SRI LANKA ACCREDITATION BOARD FOR CONFORMITY ASSESSMENT

GUIDELINE FOR USE OF REFERENCE MATERIALS IN MEDICAL TESTING

CONTENT

0. INTRODUCTION

Reference materials are important tools in realising a number of aspects of measurement quality and are used for method validation, calibration, estimation of measurement uncertainty, training, assigning values to internal quality control (IQC) and external quality assurance (EQA) (proficiency testing) purposes.

Reference materials are defined as materials, sufficiently homogeneous and stable with respect to one or more specified properties, which have been established to be fit for their intended use. Generating valid laboratory test results and making them comparable irrespective of time, place or laboratory generating the results, require standardization using reference materials.

Routine measurement procedures with calibration traceable to higher-order reference material or reference method should produce test results that are comparable. This comparability or uniformity of test results is obtained in relation to reference method or reference material used.

Harmonization of laboratory testing refers to the ability of a laboratory to achieve the same test result and the same interpretation, irrespective of the measurement procedure used, the unit or reference interval applied, or when and where a measurement is made. The lack of harmonization makes it difficult to compare lab results obtained at different laboratories or even the same laboratory over a period of time. Therefore, generating valid laboratory test results and making them comparable require standardization using reference materials and harmonization of methods used.

Validity of laboratory results can be influenced by many factors. Broadly, the validity of measurements can be assured when:

- samples fulfil predetermined quality requirements including stability
- validated analytical procedures are used
- use of traceable reference material with documented uncertainty.
- comparability of measurements with other laboratories is assured (traceability and measurement uncertainty)
- qualified and competent staff undertake the work
- independent evidence of performance verification (Proficiency Testing) is available
- well defined quality management procedures (QC and QA) are employed, preferably involving third party attestation (accreditation)
- work is carried out to a clearly defined customer requirement

Documentation of calibration traceability to a higher order reference material or reference procedure may be provided by the manufacturer of the equipment or reagents. Such documentation is acceptable as long as the manufacturer's examination system and calibration procedures are used without modification. If this is not possible or relevant, other means of providing confidence in results shall be applied.

These include, but are not limited to:

- Use of certified reference material
- Calibration by another procedure /method comparison
- Mutual consent standards or methods which are clearly established
- Methods specified, characterised or mutually agreed upon by all the parties concerned.

Tests carried out in both testing laboratories accredited to ISO/IEC 17025:2017 as well as medical laboratories accredited to ISO 15189:2012, the requirements for traceability are as follows.

ISO 15189:2012 specifies the requirements for equipment calibration and metrological traceability. Clause 5.3.1.4 of the standard specifies that the laboratories shall establish a documented procedure for calibration of equipment with recorded metrological traceability of the calibration standard /working standard. This ensures traceability of the calibration of the equipment which directly or indirectly affects examination results. Metrological traceability shall be to a reference material or reference procedure of a higher metrological order whenever possible (4.1).

The standard also specifies that where this is not possible or relevant, other means for providing confidence in the results shall be applied, including but not limited to: use of certified reference materials, examination or calibration by another procedure, mutual consent standards or methods which are clearly established, specified, characterized and mutually agreed upon by all parties concerned.

ISO/IEC 17025:2017 standard also specifies that all equipment used for tests /or calibrations, including equipment for subsidiary measurements having significant effect on the accuracy or validity of the results of a test, calibration or sampling shall be calibrated before being put into service (4.2).

Clause 6.5 of the standard specifies that the laboratory shall establish and maintain metrological traceability of its measurement results by means of a documented unbroken chain of calibrations, each contributing to the measurement uncertainty, linking them to an appropriate reference.

The laboratory shall ensure that measurement results are traceable to the international system of

Units (SI) through calibration provided by a competent laboratory using certified reference materials provided by a competent producer with stated metrological traceability to the SI, or direct realization of the SI units ensured by comparison, directly or indirectly, with national or international standards. Standard also specifies that when metrological traceability to the SI units is not technically possible, the laboratory shall demonstrate metrological traceability to an appropriate reference.

There are certain calibrations that currently cannot be strictly made in SI Units, in these cases calibration shall provide confidence in measurements by establishing traceability to appropriate measurement standards such as the use of certified materials, provided by a competent supplier to give a reliable physical or chemical characterization of a material.

1. SCOPE

This document describes reference material used in laboratory medicine. It provides guidance for selection, acceptability, traceability, clarification and use of reference material and certified reference material. It also acknowledges the limitations and restrictions analysts have to face.

2. TERMS & DEFINITIONS

In the interpretation of this document, the following definitions related to reference material shall be used [4.3, 4.4,4.5, 4.6, 4.7].

2.1 Reference Material (RM)

Material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process

2.2. Certified Reference Material (CRM)

Reference material (RM) characterised by a metrologically valid procedure for one or more specified properties, accompanied by an RM certificate that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability

2.3. Reference method

Measurement method, that has been shown to have the appropriate trueness and precision for its intended use and has been officially defined as reference method by a competent body.

2.4. Reference Material Certificate

Document containing the essential information for the use of a CRM, confirming that the necessary procedures have been carried out to ensure the validity and metrological traceability of the stated property values.

2.5. Primary measurement Standard

Measurement standard that is designated or widely acknowledged as having the highest metrological qualities whose property value is accepted without reference to other standards of the same property or quantity, within a specified context.

2.6 **Primary standard**:

Standard that is designated or widely acknowledged as having the highest metrological qualities and whose value is accepted without reference to other standards of the same quantity.

2.7 Property value

Value corresponding to a quality representing a physical, chemical or biological property of an RM

2.8 Homogeneity

Uniformity of a specified property value through out a defined portion of a reference material

2.9 Matrix reference material

Reference material that is characteristic of a real sample

2.10 Metrological traceability

Property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty.

3. ABBREVIATIONS

3.1 **APTT:** Activated Partial Thromboplastin Time

3.2 **ATCC:** American Type Culture Collection

3.3 **BAM**: Bacteriological Analytical Manual

3.4 **BCSH:** British Committee for Standards in Haematology

3.5 **CFUs (unit):** Colony-Forming Unit

3.6 **CITAC:** The Cooperation on International Traceability in Analytical Chemistry

3.7 **CLSI:** Clinical and Laboratory Standards Institute

3.8 **CRM:** Certified Reference Materials

3.9 **EQA:** External Quality Assurance

3.10 **ESR:** Erythrocyte Sedimentation Rate

3.11 **EURALAB:** European Data Laboratory for Comparative Social Research

3.12 **Hb:** Haemoglobin

3.13 **HiCN:** Cyanmethhaemoglobin method

3.14 **ICSH:** International Council for Standardization in Haematology

3.15 **ILAC:** International Laboratory Accreditation Cooperation

3.16 **IQC:** Internal Quality Control

3.17 **MRI:** Medical Research Institute

3.18 **NICC:** Northeast Iowa Community College

3.19 **NIST:** National Institute of Standards and Technology

3.20 **PCV:** Pack Cell Volume

3.21 **PT:** Proficiency Testing

3.22 **QA:** Quality Assurance

3.23 **QC:** Quality Control

3.24 **REMCO:** The ISO Committee on Reference Materials

3.25 **RM:** Reference Material

3.26 **SLAB:** Sri Lanka Accreditation Board

3.27 **SLCH:** Sri Lanka College of Haematologists

3.28 **WHO:** World Health Organization

4. REFERENCES

In the preparation of this document, the following documents were referred.

4.1 ISO 15189:2012 - Medical laboratories- Requirements for quality and competence

4.2 ISO/IEC 17025:2005 - General requirements for the competence of testing and calibration laboratories

4.3 ISO/IEC 17043:2010 Conformity assessment - General requirements for proficiency testing

4.4 ISO /GUIDE 30:2015 Reference materials - selected terms and definitions

4.5 ISO Guide 31:2015 Reference Material-Contents of certificates, labels and accompanying documentation.

4.6 ISO Guide 33 2015 Reference Material- Good Practice in using Reference Material (replaces ILAC G9)

4.7 Eurachem: Terminology in Analytical Measurement, Introduction to VIM 3: 2011

4.8 Peter Roper, Shyam Burke, Richard Lawn. 2001 Application of Reference Materials in Analytical Chemistry; Published by the Royal Society of Chemistry as a deliverable under the UK's Valid Analytical Measurement (VAM) programme.

4.9 Eurachem Guide: The fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics, 1998, www. eurachem.org.

4.10 ISO /IEC 17034: 2016 General requirements for the competence of reference material producers.

4.11 ISO Guide 35:2017: Reference materials — Guidance for characterization and assessment of homogeneity and stability.

4.12 User verification of precision and estimation of bias. CLSI- EP15-A3: (2014). Third Edition

4.13 Measurement verification in the clinical laboratory: A guide to assessing analytical performance during the acceptance testing of methods (quantitative examination procedures) and/or analysers; Zahra Khatami , Robert Hill , Catherine Sturgeon et.al. 2005

4.14 www.**comar**.bam.de/

4.15 https://www.bipm.org › committees › jctlm

4.16 www.lgcstandards.com

4.17 https://ec.europa.eu/jrc/en/**irmm**

4.18 http://www.nist.gov/srm/

4.19 http://www.eurolab.org/pub/i_pub.html

4.20 http://www.iaea.org/programmes/nahunet/e4/nmrm/index.htm

4.21 http://european-accreditation.org

4.22 Clinical and Laboratory Standards Institute :2008 Assessment of Laboratory Tests when Proficiency Testing Is Not Available; Approved Guidelines, 2nd Edition. CLSI document GP29-A2.

4.18 http://www.microbiologics.com/Products/All-Epower-CRM-Products

4.19 http://www.microbiologics.com/Products/All-Epower-CRM-Products

4.20 http://www.who.int/biologicals/reference_preparations/distribution/en/

4.21 "Clinical Laboratory Improvement Amendments of 1988"

4.22 Performance standard for Antimicrobial Disk Susceptibility Tests: Approved Standard-11th edition; Clinical and Laboratory Standard Institute (CLSI); M02-A11; January 2012.

4.23 Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement; M100-S24; Clinical and Laboratory Standards Institute; January 2014.

4.24 Applications of reference materials in Analytical Chemistry: Barwick V, Bruke S, Lawn R, Roper P, Walker R. Royal Society of Chemistry, UK.

4.25 Reference Materials in Analytical Chemistry - A Guide for Selection and Use, edited by A Zschunke, Springer, 2000.

4.26 EEE/RM/062rev3-Eurachem 2011. The selection and use of reference materials: a basic guide for laboratories and accreditation bodies.

4.27 European Accreditation Publication Reference (EA-4/14 INF:2003)

5. REFERENCE MATERIAL/CERTIFIED REFERENCE MATERIAL

5.1 What are reference materials?

Reference materials are characterized for ‘property values’ (e.g amount of specified analyte chemical/physical or biological property) or ‘identity’ (e.g. chemical structure, morphological appearance or microbiological species etc). As such, reference materials have well-established properties with sufficient stability, homogeneity, and matrix match. They are used for calibration of equipment, assessment of measurement procedures, chemical analysis and for assigning values to materials.–Reference material may be in the form of a pure or mixed gas, liquid or solid.

Certified reference Materials are reference material whose purity has been established by physical and/or chemical means and accompanied by a certificate that provides the value of the

specified property, its associated uncertainty, and a statement of metrological traceability. Metrological traceability requires an unbroken chain of calibrations to stated references, all having stated uncertainties. Traceable certified reference materials provide metrological traceability for measurement results. In the USA, NIST made reference material are call as standard reference material (SRM) and in Europe as ‘certified reference material’ (CRM).

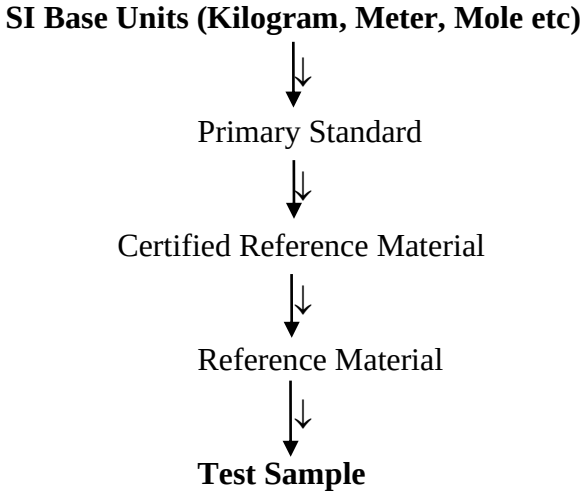
Therefore, CRM is defined as "reference material, characterized by a valid measurement procedure for one or more specified properties, accompanied by a certificate that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability". CRMs are mainly used for validation of test methods and evaluation of instrument performance.

5.2 Traceability and Hierarchy of reference Material

Reference material are important tools for the transfer of measurement accuracy between laboratories and their property values should, where feasible, be traceable to SI units. The Measurement uncertainty of the property value of a reference material employed in a measurement process will contribute to the final measurement but should contribute less than one third of overall measurement uncertainty. Traceability of a reference material is essential because it provides the linkage that ensures that measurement results obtained in different laboratories or at different times are comparable. To achieve this, it is necessary to link all the individual measurement results to some common, stable reference material. If a formally stated traceability is not available, it is necessary for the user to make judgement about implicit traceability, based on the ‘certification’ data available in reports and the technical literature. [4.8]

The different types of reference materials can be arranged between the SI base units and routine test sample measurements according to their relative metrological positions. The higher the position in the metrological order, the lower the uncertainty of the property value of the material.

The following diagram shows the hierarchy of RM [4.8].



A primary standard material should be extremely pure which means that it should be a chemical of high grade of purity, preferably 99.98%. In a chemistry lab you will come across chemicals of different grade of purity. If you check the label, you will notice a number with percentage termed as purity.

Essential characteristics of a primary standard are:

a. chemical of high chemical purity, preferably 99.98%
b. of high stability
c. homogeneous
d. non-hygroscopic and non-efflorescent
e. readily soluble
f. of high equivalent weight (to minimize weighing errors)
g. able to undergo accurate stoichiometric reaction in titration
h. are readily available commercially

Primary standards represent the top tier of chemical standards and in principle, provide a means of establishing the traceability of analytical data to the SI measurement units.

The position of a particular reference material in the hierarchy is not necessarily an indication of its suitability for a particular purpose. A matrix-based CRM would be of greater value than a primary standard consisting only of ultra-pure compound of interest in clinical chemistry analysis.

5.3 Classification of Reference Material

Reference Materials are classified according to

- Physical character - gases, liquids or solids
- supplied property -pure chemical species, physico-chemical property
- preparation method - synthetic mixtures, natural materials
- metrological qualification - primary RM, secondary RM
- intended use - calibration of instruments, validation of analytical methods

5.4. Types of Reference Material

According to the chemical composition, there are two main types of reference material [4.8].

i. **Single substance reference materials** are pure chemicals including primary standards or solutions of pure chemicals that have well characterized reference values.

   Single substance reference materials are primarily used in the measurement steps of an analytical process such as calibration of analytical instruments and as such in analytical determinations.

ii. **Matrix reference material**s contain analytes of interest in their natural environment or materials which have a matrix that closely resembles the matrix of the samples to be tested.

   The most important use of matrix reference materials is for validation of analytical methods. Matrix reference materials are introduced at the beginning of the analytical process and therefore are used to assess the quality of the entire analytical process.

The choice of the reference material will depend on a variety of factors including availability, cost, suitability, and the measurement uncertainty required for the measurement.

Reference Material are used to support measurements concerned with chemical composition, biological, clinical, physical, engineering properties and miscellaneous features such as taste and odor. They may be characterised for ‘identity’ (e.g. chemical structure, fibre type, microbiological species etc.) or for ‘property values’ (e.g. amount of specified chemical entity, hardness etc.) European accreditation publication [4.9] describes the following five types of reference materials listed below.

1. **Pure substances**; essentially pure chemicals, characterised for chemical purity and/or trace impurities
2. **Standard solutions and gas mixtures**, often prepared gravimetrically from pure substances and used for calibration purposes
3. **Matrix reference materials** characterised for the composition of specified major, minor or trace chemical constituents. Such materials may be prepared from matrices containing the components of interest, or by preparing synthetic mixtures

   Physico-chemical reference materials characterised for properties such as melting point, viscosity, or optical density
4. **Reference objects or artefacts**, characterised for functional properties such as taste, odour, octane number, flash point and hardness. This type also includes microscopy specimens characterised for properties ranging from fibre type to microbiological specimens.

In addition, other commonly encountered Reference Materials in laboratory medicine include:

5. Microorganisms with defined properties /characteristics
6. Slides in histology/ cytology /Haematology with defined properties /characteristics
7. Pure isolates of genetic material- RNA, DNA, Chromosomes with defined qualities/characteristics
8. Solutions/Mixture of artifacts/substances with defined properties/characteristics of composition similar /equivalent to human blood-matrix match

There are several organizations producing reference materials worldwide. Organizations producing Reference /certified reference material are listed in Annexure I. Section 6.7 of this document also provides information of data bases on reference material.

6.0 REFERENCE MATERIAL IN CLINICAL CHEMISTRY

6.1 Use of reference materials in Clinical Chemistry [4.8]

Reference Materials are used for

- Calibrating measurement systems/analytical instruments
- Checking instrument performance
- Checking laboratory and analyst performance
- Validating methods and estimating the uncertainty of analytical measurements
- Assessing accuracy in analytical data
- Internal quality control /Intermediate checks
- PT programs /inter laboratory comparison programs
- Development of in-house standards
- Training and evaluation of competence of laboratory personnel
- Independent evidence of performance

6.2. Selection of reference materials

In selecting a reference material for a particular application in clinical chemistry, it is the responsibility of the laboratory to assess its suitability. As such, it is necessary to consider all or some of the following factors before selecting a material.

- Measurands/analytes
- Measurement value of intended use
- Matrix match
- Potential interferences
- Traceability of CRM
- Availability of certification for traceability
- Target value, concentration range and measurement uncertainty of CRM
- Contribution of uncertainty of CRM to your measurement uncertainty
- Demonstrated competence of RM producer /Proof of accreditation certification
- Availability of material

RMs must be within the scope of the method in terms of matrix type, analyte concentration etc. and ideally a number of RMs covering the full range of the method should be tested. RMs should be characterized with respect to homogeneity, stability, and the certified property value(s).

6.3. Acceptability of Reference Material

In general, CRM/RMs should demonstrate traceability & uncertainty of the certified value. Documentary evidence on traceability & uncertainty of the certified value is mandatory.

In the absence of a certificate of analysis with documented traceability, experimental evidence of demonstrated comparability from participation in international comparisons should be provided by the supplier. Preferably, they should be produced by a facility which is accredited as per ISO 17034:2016 **(4.10)** or verification report through comparison between reference laboratories.

There must be an accompanying document stating the requirements for the use of the CRM/RMs. The advice on the storage conditions of the unopened vials and the stability once opened should be clearly stated.

Information required with regard to CRM /RM based on ISO Guide 31:2015**(4.5)** are given below.

Identification of the reference material

i. Identity of the manufacturer
ii. A description of the reference material and its intended use
iii. Instructions on the correct use of the material
iv. The assigned property values and the method used to derive these values
v. The date of certification and the period of validity of the certificate
vi. Safety instructions
vii. An indication of the level of homogeneity of the material
viii. Signature and the name of the certifying officer(s) signing the certificate of analysis (a legal consideration)
ix. In the case of certified reference materials, the traceability and a statement of the uncertainty interval at a stated level of confidence must be provided to the clients
x. Certification of the procedure used by the manufacturer of RM including documentation supporting accreditation of the said procedure in the manufacturer's laboratory
xi. Evidence from the local agent regarding proof of maintaining the integrity of the RM

6.4 Certificates and Supporting Reports on Reference Materials

A certificate providing information complying with the ISO Guide 31:2015**(4.5)** and a report covering the characterization, certification and statistical analytical procedures, complying with the ISO Guide 35:2017 **(4.11)** should be provided.

However, many RMs, particularly older materials and materials not specifically produced as RMs, may not fully comply with ISO Guides 31 and 35. Alternative, equivalent information in

whatever form it is available, that provides credible evidence of compliance can be considered acceptable.

Examples include technical reports, trade specifications, papers in journals or reports of scientific meetings and correspondence with suppliers.

6.5. Assessment of the Suitability of Reference Materials

Laboratories are responsible for the selection of RMs and any decision not to use a RM. In the absence of specific information, it may not be possible to assess the quality of a RM. It is important to demonstrate that the material meets certain specifications and is fit for the end use.

However, a RM should be verified upon reception in the laboratory and before being used in the analytical process and must be adequately documented together with relevant dates and origin of the compounds. The number of tests to be run for verification will vary depending on the source of the reference material. The rigour of an assessment of the suitability of a RM depends on the criticality of the measurement, the level of the technical requirement and the expected influence of the RM on the validity of the measurement.

6.6. Interpretation of results obtained with CRM

Comparison of actual test results obtained when using the CRM with its certified reference values and their associated uncertainties:

Normally, to obtain a reliable estimate of mean and SD, at least seven independent replicate measurements and preferably up to 20 are to be recommended if time and resources permit. (4.8, 4.12, 4.13)

6.7 Availability of Reference Material

Given below are some sources where information on the availability of reference materials for clinical chemistry can be obtained (4.14-4.21).

a. The COMAR certified reference material data base (www.**comar**.bam.de/)
b. Reference material producer catalogues
c. Websites of major reference material producers

JCTLM database: Laboratory medicine and in vitro diagnostics
https://www.bipm.org › committees › jctlm)

LGC Standards (www.lgcstandards.com)

Institute of Reference materials and Measurements (IRMM) (https://ec.europa.eu/jrc/en/irmm)

National Institute of Standards and Technology (NIST) http://www.nist.gov/srm/ http://www.eurolab.org/pub/i_pub.html

http://www.eurolab.org/pub/i_pub.html

http://www.iaea.org/programmes/nahunet/e4/nmrm/index.htm

http://european-accreditation.org>2018

6.8 non-availability of reference material /methods.

RM or EQA are not always available for all the tests performed by a laboratory due to reasons such as unstable analytes, matrix effects, testing methods which may be restricted to a small number of laboratories making it cost prohibitive to provide the facility. When reference materials /reference methods, EQA or IQC programmes are not available, the laboratory shall take measures to prove the validity of test results generated by the laboratory (ISO 15189: 2012 Clause 5.6.3.2). In such situations, alternate methods are necessary to assess test performance and to address pre- and post-analytical factors (CLSI 2008) (4.22). Some of the alternative options are given below.

- Inter-laboratory comparison: This is a widely used alternate procedure when formal proficiency assessment is not available to ensure the validity of patient results. Comprehensive documentation on the comparison of inter-laboratory test results is essential.
- Other alternate methods include:
    - A. Split-sample /exchange of samples with other laboratories
    - B. Internal split-sample
    - C. Audit-sample procedure (single patient specimen aliquots are tested by the same method and compared over time)
    - D. Analysis of manufacturer's calibrator or assayed IQC material with traceability and from different lots
    - E. Use of control materials that are tested daily in interlaboratory comparison programmes.

7. USE OF REFERENCE MATERIAL IN HAEMATOLOGY

When available, use of reference materials in Haematology shall fulfil required specifications mentioned in the general guidelines in this document.

Deviations and unavailability of reference material in Haematology testing shall be evaluated based on the following.

7.1. Automated haematology analyzers (for all the automated tests in haematology)

7.1.1 Automated haematology analyser procedures should have the calibration/method traceability as follows:

7.1.1.1 Tests/methods are calibrated against a reference material recommended by International council for standardization of Haematology (ICSH) or equivalent organization (such as WHO). When automatized analyzers are purchased, it is mandatory to obtain these data from the manufacturer by the laboratory.

7.1.1.2 Tests /equipment are calibrated using a material onsite with defined performance which had been verified against or traceable to ICSH recommended reference material.

7.1.1.3 Test methods should be ICSH/WHO defined methods or methods traceable to such methods of higher standard defined by international bodies/institutions as per the test. (eg: for ESR all methods should be traceable to Westergren method/ PCV, HCT should be traceable to ICSH defined either Wintrobe method or capillary method)

7.1.1.4 Intermediate checks/daily verification is available as follows

i. IQC and EQA material with defined traceability

ii. IQC material with defined value checked/verified against a traceable method or compared against a traceable reference material

Calibration of a test method, photoelectric system and test system when require shall be done by the supplier/manufacturer with material traceable as above and documentation should be available in the laboratory on such calibrations performed as per requirements /procedures specified by the laboratory and/or by the manufacturer at least.

7.1.2 Hb estimation

Either manual Hb estimation- HiCN method or a method traceable to HiCN method: Spectrophotometer used should have been calibrated using Hb standard solution with measurement traceability data as previously described in manual methods.

When Hb concentration is verified with automated analyzer with calibration traceable as above, working reference material can be prepared for IQC from patient samples, provided the integrity is maintained for a defined period. In such instances laboratory shall define target value (mean) and the range for the in-house QC material prepared.

7.1.3 PCV Estimation

7.1.3.1 Wintrobe method and

7.1.3.2 Capillary method (as specified by WHO/ ICSH) are recommended or method traceable to 7.1.3. 1 or 7.1.3. 2.

7.1.4 Manual platelet count verification using blood smears.

Results shall be validated for the microscope used against automated platelet count generated using an analyser with traceability of platelet count estimation as specified for automated FBC.

7.1.5 ESR

Westergren method or methods traceable to Westergren method

7.1.6 Blood Picture

7.1.6.1 When required confirmed positive (and negative) slides can be used depending on discretion of the (specialist in the) laboratory. Reporting should be adhered to National International Standard Nomenclature /Taxonomy as much as possible.

7.1.6.2 Reference slides shall be used based on the judgement of a specialist haematologist as a suitable one to compare staining quality (staining method is validated).

7.1.7 Reticulocyte count

Manual method: Supra-vital staining: White cells and platelets should be reviewed as the positive control in the same smear prepared.

Verification of count shall be performed by duplicate counting, using ocular graticule fixed to the microscope or by using commercial IQC if available.

The traceability of manual method could be achieved by comparison with calibrated automated analyser results if available.

Automated reticulocyte counting shall have traceability of method and results as described in 7.1.1.1, 7.1.1.2

7.1.8 Special stains

Use of confirmed positive and negative smears are recommended.

7.1.8.1 Both positive and negative controls shall be required when the positive and negative stain has defined different colour development.

7.1.8.2 If negative is the absence of colour development, inclusion of a positive control alone is sufficient.

7.1.8.3 Quality of the stain shall be verified as 7.1.6.2

7.1.8.4 Only validated methods of staining preparation and staining procedure shall be used.

7.1.9 PT

Reagent should have defined ISI value acceptable within standard practice defined ICSH/BCSH/WHO/WFH. For Sri Lanka Ministry of Health and SLCH defined ISI is 0.8 -1.2.

7.1.10 APTT

Reagents with specified characteristics acceptable as per the specifications of ICSH/BCSH/WHO/WFH.

For manual tilt method, all the test conditions should be well controlled including number of technical personnel performing test and their competency to assure optimization of end point detection.

7.1.11 When reference material /reference method not available /not defined, the laboratory should define how the results generated are verified for its quality as follows.

7.1.11.1 Manufacturer defined characteristics

7.1.11.2 Results compared with surrogate method (eg. Positive reaction compared with positive genetic marker) or surrogate marker acceptable for clinical decision making.

7.2. Use of IQC and EQA

IQC and EQA material should fulfil criteria specified previously when available.

Any IQC prepared in house, shall have the values generated using the standard method /method traceable to standard method. The method and statistical package used shall be documented with references.

When EQA programme is not available, a surrogate EQA can be used when scientifically acceptable. (Immunological data verified using molecular genetics studies)

In the absence of EQA programme, inter laboratory comparisons shall be done as specified by SLAB/SLCH using patient sample. The attention should be paid with regard to method and equipment comparability as much as possible. The acceptable deviations should be pre-determined using standards/ or National/International guidelines.

7.3. The tests not listed in haematology in this document shall comply with general requirements specified to achieve traceability as appropriate. Such instances, reference documents shall be made available.

8. USE OF REFERENCE MATERIAL IN CLINICAL MICROBIOLOGY AND SEROLOGY

Use of CRM products manufactured in compliance with ISO 17034:2016 (**General requirements for the competence of reference material producers)** (https://www.iso.org/standard/50174.html, https://www.iso.org/standard/29357.html) requirements accompanied by a certificate with documented property values, traceability and an assigned uncertainty levels should be used if possible.

The value may be a qualitative property such as identity or characteristics, or it may be quantitative property such as colony forming units. These CRM undergo rigorous testing by the manufacturer to verify each sample is homogeneous and stable.

8.1. Availability of CRM

CRM should be purchased from an ISO 17034 accredited manufacturer, whenever possible. This accreditation verifies whether the manufacturer is qualified to produce CRM products. A list of accredited manufacturers is in annexure I.

8.2. When to use CRM

CRM are used for method validation or development of new test methods. They are also used to calibrate laboratory equipment such as identification systems, for routine quality control in the laboratory, training or to evaluate the performance of laboratory personnel and for proficiency testing.

8.3. Properties of CRM used in Microbiology

Microorganism CRMs are highly characterized strains which are recognized as the highest quality material throughout a number of industries.

Certified Reference Material are manufactured by ISO 17034 accredited production and testing facilities. CRMs are highly characterized through an expanded measurement of uncertainty which gauges the variability within a sample. They are tested for homogeneity to verify consistency within a lot. They are also tested for stability over time and during transport.

8.4. Features of Recommended CRM

CRMs in microbiology are available in relation to different properties or features.

Microorganisms are available as CRM with qualitative as well as quantitative properties. The qualitative properties are the morphological characteristics, biochemical and other phenotypic characteristics, antimicrobial susceptibility and different resistance mechanisms. The quantitative properties are the specific number of colony forming units and concentrations.

For example, commercial sets contain the manufacturer recommended quality control strains for bacterial identification systems (e.g., Vitek®) and bacterial detection products (e.g., Readycult®). Control strains of bacteria for sensitivity testing and appropriate microorganism groupings for a wide selection of additional quality control tests Eg ATCC.

8.5. The minimum requirement expected in relation to reference material in a clinical Microbiology laboratory in Sri Lanka

8.5.1. For bacterial cultures

8.5.1.1 Suitable strains of bacteria have to be used for internal quality control (IQC) procedures.

8.5.1.1.1 For media and reagent quality control (QC):

i. Either ATCC (American Type Culture Collection) or NCTC (National Collection of type Collection) control strains with specific characteristics are preferred.

ii. If they are not available strains sent by the reference laboratories for External Quality assurance programmes (EQA) ~~or~~ and

iii. Strains identified in the laboratory using biochemical and/or other identification methods (Ex :- Genomic analysis) can be used.

8.5.1.1.2 As positive and negative controls for identification and biochemical tests:

i. Either ATCC or NCTC control strains with specific characteristics are preferred.

ii. If they are not available strains sent by the reference laboratories for External Quality assurance programmes (EQA) or

iii. Strains identified in the laboratory using biochemical and/or other identification methods can be used.

8.5.1.1.3 For antimicrobial susceptibility tests:

i. The strains recommended by the standard for the method of antibiotic susceptibility test have to be used. Eg: ATCC strains for CLSI. A certificate issued by a reliable source of reference material should be available to prove the traceability.

ii. The proper storage conditions and sub-culturing techniques have to be used to maintain the quality and integrity of the control strains. (CLSI guidelines)

iii. External Quality assurance programmes (EQA) conducted by the Medical Research Institute, Colombo, is acceptable.

8.5.2. For Serology

8.5.2.1 Kit traceability: The traceability to a recognised panel such as WHO panels, French Health Products Safety Agency (AFSSAPS) standards or to a gold standard method such as culture or PCR should be available. If there is documentary evidence to show that the manufacture of the test kit has ISO 13485 certification it can be considered satisfactory.

8.5.2.2 **For IQC**:

8.5.2.2.1 If positive and negative controls are supplied by the kits they have to be used as instructed in the manufacturer's literature.

8.5.2.2.2 If positive and negative controls are not provided, a known positive and a negative confirmed by the reference laboratory or sent for EQA can be used.

8.5.2.2.3 A third party QC should be used in addition to the controls provided by the manufacturer at pre-determined intervals, and when new batches and kits are verified.

8.5.2.2.4 In the absence of a third party QC a patient sample verified by a reference method/reference laboratory can be used for the verification process.

8.5.2.3 **For EQA:**

8.5.2.3.1 EQA programme conducted by the National Sexually Transmitted Diseases and AIDS Control Programme is acceptable, when available.

8.5.2.3.2 If local EQA programmes are not available, should participate in an International EQA programme.

8.5.2.3.3 If an international EQA programme is not available, a properly designed inter-laboratory comparison programme should be carried out between other laboratories (minimum 3) which are accredited for the relevant tests if available.(4.4)

8.5.3. Staining for AFB

8.5.3.1 The EQA programme conducted by the National TB reference laboratory is recommended.

8.5.3.2 The positive and negative samples confirmed by the National TB reference laboratory should be used as controls for internal QC.

8.5.4 Verification of equipment, including calibrated wire loops

8.5.4.1 Calibration certificate from the manufacturer should be available.

8.5.4.2 In house verification and internal quality control should be performed using CRM if possible or in house samples.

8.5.4.3 If calibrated loops are re-used it is important to use CRM for verification of measurements.

Annexure I -Organizations producing CRM in Microbiology

- Internationally renowned institutions such as NIST "Standard Reference Material SRM®" (certified reference material provided by NIST).

- Collaborative government sponsored programs such as the EU BCR program ("European Reference Material ERM®").

- Semi-commercial sectorial or trade associations such as the American Oil Chemicals Association

- Are examples of brands for certified reference materials provided by specific RM-producers.

- JCTLM database gives information on RM, CRMs. Institutions such as NIST of USA, EURALAB, BAM of Germany and the Laboratory of the Government. Chemist, U.K. etc., are major providers of reference materials. In addition, there are an increasing number of commercial sources who supply reference material.

- National Collection of Type Cultures (NCTC), a Culture Collection of Public Health England.; https://www.phe-culturecollections.org.uk/collections/nctc.aspx

- American Type Culture Collection (ATCC) P.O. Box 1549 Manassas, VA 20108 USA; https://www.atcc.org/Products/Cells_and_Microorganisms.aspx.

Annexure II – Composition of the Technical Expert Committee

1. Prof.(Ms) Sumedha Wijerathne, Professor in Reproductive Biology, Laboratory Director, Vindana Reproductive Centre.
2. Dr(Ms) Saroja Siriwardahana, former Consultant Chemical Pathologist, National Hospital, Colombo.
3. Dr(Ms) I D Sririwardhana, Consultant Chemical Pathologist, Specialist in Chemical Pathology, Senior Lecturer in Pathology, Faculty of Medicine, University of Moratuwa.
4. Dr K A C Wickreamarthne, Consultant Heamatologist, Specialist in Heamatologist, Senior Lecturer in Pathology, Faculty of Medicine, University of Ruhuna.
5. Dr (Ms) S K Jayathilake, Consultant Microbiologist, Sri Jayawardenapura General Hospital.