SRI LANKA ACCREDITATION BOARD

for CONFORMITY ASSESSMENT

SPECIFIC CRITERIA FOR

BIOLOGICAL TESTING

ABBREVIATIONS

AOAC : Association of Official Analytical Chemists

APHA : American Public Health Association

APLAC : Asia Pacific Laboratory Accreditation Cooperation

ASTM : American Society for Testing and Materials

BAM : Bacteriological Analytical Manual

CRM : Certified Reference Material

DNA : Deoxyribonucleic acid

EA : European Cooperation of Testing Authorities

e.g. : Example

FAO : Food and Agricultural Organization

GLP : Good Laboratory Practices

GMO Testing : Genetically Modified Organisms testing

GUM : Guide to the Expression of Uncertainty in Measurement

HEPA : High Efficiency Particulate Air

IEC : International Electrotechnical Committee

ILAC : International Laboratory Accreditation Cooperation

ISO : International Organization for Standardization

LIMS : Laboratory Information Management Systems

NABL : National Accreditation Board for Testing and Calibration Laboratories in India

PCR : Polymerase Chain Reaction

PT Programmes : Proficiency Testing Programmes

QC : Quality Control

RODAC : Replicate Organisms Direct Agar Contact

SI Units : International System of Units

SLAB : Sri Lanka Accreditation Board for Conformity Assessment

SLSI : Sri Lanka Standards Institution

TS : Technical Specification

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INTRODUCTION

1.1 The field of Biological Testing covers a variety of biological, microbiological, biochemical and physical tests on Food, Water, Toiletries, Genetically Modified Foods, Cosmetics, Aurvedic Products, Pharmaceuticals and other products.

1.2 The requirements for accreditation are laid down in the International Standard ISO/IEC 17025 (General requirements for the competence of testing and calibration laboratories). These requirements apply to all types of objective testing. However, in certain instances additional guidance is considered necessary to take in to account the type of testing and the technologies involved.

1.3 This document has been prepared by the Technical Advisory Committee on Biological Testing and authorized for adoption by the Council of Sri Lanka Accreditation Board (SLAB). It supplements International Standard ISO/ IEC 17025 and provides specific guidance for the accreditation of biological testing laboratories for both assessors and for laboratories preparing for accreditation. It addresses general issues covered under Good Laboratory Practices (GLPs) on safety aspects in relation to the activities in the Laboratory.

1.4 In the preparation of this document, NABL publication NABL 102 Specific Guidelines for Biological Testing Laboratories has been used extensively.

1.5 This booklet in Biological Testing covers relevant aspects relating to the application of general criteria for accreditation given in ISO/ IEC 17025: 2005, to specific fields and product groups given in Appendix A. This document should be read in conjunction with the Rules and Procedures of SLAB.

1.6 Majorities of the accredited biological testing laboratories are primarily involved in microbiological testing. Thus this document does have a bias toward these types of laboratories.

However this document can also provide guidance to laboratories using techniques in areas related to toxicology, veterinary science, biochemistry, molecular biology and cell culture, although there may be additional requirements for such laboratories.

1.7 Definitions of Terms used are given in Appendix B.

2. SCOPE OF ACCREDITATION

2.1 The Scope of accreditation of a laboratory is the formal statement of the range of activities for which the laboratory has been accredited. The scope is recorded in detail on an Accreditation Schedule if necessary attached to the accreditation certificate. A laboratory's scope should be defined as precisely as possible so that all parties concerned know accurately and unambiguously the range of tests and/or analyses covered by that particular laboratory's accreditation. The schedule format should typically define the laboratory's accreditation in terms of:

(i) Product(s) / Materials of Test

(ii) Specific tests performed

(iii) Test method / Standard against which tests are performed

(iv) Range of testing / Limits of detection

(v) Uncertainty

2.2 Where routine testing is carried out, it is recognized that must be as specific as is feasible and the quality assurance system maintained by the laboratory must ensure that the quality of the results is under control.

2.3 Where a laboratory uses automated test equipment or commercial test systems, the responsibility will be on the laboratory to demonstrate to the assessors that in using these techniques, it is meeting all of the criteria for accreditation. In particular, the experience, expertise and training of the staff carrying out the tests and those interpreting the data involved will be a major factor in determining whether or not such analyses can be accredited.

It is accepted that sometimes it is not practicable for laboratories to use a (fully documented) standard method in the conventional sense, which specifies each sample type and determinant. In this case, the laboratory must have its own method or procedure for use, which includes a protocol defining the approach to be adopted when different sample types are analyzed. Full details of the procedures used must be recorded at the time of each analysis so as to enable the procedure to be repeated in precisely the same manner at a later date. Where a particular analysis subsequently becomes routine, a full method as required for accreditation must be written and followed. Whenever there are deviations from standard method or inadequate clarification in Standard Method, the laboratory needs to develop effective procedure for ensuring the quality of results.

2.4 The approach to extending or amending the scope of accreditation should be as flexible as possible. Normally the laboratory will give written notice to SLAB of the tests, which it wishes to add to its scope, quoting Standard method references (where applicable) and providing copies of documented validated in-house methods before surveillance and re-assessment.

3.0 TECHNICAL REQUIREMENTS (ISO/IEC 17025: clause 5)

PERSONNEL (ISO/IEC 17025: clause 5.2)

The laboratory shall employ competent personnel to operate specific equipment, perform tests, evaluate results and sign test reports. The competence of personnel shall be determined on the basis of appropriate education, training, experience and demonstration of skills. The technical staff of the laboratory shall comprise of at least one person as authorized signatory and at least one technician/ or laboratory assistant.

3.1 Technical Management

The officer in charge of a laboratory, and section leaders in larger laboratories, must be suitably qualified with a sound knowledge of the principles of the test, be able to provide adequate supervision and have the ability to make critical evaluations of the test results. Laboratory management should also be knowledgeable about biological safety measures to be maintained in the laboratory.

3.2 Quality Manager

The technical management shall appoint a suitable senior member of the staff as Quality Manager, to establish, document, maintain and improve the management system, irrespective of other duties and responsibilities assigned to him/her. The quality manager shall be a Graduate in Science having adequate experience and direct access to the highest level of the management.

3.3 Authorized Signatory

The role of a signatory is to ensure the reliability and completeness of the test document. Any officer of an accredited laboratory who is competent to make a critical evaluation of test results, and occupies a position in his/her organization's staff structure, which makes him/her responsible for the adequacy of test results, may be appointed as authorized signatory. All authorized signatories must be fully aware of the requirements for test documents detailed in the rules and procedures for accreditation.

Authorization as a signatory may be limited to specific tests, or may be granted for all tests for which the laboratory is accredited. It should be noted that such authorization are only granted in the context of the tests carried out by that laboratory.

The following attributes should be taken in to account when assessing the suitability of a staff member as an authorized signatory:

a) Qualification and experience

b) Position in the staff structure

c) Familiarity with test procedures and their limitations

d) Knowledge of the procedures for recoding, reporting, and checking test results;

e) Awareness of the needs for periodic recalibration of equipment

f) Awareness of regulations of SLAB in general and regulations with regard to test documents in particular.

The minimum Qualifications of an authorized signatory shall be a Graduate in Science with biology as one of the subjects or equivalent qualifications with 3 years experience in the particular field.

Microbiological testing laboratories carrying out analysis of pathogens (Risk Group 2 or above) shall have a qualified microbiologist (graduate/post graduate in Microbiology with the above-mentioned experience) as the authorized signatory.

In situations where laboratory is requested to give opinions and interpretations of test reports the laboratory may do so and for that purpose suitable personnel having relevant knowledge for manufacturing of relevant products, knowledge on legislative requirements and understanding of the significance of deviations found shall be identified. In addition such persons should possess appropriate qualifications, training, and experience.

3.4 Technical Staff

Microbiological testing should be either performed or supervised by an experienced person qualified to,

a) degree with microbiology or an equivalent degree with 1 years experience in microbiological work relating to the laboratory's scope of accreditation.

b) G.C.E. Advanced Level examination with biology as a subject and Diploma in Laboratory Technology in Biology/Microbiology with 3 years relevant experience relating to the laboratory's scope of accreditation.

c) G.C.E. Advanced Level examination with biology as a subject and advance certificate course in Laboratory Technology in Biology/Microbiology with 5 years relevant experience relating to the laboratory's scope of accreditation.
d) G.C.E O/L examination with Science as a subject with minimum of 10 years relevant experience relating to the laboratory's scope of accreditation.

Staff should have relevant practical work experience before being allowed to perform work covered by the scope of accreditation without supervision, or before being considered as experienced for supervision of accredited work.

Such subordinate staff members must be engaged only in work commensurate with the extent of their training and qualifications.

3.5 Supervision of staff

Adequate supervision shall be exercised on laboratory testing and the ratio of supervisory to other staff must be sufficient to ensure quality of test results. In case when an officer exercising technical control is relatively inexperienced with respect to one facet of the laboratory's work, adequate technical control may be provided by a combination of staff under proper supervision by the technical management.

In small laboratories, the officer in charge must decide who can work under direction and who requires supervision. Each must be fully briefed or instructed. Adequate supervision must be provided at each level of the staff structure to ensure close adherence to laboratory procedures and accepted techniques at all times.

3.6 Consultants and Part – time Staff

A person engaged by the Laboratory Management as a Consultant may be appointed as authorized signatory provided that there is a written agreement between the parties setting out the extent of the authority and responsibility of the consultant in relation to the laboratory. The consultant's position in relation to the laboratory should be that the person can perform the role of a supervisory officer as effectively as an employee.

3.7 Training of Staff

The laboratory management shall ensure that all personnel have received adequate training for the competent performance of tests and the operation of equipment. All laboratories should have proper procedures for training of new staff and developing the expertise of existing staff in new and rarely used techniques. Initial training procedures should include basic techniques, e.g. plate pouring, counting of colonies, aseptic technique, etc., with acceptability determined using objective criteria. New staff shall be monitored for the validity of results particularly in the early stages after completing of training in new techniques.

3.8 Competence evaluation of staff

Tests on samples should be performed only by personnel who are either recognized as competent to do so, or if not competent should be performed under adequate supervision.
The competence of personnel to perform tests shall be evaluated and documented in relation to the results of internal and external quality control. The effectiveness of the training programme, as well as the identification of further training needs, should also be evaluated based on these results.

Ongoing competence should be monitored objectively with provision for retraining where necessary. Where a method or technique is not in regular use, verification of personnel performance before testing is undertaken may be necessary. The critical interval between performance of tests should be established and documented. The interpretation of test results for identification and verification of micro-organisms is strongly connected to the experience of the performing analyst and should be monitored for each analyst on a regular basis.

4.0 ACCOMMODATION AND ENVIRONMENTAL CONDITIONS

(ISO/IEC 17025: clause 5.3)

4.1 Samples, reagents, media, cultures and standards should be labelled and stored so as to ensure their integrity. The laboratory should guard against deterioration, contamination and loss of identity of such material.

4.2 The Laboratory shall meet the safety requirements applicable to the test procedure wherever the published standard specifications describe the requirements. In case of Microbiology the laboratory premises shall be separated for specific functions namely testing, media preparation and disposal of samples. Depending on the level of work each demarcated areas shall be arranged in such a way to open to a common area. The area reserved for administrative work shall be outside the testing premises.

4.3 It may be necessary to restrict access to particular areas of laboratory because of the nature of the work carried out there. Restrictions might be made because of security, safety, or sensitivity to contamination. Typical examples might be work involving Pathogenic microorganisms, Reference stock cultures etc. Where such restrictions are in force, staff should be aware of:

i. the intended use of a particular area;

ii. the restrictions imposed on working within such areas;

iii. the reasons for imposing such restrictions

iv. The appropriate containment levels

4.4 Frequently, it will be necessary to segregate certain types of work which are prone to interferences from other work, or which present particular problems or hazards. When selecting designated areas for special work, account must be taken of the previous use of the area. Before use, checks should be made to ensure that the area is free of contamination. Once in use, access to such areas should be restricted, and the type of work undertaken there should be carefully controlled.

4.5 The laboratory shall provide appropriate environmental conditions and controls necessary for particular tests, including temperature, humidity, free from vibration, free from air-borne and dust- borne microbiological contamination, special lighting, radiation screening. Critical environmental conditions should be monitored and recorded.

4.6 One key responsibility of the laboratory management is to provide an adequate and safe working environment. Laboratory facilities should reflect due consideration of space, design, security, health and safety. It is recognized that laboratories will be required to comply with relevant building and safety legislation. The provisions of such legislation shall be considered as additional essential requirements.

4.7 Design

The relative locations of functional areas should facilitate the use of equipment and instruments.

Adequate and proper lighting of minimum 100 lumen must be available for personnel to carry out assigned tasks. Adequate and proper plumbing and wiring must be available and accessible.

The laboratory must have proper ventilation, adequate heating, cooling and humidity control as per the requirements. Bench and floor surfaces must be appropriate for the work being performed. The design should maximize laboratory functions and activities, safeguard the physical evidence, protect the confidential nature of the laboratory operations and provide a safe and healthy environment.

4.8 Space

4.8.1 Each employ must have adequate work space to accomplish assigned tasks. Sufficient space must be provided for storage of supplies, equipment and tools. Analysts/examiners must have space available for writing reports and other official communications. There must be a clear delineation of areas used for the clerical aspects of laboratory work and the areas used for testing/examinations. Adequate and appropriate space must be available for records, reference work and other necessary documents. Sufficient space must be available for each instrument to facilitate its operation.

Accessories should be preferably stored near each instrument to facilitate its use and operation.

In designing and planning for additional space or a new facility, future space requirements should also be projected. Labs in which usable space falls below adequate levels may experience health and safety problems, compromised efficiency, adversely affected morale and productivity and an increased risk of mishandling and contamination.

4.8.2 The laboratory should be arranged in such a way so that the risks of cross contamination can be

reduced. This can be achieved by carrying out the test procedures in a sequential manner using appropriate precautions to ensure test and sample integrity and by segregating the activities by time or space. It is generally considered as a good practice to have separate areas for:

• Sample receipt and sample storage

• Sample preparation

• Examination of samples, including incubation

• Handling and storage of reference cultures/Reference materials

• Media preparation and Sterilization

• Sterility testing

• Decontamination

• Animal House

• Incompatible activities

- Cleaning glassware and other equipment
- Storage of hazardous chemicals

4.8.3 Reduction of contamination may be achieved by having:

• smooth surfaces on walls, ceilings, floors and benches (the smoothness of a surface is judged on how easily it may be cleaned). Tiles are not recommended as bench covering material;

• concave joints between the floor, walls and ceiling;

• minimal opening of windows and doors while tests are being carried out;

• sun shades placed on the outside;

• easy access for cleaning of internal sun shades if it is impossible to fit them outside;

• fluid conveying pipes not passing above work surfaces unless placed in hermetically sealed casings;

• a dust-filtered air inlet for the ventilation system;

• separate hand-washing arrangements, preferably non-manually controlled;

• cupboards up to the ceiling;

• no rough and bare wood;

• wooden surfaces of fixtures and fittings adequately sealed;

• stored items and equipment arranged to facilitate easy cleaning;

• no furniture, documents or other items other than those strictly necessary for testing activities.

This list is not exhaustive, and not all examples will apply in every situation. Ceilings, ideally, should have a smooth surface with flush lighting. When this is not possible (as with suspended ceilings and hanging lights), the laboratory should have documented evidence that they control any resulting risks to hygiene and have effective means of overcoming them, e.g. a surface-cleaning and inspection programme.

4.8.4 When PCR primers and probes are prepared, suitable segregation of these tasks should be ensured to minimize DNA cross-contamination. DNA amplification should be conducted in a dedicated section of the laboratory in a positive pressurized room.

4.9 Laboratories located in facilities where Products or ingredients are processed or manufactured shall not test for infectious pathogens (such as *Listeria monocytogenes, Salmonella* species, *Escherichia coli* O157: H7, *Shigella* species, *Campylobactor* species, *Vibrio cholerae, Vibrio parahaemolyticus, Clostridium perfringens*) unless the laboratory is physically separated with limited access, equipped with bio-safety cabinets and is supervised by a qualified microbiologist.

For procedures that involve the handling of pathogens and reference stock cultures, they shall be operated within a safety cabinet of a class commensurate with the risk level of the microorganism handled.
Most of the microbes encountered in a non-clinical testing laboratory belong to Risk Group 2 microorganisms e.g. *Salmonella, Staphylococcus aureus*. When working with samples containing microorganisms transmissible by the respiratory route e.g. *Legionella* or when the work produces a significant risk from aerosol production, a biological safety cabinet of Class II shall be used.

4.10 Laboratories should devise appropriate environmental monitoring programme with respect to the type of tests being carried out. Records shall be maintained for it.

The laboratory environment, where relevant, shall be microbiologically monitored for trends and anomalies. For example, air-borne contamination can be monitored by exposure plates. Surface swabbing of sampling and testing benches, utensils, balances stomachers etc is also recommended and considered as essential in environmental monitoring of microbiological laboratories.

Acceptable backgrounds should be assigned and there shall be a documented procedure for dealing with situations in which these limits are exceeded. Records of such situations, evaluation of the effects, if any, on the test results and corrective actions taken should be documented. Environmental contamination by microorganisms can be controlled by appropriate air-filters and air-exchange systems and supervision by a qualified microbiologist.

4.11. Hygiene

Where molecular techniques are undertaken, monitoring for DNA contaminants should be undertaken by employing a No Template Control (NTC).

4.11.1 There shall be a documented cleaning programme for laboratory fixtures, equipment and surfaces. It should take into account the results of environmental monitoring and the possibility of cross-contamination. There should be a procedure for dealing with spillages.

4.11.2 Measures should be taken to avoid accumulation of dust, by the provision of sufficient storage space, by having minimal paperwork in the laboratory and by prohibiting plants and personal possessions in the laboratory work area.

4.11.3 Protective clothing appropriate to the type of testing being performed (including, if necessary, protection for hair, beard, hands, shoes, etc.) should be worn in the microbiological laboratory and removed before leaving the area. This is particularly important in the molecular biology laboratory, where for example, movement from an area of high DNA load to one of low DNA load may unwittingly introduce cross-contamination. A change of the laboratory coat may suffice when moving between areas.

4.11.4 Adequate hand washing facilities should be available and a policy regarding appropriate glove use should be in place to avoid the spreading of micro-organisms in the laboratory.

4.12. Pest Control

The laboratory shall have pest control programme /schedule.

4.13. Laboratory safety

Where a laboratory exists within a host agency facility, documented procedures may be required to permit entry during off hours for emergencies.

The laboratory should have a fire detection system wherever applicable. In keeping with any relevant statutory requirements appropriate fire extinguishing devices must be available and policies and procedures of laboratory security must be clearly documented. Laboratory personnel should be trained in fire fighting.

5. TEST AND CALIBRATION METHODS AND METHOD VALIDATION (ISO/IEC 17025: clause 5.4)

The laboratory shall preferably use internationally or nationally accepted standard test procedures or non-standard procedures (in-house methods) that have been appropriately validated and which are performed regularly.

A laboratory seeking accreditation for a more open set of terms of accreditation must have fully documented procedures covering elements such as: method selection, method development, method validation or verification, acquisition of appropriate reference standards or reference materials and staff training. Records of the application of these procedures should be maintained.

5.1.1 Standard Methods

Where standard methods are prescribed and followed, the laboratory is expected to maintain current versions of the standard methods (reference texts) and up-date laboratory bench methods in accordance with these. Although full validation is not required, a laboratory must verify that it can properly operate the method, and can demonstrate (where specified) the limits of detection, selectivity, repeatability and reproducibility. Laboratories shall pay attention to the limitations, concentrations range and sample matrix specified in the test standards.

5.1.2 In-house Methods

In-house methods are,

- Methods developed in the laboratory
- Methods developed by a customer
- Methods developed for an industry group
- Modified standard test methods
- Methods from scientific publications

All the In-house methods stated above should be validated as per the ISO/IEC 17025, Clause 5.4.2.

If the laboratory is followed in-house test method, the following information is required,

- Reason for its development and applications
- Details of the in-house test method

5.1.3 Verification

In the preferable case of standard and validated methods being used, the laboratory is still required to prove that it can implement them in a reliable way. For verification of quantitative methods the laboratory has, in most cases, to determine repeatability, measurement uncertainty and limit of quantitation, and for qualitative methods the limit of detection.

5.1.4 Commercial test systems **(Kits)**

The use of commercial test systems (kits) will require further validation if the laboratory is unable to provide details of the validation data. When the manufacturer of the test kits supplies validation data, the laboratory will only perform secondary validation (verification).

Laboratories shall retain validation data on commercial test systems (kits) used in the laboratory. These validation data may be obtained through collaborative testing, from the manufacturers and subjected to third party evaluation (e.g. AOAC. Refer www.aoac.org for information on methods validation). If the validation data is not available or not applicable, the laboratory shall be responsible for completing the primary validation of the method.

It has been found in some cases (e.g. veterinary microbiological testing) that a specific test kit performs differently under local environmental conditions, to that of the original environmental conditions it was subjected to primary validation. In such cases the laboratory should conduct the validation to prove that the kit performs under local environmental conditions.

5.1.6 **Validation of Test Methods** (ISO/IEC 17025 Clause 5.4.3)

5.1.6.1 The validation of microbiological test methods should reflect actual test conditions. This may be achieved by using naturally contaminated products or products spiked with a predetermined level of contaminating organisms.The spiking should be carried out as per the documented procedure. The analyst should be aware that the addition of contaminating organisms to a matrix only mimics in a superficial way the presence of the naturally occurring contaminants. However, it is often the best and only solution available. The extent of validation necessary will depend on the method and the application. The laboratory shall validate standard methods applied to matrices not specified in the standard procedure.

5.1.6.2 Qualitative microbiological test methods, confirmation and identification procedures should be validated by determining specificity, relative trueness, positive deviation, negative deviation, limit of detection, matrix effect, repeatability and reproducibility, if appropriate. (See Appendix -B for definitions).

5.1.6.3 For quantitative microbiological test methods, the specificity, sensitivity, relative trueness, positive deviation, negative deviation, repeatability, reproducibility and the limit of determination within a defined variability should be considered and, if necessary, quantitatively determined in assays. The differences due to the matrices must be taken into account when testing different types of samples. The results should be evaluated with appropriate statistical methods.

5.1.6.4 If a modified version of a method is required to meet the same specification as the original method, then comparisons should be carried out using replicates to ensure that it is comparable. Experimental design and analysis of results must be statistically valid. Even when validation is complete, the user will still need to verify on a regular basis that the documented performance can be met, e.g. by the use of spiked samples or reference materials incorporating relevant matrices.

5.1.6.5. Even when validation is completed, the user will still need to verify, as appropriate, (e.g. when there is a change in the critical factors) that the documented performance can be met. This can be accomplished by the use of spiked samples or reference materials incorporating relevant matrixes.

Appendix- C provides some guidelines for method validation in microbiology

5.2 Uncertainty of Measurement (ISO/IEC 17025 Clause 5.4.6)

5.2.1 Laboratories need to make a formal estimate of measurement uncertainty for all tests in the scope of accreditation that provide numerical results. Where the test results are not based on the numerical data, e. g. detected/not detected, pass/fail, negative / positive or based on visual/tactile or other qualitative examinations uncertainty estimation is not required. Nevertheless the individual sources of variability, e.g. consistency of reagent performance and analyst interpretation should be identified and demonstrated to be under control.

5.2.2 Where the laboratory needs to estimate the measurement uncertainty, it is required to document the procedures and processes on how this is to be done. The uncertainty estimation methods given by reputable professional and standard writing bodies can be accepted within the testing discipline and may be used. ISO/IEC 17025 does not specify any particular approach.

5.2.3 Once a documented procedure is established, the laboratory needs to develop and commence implementation of a programme for applying this procedure to all relevant tests within the scope.

Microbiology of food and animal feeding stuffs – Guidelines for the estimation of measurement uncertainty for quantitative determinations) published by LGC, UK and ISO /TS 19036: 2006, ISO 29201:2012

5.2.4 In the case of microbiological laboratories performing molecular testing for the detection and quantification of genetically modified organisms (GMOs), measurement uncertainty is estimated according to JRC/IRMM Guidance EUR 22756 EN (19)

5.3 Use of automated system / Computer (ISO/IEC 17025: Clause 5.4.7)

5.3.1 The biological testing environment creates particular hazards for the operation of computers and storage of computer media. Advice can usually be found in the operating manuals, however particular care should be taken to avoid damage due to chemical, microbiological or dust contamination, heat, damp and magnetic fields.

If a testing instrument cannot be isolated from the data processing system, the system as a whole must be calibrated either statically or dynamically. Each such system will have to be examined individually.

If the testing instrument can be isolated from the data processing system, the opportunity is available to calibrate each component of the system separately. The testing instrument can be calibrated (again, statically or dynamically) in the conventional manner and a separate verification of the data processing system can be undertaken incorporating the A/D converters and interfacing systems.

5.3.2 Computer controlled automated system

Such systems will normally be validated by checking for satisfactory operation (including performance under extreme circumstances) and establishing the reliability of the system before it is allowed to run unattended. An assessment should be made of the likely causes of system malfunction. Where possible the controlling software should be tailored to identify and highlight any such malfunctions and tag associated data. The use of quality control samples and standards run at intervals in the sample batches should then be sufficient to monitor correct performance on a day-to-day basis. Calculation routines can be checked by testing with known parameter values.

Electronic transfer of data should be checked to ensure that no corruption has occurred during transmission. This can be achieved on the computer by the use of `verification files' but wherever practical the transmission should be backed up by a hard copy of the data.

5.3.4 Laboratory information management systems (LIMS)

LIMS systems are increasingly popular as a way of managing laboratory activities using a computer. A LIMS is a software package allowing the electronic collation, calculation and dissemination of analytical data, often received directly from other instruments and it incorporates word-processing, database, spreadsheet and data processing capabilities. It can perform a variety of functions, typically sample registration and tracking; processing captured data; quality control; financial control; report generation.

All software using to be validated. Particular validation requirements include control of access to the various functions and audit trials to catalogue alterations and file management.

6. EQUIPMENT - MAINTENANCE, CALIBRATION AND PERFORMANCE

(ISO/IEC 17025: Clause 5.5)

As part of its quality system, all accredited laboratories are required to maintain a documented programme for the maintenance, calibration and performance verification of its equipment necessary to carry out the tests included in the scope of accreditation.

6.1 Maintenance

6.1.1 Maintenance of essential equipment used in the laboratory shall be carried out at specified intervals as determined by factors such as the rate of use. Detailed records shall be kept.

If a test method or operating environment requires a more stringent calibration/verification interval than that set by the laboratory, more frequent calibration will apply.

6.1.2 Commonly used equipment for biological tests include balances, thermometers, pH meter, timer, ovens, incubators, autoclaves, water bath, Laminar Flow chamber, Bio-safety cabinets, thermocycler and volumetric glassware.

6.1.3 Attention should be paid to the avoidance of cross-contamination arising from the equipment used to perform the tests, e.g. Disposable petri dishes

6.1.4 Typically, the following items of equipment will be maintained by cleaning and servicing, inspecting for damage, general verification and, where relevant, sterilizing:

- general service equipment - filtration apparatus, glass or plastic containers (bottles, test tubes), glass or plastic Petri-dishes, sampling instruments, wires or loops of platinum, nickel/chromium or disposable plastic;
- water baths, incubators, microbiological cabinets, autoclaves, bio safety cabinets, homogenizers, fridges, freezers;
- volumetric equipment - pipettes, automatic dispensers;
- measuring instruments - thermometers, timers, balances, pH meters, colony counters.

6.1.5 Apparatus, including validated computerized systems, used for the generation, storage and retrieval of data, and for controlling environmental factors relevant to the toxicological study should be suitably located, and of appropriate design and adequate capacity.

6.2 Calibration and Performance Verification

Commonly used equipment for biological tests that requires calibration and/or performance verification include balances, thermometers, pH meter, timer, ovens, incubators, autoclaves, water bath, Laminar Flow chamber, Biosafety cabinets, thermocycler, refrigerators used to store sample/media etc., micropipettes and volumetric glassware.

6.2.1 Autoclave

6.2.1.1 Autoclave shall not be used to sterilize clean equipment and to decontaminate used equipment during the same sterilization cycle. Ideally the laboratories should have separate autoclave for these two purposes. Records of autoclave operations including temperature and time shall be maintained. Acceptance and rejection criteria for operation conditions shall be set and implemented.

6.2.1.2 Pressure measurements alone cannot guarantee that appropriate temperature has been attained through the sterilization cycle. Measurement of temperature is essential for each autoclave cycle to ensure that the unit has been correctly vented. Autoclaves therefore need to incorporate a temperature recording device.

6.2.1.3 Temperature controllers, temperature recording device and thermocouples need to be calibrated initially and every six months using a reference thermometer or thermocouple, which in turn an accredited calibration laboratory, has calibrated. The temperature calibration results will reveal the pressure gauge deficiencies.

6.2.1.4 In addition to monitoring the temperature, the effectiveness of sterilization can be checked with biological indicators and chemical indicators. Temperature sensitive tape or indicator strips shall be applied for each load. However they are used only to show that the load has been processed but not as a monitor of the actual process applied.

6.2.1.5 Validation of autoclaves enables laboratories to demonstrate acceptable and consistent temperature of sterilization. The main thrust of the need to validate autoclaves is to ensure that the media used for microbiological analysis are not being "over cooked" in the autoclaves. In particular the temperatures should not exceed 121°C and that media are not exposed to a high temperature for too long a time. Sufficient heat is needed to kill all spores whilst protecting the media from excessive heat input thereby "overcooking".

6.2.1.6 Monitoring

Monitoring may be achieved by one of the following:

(i) using a thermocouple and recorder to produce a chart or printout;

(ii) direct observation and recording of maximum temperature achieved and time at that temperature.

In addition to directly monitoring the temperature of an autoclave, the effectiveness of its operation during each cycle may be checked by the use of chemical or biological indicators for sterilization/decontamination purposes.

Autoclave tape or indicator strips should be used only to show that a load has been processed, not to demonstrate completion of an acceptable cycle.

Performance of autoclaves shall be checked periodically with biological indicators (e.g. *Bacillus stearothermophilus*)

6.2.2 Incubators, Water Bath, Hot Air Ovens

6.2.2.1 The stability of temperature, uniformity of temperature distribution and time required to achieve equilibrium conditions in incubators, water baths ovens and temperature controlled rooms shall be established initially and documented. Temperature of incubators shall be verified against the specifications of the test standards and checks on the shelves shall be recorded. Temperatures at different levels and different positions at same level inside the incubator shall be verified at defined time intervals and at least annually against the temperature specifications of the tests.

6.2.2.2 Performance of ovens shall be checked periodically with biological indicators. Eg. *Bacillus subtilis*

6.2.2.3 When there is a direct effect on results, a continual power supply to such equipment should be ensured.

6.2.3 Temperature Monitoring Devices

Where the accuracy of the temperature measurement has a direct effect on the result of the analysis, the temperature measuring devices used in incubators and autoclaves shall be of appropriate quality to achieve the specifications in the test methods. The graduation of the device shall be appropriate for the required accuracy and the traceability of the temperature measurement device has to be established. The overall uncertainty of measurement shall be estimated and appropriate for the measurement.

6.2.4 Refrigerator, Freezer or Cold Storage Room

Permissible ranges of operation shall be specified and records of temperature checks shall be maintained.

6.2.5 Weights and Balances

Weights and balances shall be calibrated traceably at regular intervals according to their intended use.

6.2.6 Volumetric Equipment

(a) Volumetric equipment such as automatic dispensers, dispenser /diluters, mechanical hand pipettes and disposable pipettes may all be used in the biological laboratory. Laboratories should carry out initial verification of volumetric equipment and then make regular checks to ensure that the equipment is performing within the required specification. Verification should not be necessary for glassware, which has been certified to a specific tolerance. Equipment should be checked for the accuracy of the delivered volume against the set volume (for several different settings in the case of variable volume instruments) and the precision of the repeat deliveries should be measured.

(b) For ‘single-use’ disposable volumetric equipment, laboratories should obtain supplies from companies with a recognized and relevant quality system. After initial validation of the suitability of the equipment, it is recommended that random checks on accuracy are carried out. If the supplier does not have a recognized quality system, laboratories should check each batch of equipment for suitability.

6.2.7 Bio-safety Cabinet / *Laminar Flow Hoods*

- Biosafety cabinet/ laminar flow hoods shall be used for personnel protection when testing for hazardous microorganisms. It shall be maintained monthly, quarterly, or annually depending on the class of the cabinet. Parameters monitored such as final filter and exhaust filter integrity, air velocity and uniformity, air barrier containment, induced air leakage, UV radiation, light intensity and noise level shall be monitored.
- It is important that laminar flow hoods are serviced annually. High Efficiency Particulate Air (HEPA, 99.9%) filters shall be checked and cleaned or replaced as needed.

- Airflow rate shall be monitored regularly or at-least annually with a calibrated Velometer, anemometer or other appropriate flow instrument, to ensure that the exhaust system functions properly. Particle count shall also be checked on a routine basis to comply with relevant standard.
- Cleanliness of hood surfaces shall be maintained before and after each use. They shall be routinely monitored using appropriate method. E.g. the use of Replicate Organisms Direct Agar Contact (RODAC) plates or by surface swabbing method.
- During operation the aerial microbial contamination shall also be checked. E.g. agar plates or air sampler technique.
- Appropriate disinfection of the Laminar Flow Hood shall be carried out before and after use.

Biosafety levels are explained in Appendix- E

6.2.8 PCR Equipment

The performance of the PCR equipment such as thermocycler and the built in spectroscopic components of PCR equipment shall be verified regularly.

6.2.9 Other equipment

Conductivity meters, oxygen meters, pH meters and other similar instruments should be verified regularly or before each use. The buffers used for verification purposes should be stored in appropriate conditions and should be marked with an expiry date. Where humidity is important to the outcome of the test, hygrometers should be calibrated, the calibration being traceable to national or international standards. Timers, including the autoclave timer, should be verified using a calibrated timer or national time signal.

Where centrifuges are used in test procedures, an assessment should be made of the criticality of them centrifugal force. Where it is critical, the centrifuge will require calibration.

6.3 Reference Standards and Reference Materials

6.3.1 Reference Standards

6.3.1.1 Reference standards, if available should be used to provide essential traceability in measurements and are used, for example;

- to demonstrate the accuracy of results,
- to calibrate equipment
- to monitor laboratory performance,
- to validate methods and
- to enable comparison of methods

If possible, reference materials should be used in appropriate matrices.

6.3.1.2 Biological testing laboratories are expected to source their reference standards (particularly when biochemical or immunological in nature) from the following possible sources (generally in decreasing order of preference) where availability permits:

a) Reference standards from national measurement institute, from accredited (to ISO guide 34) reference standard products.

b) Customer supplied reference standards, preferably with certification.

6.3.2 Reference materials

6.3.2.1 Reference materials – Except Reference Cultures

6.3.2.1.1 Regardless of the source of the certified reference materials, care shall be exercised to see that they are packaged stored and handled to prevent deterioration. This means that efforts shall be made to minimize exposure to moisture air, heat and light which are the primary causes of deterioration. They shall be kept under secure and appropriate storage conditions, and records shall be maintained of receipt and use.

6.3.2.1.2 The laboratory shall assign suitable staff as monitor(s) for certified reference materials. Their duties shall include ordering or assisting in the ordering of new reference materials, checking calculations of assays, refilling empty working supply containers, keeping lists of laboratory-available certified reference materials up to date, properly identifying reference material containers, maintaining reference materials in their proper location, disposing old or outdated reference materials, and so forth.

6.3.2.1.3 It is preferable that records are kept in sign-in; sign-out logbooks located near the storage areas. Each analyst using a certified reference material shall be required to enter the name of the reference material in the log book, the date and time it is taken and returned, and his or her initials.

6.3.2.1.4 Analysts shall be instructed in the care of certified reference materials and procedures for handling them.

6.3.2.1.5 Biological testing laboratories are expected to source their reference materials (particularly when biochemical or immunological in nature) from the following possible sources (generally in decreasing order of preference) where availability permits:

- Reputable chemical supply houses (particularly kit manufacturers and for pure biochemical standards or reagents);
- Customer supplied reference standards, preferably with certification;
- In-house produced reference standards with verified performance for the intended use.

6.3.2.1.6 Laboratories shall demonstrate traceability by use of certified reference materials obtained from a recognized national / international institute.

If laboratory uses standard materials for quantitative tests in molecular biology- refer appendix B

6.3.2.1.7 Nucleic acid extracted from certified reference materials are stored to provide reference stocks. Reference stocks shall be stored at a condition to minimize nucleic acid degradation. Laboratories shall have a policy and procedures for purchase, handling, storage, maintenance and use of certified reference materials and stocks.

6.3.2.1.8 Reference stocks should be aliquoted to minimize damage due to freezing and thawing. Laboratories should verify stability of stock DNA. Procedures for verification of stocks should be documented.

6.3.2.1.9 The following records shall be maintained:

a) the sources, lot numbers, dates of receipt and expiry, dates put in use, conditions and integrity of packaging of certified reference material;
b) preparation records of reference stocks with dates of preparation, expiration, and name of operator;
c) verification records of reference stocks; and
d) records of monitoring of environmental conditions for storage of reference stocks.

6.3.2.1.10 Normally, at least three standards (excluding zero concentration) shall be used to establish a linear calibration graph for quantitative tests. The standards used shall cover the concentration range of the test samples. The lowest standard shall be at a detection level close to the limit of the test method. Criterion of the correlation coefficient of linear calibration graph shall be defined and implemented.

6.3.2.1.11 Positive DNA reference materials/plasmids/vectors shall be verified by checking with at least one reference material from a different manufacturer or source, if available, before use. The same requirements as 5.3.1.10 should be applied.

6.3.2.1.12 Calibration of equipment critical to test results should be traceable to the International System of Units (SI). Where traceability of measurements to SI units is not possible, traceability to agreed methods and/or consensus standards are required (for example, certified reference materials, certified reference cultures).

6.3.2.2 Reference materials -Reference Cultures

6.3.2.2.1 Reference cultures are required for establishing acceptable performance of media (including test kits), for validating methods and for assessing/evaluating on-going performance. Traceability is necessary when establishing media performance for test kit and method validations etc.

6.3.2.2.2. Microbiological testing laboratories are expected to source their reference cultures from the following possible sources (generally in decreasing order of preference) where availability permits:

- Directly from a recognized national or international collection,
- Customer supplied reference organisms preferably with certificates
- In-House produced reference cultures with verified performance for the intended use.
- Reference cultures can be obtained from the standard culture collections. For example, American Type Culture Collection (ATCC), National Culture Type Collection (NCTC),National Collection of Industrial Microorganisms (NCIM), etc.

6.3.2.2.3. Laboratories shall demonstrate traceability by use of certified reference cultures obtained from a reputed culture collection.

For in-house produced reference cultures it is laboratory's responsibility to demonstrate that the micro organisms are fit for their intended purpose.

6.3.2.2.4 Reference cultures may be sub-cultured once to provide reference stocks. Reference stocks shall be preserved by a technique such as freeze-drying, liquid nitrogen storage, frozen beads storage etc., which maintains desired characteristics of the strains. Laboratories shall have a policy and procedures for purchase, handling, storage, preservation, maintenance and use of reference cultures and stocks.

6.3.2.2.5 Reference stocks shall be used to prepare working stocks of routine work. Bacterial working stocks should not normally be sub-cultured. However, working stocks may be sub-cultured up to a defined number of generations, (normally no more than five passages from the original national collection culture) provided that it is required by test standards or documentary evidence demonstrating that there has been no loss of viability, no changes of biochemical activity and/or no change in morphology.

Procedures for preparation and verification of working stocks shall be documented.

Desired characteristics of the strains shall be verified by serological, biochemical and/or morphological tests. Verification procedures shall be enhanced, if the reference cultures are used beyond the recommended five passages.

6.3.2.2.6 Reference cultures of microorganisms available not directly from, but claimed to be traceable to a reputed culture collection may be used for quality control checks, but the requirements on number of passages and the relevant verification procedures required as mentioned in 6.3.2.2.2 shall also be observed. They shall not be further sub-cultured, if no information on passage number is available from the supplier.

6.3.2.2.7 The following records shall be maintained:

a) the sources, lot numbers, dates of receipt and expiration, dates put in use, conditions and integrity of packaging of reference cultures;
b) verification records of working stocks;
c) history of subculture from reference stocks with dates of preparation and expiration, and name of operator;
d) methods used for preservation of reference stocks and records of monitoring of environmental conditions for storage of reference cultures, reference and working stocks.

Details on control of reference culture is given as Appendix-D

6.4 Reagents and Culture Media

6.4.1 Laboratories should ensure that the quality of reagents, dyes and stains used is appropriate for the test concerned. They should verify the suitability of each batch of reagents critical for the test, initially and during its shelf life, using positive and negative control organisms, which are traceable to recognized national or international culture collections.

6.4.2 Laboratories shall have a policy and procedure(s) for the selection and purchasing of services and supplies. Quality and grade of reagents including detergent, dyes and stains should be appropriate for the tests concerned. They shall not contain any impurities that may inhibit bacterial growth. Guidance on precautions, which should be observed in the preparation or use of reagents, should be documented.

These precautions relate to toxicity, flammability, stability to heat, air and light, reactivity to other chemicals, etc. Reagents prepared in laboratories shall be labelled to identify substance, strength, solvent, any special precautions or hazards, any restrictions on use, and date of preparation and/or expiration.

Persons responsible for preparation of reagents shall be identifiable from records.

6.4.3 The sources and history of consumables having an effect on the validity of tests such as media, antisera, biochemical kits and membrane filters shall be recorded. A logbook shall be maintained to record all such materials received at laboratories. This logbook shall include information such as supplier, lot number, date received, date put in use, date of verification and date of expiration.

6.4.4 Media, supplements and additives

6.4.1 All dehydrated complete or pre-prepared media and purified agars shall be checked for their physical states and verified for their microbiological performance prior to release for use.

A media quality control programme needs to cover all media whether it be purchased prepared media, in house prepared media from basic ingredients, in house prepared media from commercially available dehydrated products.

Schedules for checking media for decomposition, discoloration, deterioration and caking shall be documented. It is important to prevent dehydrated culture media from absorbing moisture during storage. Dehydrated media should be stored in a dry, cool and dark environment. Acceptance ranges of storage conditions and criteria for rejecting media should be documented. Records of monitoring the storage conditions and checks of media shall be maintained.

All media shall be checked

- using positive and negative control organisms with typical characteristics and
- for their quantitative and /or qualitative performances.
- Criteria of recovery and records of verification shall be maintained

6.4.1. The suitable performance of culture media, diluents and other suspension fluids prepared in-house should be checked, where relevant, with regard to:

• recovery or survival maintenance of target organisms;

• inhibition or suppression of non-target organisms;

• biochemical (differential and diagnostic) properties;

• physical properties (e.g. pH, volume and sterility).

Quantitative procedures for evaluation of recovery or survival should be performed according to ISO 11133.

Laboratories should establish and record an appropriate re-ordering schedule to prevent the holding of stocks beyond their expiry dates.

6.4.2 All media recipes and procedures for preparation shall be fully documented and authorized. Records shall be kept of all relevant details of each batch of medium prepared. The records should include medium name, lot number, manufacturer, ingredient quantities (if applicable), final pH, sterilization process, date of preparation and name of operator.

Prepared media not put in use immediately shall be labelled with medium names or codes, date of preparation, and date of expiration if applicable. Information on the life expectancy of prepared media under specific storage conditions shall be specified and documented.

(Guidance on the preparation, sterilization of media and recommended storage times can be found in ISO 7218 Microbiology of Food and Animal Feeding Stuffs – General rules for microbiological examinations and American Public Health Association Standard Methods for the Examination of Water and Wastewater (APHA) section 9020 B).

6.4.3 Supplements and additives should be stored as directed by the supplier, or as determined by in-house storage procedures.

6.4.4 Quality of reagent water used for critical processes should be specified and checked regularly for compliance against the requirements.

6.4.5 Serological and biochemical kits shall be verified with positive and negative control organisms.

6.4.6 Chemicals and reagents involved from sample preparation to PCR testing shall be molecular biology grade or equivalent and free from contaminating nucleic acids or nucleases (both DNase and RNase). Extraction buffer or solution has to be autoclaved prior to use. Any special precautions in preparation or use of the reagents shall be documented. Stability of the reagents to heat, air, light and other chemicals etc should be included, if it is applicable and relevant.

6.4.7 The consumables having an effect on the validity of tests such as taq polymerase shall be documented as well as their sources if relevant.

6.4.8 All taq polymerase /master mix/kits/primers and probes shall be checked and verified for their performance using standard materials prior to release for their use. Verification procedures, criteria for acceptance, shelf lives and special storage conditions shall be documented.

Records shall be maintained for verification and monitoring of the storage conditions.

6.4.9 Apart from reagents, laboratories shall ensure that labware such as culture dishes, culture tubes, sample containers, sample bags, spatula, pipettes and pipette tips shall be pre-sterilized or sterilizable.

6.4.10 Membrane filtration units shall be stainless steel, glass, or autoclavable plastic, not scratched or corroded and shall not leak. Diameter and pore size of membrane filters, and diameter and absorption capability of absorbent pads shall meet the requirements specified in the test standards. They shall be confirmed of their sterility prior to release for use.

6.4.11 Sterile metal or disposable plastic loops, wood applicator sticks, sterile swabs, spreaders etc. should be used as inoculating equipment. When wood applicator sticks are used, they should be sterilized by dry heat. The metal inoculating loops should be made of alloys that do not interfere with any biochemical tests.

7. SAMPLING (ISO/IEC 17025: Clause 5.7)

7.1 In many cases, testing laboratories are not responsible for primary sampling to obtain test items. When testing laboratory is responsible, it is strongly recommended that this sampling be covered by quality assurance and ideally by accreditation. Customers taking their own samples should be made aware of proper sampling, storage and transportation facilities.

Customers should be informed if the sample received is too small for meaningful analysis and/or not meeting sample acceptance criteria.

7.2 Transport and storage should be under conditions that maintain the integrity of the sample (e.g. chilled or frozen where appropriate). The conditions should be monitored and records kept. Where appropriate, responsibility for transport, storage between sampling and arrival at the testing laboratory shall be clearly documented. Testing of the samples should be performed as soon as possible after sampling and should conform to relevant standards and/or national/international regulations.

7.3 Laboratories shall document the sampling procedures for taking test portions from laboratory samples and shall have measures to ensure that the test portion is as representative of the sample as possible, and the composition of the sample would not be altered in a way that would affect the concentration or identification of the organisms/ targeted DNA being determined. In GMO testing, for cases of whole beans or grains, sample shall be sufficiently large to provide meaningful statistical data at the limit of detection of the method. The processed foods, canned and bottled products, etc. could be collected in sufficient numbers belonging to the same batch for analysis. In case different batches are used, details should be recorded and retained for reference.

7.4 Special sampling procedures should be established for special/non-routine samples and made available to the samplers as well as laboratory personnel. A copy of such documented procedure shall be maintained with the raw data and retained for future reference.

7.5 Sampling should only be performed by trained personnel. It should be carried out aseptically using sterile equipment. Environmental conditions for instance air contamination and temperature should be monitored and recorded at the sampling site. Time of sampling should also be recorded. Sampling procedure can form part of the test methods and shall include procedures for sterilization of sampling equipment and precautions in performing aseptic techniques.

7.6 In the case of seed testing laboratories sampling is the key activity and the laboratory management must appoint specific personnel to perform particular types of sampling and seed testing.

7.7 Seed testing laboratories must be able to demonstrate that it has a system for the approval of lot identification, licensing of the seed samplers including the approval and /or provision of sampler training programmes, and arrangements for maintaining and distributing up-to-date lists of licensed seed samplers.

7.8 Seed testing laboratories should have procedures and practices to monitor the uniformity of seed lots and to refuse the sampling and testing where doubt exists concerning uniformity.

8. **SAMPLE HANDLING** (ISO/IEC 17025: Clause 5.8)

8.1 Laboratories shall examine and record the conditions and appearance of samples upon receipt. Where appropriate (e.g. environmental samples for quantitative results), the time of sampling should also be recorded.

Items to be checked include nature and characteristics of sample, volume/amount of sample, storage temperature of sample on receipt, conditions of sample container i.e. whether it has been sterilized before sampling, characteristics of the sampling operation (sampling date and condition), etc.

If there is insufficient sample or the sample is in poor condition due to physical deterioration, incorrect temperature, torn packaging or deficient labelling, laboratories should either refuse the sample or carry out the tests as instructed by the customers and shall indicate the conditions on test reports.

8.2 Samples awaiting test shall be stored under suitable conditions to minimize any modifications to any microbial population present. Storage conditions and maximum holding times for different samples shall be documented and shall fulfill the requirements of test standards. Where a sample has to be held secure, the laboratory must have arrangements for storage and security that protect the condition and integrity of the secured samples concerned.

8.3 Frequently, it is necessary to split or transfer samples for testing of different properties. Sub-sampling by the laboratory immediately prior to testing is considered as part of the test method. It should be performed as per national/international standards, where they exist, or by validated in-house methods. It is essential that procedures are available for preventing spread of contamination, delivery of samples including special transportation such as refrigeration and exclusion of light, disposal and decontamination processes and unbroken chain of identification of the sub-samples/samples shall be provided.

8.4 There shall be a written procedure and defined period for the retention and disposal of the samples in the laboratory.

Samples should be stored until the test results are obtained, or longer if required. Laboratory sample portions that are highly contaminated should be decontaminated prior to being discarded. Seed sample retention must be for not less than one year after testing has been completed.

9. DISPOSAL OF CONTAMINATED WASTE

9.1 The correct disposal of contaminated materials may not directly affect the quality of sample analysis, although procedures should be designed to minimize the possibility of contaminating the test environment or materials. Hazardous waste (biological, chemical or toxic) shall be disposed off by documented procedures based on the type and nature of hazard/level of toxicity.

9.2 Handling and disposal of wastes in the toxicological laboratories should be carried out in such a way as not to jeopardize the integrity of studies. This includes provision for appropriate collection, storage and disposal facilities, decontamination and transportation facilities.

Conventional, biological and hazardous waste should be removed and disposed off regularly and safely. On site incineration, landfill, neutralization and sterilization before disposal or pick-up by a licensed contractor are acceptable means of disposal subject to local regulations. Adequate number of properly labeled waste receptacles should be strategically placed throughout the facility, they should be leak proof with tight fitting lids and disposable liners to collect the waste.

Waste storage area should be marked and kept free from insects and vermin till pick-up or final disposal.

However, it is a matter of good laboratory management and should conform to national/international environmental or health and safety regulations (see also ISO 7218).

10. ASSURING QUALITY OF TEST RESULTS (ISO/IEC 17025: Clause 5.9) 10.1

Laboratories shall establish and implement quality control plans to ensure and demonstrate that the measurement process is in-control and test results generated are accurate and reliable. The plans shall include types of quality control checks, their frequency and acceptance criteria, and actions to be taken when results will be outside the defined acceptance criteria.

10.2 Internal Quality Control

10.2.1 Internal quality control consists of all the procedures undertaken by a laboratory for the continuous evaluation of its work. The main objective is to ensure the consistency of day-to-day results and their conformity with defined criteria.

10.2.2 Programme of periodic checks is necessary to demonstrate that variability (i.e. between analysts and between equipment or materials etc.) is under control. All tests included in the laboratory's scope of accreditation need to be covered. This can be achieved by:

- Sterility controls - Uninoculated samples shall be run at a minimum of once for every test run. Sterility controls are used to detect the presence or absence of possible laboratory contamination.
- Split samples (Duplicates) for quantitative tests - Split samples comprise a sample divided into 2 sub-samples. Analyses of spilt samples are normally expects to be conducted at a frequency of once per test run.
- Confirmation/verification of presumptive positive samples

Positive and negative characteristic strains, if applicable, shall be tested concurrently with any biochemical, serological and morphological tests for confirmation of presumptive microorganisms. The number or percentage of colonies that stipulated in test standard required for confirmation process shall be followed. Laboratories can also define the minimum number of colonies for confirmation if such requirements are not specified.

- Establish Precision of Test Method/Use of spiked samples

The laboratories shall establish the precision of test methods. Acceptance limits for precision can be established by running spiked samples of cell suspension in duplicate or triplicate, using two or more operators. Criteria used to set acceptance limits for precision (for example relative standard deviation or range) shall be based on statistical principles and clearly presented for each test method.

The laboratory shall also document the application of the precision criteria in monitoring acceptance of daily test results

- Verification of continuing competence

Laboratories shall establish schedules, in compliance with the verification frequency stipulated in test standards, for checking the continuing competence to perform positive tests for each test method if no positive samples are encountered. Reference stocks shall be maintained for all tests conducted, and suitable suspensions of fresh subcultures shall be spiked into appropriate matrix and run through each entire test procedure. The analyst is required to make parallel analyses with another analyst. Criteria shall be set for maximum allowance difference between the counts based on precision of test methods. Control charts should be used, where appropriate, to monitor the performance of the laboratory. Furthermore, full confirmation of few suspected colonies shall be conducted using appropriate biochemical or serological tests. (Appendix) -D

* Replicate Testing

* Replicate evaluation of test results

10.2.3 The interval between these checks will be influenced by the construction of the programme and by the number of actual tests. It is recommended that, where possible, tests should incorporate controls to monitor performance.

10.2.3 In some cases, a laboratory may be accredited for a test that it is rarely called on to do. It is recognized that in such cases an ongoing internal quality control programme may be inappropriate and that a scheme for demonstrating satisfactory performance, which is carried out in parallel with the testing, may be more suitable.

10.3 The following can be practiced as a quality control measure in testing laboratories where PCR technique is being used. Example - GMO testing labs.

10.3.1 **In-process Control Check**

The following controls shall be run at a minimum of once for every test run as shown below: -

10.3.1.1 Extraction negative (or blank) control

The extraction buffer employed for DNA extraction shall be prepared from sterile water and shall be autoclaved prior to use.

10.3.1.2 Negative PCR control by use of sterile water and non-GM material (0% GM content) exactly in the same manner as the samples.

10.3.1.3 Detection limit control

A sample of known GM content or CRM can be used to establish the detection limit meeting the limit of detection of the method. In the absence of a GM-CRM, the laboratory can spike appropriate amount of DNA enabling to achieve the desired detection limit.

10.3.1.4 Positive PCR amplification control

Reference DNA or DNA extracted from a CRM or a known positive sample representative of a gene sequence under study shall be incorporated to demonstrate the unique performance of the PCR assay.

10.3.1.5 Replicate analyses

PCR test samples shall be analyzed in at least duplicate for quantitative, semi quantitative and qualitative testing. Because duplicate extractions and PCR of the same sample can give qualitatively different results, one positive, one negative. In situations where false positive results occur due to contamination, rules out false negative results. This situation is most likely to occur in cases where the test is working at concentrations close to the limit of detection and/or there is some degree of inhibition of PCR due to co-extractives from the sample.

10.3.1.6 Number of primer sets

It is normally expected that test results are based on the results of at least two, different GM-specific primer sets, each providing consistent result. The requirement of using at least two primer sets may be relaxed provided that other options for confirming the identity of an amplicon on a gel, e.g. restriction enzyme cutting to produce fragments of the expected size, shall be established to confirm test results.

10.4 External Quality Assessment Programmes

10.4.1 Proficiency testing is defined as the *"determination of laboratory testing performance by means of inter-laboratory comparison*s" (ISO/IEC 17043:2010) and is thus a very important tool in a laboratory's quality control programme to demonstrate the validity and comparability of results.

10.4.2 Proficiency testing programme shall be scheduled and implemented on a regular basis relevant to their scope of accreditation. Preference should be given to proficiency testing schemes, which use appropriate matrices. In specific instances, participation may be mandatory.

10.4.3 Laboratories should use external quality assessment not only to assess laboratory bias but also to check the validity of the whole quality system.

10.4.4 SLAB as an independent accreditation authority, a signatory to the Mutual Recognition Arrangement (MRA) of Asia Pacific Laboratory Accreditation Co-operation (APLAC), in order to earn international recognition in accreditation services. To comply with above requirements in accordance with the policy of APLAC, applicant/accredited biological testing laboratories shall demonstrate their technical competence by the satisfactory participation in proficiency testing activity where such activity is available and feasible.

The minimum amount of appropriate proficiency testing required per laboratory is one activity prior to gaining accreditation. Accredited laboratories have to cover the major groups in the scope by proficiency testing programme within a span of three years of accreditation cycle. (TL-RG(P)-03 Section 16)

10.4.5 Laboratories are expected to select the proficiency testing activities according to the following criteria (in a generally decreasing order of preference): .Refer (TL-RG(P)-03 Section 16)

(a) Mandated programmes. In some areas of biological testing, participation in a particular programme may be mandatory.

(b) International inter-laboratory comparison/PT programmes.

(c) National inter-laboratory comparison programmes.

(d) Proficiency testing programmes operated in accordance with ISO/IEC 17043:2010.

(e) Formal inter-laboratory comparison programmes involving several independent laboratories.

(f) Less formal inter-laboratory comparison programmes between two or more laboratories.

(g) Where none of the above is neither available nor applicable, intra-laboratory comparisons between technicians within the same laboratory could be considered a valid proficiency testing activity.

10.4.6 Laboratories shall document procedures for rectifying unsatisfactory performance in proficiency testing programmes.

If unsatisfactory results are obtained, laboratories shall be able to show that the problems are promptly investigated and rectified, and satisfactory performance for the test/method in question can be achieved afterward. All findings in connection with unsatisfactory performance shall be recorded.

11. REPORTING THE RESULTS (ISO/IEC 17025: Clause 5.10)

11.1 Test Records

An adequate test record system in accordance with the various clauses of ISO/IEC 17025, e.g. 4.12, 5.4.7 is essential. Most laboratories have developed forms (proforma sheets) for all their routine testing. These are generally the preferred option as their use prompts the recording of all the required information, maintains consistency and increases recording efficiency.

11.1.1 Test records may also be contained in personal or test specific workbooks. Where such workbooks are free text (i.e. not bound proforma sheets), this type of records system is generally less efficient and requires a great level of management to ensure that records are not lost.

11.2 Test Reports

11.2.1 Clause 5.10 of ISO/IEC 17025:2005 standard sets out the requirements for test report issued by testing laboratories.

11.2.2 Test reports must give the customer all relevant information and every effort should be made to ensure that the test report is unambiguous. All information in a test report must be supported by the records pertaining to the test. All information required to be reported by the test specification must be included in the report.

11.2.3 It is important to note that in many instances the test standards, regulatory requirements and industry accepted practice will determine the report format and content.

11.2.4 Laboratories must retain an exact copy of all reports issued. These copies must be retained securely and be readily available for the time specified in the laboratory's documented policies.

11.2.5 In microbiological testing if the result of the enumeration is negative, it should be reported as "not detected for a defined unit" or less than the detection limit for a defined unit". Qualitative test results should be reported as "detected/not detected in a defined quantity or volume".

11.2.6 Where an estimate of the uncertainty of the test result is expressed on the test report on demand, any limitations (particularly if the estimate does not include the component contributed by the distribution of microorganisms within the sample) have to be made clear to the customer.

11.2.7 When test results are below the reporting limits, an indication of the reporting limits shall be given in test reports.

11.2.8 Accredited Laboratories are permitted to use SLAB symbol in the test reports (Refer AC-RG(P)-01, Terms & Conditions for use of SLAB symbol).

11.2.9 For biological testing laboratories, all test reports carrying SLAB symbol must be signed by the authorized signatory approved by SLAB for biological testing.

11.3 **Electronic Reporting**

Traditionally, laboratories issued test reports in hard copy format with manual signatures. With increased use of electronic media such as email and the Internet, and the use of electronic databases, laboratories are now being required to report electronically. Such practices challenge the generally accepted reporting criteria for accredited laboratories.

11.3.1 ISO/IEC 17025 clause 5.10.7 attempts in a general way to specify the specific requirements for electronic reporting. While it is difficult to specify in detail a set of requirements to address every eventuality (as laboratories will tend to develop electronic reporting systems to suit their own circumstances and those of their customers), the following is intended to provide guidance on common issues of concern.

11.3.1.1 **Transmission of Report**

It is the responsibility of the issuing laboratory to ensure that what was transmitted electronically is what the customer received.

Email systems have proven to be robust in this regard, but laboratories need to consider whether customers will have the appropriate software and version to open attachments without corruption.

Laboratories should verify (at least initially, and periodically thereafter is recommended) the integrity of the electronic link e.g. by asking the customer to supply a copy of what was received and comparing it with what was transmitted. It is also important that the laboratory and its customer agree as to which parts of the electronic transfer system they are responsible for and the laboratory must be able to demonstrate data integrity at the point the data comes under the control of the customer.

11.3.1.2 **Security**

Laboratories should avoid sending test reports in an electronic format that can be readily amended by the recipient. Where possible, reports should be in a read only format e.g. pdf files. Where this is not possible e.g. the customer may wish to transfer the reported results file into a larger database, then laboratories are recommended to indicate these electronic reports have an interim status and are followed-up by a hard copy (or more secure) final report.

Laboratories must retain an exact copy of the report that was sent to the customer. This may be a hard copy (strongly recommended) or an electronic copy. These copies must be retained securely and be readily available for the time specified in the laboratory's documented policies.

11.3.1.3 **Electronic Signatures**

The reports must not be released to the customer until authorized by individuals with the authority to do so. For electronic reports there must be a clear audit trail with a positive authorization record to demonstrate this is the case. Where this is managed through password access levels in the laboratory's electronic system, appropriate procedures should be in place to prevent abuse of password access.

The electronic report should show the identity of the individual releasing the report (authorized signatory approved by SLAB). This may involve an electronic signature. The security of these signatures should be such as to prevent inadvertent use or misuse.

APPENDIX- A

CLASSES OF TESTS IN BIOLOGICAL FIELD

The field of Biological Testing is divided into several classes of test. Application for accreditation may be made for one or more classes of test or for one or more items or specific tests within a class of test. Consideration of an application is simplified if it is specific and in the form of the list of classes of test.

The tests carried out by laboratory will be given accreditation on the basis of the classes of test given below. Most accreditations will be described in more explicit detail and usually will include reference to specific determinations, analytical techniques, relevant standard test methods and specifications, and in some areas will include analytical ranges and limits of detection.

Accreditation for these tests may be granted in whichever field of testing best suits the testing laboratory concerned.

The terms of accreditation may be extended provided that the laboratory complies with conditions for accreditation for the classes of test or specific tests involved.

Accreditation may be granted for tests performed in mobile laboratories, field laboratories or locations as well as in formal laboratory accommodation.

A. 1 Food and Agricultural Products

Sampling
Animal Feeds
Bakery & Confectionery Products
Beverages (Alcoholic / Non-Alcoholic)
Canned & Processed Foods
Cereals, Pulses & Cereal Products
Coffee & Cocoa Products
Edible Colours & Flavours
Edible Oils & Fats
Eggs & Egg Products
Essential Nutrients Including Vitamins
Fish & Sea Foods
Food Additives & Preservatives
Fruit & Fruit Products
Gelatin and Other Gums
Genetically Modified Foods and agricultural products
Herbs, Spices & Condiments
Honey & Honey Products
Infant Foods
Jams, Juices, Sauces & Concentrates

Meat & Meat Products
Milk & Dairy Products
Margarine
Natural Waxes
Nutritional Supplements
Nuts & Nut Products
Oil Seeds & By-Products
Pet Foods
Poultry & Poultry Products
Starch & Starch Products
Sugar & Sugar Products
Tea
Tobacco & Tobacco Products
Vegetables & Vegetable Products
Other Specified Food Items
Sensory Evaluation Tests on Foods

A. 2 Drugs and Pharmaceuticals

Antibiotics
Ayurvedic Drugs
Biotechnology derived pharmaceuticals
Chemotherapeutic Agents
Drug Intermediates & Raw Materials
Endotoxins
Enzymes
Filtrable Solutions & Soluble Preparations
Hormones
Herbal drugs
Immunological Products
Microbial limit tests
Natural Drugs
Non-Filterable Preparations Including Ointments
Preservative efficacy
Pyrogen tests
Sterility tests
Surgical Dressings
Synthetic Drugs
Vaccines
Veterinary Drugs

Vitamins
Bioassays of Other Products *(Other Than Those Products Mentioned Above)*
Tests for Medical devices and Medical Textile
Specific Pathogens
Sterility
Other Specified Tests

A. 3 Water

Drinking water
Potable water
Packaged Drinking Water
Packaged Natural Mineral Water
Water for Swimming Pool and Spas
Water for Construction Purpose
Water Purifiers
Ground Water/ Surface Water
Water for Medicinal Purposes
Distilled /Demineralised Water
Water for Processed Food Industry
Water for industrial purpose
Ice
Test for Efficacy of Water Filtering Plants

A. 4 Pollution and Environment

Air
Effluents
Solid waste
Sewage
Soil

A.5 Biocides

Algacides
Bactericides
Fungicides
Herbicides
Insecticides
Sporicides
Viricides
Weedicides

Antiseptics,
Disinfectants

A.6 Cosmetics, Perfumes and Essential Oil

Gram negative Pathogens
Microbial Count
Preservative Efficacy
Sterility Tests

A.7 Industrial Cultures

Dairy Starter Cultures
Rizhobial Cultures
Yeast and Other Ferments
Mushroom Spawn
Meat Starter Cultures
Other specified cultures

A.8 Seed Testing

Sampling
Moisture
Purity
Germination
Tertazolium
Fluorescence
Other Specified Tests

A.9 Plants and Plant Materials for Presence of Disease

Identification of Bacterial Pathogens
Identification of Fungal Pathogens
Identification of Viral Pathogens
Other Specified Tests

A.10 Molecular Biology

(Tests for Various Matrices)
Genotyping
Promoter/Terminator Screening
Pathogen Detection
Gene Expression
Gene Copy Number

Bacterial Mutagenicity Tests
Sister Chromatid Exchange Tests
Transformation Assays In Cell culture
Other Specified Tests

A.11 GMO Testing

Detection by DNA
Detection by Protein

A.12 Cell Culture

Cytotoxicity
Cytogenetics
Cell permeability test

A.13 Resistance to Microbial Attack

Textiles and Fabrics
Paints and surface coatings
Adhesives, glues and other bondings
Paper and paper pulp
Electrical Components
Timber and Allied Material
Other Specified Materials

A.14 Biological Tests on Other Miscellaneous Test Items

Adhesives Glues and Sealant
Fuels and Oils
Lubricants
Packaging Materials
Paints & Surface Coatings
Pulp & Paper
Soaps & Detergents
Textiles & Fabrics
Wood & Wooden Products
Toys and Other Children's Products
Biofertilizer
Soil Conditioners and Organic Fertilizer

A.15 Factory Hygiene Purposes

Surfaces
Air
Water

A.16 Toxicology

Acute Toxicity
Subacute Toxicity
Neurobehavioral Toxicity Evaluation
Promoter screening
Reproductive Toxicity
Chronic toxicity
Generation Study
Mucous membrane irritation test
Skin sensitization test
Hypersensitivity/allergenicity test
Eye irritation test
Neurotoxicity
Carcinogenicity
Environmental toxicity
Mutagenicity
Teratogenicity
Fish Toxicity Studies
Bird Toxicity

A.17 Identification of Bacterial and Viral Pathogens in Food Items by:

Test Kits
ELISA
PCR

A.18 Residue Analysis

Antibiotic residue analysis by ELISA

A.19 Veterinary Testing

Specified tests in biochemistry, haematology, cytopathology, histopathology etc

A.20 Phytosanitory Tests

Testing of quarantine pathogens

APPENDIX -B

GLOSSARY OF TERMS

Calibration

Set of operations that establish, under specified conditions, the relationship between values of quantities indicated by a measuring instrument or measuring system, or values represented by a material measure or a reference material, and the corresponding values realized by standards.

NOTES

1 The result of a calibration permits either the assignment of values of measurands to the indications or the determination of corrections with respect to indications.

2 A calibration may also determine other metrological properties such as the effect of influence quantities.

3 The result of a calibration may be recorded in a document, sometimes called a calibration certificate or a calibration report.

[VIM: 1993 ISO International vocabulary of basic and general terms in metrology]

B.1Certified Reference Material

Reference material, accompanied by a certificate, one or more of whose property values are certified by a procedure, which establishes traceability to an accurate realization of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence.[ISO Guide 30:2015]

B.2 Limit of Determination

Applied to quantitative microbiological tests - The lowest number of micro-organisms within a defined variability that may be determined under the experimental conditions of the method under evaluation.

B.3 Limit of Detection

Applied to qualitative microbiological tests- The lowest number of micro-organisms that can be detected, but in numbers that cannot be estimated accurately.

B.4 Negative Deviation

Occurs when the alternative method gives a negative result without confirmation when the reference method gives a positive result. This deviation becomes a false negative result when the true result can be proved as being positive.

B.5 Positive Deviation

Occurs when the alternative method gives a positive result without confirmation when the reference method gives a negative result. This deviation becomes a false positive result when the true result can be proved as being negative.

B.6 Reference Cultures

Collective term for reference strains, reference stocks and working cultures. Microorganisms defined at least to the genus and species level, catalogued and described according to its characteristics and preferably stating its origin.[ISO 11133-1:2014] Normally obtained from a recognized national or international collection.

B.7 Reference Material

Material or substance one or more of whose property values are sufficiently homogeneous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials.

[ISO Guide 30:2015]

B.8 Reference Method

Thoroughly investigated method, clearly and exactly describing the necessary conditions and procedures, for the measurement of one or more property values that has been shown to have accuracy and precision commensurate with its intended use and that can therefore be used to assess the accuracy of other methods for the same measurement, particularly in permitting the characterization of a reference material.

Normally a national or international standard method.

B.9 Reference Stocks

A set of separate identical cultures obtained by a single sub-culture from the reference strain. [ISO 11133-2:2014]

B.10 Relative Trueness

The degree of correspondence of the results of the method under evaluation to those obtained using a recognized reference method.

B.11 Repeatability

Closeness of the agreement between the results of successive measurements of the same measurand under the same conditions of measurement.

[VIM: 1993 ISO International vocabulary of basic and general terms in metrology]

B.12 Reproducibility

Closeness of the agreement between the results of measurements of the same measurand carried out under changed conditions of measurement. [ISO VIM: 1993 ISO International vocabulary of basic and general terms in metrology]

B.13 Sensitivity (applied to microbiological tests)

The fraction of the total number of positive cultures or colonies correctly assigned in the presumptive inspection. [ISO/TR 13843:2000]

B.14 Specificity (applied to microbiological tests)

The fraction of the total number of negative cultures or colonies correctly assigned in the presumptive inspection. [ISO/TR 13843:2000]

B.15 Working Culture

A primary sub-culture from a reference stock. [ISO 11133-2014]

B.16 Validation

Confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled. [ISO 9000: 2005]

B.17 Verification

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

APPENDIX -C

METHOD VALIDATION

Laboratories with the appropriate knowledge, skills, experience and resources to do so in a competent and thorough manner should only carry out validation of biological testing methods. The requirements for method validation are detailed in Clause 5.4.5 of ISO/IEC 17025:2005.

The diagram on the following page (Figure 1) provides a very generalized approach to method validation

It is not intended to be a comprehensive reference to validation requirements, but rather a starting point to assist laboratories to ensure the key components are considered. In some instances laboratories may need to do more to demonstrate full validation; in other instances, some of the elements may not need to be considered - depending on the purpose to which the method is to be applied.

APPENDIX -D

CONTROL OF REFERENCE ORGANISMS

Cultures of microorganisms with defined characteristics are required for most microbiological tests performed by SLAB accredited laboratories .For e.g. reference organisms are used in a wide range of determinations and organisms with known properties may be used in proficiency testing. A well-maintained culture collection is an essential element of good laboratory practice.

Verification of Reference Organisms

Reference cultures obtained from a recognized national or international collection need to be verified for their purity and identity on receipt. The level of verification of identity should be based around "fitness-for purpose" principles laboratory i.e. does the organism display the typical characteristics expected in its usual everyday use in the particular laboratory. Gram stain and biochemical reactions should also be used where the laboratory to conduct such checks.

Maintenance Guidelines

Microorganisms have an inherent tendency to mutate in laboratory culture. It is essential then that laboratories use procedures to maintain their cultures in a viable and genetically stable state. Various methods have been established to preserve cultures so that minimum genetic drift occurs.

Microbiological laboratories routinely require easy access to actively growing cultures. They are required on a day to day basis for quality control, comparative testing, inocula for bioassays and for various other reasons.

The following guidelines are to provide guidance to laboratories on the general principles involved on culture maintenance. They are generally applicable to most aerobic organisms in common use. However it is to be noted that culture conditions for anaerobic organisms are significantly different.

Reference cultures may be sub-cultured once to provide **reference stocks.** The reference stocks must be used to prepare **working stocks** for routine works and they must not be refrozen and reused once thawed. Working stocks shall not be sub cultured to replace reference stocks. Records of sub-culturing shall be kept.

Appropriate technique shall be used to preserve the reference microorganism so that the desired characteristics of the strains are maintained. The laboratory shall assign suitable staff for maintenance of reference culture. Written protocols for culture maintenance shall be available in the laboratory.

Authenticated organism from the Reference Culture Collection

Tier-1 ***cultured once****

Reference stocks

Tier-2

cultured once*

Working stock

Cultured once

Tier-3

Daily QC use

***** Purity checks and biochemical tests as appropriate

In the majority of laboratories, the following techniques are used:

(i) ***Refrigerated Storage***

The reconstituted authenticated organism is maintained at 4°C on an appropriate medium and at 3 - 6 monthly intervals is used to prepare a second tier of organisms which in turn is used at 1 - 2 weekly intervals to prepare a third tier of 'working' organisms for day to day quality control use. All organisms are stored at 4°C. Tier 1 is replaced at 1 – 2 yearly intervals.

Storage of reference cultures must be appropriately segregated from test samples.

(ii) Freezing on Beads

There are a number of preservation methods which employ the drying of organisms from the liquid state on inert substrates such as sterile soil, gelatin discs, porcelain beads, silica gel or paper discs. These methods are suitable for short to medium term preservation at -18°C to -70°C for periods not exceeding 2 or 5 years respectively, with good genetic stability.

The procedure essentially consists of taking a pure culture from solid media and inoculating into a suitably prepared vial containing appropriate broth medium and unglazed porcelain beads. After agitating the beads in the broth, all excess fluid is removed from the vial with a fine tip Pasteur pipettes. The vial is stored at -18°C to -70°C. With reference to the above diagram, the frozen beads are essentially acting as Tier 1. Recovery is effected by removing a single bead aseptically from the vial and inoculating it directly onto solid media or into broth i.e. from Tier 1 directly to Tier 3. The remaining beads are available for later use Laboratories may choose to insert a second Tier of refrigerated storage (see below), using the beads for Tier 1 maintenance only.

(iii) Lyophilization or Freeze – drying

Lyophilization, or freeze-drying, involves the freezing of a culture followed by its drying under vacuum which results in the sublimation of the cell water. The technique involves growing the culture to the maximum stationary phase and resuspending the cells in a protective medium such as milk, serum or sodium glutamate. A few drops of the suspension are transferred to an ampoule, which is then frozen and subjected to a high vacuum until sublimation is complete, after which the ampoule is sealed. The ampoules may be stored in a refrigerator and may remain viable for 10 years or more. Lyophilization is probably the most popular preservation technique and may be used for a wide range of microorganisms.

General

The laboratory's documented procedures need to include a section on reference organisms, which must include:

- Details of the organisms held in the laboratory, their source and identification, and the purposes for which they are used;
- Procedures for the verification of identity and purity of each organism;
- Details on the maintenance program used for each organism and records maintained. Laboratories are expected to maintain records of all their reference culture maintenance activities, including certificates from the reference culture Collection, verification records, and sub-culturing records for all tiers including any purity/verification checks

APPENDIX – E

BIOSAFETY

E.1: **Risk Groups of Microorganisms**

Microorganisms are classified into 4 risk groups according to the risks posed by them to the handlers, and the ease of their transmission to the society.

Risk Group 1 (no or low individual and community risk). A microorganism that is unlikely to cause human disease or animal disease

Risk Group 2 (moderate individual risk, low community risk). A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventative measures are available and the risk of spread of infection is limited.

Risk Group 3 (high individual risk, low community risk). A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.

Risk Group 4 (high individual and community risk). A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.

NOTE: A classification of microorganisms on the basis of risk groups is given in Guidelines for the Safe use of Recombinant DNA technology in the Laboratory published by the National Science Foundation (NSF) of Sri Lanka.

E.2: Biosafety Levels

There are four levels of biosafety precautions adopted for biological agents according to the risk groups.

Biosafety level 1 is suitable for work involving well characterized agents not known to consistently cause disease in healthy adult humans and of minimal potential hazard to laboratory personnel and the environment (Risk Group 1).

Work is generally practiced on open bench tops using standard microbiological practices. Special containment equipment or facility design is neither required nor generally designed. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a qualified and trained person in the area of microbiology or related science.

Biosafety Level 2 is similar to Biosafety Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment (Risk Group 2). It differs from the level 1 by

1) Laboratory personnel have specific training in handling pathogenic agents and are directed by competent personnel.
2) Access to the laboratory is limited when work is being conducted
3) Extreme precautions are taken with contaminated sharp items and ;
4) Certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment

Biological Safety Cabinets of Class I or Class II are recommended for use.

Biosafety Level 3 is applicable to clinical, diagnostic, teaching research or production facilities in which work is done with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route (Risk Group 3).

Laboratory personnel have specific training in handling pathogenic and potentially lethal agents, and are supervised by competent scientists who are experienced in working with these agents.

All procedures involving the manipulation of infectious materials are conducted within biological safety cabinets or other physical containment devices or by personnel wearing appropriate, personal protective clothing and equipment. The laboratory has special engineering and design features.

It is recognized however that some existing facilities may not have all the facility features recommended for Biosafety Level 3 (i.e., double door access zone and sealed penetration). In this circumstance, an acceptable level of safety for the conduct of routine procedures, (e.g., diagnostic procedures involving the propagation of an agent for identification, typing, susceptibility testing, etc.) may be achieved in a Biosafety Level 2 facility providing:

1) The exhaust air from the laboratory room is discharged to the outdoors via HEPA filters
2) The ventilation to the laboratory is balanced to provide directional airflow into the room
3) Access to the laboratory is restricted when work is in progress
4) Recommended Standard Microbiological Practices, special practices and safety equipment for Biosafety level 3 are rigorously followed.

The decision to implement Biosafety level 3 recommendations should be made only by the laboratory director.

Biological Safety Cabinets of Class I or Class II are recommended for use.

Biosafety Level 4 is required for work with dangerous and exotic agents that pose a high individual risk of aerosol transmitted laboratory infections and life threatening disease (Risk Group 4).

Agents with a close or identical antigenic relationship to Biosafety Level 4 agents are handled at this level until sufficient data are obtain either to confirm continued work or to work them at a lower level.

Members of the laboratory staff have specific and thorough training in handling extremely hazardous infectious agents and they understand the primary and secondary containment functions of the standard and special practices, the containment equipment and the laboratory design characteristics. They shall be supervised by competent scientists who are trained and experienced in working with these agents. The laboratory director should strictly control access to the laboratory.

The facility is either a separate building or in a controlled area within a building, which is completely isolated from all other areas of the building. A specific operation manual is prepared or adopted.

Within work areas of the facility all activities are confined to Class III biological safety cabinets or Class II biological safety cabinets used with one-piece positive pressure personnel suits ventilated by a life support system.

The Biosafety Level 4 laboratory has special engineering and design features to prevent microorganisms from being disseminated into the environment.

E.3: **rDNA Technology in the Laboratory** – Safety precautions given in Guidelines for the Safe use of Recombinant DNA technology in the Laboratory published by the National Science Foundation (NSF) of Sri Lanka Should be followed in application of rDNA Technology in the Laboratory.

Appendix F

Guidance on calibration and calibration checks

This information is provided for guidance purposes and the frequency will be based on the need, type and previous performance of the equipment.

Type of equipment	Requirement	Suggested frequency
Reference thermometers (liquid-in-glass)	Full traceable re-calibration Single point (e.g. ice-point check)	Every 5 years Annually
Reference thermocouples	Full traceable re-calibration Check against reference thermometer	Every 3 years Annually
Working thermometers & Working thermocouples	Check against reference thermometer at icepoint and/or working temperature range	Annually
Balances	Full traceable calibration	Annually in the first 3 years, followed by less frequently, based on satisfactory performance
Calibration weights	Full traceable calibration	Every 5 years
Check weight(s)	Check against calibrated weight or check on balance immediately following traceable calibration	Every two years
Volumetric glassware	Gravimetric calibration to required tolerance	Annually
Pipettors/pipettes	Full traceable calibration	Annually
Microscopes	Traceable calibration of stage micrometre (where appropriate)	Initially
Hygrometers	Traceable calibration	Annually
Centrifuges	Traceable calibration or check against an independent tachometer, as appropriate	Annually

Appendix F

Guidance on equipment validation and verification of performance

This information is provided for guidance purposes and the frequency will be based on the need, type and previous performance of the equipment.

Type of equipment	Requirement	Suggested frequency
Temperature controlled equipment (incubators, baths, fridges, freezers)	(a) Establish stability and uniformity of temperature (b) Monitor temperature	(a) Initially, periodically, at documented frequency, and after repair/ modification (b) Daily .each use
Sterilizing ovens	(a) Establish stability and uniformity of temperature (b) Monitor temperature	(a) Initially, periodically, at documented frequency, and after repair/ modification (b) Daily/each use
Autoclaves	(a) Establish characteristics for loads/cycles (b) Monitor temperature/time	(a) Initially, periodically, at documented frequency, and after repair/ modification (b) Daily/each use
Safety cabinets	(a) Establish performance (b) Microbiological monitoring (c) Air flow monitoring	(a) Initially, every year and after repair/ modification (b) Weekly (c) Daily/each use
Laminar air flow cabinets	(a) Establish performance (b) Check with sterility plates	(a) Initially, and after repair/modification (b) Weekly
Timers	Check against national time signal	Annually
Microscopes	Check alignment	Daily/each use
pH meters	Adjust using at least two buffers of suitable quality	Daily/each use
Balances	Check zero, and reading against check weight	Daily/each use
De-ionisers and reverse osmosis units	(a) Check conductivity (b) Check for microbial contamination	(a) Weekly (b) Monthly
Gravimetric diluters	(a) Check weight of volume dispensed (b) Check dilution ratio	(a) Daily/each use (b) Daily/each use
Media dispensers	Check volume dispensed	Each adjustment or replacement

Type of equipment	Requirement	Suggested frequency
Pipettors/pipettes	Check accuracy and precision of volume dispensed by gravimetric method	Regularly (to be defined by taking account of the frequency and nature of use)
Spiral platers	(a) Establish performance against conventional method (b) Check stylus condition and the start and end points (c) Check volume dispensed	(a) Initially and annually (b) Daily/each use (c) Monthly
Colony counters	Check against number counted manually	Annually
Centrifuges	Check speed against a calibrated and independent tachometer	Annually
Anaerobic jars/ incubators	Check with anaerobic indicator	Daily/each use
Laboratory environment	Monitor for airborne and surface microbial contamination using, e.g. air samplers, settle plates, contact plates or swabs	Weekly for total count and moulds: Biannually for pathogens or as otherwise decided by the laboratory based on activities and historical trends and results

Appendix G

Guidance on maintenance of equipment

This information is provided for guidance purposes and the frequency will be based on the need, type and previous performance of the equipment.

Type of equipment	Requirement	Suggested frequency
(a) Incubators (b) Fridges (c) Freezers, ovens	Clean and disinfect internal surfaces	(a) Monthly (b) When required (e.g. every 3 months) (c) When required (e.g. annually)
Water baths	Empty, clean, disinfect and refill	Monthly, or every 6 months if biocide used
Centrifuges	(a) Service (b) Clean and disinfect	(a) Annually (b) Each use
Autoclaves	(a) Make visual checks of gasket, clean/drain chamber (b) Full service (c) Safety check of pressure vessel	(a) Regularly, as recommended by manufacturer (b) Annually or as recommended by manufacturer (c) Annually
Safety cabinets Laminar flow cabinets	Full service and mechanical check	Annually or as recommended by manufacturer
Microscopes	Full maintenance service	Annually
pH meters	Clean electrode	Each use
Balances, gravimetric diluters	(a) Clean (b) Service	(a) Each use (b) Annually
Stills	Clean and de-scale	As required (e.g. every 3 months)
De-ionisers, reverse osmosis units	Replace cartridge/membrane	As recommended by manufacturer
Anaerobic jars	Clean/disinfect	After each use
Media dispensers, volumetric equipment, pipettes, and general service equipment	Decontaminate, clean and sterilize as appropriate	Each use
Spiral platers	(a) Service (b) Decontaminate, clean and sterilise	(a) Annually (b) Each use
Laboratory	(a) Clean and disinfect working surfaces (b) Clean floors, disinfect sinks and basins (c) Clean and disinfect other surfaces	a) Daily, and during use (b) Weekly or more frequently if required (c) Every 3 – 12 months depending on type of laboratory work

COMPOSITION OF THE TECHNICAL COMMITTEE

1	Prof (Ms) Chandrani Wijeratne, Senior Lecturer Department. of Botany University of Sri Jayawardenepura Gangodawila Nugegoda	Chairman
2	Dr. (Ms) S I Abeygunawardhane Senior Lecturer Dept. of Microbiology, University of Kelaniya, Kelaniya	Member
3	Dr (Ms) Chandrika Nanayakkara Senior Lecturer Department of Plant Science University of Colombo	Member
4	Ms. S G S Sooriyaarachchi Director (Product Certification) Sri Lanka Standards Institution Elvitigala Mawatha Colombo 08	Member