrective measures, preventive measure reports & follow up audits be performed and documented.

A simple approach would be to classify the problem:

i. Clerical error

1. Transcription error (may be pre- or post-analytical factors).
2. Situations where wrong method has been registered for analysis or method change not updated.

ii. Methodological problem

1. Instrument function checks (e.g., temperatures, blank readings, pressures) not performed as necessary, or results not within acceptable range.
2. Scheduled instrument maintenance not performed appropriately.
3. Incorrect instrument calibration.
4. Standards or reagents improperly reconstituted and stored, or inadvertently used beyond expiration date.
5. Instrument probes misaligned.
6. Problem with instrument data processing functions. The laboratory may need to contact the manufacturer to evaluate such problems.
7. Problem in manufacture of reagents / standards, or with instrument settings specified by manufacturer
8. Carry-over from previous specimen.
9. Automatic pipettor not calibrated to acceptable precision and accuracy.
10. Imprecision from result being close to detection limit of method.
11. Instrument problem not detected by quality control:
  - a) QC material not run within expiration date, or improperly stored
  - b) QC material not run at relevant analyte concentration
12. Result not within reportable range (linearity) for instrument / reagent system.
13. Obstruction of instrument tubing / orifice by clot or protein.
14. Incorrect incubation times.

iii. Technical problem

1. EQA material improperly reconstituted.
2. Testing delayed after reconstitution of EQA material (with problem from evaporation or deterioration).
3. Sample not placed in proper order on instrument.
4. Result released despite unacceptable QC data.
5. QC data within acceptable limits but showed trend suggestive of problem with the assay.
6. Inappropriate quality control limits / rules. If the acceptable QC range is too wide, the probability increases that a result will fall within the acceptable QC range yet exceed acceptable limits for EQA.
7. Manual pipetting / diluting performed inaccurately, at an incorrect temperature or with incorrect diluent.
8. Calculation error or result reported using too few significant digits.
9. Secondary specimen tubes incorrectly labeled.
10. In addition to above discipline specific errors may also occur.

iv. Problem with proficiency testing materials

1. Matrix effects: The performance of some instrument / method combinations may be affected by the matrix of the PT sample. This can be overcome to some extent by assessing participants in peer groups – to be done by the PT provider.
2. Non-homogenous test material due to variability in fill volumes, inadequate mixing, or inconsistent heating of lyophilized specimens.
3. Non-viable samples for microbiology PT program.
4. Haemolysis on an immune-haematology program samples.

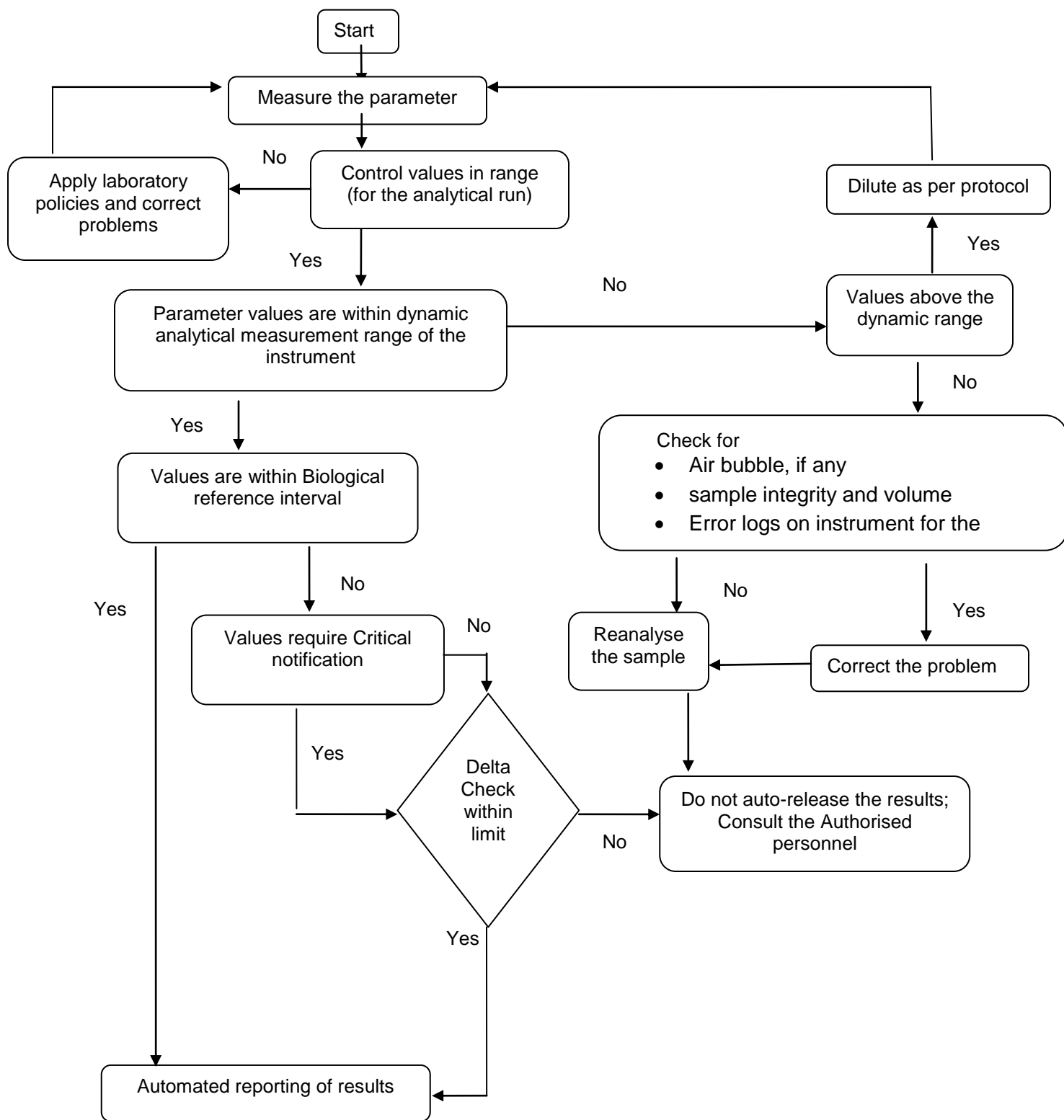
v. Problem with evaluation of results by the PT provider

1. Peer group not appropriate.
2. Inappropriate target value: Target values developed from participant consensus can be inappropriate from non-homogeneous testing material or lingering ("masked") outliers. However, occasional inappropriate target values occur in every PT program.  
Inappropriate evaluation interval: An evaluation interval may be inappropriately narrow e.g. if  $\pm 2$  standard deviation units are used with an extremely precise method, the acceptable range may be much narrower than needed for clinical usefulness.
3. Incorrect data entry by PT provider.

vi. No explanation after investigation

When all identifiable sources of error have been excluded, a single unacceptable result may be attributed to random error, particularly when the result of repeat analysis is acceptable. In such cases, no corrective action should be taken; as such an action may actually increase the probability of a future unacceptable result.

Guidelines algorithm for Automated Selection and Reporting of Results



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