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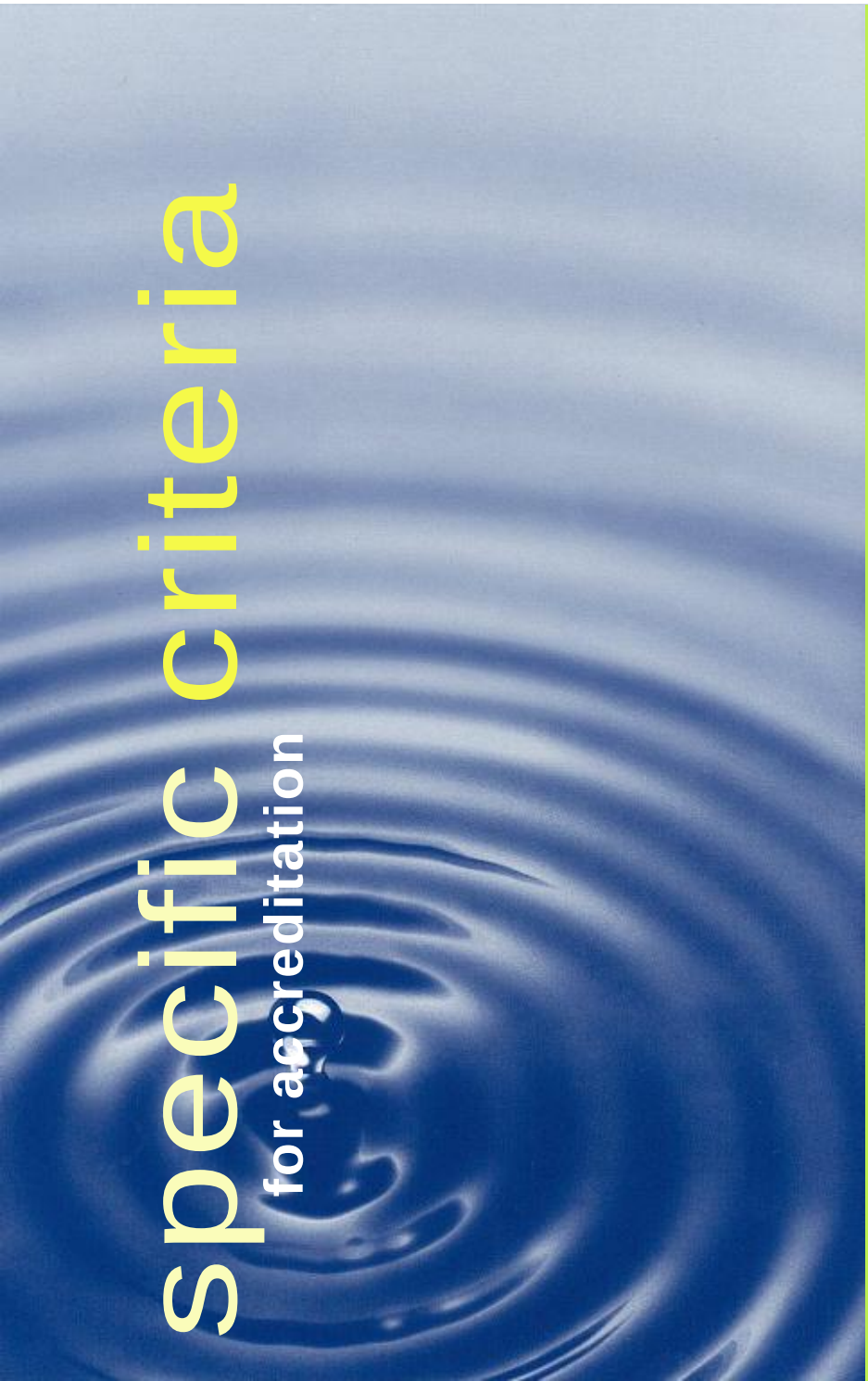


# Biological Testing

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# specific criteria for accreditation

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## 1

Fourth edition May 2011

# specific criteria for accreditation

## Biological Testing

## AS LAB C 1

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## Contents Page

## 1 Introduction

International Accreditation New Zealand's (IANZ) Specific Criteria are an elaboration of the general Criteria for Accreditation for specific fields of test and calibration, test technologies, products or materials. They address items that are essential or most important for the proper conduct of a test or calibration. Specific Criteria provide detail or add extra information to the generally stated requirements of the IANZ General Criteria for Accreditation, which remains the governing document. A list of all published Specific Criteria is available from IANZ on request.

This criteria document must be read in conjunction with current issues of NZS ISO/IEC 17025 and the IANZ publication "*Procedures and Conditions of Accreditation*", the latter document describing the organisation and operation of the IANZ Laboratory Accreditation Programme.

NZS ISO/IEC 17025 is a general document designed to apply to all types of testing and calibration laboratories. This criteria document, on the other hand, provides information and interpretation on classes of test, staff, accommodation, equipment and other aspects of good laboratory management practice which are considered to be minimum standards for biological testing laboratories being accredited against NZS ISO/IEC 17025.

## 2 Scope

This document sets out the specific requirements a biological testing laboratory has to meet, in addition to the general requirements of NZS ISO/IEC 17025, if it is to be accredited by IANZ. A significant majority of accredited biological testing laboratories are primarily involved in bacteriological testing. Thus this document does have a bias toward these types of laboratories. However, the principles embodied within these criteria can be applied equally, where relevant, to other types of biological testing laboratories.

In addition to this document, there are Supplementary Criteria documents applicable to biological testing laboratories working in specialised areas of testing which have their own set of unique criteria. At the time of publication the following additional criteria documents have been published:

| | |
|---|---|
| AS LAB C9 | *Specific Criteria for Accreditation – Dairy Testing* |
| AS LAB C1.2/C2.2 | *Supplementary Criteria for Accreditation – Ministry of Health Register of Water Testing Laboratories* |

Please contact IANZ for more details.

## 3 Classes of Test

IANZ accreditation does not constitute a blanket approval of all a laboratory's activities. Therefore, a means of identifying those activities for which accreditation is granted is necessary. The classes of test given in Appendix 1 provide the framework within which the scope of accreditation is expressed for biological testing laboratories.

These classes are an arbitrary subdivision of the potential range of activities involved in biological testing laboratories on the basis of the types of samples being tested, the scientific disciplines involved and the test methods employed. These classes and subclasses do not, however, constitute any restriction on the work a laboratory can perform but provide a convenient means of expressing an accredited laboratory's capabilities.

## 4 Laboratory Accommodation

Accommodation requirements for biological testing vary widely depending on the nature of the testing involved.

Irrespective of where tests and measurements are performed there must be adequate space and storage facilities for carrying out the tests, recording of test data, report preparation, etc. The internal layout should generally provide for sample receipt, washing-up and sterilisation, media preparation, general testing and incubation areas. A distinct space, if not a separate room, should be used for microbiological testing in a

laboratory complex. Where specialist testing e.g. sterility, pathogens, is involved this would normally be mandatory. The laboratory layout should be designed to minimise potential contamination and to ensure protection of personnel.

Formal laboratory areas must have good lighting, adequate bench space, freedom from excessive dust and fumes, freedom from unwanted vibration and acoustic noise and, for some tests, control of temperature and humidity. For the majority of laboratories, air conditioning is considered essential. The extent to which these environmental factors apply will vary according to the type and precision of the testing. Factors that may need to be considered include but are not necessarily restricted to:

(a) Isolation from sources of stray electric and magnetic fields, mechanical vibration and shock likely to have a detrimental effect on sensitive instruments (e.g. high accuracy balances)
(b) Adequate ventilation when fumes are created during the testing procedure. This includes adequate ventilation during autoclaving activities
(c) Suitable equipment and areas for the preparation of test samples.

Storage facilities must be sufficient to allow for the retention and, where relevant, segregation of samples for designated periods and provide conditions that maintain sample integrity. Refrigerators or freezers must have adequate capacity when samples require refrigeration before or after testing.

The design of workbenches, cupboards, shelves and the finish of all surfaces (i.e. benches, floors, ceilings, walls and windows) must facilitate cleaning and sterilisation. Walls, floors, ceilings and work surfaces shall be non-absorbent and easy to clean and disinfect. Wooden surfaces of fixtures and fittings shall be adequately sealed. Measures should be taken to avoid accumulation of dust (e.g. sufficient storage space, minimal paperwork, documented cleaning programme for laboratory areas, fixtures and equipment).

High standards of housekeeping are essential, and routine housekeeping procedures should be documented. Since all analyses are susceptible to contamination, the laboratory should document and implement procedures and precautions to be taken to prevent contamination from the air, the personnel, aerosols and dust. Instructions must be available for procedures such as washing glassware, generating distilled, deionised or reagent water, sterilisation and wiping down of bench tops, etc.

### 4.1 Monitoring of the Environment

The laboratory environment, where relevant, shall be microbiologically monitored for trends and anomalies and records shall be kept. Laboratories should devise appropriate programmes of monitoring with respect to the type of testing being carried out. As a minimum, monitoring should be of airborne contamination e.g. exposure plates. Swabbing of critical surfaces such as sampling and testing benches, utensils, balances, stomachers, etc. are also recommended, and in pathogen testing laboratories this would be considered essential. Acceptable background counts shall be assigned and there shall be a documented procedure for dealing with situations in which these limits are exceeded.

Where necessary, appropriate pest and vermin control measures are expected to be in place. The suitability of the accommodation will be judged on whether it is likely to adversely affect the samples, equipment, staff performance or final test results.

References such as AS 2243, or an appropriate Code of Practice registered with the Department of Labour, should be consulted when Laboratory Safety Procedures are being prepared and implemented.

## 5 Traceability of Measurement

Traceability of measurement in biological testing is the subject of much discussion and debate in the international testing community and readers are encouraged to familiarise themselves with current developments through sources such as those detailed in the References (5 & 6). The following discussion is provided to summarise the key issues associated with current approaches and provides laboratories with guidance on where to focus their efforts to improve the traceability of their biological measurements.

The "*International Vocabulary for Metrology (VIM)*" defines traceability as the:

*"…property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties."*

This definition is well understood and effectively applied in the traditional metrological areas where physical, electrical and other measurements can demonstrate traceability to "Systeme Internationale" (SI) units such as the kilogram (mass), the meter (length), the second (time), the amp (electric current) and the like.

### 5.1 Traceability of Biological Substances or Organisms

The SI unit for the amount of substance (e.g. for chemical testing) is the mole. To fully realise the mole as a base unit, it is necessary to specify not only the amount but also the specific substance referred to. For biological testing where the "amount of substance" is the biological entity being measured, the same principles apply i.e. both the base unit of the organism has to be realised along with its unique identity. This can provide considerable practical difficulties and therefore alternative approaches to establishing traceability in biological measurement need to be applied.

The fundamental motivation for establishing and demonstrating traceability in biological measurement is to ensure (or at least improve) comparability of results from different laboratories across both space and time. In order for biological measurements to be comparable, there are several critically important components of traceability.

The first is the organism or biological entity to be measured (the "measurand"), which must be clearly and unambiguously defined i.e. to ensure that the results being compared are of the same biological entity. Most often in biological measurement the measurand is defined by the methods of measurement used e.g. aerobic plate count, organisms to species level only, sterility testing, etc. Such methods that define the measurand are often called empirical methods.

The next component in biological traceability is the defined measurement or test method used to provide an estimate of the measurand quantity. The development of a method leads to:

(a) A set of instructions for the conduct of the test
(b) A set of test conditions detailing the parameters that must be kept fixed or stable e.g. growth media, incubation times and temperatures, incubation environmental conditions (such as anaerobic conditions), etc.
(c) A method equation from which the result or quantity of the measurand is calculated using the values of the other measured parameters.

Validation of this test method plays a key role in establishing traceability as it determines whether the assumptions made in setting the method conditions are valid. Validation determines the relative importance of these conditions and rigour to which they need to be monitored and controlled.

Where validation shows that a particular condition is critical to the validity of the test result, then the monitoring and control requirements of this condition are part of the defined traceability of the measurand. For example, where incubation times and temperatures are critical to the outcome of the test, these parameters will need to be measured and these measurements themselves need to be traceable.

In essence, therefore, a biological measurement can be considered traceable when

(a) The defined test method is followed as prescribed, and
(b) Each of the measured parameters in the method conditions are traceable to appropriate standards, and
(c) Each of the measured parameters in the method equation is also traceable to appropriate standards.

The traceability of each of these measured parameters is achieved through calibration. Where these parameters are defined physical measurements – and this is typically the case for the test method conditions in point (b) above, e.g. time and temperature previously mentioned, and mass (of the sample tested for example) - the traceability chains are well established and requirements for these are given in Section 5.2 below.

Often however, and especially for the parameters associated with the method equation in point (c) above, the parameter is a visual or instrument response (such as typical colonies or plaques on a plate of medium, a biochemical or immunoassay response, etc.). These often are or can be compared with a "known" response of the pure biological entity – usually a reference standard or organism but may also be reference data from texts.

### 5.1.1 Biochemical Traceability

Particularly where a reference standard is biochemical or immunological in nature, the mechanisms to ensure traceability of such reference material are not well developed. It is also recognised that availability of reference material complying with the generally accepted mechanisms to ensure traceability is limited.

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:

(a) Reference standards from national measurement institutes, from accredited (to ISO Guide 34) reference material producers, or from reputable reference material producers
(b) Reputable chemical supply houses (particularly kit manufacturers, and for pure biochemical standards or reagents)
(c) Customer supplied reference standards, preferably with certification
(d) In-house produced reference standards.

### 5.1.2 Biological Organism Traceability

As discussed in the first paragraph of Section 5.1, the traceability of these pure biological entities to an accepted universal standard such as an SI unit involves realisation of both identity and quantity.

(a) Traceability of Identity

Microbiological testing laboratories have access to international or national culture collections to source their reference strains of organisms, including bacteria, viruses and the like. Such testing laboratories are expected to source their reference materials or organisms from the following possible sources (generally in decreasing order of preference) where availability permits:

(i) For microbiological laboratories, directly from national or international type culture collections e.g. The New Zealand Reference Culture Collection (ESR Kenepuru Science Centre), ATCC (USA), NCTC (UK)
(ii) Reference standards (particularly when biochemical or immunological in nature) from national measurement institutes, from accredited (to ISO Guide 34) reference material producers, or from reputable reference material producers
(iii) Reputable chemical supply houses (particularly kit manufacturers, and for pure biochemical standards or reagents)
(iv) Customer supplied reference standards/organisms, preferably with certification
(v) In-house produced reference standards/organisms.

Without formal evidence of traceability it remains the laboratories' responsibility to demonstrate the materials/organisms are fit for their intended purpose.

(b) Traceability of Quantity

Any test method involving the measurement of parameters associated with a living biological organism will by its very nature be empirical, in that the result obtained will be dependent on the defined method conditions and how the organism of interest is treated. Comparability of results (and particularly quantitative measurements), and thus measurement traceability, is entirely dependent on laboratories complying in full with the detail of the defined method as published.

While other methods to determine **the defined measurand** are possible, these must be validated or "calibrated" against the primary reference method defining the measurand e.g. when changing culture media or incubation time and/or temperatures; when adopting non-culture methodology for traditional culture methods, etc.

### 5.2 Traceability of Physical Measurements

Traceability requires that there is a chain of equipment whose calibrations to known levels of uncertainty are traceable from one item to the next and eventually to a national standard of measurement. The concept of traceability also includes the competence of all the people involved, the fitness of each measurement environment, the suitability of the methods used and all other aspects of the quality management systems involved at each step in the chain of measurements.

Traceability must be established for all critical* measurement and calibration equipment either:

(a) Directly to the national standards laboratory (IRL - Measurement Standards Laboratory) or another such national body (e.g. NPL-UK, NMI-Australia, etc) acceptable to the Measurement Standards Laboratory, or

(b) From a third party accredited calibration laboratory that is accredited by IANZ or an organisation with which IANZ has a mutual recognition arrangement.

The calibration certificates issued by accredited calibration laboratories must be endorsed in accordance with the requirements of the accreditation bodies concerned. This constitutes proof of traceability to national standards. Endorsement to ISO 9000 standards only is not considered acceptable.

**Critical measurements/calibrations are those that will significantly affect the accuracy or proper performance of tests.*

IRL = Industrial Research Ltd
NPL = National Physical Laboratory
NMI = National Measurement Institute

## 6 Equipment Management and Calibration

Laboratory equipment, and its suitability, ranks on a level equal to the competence of the staff using it. An accredited laboratory will be expected to possess and maintain under a documented management system, all equipment necessary to carry out the tests requested for inclusion in the scope of accreditation.

Guidelines on calibration requirements and re-calibration intervals for specific items of equipment are detailed in Appendix 3. The guidelines set out **maximum** periods of use before equipment must be re-calibrated. These periods have been established by accepted industry practice and, in most instances, are the maximum permitted re-calibration intervals as laid down by international convention. Where a test method or operating environment requires a more stringent recalibration period than that given here, the more frequent calibration will apply.

IANZ may accept **reduced** or **extended** calibration intervals based on factors such as history of stability, accuracy required and ability of staff to perform regular checks. It is the responsibility of the laboratory to provide clear evidence that its calibration and maintenance system will ensure that confidence in the equipment can be maintained.

Precision balances that are being used to their full readability (i.e. to the last place showing) will also require full re-calibration by an appropriate calibration authority (i.e. external calibration) if they are moved to a different location. Balances being used for less than their accuracy limit may be re-validated using appropriate QC methods (i.e. single point and repeatability checks with standard check masses).

Records of calibrations carried out in-house must confirm traceability of measurement (see Section 5.2 above). This is normally achieved by the record specifically identifying the reference item used, the date and the person performing the work using the documented procedure.

### 6.1 Measurement Uncertainty in Calibration

Clause 5.4.6.1 of NZS ISO/IEC 17025:2005 requires testing laboratories which perform their own calibrations to have and apply a procedure to estimate the uncertainty of measurement in all calibrations. The full rigour of this requirement will be expected to be applied where the equipment item being calibrated has performance (accuracy and precision) requirements that are critical to the accuracy or proper

performance of the test and which are approaching the performance specification of the equipment item. Examples would include the calibration of analytical balances, thermometers requiring a high level of (relative) accuracy, and the like.

Biological testing laboratories are recommended to have these items calibrated by an accredited external agency (see Section 5.2 above). If biological testing laboratories wish to calibrate these items themselves a full measurement uncertainty budget is expected to be estimated. This would normally be expected to be estimated in accordance with the "*Guide to the Expression of Uncertainty of Measurement*" (ISO, 1995). The IANZ "*Specific Criteria for Accreditation in Metrology and Calibration" (AS LAB C5)* should be consulted for further information.

Uncertainty of measurement estimations for periodic checks conducted in-house on calibrated equipment (i.e. conducted between full calibrations) are not required.

## 7 Staff and Key Technical Personnel

An accredited laboratory must have at least one staff member who is competent in the testing being undertaken.

Prior to 31 December 2003, IANZ recognised such competency in biological testing laboratories through the awarding of Approved Signatory status to individuals in the laboratory. This involved laboratories submitting Applications for Signatory Approval for each of the applicant Signatories nominated by the laboratory, followed by an assessment by an IANZ Assessment Team of the individual at the laboratory site. Laboratories were required to maintain at least one Approved Signatory for each of the tests in the scope of accreditation. The requirements for Approved Signatories were very similar to those detailed in Appendix 2.

Historically, the Approved Signatory concept and processes have provided IANZ with an effective mechanism to ensure accredited biological testing laboratories have the necessary competencies in terms of technical supervision of the testing. However, the nature of biological testing laboratories has changed significantly since laboratory accreditation was first offered in New Zealand. Laboratories have tended to become both larger, and technologically more advanced. This has meant increased specialisation of staff within the laboratory operation, and has resulted in a significant increase in the number of Signatory applicants, many of whom seek approval in a limited range of specialised testing relative to the full scope of accreditation. Many laboratories are also now operating outside of normal working hours and this has resulted in further Signatory applications to give such laboratories the necessary Approved Signatory cover.

From 1 January 2004, IANZ will no longer assess and grant Approved Signatory status to individuals within biological testing laboratories. Rather, the qualification and appointment of approved signatories will be an internal process in the laboratory under the responsibility of the laboratory management. To distinguish these individuals from the traditional IANZ “Approved Signatories”, the internal appointments will be known as “Key Technical Personnel”.

The expected roles and qualifications of a Key Technical Person are given in Appendix 2. IANZ will no longer be assessing each appointed individual against neither these requirements nor issuing a Certificate of Signatory Approval to those who are considered to meet these requirements.

The following requirements in regard to Key Technical Personnel will take affect from 1 January 2004:

(a) Appointment of Key Technical Personnel will be the responsibility of a designated senior laboratory officer who is a member of the laboratory’s senior management team. Laboratories are required to have a documented person/position specification for Key Technical Persons and a documented and formal process for their qualification and appointment

(b) The laboratory will maintain a list of current Key Technical Personnel, including the technical scope of their areas of responsibility. This list may be included in the laboratory’s quality manual or as a separate document, but must be maintained up-to-date at all times. The technical scope for each individual will be described in a manner to suit the laboratory’s circumstance and organisational structure, but there must be at least one Key Technical Person appointed for each test, or group of tests in the laboratory’s scope of accreditation. The laboratory may choose to use the Classes of Test detailed in Appendix 1 with additional qualifiers as appropriate, but this is not mandatory

(c) The list of Key Technical Personnel and their individual scope of responsibility must be notified to IANZ who will maintain this listing for each accreditation. IANZ will request this information in the Application for Accreditation or Reassessment documentation provided prior to the three-yearly full reassessment. The list will also be reviewed with laboratories during their annual surveillance assessment

(d) Changes to Key Technical Personnel listings (including individuals who have left the laboratory, new Key Technical Person appointments, or changes in the technical scope of responsibility) made between annual on-site assessments must also be notified to IANZ. This is the responsibility of the laboratory’s Authorised Representative

(e) In addition to the laboratory’s usual training records, each Key Technical Person is required to have a brief CV-type summary of qualifications and experience. This CV information will be requested to

be provided to IANZ for each appointed Key Technical Person in the Application for Accreditation/Reassessment documentation described above. This information is also expected to be provided to IANZ when new Key Technical Personnel are appointed and notified to IANZ outside of annual assessments

(f) Where a laboratory loses the sole Key Technical Person for all or part of their scope of accreditation and no new appointment is made by the laboratory management then the laboratory's accreditation (or part thereof) will be suspended until such time as a new appointment is notified to IANZ. Where new Key Technical Personnel appointments are made outside of routine reassessments, and particularly when a new appointment is the sole Key Technical Person for all or part of the accreditation, IANZ reserves the right to conduct an on-site assessment of the laboratory to be assured the laboratory's systems and integrity of the laboratory's tests results will continue to be maintained

(g) All IANZ-endorsed test reports issued by an accredited laboratory must be signed or authorised by a Key Technical Person nominated by the laboratory. See Section 10.2.1.

The move from IANZ Approved Signatories to Key Technical Personnel effectively means the responsibility for qualification of key individuals within a laboratory has shifted from IANZ Assessment Teams to laboratory management. IANZ Assessment Teams will no longer feel obliged to interview all appointed Key Technical Personnel. The Key Technical Personnel will still generally be expected to be the escorts for IANZ assessment teams during the course of an on-site assessment, with any of the appointed individuals being selected for the particular part of the scope of accreditation being assessed. The team may also choose to interview other levels of technical staff. In the case where a particular Key Technical Person is not able to demonstrate to the assessment team that the laboratory is continuing to maintain the requirements for accreditation, it is not the individual who is considered to have "passed" or "failed" but rather the laboratory as a whole on the grounds of inadequate continuous technical supervision. Rather than an individual not being granted "IANZ Signatory Approval", it may be that the affected part of the scope of accreditation is suspended.

# 8 Test Methods

Accreditation is normally granted only for internationally or nationally accepted standard test procedures or non-standard procedures (in-house methods) that have been appropriately validated and which are performed regularly.

## 8.1 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 can be obtained.

## 8.2 Kits

Commercial test systems (kits) will require further validation if the laboratory is unable to source the validation data from manufacturers with a recognised quality assurance system; reputable validation based on collaborative testing, e.g. AOAC Official Methods and associated JAOAC Publications; or independently reviewed methods e.g. AOAC Performance Tested Methods.

## 8.3 In-house methods

In-house methods could include but not be restricted to:

(a) Methods developed in the laboratory
(b) Methods developed by a client
(c) Methods developed for an industry group
(d) Modified standard test methods
(e) Methods from scientific publications, but which have not been validated.

Validation procedures shall involve, as appropriate, the aspects referred to in Clause 5.4.5 of NZS ISO/IEC 17025:2005. Appendix 4 provides some guidelines for method validation in microbiology.

Standard test methods should be used whenever possible in order to ensure inter-laboratory reproducibility of test results. Laboratories are discouraged from seeking accreditation for test methods that depart from recognised published standards. If however, approval of an in-house test method is required the following information must be provided:

(a) A copy of the fully documented test method
(b) Details of the origin of the in-house test method
(c) Details of the reason for its development and application
(d) The results of comparative tests with standard methods (if possible) and other laboratories
(e) Full details of test method validation as described in Clause 5.4.5 of NZS ISO/IEC 17025:2005.

Once a laboratory is accredited for a specific test method the detailed procedures of that method must be adhered to at all times. Occasionally it may be necessary to deviate from the documented test method. Any departures must be reported with the test results, and may invalidate accreditation status of that particular test.

Accreditation for opinions and interpretations in biological testing is not offered under the IANZ Biological Testing Laboratory Accreditation Programme.

## 9 Uncertainty of Measurement

It is important for testing laboratories to understand the concept of uncertainty of measurement. Laboratory management should be aware of the effect that their own uncertainty of measurement will have on test results produced in their laboratory.

Clause 5.4.6 of NZS ISO/IEC 17025:2005 requires testing laboratories to estimate their measurement uncertainty in the testing they conduct. While the concept and application of measurement uncertainty estimations have been well established in metrology and calibration laboratories, the same cannot be said for testing laboratories. The publication of NZS ISO/IEC 17025:2005 has prompted rigorous discussion internationally on uncertainty of measurement in biological testing and, as at the time of publication, a consensus agreement on the definitive methodology to be used for estimating uncertainty is still some way off. Readers are encouraged to familiarise themselves with current developments through sources such as those detailed in the References (8, 9, 11 & 15).

The following details the current requirements for laboratories accredited under the Biological Testing Programme:

(a) Laboratories need to make a formal estimate of measurement uncertainty for all tests in the scope of accreditation that provide numerical results. Where results of tests are not numerical or are not based on numerical data e.g. detected/not detected, pass/fail, positive/negative, or based on visual, tactile or other qualitative examinations, estimates of uncertainty are not required

(b) Where an estimate of measurement uncertainty is required, laboratories need to document their procedures and processes on how this is to be done.

    There are various published approaches to the estimation of uncertainty in testing. NZS ISO/IEC 17025 does not specify any particular approach. All approaches which give a reasonable estimate and are considered valid within the biological testing community are equally acceptable and no one approach is favoured over others. For guidance, Appendix 5 sets out a possible approach which IANZ would suggest as being consistent with approaches internationally. This approach is not mandatory but alternative approaches would be expected to address the principles embodied within it.

    Laboratories are referred to the References (9, 11, 15 & 24) for further information.

    What is important is that laboratories document, with reference to published procedures, what their approach to estimating uncertainty in measurement will be. IANZ assessment teams will assess the suitability and rigour of these approaches during annual assessments

(c) 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 of accreditation.

It is recognised that in some instances this may take some time as the procedures in (b) above may require a redesign of current quality control programmes, and data may need to be collected over a reasonable length of time in order to make a sufficiently rigorous assessment of measurement uncertainty. Laboratories will need to maintain records of each test or type of tests to demonstrate full implementation of the procedure required by (b) above.

### 9.1 Reporting Measurement Uncertainty

Biological testing laboratories are not required to report their estimated measurement uncertainty on test reports as a matter of routine.

However, Clause 5.10.3.1(c) of NZS ISO/IEC 17025:2005 requires reporting of measurement uncertainty when it is required for the correct application or interpretation of the test result. One such instance is where test results are used to determine if a sample conforms to a required numerical specification, and where the specification limit falls within the limits of measurement uncertainty associated with the test result obtained.

## 10 Test Records and Reports

### 10.1 Test Records

An adequate test records system in accordance with the various clauses of NZS ISO/IEC 17025, e.g. 4.13, 5.4.7 is essential.

Most laboratories have developed forms (proforma sheets) for all of 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.

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 greater level of management to ensure that records are not lost. For these reasons free text recording systems are now usually found only where a high level of non-routine testing is carried out, e.g. in research organisations.

### 10.2 Test Reports

Clauses 5.10.1, 2, 3, 6, 7, 8, and 9 of NZS ISO/IEC 17025:2005 set out the requirements for test reports issued by testing laboratories.

Test reports must give the client 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.

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.

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.

#### 10.2.1 IANZ-Endorsed Test Reports

Accredited laboratories are permitted to include reference to their accreditation in the test reports they issue. The general rules governing the use of IANZ endorsements are detailed in Appendix 1 of the IANZ publication "*Procedures and Conditions of Accreditation" (AS 1)*. For biological testing laboratories, all test reports carrying the IANZ endorsement must be formally authorised by at least one of the laboratory's

nominated Key Technical Personnel (see Section 7 and Appendix 2). This would normally be by a signature on the report itself (see also Section 10.2.2 below).

It is recognised that many of today's laboratories are multi-disciplinary in nature and in some cases, very specialised within disciplines. Test reports pertaining to a particular sample or set of samples may include test results from several specialist areas and/or disciplines.

While the technical scope of nominated Key Technical Persons is expected to match their expertise in various specialist areas and/or disciplines, it is not practical to expect a number of Key Technical Personnel to sign a test report to cover each of the results that may be reported therein. In these instances it is acceptable that a multi-disciplinary test report is signed by only one of the laboratory's Key Technical Persons under the following conditions:

(a) The individual authorising the test report is responsible for ensuring all results which are outside their technical scope as a Key Technical Person (and that are included in the test report) have been authorised or released internally by a Key Technical Person (or delegated staff – see Appendix 2, point (c)) with these tests in their technical scope
(b) There is a clear audit trail within the laboratory's system to demonstrate this.

#### 10.2.1.1 Opinions and Interpretations

Clause 5.10.5 of NZS ISO/IEC 17025:2005 allows for test reports to include statements of opinion and interpretation related to the test results. In biological testing, it is the policy of IANZ that accreditation is not granted to laboratories for providing statements of opinion and interpretation of test results.

Except where an interpretation is clearly factual (e.g. a statement of compliance or otherwise with a specification), opinions and interpretations cannot be implied as being within the scope of the laboratory's accreditation on an IANZ-endorsed test report.

This does not preclude accredited laboratories from making such statements as an added value service to their clients. However, they should either be given in a (non-IANZ endorsed) separate document to the test report, or if included directly in IANZ-endorsed reports, a clear disclaimer made that the statements made are outside the laboratory's scope of accreditation.

### 10.2.2 Electronic Reporting

Traditionally, laboratories issued test reports in hard copy format with manuscript signatures (from IANZ Approved Signatories if the test report was IANZ-endorsed). 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.

Clause 5.10.7 of NZS ISO/IEC 17025:2005 attempts in a very general way to specify the 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 clients), the following is intended to provide guidance on common issues of concern.

#### 10.2.2.1 Transmission of Reports

It is the responsibility of the issuing laboratory to ensure that what was transmitted electronically is what the client received. Email systems have proven to be robust in this regard, but laboratories need to consider whether clients 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 client to supply a copy of what was received and comparing it with what was transmitted. It is also important that the laboratory and its client 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 client.

#### 10.2.2.2 Security

Laboratories should avoid sending test reports in an electronic format that can be readily amended by the recipient. Examples would be in word processing or spreadsheet software. Where possible, reports should be in a read only format e.g. pdf files.

Where this is not possible e.g. the client 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 what was sent. This may be a hard copy (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.

#### 10.2.2.3 Electronic Signatures

All reports (whether hard copy or electronic) must not be released to the client until authorised by individuals with the authority to do so. For electronic reports there must be a clear audit trail with a positive authorisation 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 (a nominated Key Technical Person in the case of IANZ-endorsed reports). This may involve an electronic signature. The security of these signatures should be such as to prevent inadvertent use or abuse.

#### 10.2.2.4 Electronic Report Formats

Clause 5.10.1 of NZS ISO/IEC 17025:2005 allows for simplified report formats for internal clients or in the case of written agreement from the client. This is often the case for electronic reports. While the laboratory may be accredited for the testing, it is usual such reports would not normally carry the formal IANZ-endorsement.

IANZ-endorsed reports, whether electronic or not, would normally be expected to comply with the requirements of Clause 5.10.2 and 5.10.3 (as appropriate) of NZS ISO/IEC 17025:2005.

## 11 Quality Control

It is essential that accredited biological testing laboratories have developed, documented and implemented an appropriate quality control (QC) programme.

Clauses 5.9 and 5.4.7.1 of NZS ISO/IEC 17025:2005 suggest various quality control procedures that can be included in a laboratory's day-to-day activities and each laboratory is expected to implement the procedures most appropriate to their circumstances. Where relevant, quality control data should be analysed, and where it is found to be outside pre-defined action criteria, the defined action shall be taken to correct the problem and to prevent incorrect results from being reported.

It is important for laboratories to understand where tests can go wrong so that steps can be taken to either eliminate the potential error point, or put in an appropriate QC step for alerting the operator when the test has gone wrong. Quality control in some form is possible with any test being performed. A disciplined approach is required for the development of a suitable QC plan and this approach should be applied on a test-by-test basis.

The quality control programme should be designed in such a way as to demonstrate that the on-going control of both the accuracy and precision of each test is being maintained.

Where tests are performed infrequently, the laboratory should carry out regular performance checks to demonstrate its continuing competence to perform them, or have in place a system for demonstrating proficiency prior to performing the test on a client sample.

### 11.1 Quality Control in Microbiology Laboratories

As discussed in Section 5, microbiological testing methods are for the most part empirical in nature. The result obtained (and its associated traceability) is dependent on adherence to the method used, including the method conditions specified. Short of revalidating the method conditions every time the test is conducted (e.g. by the quantitative assessment of the recovery of a reference organism from the sample matrix for each sample tested – see Appendix 4), laboratories need to implement a quality control programme that ensures the method conditions are adequately controlled. This control procedure will also ensure the method conditions (or parameters contributing to the measurement uncertainty – see Section 9 and Appendix 5) operate within defined parameters and thus have a predictable and consistent contribution to the uncertainty in the measurement results.

In essence, all possible inputs into the testing system need some level of quality control to ensure consistency to the quality of the results produced.

Many published method texts have chapters which detail such quality control programmes suited to their particular application (e.g. References 16, 17, 18 & 19). The guidelines in this document are not intended as a replacement for these, but rather a summary of the key issues and as a resource for laboratories which do not have ready access to such texts.

Some of the key inputs common to most microbiological testing and which need to be subject to such a quality control programme can be summarised as follows:

(a) Personnel (Section 7 and Appendix 2)
(b) Valid test methods (Section 8 and Appendix 4)
(c) Laboratory accommodation and environment (Section 4)
(d) Equipment and its calibration (Section 6 and Appendices 3 and 5)
(e) The authenticity and maintenance of reference organisms to ensure their validity and viability (Appendix 6)
(f) Consumables used in the conduct of the tests; including media, reagents and diluents (Appendix 8) and their preparation (also Appendix 5)
(g) Laboratory supplies and equipment having direct contact with the samples/organisms under test (Appendix 9).

This list is not necessarily exhaustive and additional quality control requirements may be required by method specifications, industry standards or general good practices. Similarly, the application of these particular requirements may not be necessary in all instances.

## 12 Proficiency Testing

Proficiency testing is defined as the "*determination of laboratory testing performance by means of inter-laboratory comparisons*" (ISO Guide 2:1996) and is thus a very important tool in a laboratory's quality control programme to demonstrate the validity and comparability of results.

In accordance with the policy of the Asia Pacific Laboratory Accreditation Co-operation (APLAC), to which IANZ is a full member of their Mutual Recognition Arrangement (MRA), (see Reference 14), it is IANZ policy that applicant/accredited biological testing laboratories shall:

(a) Demonstrate their technical competence by the satisfactory participation in proficiency testing activity where such activity is available, and that:
(b) The minimum amount of appropriate proficiency testing required per laboratory is one activity prior to gaining accreditation, followed by:
    (i) Participation in as many inter-laboratory comparison programmes (where available) required to cover the scope of accreditation, and
    (ii) For the programmes selected, to participate in all relevant rounds that are available. Where multiple programmes exist covering the same methodologies on similar sample types, participation in all rounds may be relaxed. This would need to be justified on performance-based criteria, and each case will be treated on its merits. The overall frequency must still be such as to demonstrate on-going proficiency.

Aside from the issues of coverage and frequency, laboratories are expected to select proficiency testing activities according to the following criteria (in a generally decreasing order of preference):

(a) Mandated programmes. In some areas of biological testing, participation in a particular programme may be mandatory e.g. Laboratory Approval Scheme testing laboratories
(b) International inter-laboratory comparison programmes
(c) National inter-laboratory comparison programmes
(d) Proficiency testing programmes operated in accordance with ISO Guide 43: Part 1
(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.

The participation in a programme is of little value without the combined results being analysed to determine the nature of any discrepancies and the effect of this on any routine test results. Discrepancies may be in the order of expected uncertainty, or they may indicate a serious shortcoming in a laboratory's procedure. It is important for laboratories to have undertaken this analysis and to have adequately determined and implemented appropriate corrective action.

Records of the above analysis, and any action taken, of all proficiency testing results are required, including those for which no further action is considered appropriate i.e. satisfactory results.

The results from proficiency testing activities and their analysis will be viewed by IANZ at each assessment.

### 12.1 APLAC Proficiency Testing Programmes

From time to time APLAC arranges for proficiency testing programmes to be run and expects accredited laboratories in all economies which are members of the MRA to participate.

On receipt of an invitation to participate, IANZ nominates (usually to a maximum of four) accredited laboratories to participate, provided the programme is relevant to their scope of accreditation. Nominated laboratories are expected to participate (usually no fee is charged) unless there are valid reasons for not doing so.

The results from these APLAC Proficiency Testing Programmes are required to be treated by IANZ in a formal manner. Both the participating laboratories and IANZ receive a copy of the report. Where a particular laboratory has outlier or non-conforming results they will be required to submit to IANZ detail on the investigations conducted and any corrective action taken.

*It should be noted that all accredited laboratories in any inter-laboratory comparison programme are expected to do this, but would not normally report it to IANZ. Such records would be reviewed at the next on-site visit.*

IANZ staff will review the response and comment where appropriate. The records will also be reviewed at subsequent on-site assessments – particularly by a technical assessor where appropriate.

It should be noted that APLAC Proficiency Testing Programmes are as much a measure of the IANZ performance in accrediting laboratories as they are a measure of the participating laboratories' performance. The co-operation of the nominated laboratories is appreciated by IANZ.

## 13 References

1. NZS ISO/IEC 17025:2005 – *General requirements for the competence of testing and calibration laboratories*

2. *Procedures and Conditions of Accreditation (AS 1)*, IANZ

3. AS/NZS 2243 – *Safety in Laboratories*

4. *International Vocabulary of Basic and General Terms in Metrology (VIM)*, 2nd Ed.(1993), ISO/BIPM/IEC/IFCC/IUPAC/IUPAP/OIML

5. *Eurachem/CITAC Guide: Traceability in Chemical Measurement – A guide to achieving comparable results in chemical measurement*, Voting Draft, March 2003 (see www.eurachem.ul.pt)

6. King, B. *Meeting ISO/IEC 17025 Traceability Requirements: A new guide with worked examples*, CITAC News (February 2003), p.8

7. ISO Guide 34: 2000 – *General requirements for the competence of reference material producers*

8. *Guide to the Expression of Uncertainty in Measurement*, 1st Ed.,(1995), ISO/BIPM/IEC/IFCC/IUPAC/IUPAP/IOML

9. *Eurachem Guide: Quantifying Uncertainty in Analytical Measurement*, 2nd Ed.,(2000) (see www.eurachem.ul.pt)

10. ISO/IEC 5725:1994 – *Accuracy (trueness and precision) of Measurement Methods and Results*

11. APLAC TC005: *Interpretation and Guidance on the Estimation of Uncertainty of Measurement in Testing*, Asia Pacific Laboratory Accreditation Cooperation (APLAC), (see www.aplac.org)

12. ISO Guide 2: 1996 – *Standardisation and related activities – General vocabulary*

13. ISO/IEC Guide 43-1: 1997 – *Proficiency Testing by Inter-laboratory Comparisons – Part 1: Development and operation of proficiency testing schemes*

14. APLAC MR-001: *Procedures for Establishing and Maintaining Mutual Recognition Arrangements amongst Accreditation Bodies*, (see www.aplac.org)

15. Nordic Committee on Food Analysis (NMKL) Procedure No.8, *Measurement of Uncertainty in Microbiological Examination of Foods* (1999)

16. APHA *Standard Methods for the Examination of Water and Wastewater* (latest edition)

17. APHA *Compendium of Methods for the Microbiological Examination of Foods"*(latest edition)

18. *NZTM 1: New Zealand Dairy Industry Good Laboratory Practice Manual* (latest edition)

19. AgResearch, *Microbiological Methods for the Meat Industry* (4th edition)

20. USEPA, *Microbiological Methods for Monitoring the Environment*

21. Cosmetic and Toiletry Manufacturers' Code of GMP

22. IANZ Technical Guide 2: *Laboratory Balances Calibration Requirements* (AS TG2)

23. IANZ Technical Guide 3: *Working Thermometers Calibration Requirements* (AS TG3)

24. IANZ Technical Guide 5: *Uncertainty of Measurement, Precision and Limits of Detection in Chemistry and Microbiology Testing Laboratories* (AS TG5)

# Appendix 1

## Classes of Test
## Biological Testing

1.02 Diagnostic Tests, Veterinary
- (a) Biochemistry
- (b) Haematology
- (c) Parasitology
- (d) Endocrinology
- (e) Histology
- (f) Microbiology
- (g) Serology/Immunology
- (h) Virology
- (i) Cytology

1.03 Drugs and Pharmaceuticals
- (a) Microbiological quality (including sterility)
- (b) Efficacy of therapeutic substances (including potency and bioavailability)
- (c) Safety of therapeutic substances
- (d) Toxicity tests
- (e) Bioassays of hormones
- (f) Bioassays of vitamins
- (g) Bioassays of enzymes
- (h) Bioassays of immunological products
- (i) Bioassays of chemotherapeutic agents and antibiotics
- (j) Other specified tests

1.11 Foods
- (a) Cereals and cereal products
- (b) Edible oils, fats and derived products
- (c) Nuts, fruits, vegetables and derived products
- (d) Sauces, herbs, spices and condiments
- (e) Sugar and sugar confectionery
- (f) Dairy products
- (g) Meat, poultry and derived products
- (h) Fish and fish products
- (i) Eggs and egg products
- (j) Alcoholic beverages
- (k) Non alcoholic beverages
- (l) Food additives and supplements
- (m) Essential nutrients including vitamins
- (n) Other specified fresh foods
- (o) Other specified preserved foods
- (p) Canned foods
- (q) Animal feeds

1.12 Waters
- (a) Potable waters
- (b) Non-potable waters e.g. receiving waters, ground waters
- (c) Sewage
- (d) Effluents and trade wastes
- (e) Cooling tower and industrial waters
- (f) Swimming and spa pools
- (g) Marine waters

1.13 Cosmetics, Perfumes and Essential Oils
(a) Microbiological quality (including sterility)
(b) Other specified tests

1.14 Specified Miscellaneous Materials
(a) Microbiological quality
(b) Bioassays
(c) Toxicity tests
(d) Other specified tests

1.21 Materials for Microbial Deterioration
(a) Biodegradability
(b) Resistance to fungal attack

1.22 Resistance to Insect Attack
(a) Textiles and fabrics
(b) Timber and allied materials
(c) Other specified materials

1.31 Biocides
(a) Antiseptics, disinfectants, bactericides, fungicides, algaecides, viricides
(b) Insecticides
(c) Herbicides
(d) Rodenticides

1.41 Industrial Cultures
(a) Dairy starter cultures
(b) Rhizobium inoculum
(c) Mushroom spawn
(d) Yeasts and other ferments
(e) Other specified cultures

1.51 Plant Growth Regulating Substances

1.52 Seeds for Purity and Germination

1.53 Plants and Plant Materials for Freedom from Disease

1.60 New Zealand Shellfish Quality Assurance Programme
(a) Seawater
(b) Raw shellfish
(c) Processed shellfish
(d) Shellfish toxin bioassay

1.61 Animals for Freedom from Disease
In accordance with the MAF Biosecurity New Zealand Export Laboratory Programme
(a) Antibody / Antigen detection systems
(b) Molecular biology detection systems
(c) Propagation based assays
(d) Pathology diagnostic systems
(e) Visualisation diagnostic systems

1.62 Molecular Biology
(a) Physiological fluid identification
(b) DNA typing
(c) Parentage testing

1.71 Biological Condition
(a) Air
(b) Plant hygiene evaluation
(c) Other specified tests

1.81 Environmental Biology
(a) Fish toxicity studies
(b) Identification of macro-organisms
(c) Identification of micro-organisms
(d) Other specified tests

1.91 Sensory Evaluation

1.92 Prepared Microbiological Media Performance Evaluation
*This class of test applies to pre-prepared ready-to-use media, not dehydrated media. While evaluation certificates may be available for dehydrated media, the purchasing laboratory still needs to evaluate the performance of the media in its own operating environment.*

# Appendix 2

## Key Technical Personnel

Supervisory staff in accredited laboratories must be competent and experienced in the technical areas covered by their accreditation. They must be able to oversee the operations and cope with any problems that might arise in their work or that of their colleague or subordinates. Such staff members, formally appointed by the senior management of the laboratory, are referred to as Key Technical Personnel.

The following sets out IANZ's expectations in relation to who the laboratory management should be appointing as Key Technical Persons:

(a) Key Technical Persons would be expected to have:
- (i) A tertiary qualification or equivalent professional recognition in the relevant discipline. Laboratories engaged in a restricted range of repetitive work may be able to appoint Key Technical Personnel with appropriate practical experience and specific training in that work, but without formal qualifications.
- (ii) A position in the staff structure which provides for the authority to implement necessary changes in the laboratory operation to ensure the integrity of test results is maintained. The position in the staff structure should ensure the individual can maintain a working knowledge of the quality assurance and technical systems in operation in the laboratory on a day-to-day basis.
- (iii) A working knowledge of and commitment to the requirements for IANZ accreditation, including the quality and technical management principles embodied in NZS ISO/IEC 17025 and relevant Specific Criteria.
- (iv) The necessary scientific expertise and experience to be aware of and understand any limitations of the test procedures, and to fully understand the scientific basis of the procedures.
- (v) Sufficient experience in the accredited laboratory to address all of the above points.

(b) Key Technical Personnel are those individuals who are given both the responsibility and authority to:
- (i) Develop and implement new operational procedures.
- (ii) Design quality control programmes, set action criteria and take corrective action when these criteria are exceeded.
- (iii) Identify and resolve problems
- (iv) Take responsibility for the validity of the outputs.

(c) Key Technical Personnel would normally be those individuals who authorise the release of all test results. However in large laboratories such authorisations may be delegated to other supervisory staff on a day-to-day basis provided the delegations and the basis for them are clearly documented. Such delegation of authority does not absolve the Key Technical Person from taking full responsibility for the validity of the work. The authority to release results should not be confused with the authority to issue formal test reports. See Section 10.

(d) Laboratory management may choose to appoint an individual engaged by the accredited laboratory as a consultant, where their Key Technical Person responsibilities relate to work done within the scope of accreditation. There is an expectation that there would be a written agreement between the parties setting out the extent of the authority and responsibility of the consultant in relation to the services provided. *The consultant's position in the laboratory organisation should be such that they can perform their role as a technical decision maker as effectively as if they were an employee.*

(e) Staff members of an accredited laboratory who are not engaged full-time could also be appointed as Key Technical Persons. However, the circumstances in which they are called upon to exercise their Key Technical Person responsibilities and their access to and knowledge of the technical operations should be such that they are able to take full responsibility for the work they authorise or oversee.

## Appendix 3

## Recommended Calibration Intervals

The following table sets out the normal periods between successive calibrations for a number of reference standards and measuring instruments. It must be stressed that each period is generally considered to be the maximum appropriate in each case providing the other criteria as specified below are met:

(a) The equipment is of good quality and of proven adequate stability, and

(b) The laboratory has both the equipment capability and staff expertise to perform adequate internal checks, and

(c) If any suspicion or indication of overloading or mishandling arises, the equipment is checked immediately and thereafter at frequent intervals until it can be shown that stability has not been impaired.

Where the above criteria cannot be met, appropriately shorter intervals may be necessary. IANZ is, however, prepared to consider submissions for extension of calibration intervals based on the factors outlined in Section 6.

Items marked (*) in the table are those which may be calibrated by staff of a laboratory, if it is suitably equipped and the staff are competent to perform such recalibrations. Where the staff of a laboratory have performed calibrations, adequate records of these measurements must be maintained.

IANZ has produced a number of Technical Guides with further information on some calibration procedures (e.g. balances, thermometers). Contact IANZ for further details.

| Type of equipment | Maximum period between successive calibrations | Procedures |
|---|---|---|
| Anaerobic Jars or Cabinets | *Each use | Check condition by suitable means such as an indicator, vacuum gauge, growth of known anaerobes, etc. |
| Automatic Burettes, Dispensers and Pipettors | *Initial and three months | Accuracy of and repeatability at volumes in use. |
| Balances | Initial calibration and three yearly recalibrations | By an accredited calibration laboratory; or<br>*Calibration using traceable certified masses.<br><br>Refer CSIRO Division of Applied Physics paper *Calibration of Balances* and IANZ Technical Guide AS TG 2.<br><br>Staff performing calibrations need to be formally trained.<br><br>Annual servicing is recommended. |

| Type of equipment | Maximum period between successive calibrations | Procedures |
|---|---|---|
| Balances (*continued*) | Accompanied by:<br>(a) *Each weighing<br>(b) *One Month<br>(c) *Six months | Zero check.<br>One point check using a known mass close to balance capacity. (CSIRO paper).<br>Repeatability checks at the upper and lower ends of the scale. (See CSIRO paper). The standard deviation of the results can be compared against the results recorded on the last external calibration certificate. |
| Biological Safety Cabinets | One year | By an accredited laboratory. Documented procedures need to be in place for on-going monitoring. |
| Centrifuges | *One year (where the operating speed is specified) | Calibrated tachometer (mechanical stroboscope or light cell type). |
| Computerised Systems | *Instruments with electronic readouts must be calibrated as a system, including the electronic readout. The period between calibrations will depend entirely upon the nature of the instrument and the use it is being put to.<br>*Computer programmes used to manipulate data into test results must be validated against manually calculated data upon commissioning. The results of this validation must be retained on file in the same manner as a calibration record and may be used for on-going QC checks. The programmes need revalidation if the programme is reloaded, subjected to a voltage spike, or if doubt of the integrity exists. In any event it is recommended that they be revalidated periodically.<br>It is insufficient for the laboratory to assume that proprietary programmes, or programmes adopted from another accredited laboratory are inherently correct. The laboratory will need to run its own commissioning validations and subsequent QC checks. | |
| Conductivity Meter | *Each use<br>*If a temperature compensation probe is used, it must be calibrated. See Thermometers.* | Checked using appropriate standards in each of the scale ranges of the meter in use. |
| | | |

| Type of equipment | Maximum period between successive calibrations | Procedures |
|---|---|---|
| Filters (membrane) | Each manufacturer's batch | Manufacturer's certification of conformance to USEPA standards; and/or<br>*verification checks as per Reference 16 |
| Masses<br>(Integral, stainless steel, or nickel-chrome alloys) | Initial calibration<br><br>Three years (first recalibration)<br><br>Five years (successive recalibrations) | By an accredited calibration laboratory.<br><br>By an accredited calibration laboratory.<br><br>By an accredited calibration laboratory. |
| pH meter | *Daily or before use<br><br>*Note: If a temperature compensation probe is used, it must be calibrated. See thermometers.* | Calibrate using at least two appropriate standard buffers. Buffers need to be stored in appropriate containers and marked with an expiry date. |
| Refrigerators | *Daily | Monitor the temperature and record. |
| Sterilisers<br>Autoclaves | Initial and following repair or maintenance<br><br>*Each use | Check heating profiles of typical loads with respect to chamber temperatures to determine lag times (see Appendix 6), by an accredited calibration laboratory; or<br>*Using appropriately calibrated equipment following a fully documented procedure.<br><br>Annual servicing of steam sterilisers is strongly recommended.<br><br>Check the time and temperature of the cycle. Discard loads should be autoclaved for at least 30 minutes at 121°C. |
| Hot Air Sterilising Ovens | *Each use | Check of time and temperature. At least 160°C for 2 hours. |
| Tachometers | Five years | By an accredited calibration laboratory. |
| | | |

| Type of equipment | Maximum period between successive calibrations | Procedures |
|---|---|---|
| Thermocouples<br>Reference | Three years or 100 hours use (whichever is sooner) | By an accredited calibration laboratory. |
| Working | *Six months | Single point within the working range against a reference thermometer or thermocouple. |
| Thermometers (Liquid in glass)<br>Reference | Five years (complete) | By an accredited calibration laboratory, followed by an ice point check on receipt. |
| | *Six months | Ice point (see IANZ Technical Guide AS TG 3). |
| Working | *Initial | Check against reference thermometer / thermocouple across working range or at points of use. (See IANZ Technical Guide AS TG 3). |
| | *Six months | Check at ice point or at points of use. |
| Thermometers (Resistance)<br>Reference | Five years | By an accredited calibration laboratory, followed by an ice point check on receipt. |
| | *Six months | Ice point. If outside five times the uncertainty of the calibration, complete recalibration is required. |
| Working | *Initial | Check against reference thermometer / thermocouple across working range or at points of use. (See IANZ Technical Guide AS TG 3). |
| | *Six months | Ice point. If outside five times the uncertainty of the calibration, complete recalibration is required. |
| Thermometers (Handheld non-resistance electronic)<br>Working | *One year | Check against reference |

| Type of equipment | Maximum period between successive calibrations | Procedures |
|---|---|---|
| *Note: Handheld non-resistance working thermometers are generally considered of insufficient quality to be used as reference thermometers* | | thermometer/thermocouple across working range or at points of use (see IANZ Technical Guide AS TG 3). |
| Thermostatically Controlled Equipment<br>(Incubators, water baths, ovens) | *Daily<br><br>*Two years | Monitor the temperature and record.<br><br>Temperature variation within the working space by an accredited calibration laboratory; or<br>*Using appropriately calibrated equipment following a fully documented procedure |
| Timers<br>(Stopwatches, chart recorders)<br><br>Mechanical<br><br><br>Electronic | *Three months<br><br><br>*One year | Comparison against radio time “pips” or similar e.g. IRL Talking Clock (0900) 45678.<br><br>Comparison against radio time “pips” or similar e.g. IRL Talking Clock (0900) 45678. |
| Volumetric glassware (flasks, pipettes, burettes) | *Initial only | Using distilled water at critical graduations. |

# Appendix 4

## Method Validation

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

The diagram on the following page (Figure 1) provides a much generalised approach to method validation that IANZ adopts when assessing the in-house validation of chemical and/or biological testing methods by individual laboratories and is considered to be consistent with the "fitness for purpose" principles embodied in Clause 5.4.5 of NZS ISO/IEC 17025:2005. 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.

Microbiological Method Validation
Most well known texts (particularly in bacteriology) offer an array of internationally recognised standard test methods which can be employed in the analyses of particular sample types and in most cases these will be appropriate. Laboratory staff must be aware, however, the method will not always be appropriate for all of their sample types.

Inhibitory foodstuffs such as spices are examples of samples where the recovery of bacteria using standard test procedures may well be prevented. Other samples may contain chemical preservatives or have pH characteristics that prohibit the growth of the organisms being analysed for, especially at low dilutions.

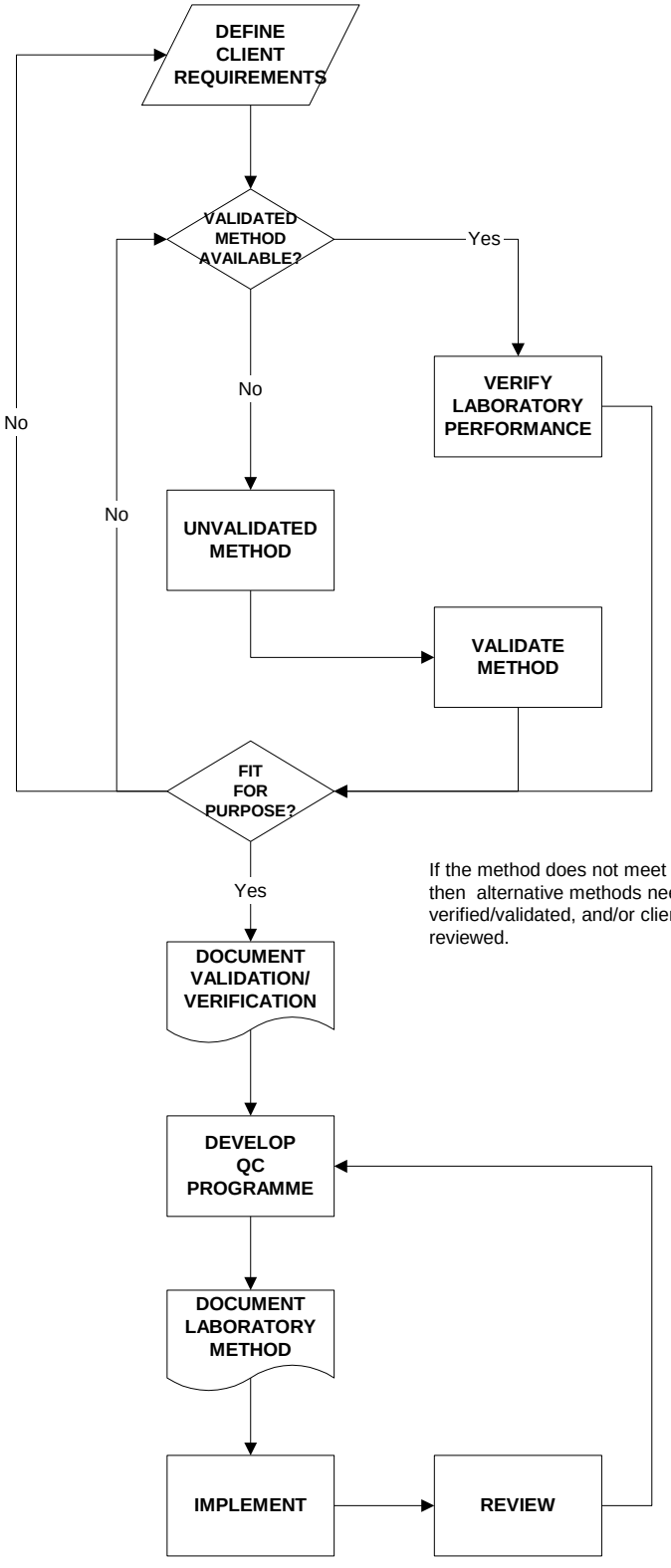
Methods may need modification to include neutralisers or dilution techniques, etc. to remove or reduce the inhibitory influences and allow the recovery of stressed cells. Method validation procedures are therefore essential in these circumstances to ensure that the procedure selected for a particular analysis of a particular kind of product is appropriate.

Modified methods should be compared against the original method if the same specifications are required to be met. This is normally achieved by analysing a sample before and after spiking with a known low number of the appropriate target organism(s), and then checking recovery. Should the expected sample types be likely to contain stressed organisms, then the use of a stressed control organism in the recovery checks should be considered. The resuscitation procedures for recovering these stressed cells may also need to be considered.

For sample analysis involving membrane filtration techniques, comparative data can be obtained using most probable number (MPN) techniques. For example, APHA "Standard Methods for the Examination of Water and Wastewater" states that for new water sources, it could be expected that 80 % of the membrane filter test results would be within the 95 % confidence limits of the multiple tube test results.

Validation of test methods should be performed under the same conditions as those of a real assay, by using a combination of naturally contaminated products and spiked products. All validation data must be recorded and stored for at least as long as the method is in force and as long as necessary to ensure adequate traceability of raw data and results. Proficiency testing or a collaborative trial can be used to check the validity of methods, but this may not always be feasible. Analysis of samples by the proposed new method and any existing methods for the same determination is also beneficial.

**Figure 1: General processes for method validation in chemical and biological testing laboratories**




**Client requirements** need to be defined and should include but not be limited to:
- Why is testing being done?
- Is there a specification limit?
- What accuracy is required?
- What detection limit/precision is required?
- Turnaround time?
- Cost (including development)?

Source a **validated method** from:
- International standards
- National standards
- Other validated methods
  e.g. ASTM, AOAC, AOCS, APHA, etc

**Verify laboratory performance** through:
- proficiency testing
- reference materials
- detection limit determination
- repeatability determination
- reproducability determination
- consumables verified

**Unvalidated methods** may be available from;
- journals
- customers
- in house

All methods need **validation**, for example by:
- proficiency testing
- reference materials
- linearity confirmation
- specificity confirmation
- robustness assessment
- matrix effects/spiking
- detection limit determination
- repeatability/reproducability determination
- consumables verified

If the method does not meet client requirements then alternative methods need to be sourced and verified/validated, and/or client requirements reviewed.

Develop **routine quality control programme**: e.g
- duplicates
- spikes
- reference materials
- proficiency testing

Following implementation a review programme should be instigated.

# Appendix 5

## Uncertainty of Measurement

Section 9.1 sets out the IANZ policy for accredited biological testing laboratories to make estimates of the uncertainty of measurements in their test results.

The following approach to estimating uncertainty of measurement is one that IANZ would suggest as being consistent with current published approaches in the international literature. It is not a mandatory specification and other approaches will be considered as equally valid provided they are sourced from published guidelines and meet the underlying principles of this process.

(a) For each of the methods in the scope of accreditation providing numerical results, the laboratory should identify all components of the testing process which will contribute to the uncertainty in the final result. At this stage it should not be necessary to quantify each component but rather just identify that it exists. Possible approaches to doing this exercise are:

(i) By critically evaluating each step in the documented method to identify those actions/equipment etc. (i.e. components) that may affect the result.

(ii) Using the method equation and critically evaluating each variable to identify the components that will affect its value.

The use of fish-bone diagrams and the like may be a useful tool in this regard.

(b) Identify and gather or collate all available data relating to the performance of the method. The sources of such data may be external to the laboratory or data generated internally i.e.:

(i) External data such as:

- Published validation data for the standard method (which may be published in the method itself or as a separate publication), including MPN tables
- Results from formal proficiency testing or inter-laboratory comparison programmes e.g. reproducibility (R) figures

(ii) Internal data such as

- In-house validation studies
- Precision or repeatability (r) data from duplicates
- Uncertainty of measurement values from calibration certificates
- Variability in spike recovery data.

Also possible but less likely in a microbiology laboratory:

- Standard reference material results
- In-house reference material results

(c) Conduct a gap analysis to assess which of the components identified in (a) are incorporated in the data collected in (b). Care needs to be taken in this exercise. It is important to have a clear understanding of how the data collected in (b) are generated and what they mean. The following are a few examples which illustrate the type of issues that need to be considered:

(i) Data from true duplicate sample testing will include components associated with taking the test portion from the submitted test sample (normally the taking of the test sample from the bulk is outside the control of the testing laboratory and thus the uncertainty component associated with sampling would not be considered) but will not normally include components of uncertainty associated with different equipment, different operators, different batches of media and reagents, etc. The precision data from duplicates would in itself give an under-estimation of the overall uncertainty.

However, if as many of the testing variables (the components identified in (a) above) were to be varied in the analysis of each duplicate of each sample (i.e. different analysts; different batches of diluents, media, etc; different pipettors, incubators, etc.), then this data (another form of intermediate precision) will provide a more realistic assessment of the measurement

uncertainty. For many biological testing laboratories where the sample is not stable, this approach may be the only realistic one to estimating measurement uncertainty.

Precision data from true duplicates gathered over a long period of time in which each of the components were varied, may provide (following appropriate statistical analysis – details of which are outside the scope of this guidance) a possible estimate of uncertainty.

(ii) Data from duplicate plating alone will not provide an adequate estimation of measurement uncertainty as this is generally done only on the last dilution and thus is only a measure of an individual's ability to repeatably plate and count. It will not include the majority of other major components of uncertainty (e.g. sub-sampling, initial dilution, diluents, dilution equipment, enumeration media performance, etc, etc.)

(iii) Intermediate precision from reference materials analysed repeatedly over time would include the components associated with different operators, different time, different equipment (if relevant), different media and reagents, etc. However by their very nature, reference materials are homogenous and stable and thus this intermediate precision data would not include uncertainty components associated with sub-sampling test portions from real test samples.

(iv) Reproducibility (R) data would generally give an over-estimation of an individual laboratory's uncertainty of measurement as it includes many different operators, types of equipment, batches of media and often different methods and some of these components are not relevant to a particular laboratory's circumstance. Balancing this is the possibility that reproducibility may actually be an under-estimate as such data is normally generated from homogenous and stable samples, which may not reflect actual practices in working laboratories.

(v) Spike recovery data needs to be carefully considered. The actual recovery itself is not a component of measurement uncertainty as it can be corrected for. However, variability in recoveries achieved is. Over time, this data will incorporate much of the measurement uncertainty components. Spiking may also be required should the intermediate precision approach outlined in a) be considered in order to obtain statistically significant counts.

(d) Where there are components identified in (a) which are not incorporated into the data collated in (b), these need to be independently estimated and their significance assessed.

If they are significant, laboratories will need to review and redesign their quality control data collection programmes in order to incorporate as many of these additional components of uncertainty as possible. Components of uncertainty which cannot be incorporated into the quality control data generated can be estimated by separate experiment, from published data, from calibration certificates, certificates of analysis or by professional judgement.

Statistical methods for the combination of components of uncertainty of measurement are outside the scope of this guidance document and readers should consult the referenced texts for further information. However, as precision data for microbiological assays are calculated from log and anti-log transformations, the combination of this data with other uncertainty components is mathematically very complex and is usually not necessary where intermediate precision experiments have been well designed.

The examples in (c) above suggest laboratories should be able to obtain data to sufficiently cover all significant identified components of uncertainty, but these may come from different sources. It is important to ensure all major components of uncertainty are not double accounted.

## Discussion

In the vast majority of tests of a biological nature, the methodology used is of an empirical nature (where the result is dependent on the method used). Therefore, if the method is followed, method bias does not contribute to the measurement uncertainty. The empirical nature of the methods arises because the measurand cannot be realised in its pure form and thus like traceability of measurement (see Section 5)

the uncertainty associated with an actual measurement cannot also be realised. The best estimate of the uncertainty of a measured result will therefore come from the uncertainty associated with the performance of the method used.

The methodology suggested above for estimating measurement uncertainty will generally provide appropriate consideration of these issues, and result in a reasonable estimate, provided the data are generated from samples of the same or similar matrix. Laboratories are reminded that results from plate count tests have a skewed distribution and require log transformation to approximate normal distribution statistics. Log standard deviation/confidence limits should then be calculated before anti-logging each limit independently.

In quantitative biological testing, it is ideal if the uncertainty estimation is evaluated at selected levels across the range of application of the method. However, often a test is conducted to assess compliance with a particular specification, regulatory limit or the like. In these instances, laboratories should at least estimate an uncertainty value attributable to measurement results close to the specification limit i.e. to use the specification limit as the value at which the uncertainty is estimated.

Except in the case of test results obtained from MPN tables where the significant components of uncertainty are already built into the MPN table's values, the "number of significant figures" approach and that of Note 2 in Clause 5.4.6.2 of NZS ISO/IEC 17025:2005 should not be used as a substitute for evaluating measurement uncertainty in biological testing. For MPN results, IANZ will accept laboratories using the values from the 95% confidence column of the tables as a reasonable estimate of uncertainty of these results, provided laboratory estimates of precision (i.e. duplicate assays) fall within these values.

# Appendix 6

## Autoclaves

The basic requirement for sterilisation in an autoclave is that the contents whether liquid or solid, be exposed to saturated steam at the required temperature and for the predetermined length of time. Pressure serves as the mechanism for attaining steam temperatures above 100°C, but plays no part itself in the sterilisation process.

Sterilisation failure can occur, for example, where steam-air mixtures are present (i.e. steam saturation is not achieved). If air is not completely removed from the sterilising chamber or its contents, the residual air will contribute to the pressure indicated on the gauge, but the temperature will be lower than that expected at the pressure shown.

Pressure measurements alone, therefore, cannot guarantee that the appropriate temperature has been attained through the sterilisation cycle. Measurement of temperature is therefore essential for each autoclave cycle to ensure that the unit has been correctly vented.

**Autoclaves, therefore, need to incorporate a temperature recording device.** This device may be a fixed or flexible probe and may be sited either in the chamber or the drain.

Temperature controllers, temperature recording charts and thermocouples need to be calibrated initially and every 6 months using a reference thermometer or thermocouple which in turn an accredited calibration laboratory has calibrated. Such secondary temperature calibration can be performed by the laboratory itself or by an agency accredited to perform such calibrations.

Chart recorded times and timers also need checking for accuracy. Many media contain ingredients or carbohydrates that are adversely affected by exposure to heat over time.

Pressure gauges need not be traceably calibrated but ideally should read true with respect to the required pressure at the nominated calibration temperature. Temperature calibration results will reveal deficiencies in pressure gauge readings. Biological and chemical indicators can be used to monitor the sterilisation process but they cannot give the same level of assurance as above and therefore cannot alone be relied upon.

Maximum temperature registering thermometers are also designed to be indicators of temperatures achieved, but not, however, of the temperature profile for any particular sterilisation cycle. Laboratories are strongly discouraged from relying on these thermometers as they cannot be calibrated under conditions of use, so there is not assurance of the accuracy of the temperature indicated. In addition, they can be easily broken causing contamination of the autoclave with mercury, with subsequent health dangers.

Domestic pressure cookers fitted with only a pressure gauge are not regarded as being suitable for sterilisation of media or decontamination of wastes because of the difficulty in adjusting and maintaining the sterilisation temperature.

### Validations

Validation of autoclaves enables laboratories to demonstrate acceptable and consistent temperature of sterilisation. Heating profiles of typical loads need to be studied in relation to chamber temperatures. Placement of a thermocouple at the centre of loads and inside large volumes of liquid allows time lags to be determined when monitored with respect to the chamber temperature. Instructions for the operation of the autoclave under various load conditions can then be compiled.

The main thrust of the need to validate autoclaves is to ensure that microbiological media are not being "over-cooked" in the autoclaves. In particular, those temperatures do not exceed 121$^{o}$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 “over-cooking”.

Please note that IANZ accredited Medical Testing Laboratories have less stringent requirements as they are concerned with killing microbes rather than over sterilizing media.

Putting the specific requirements of the British Standard (BS 2646:1993) aside for the moment, the following is essentially the validation data that are required:

### (a) Empty Cycles

A temperature profile should be conducted on an empty chamber and not during a routine sterilisation run. This is to check that 121$^{o}$C is not exceeded, and that the temperature is uniform throughout the load space. The latter may not be met if not all air is expelled and replaced with saturated steam. While useful, this exercise is not as important as the following.

### (b) Media Cycles

The laboratory needs to determine what its common or standard load compositions are, i.e. liquid diluents in tubes/bottles, broths, agars, equipment, etc., or a combination of any of the above.

Temperature profiles *for each load type* need to be conducted to determine the temperature profile *within the media container* relative to the profile within the chamber or more particularly, at the temperature monitoring probe in the chamber/drain (see point (d) below).

As a rule of thumb, mixed volume loads should be avoided, as it is impractical to properly control their heating cycle in a consistent manner.

The most important information gained from this data is:

(i) Heat-up times for the actual media. As a rule of thumb these should not exceed 30 minutes to reach 121$^{o}$C. Obviously 500 ml bottles of agar will take a lot longer than 9 ml diluent tubes for example.

(ii) The lag time between the chamber reaching 121°C and the media reaching 121°C. Again, this would be expected to be longer for say 500ml agar than for 9ml diluents.

This lag time may be added onto the sterilisation time to ensure that the media is at say 121$^{o}$C for 15 minutes. However, it is acknowledged that laboratories may choose not to add this on if they have data to demonstrate media sterility is not a problem, and to prevent the risk of "over-cooking".

Once the data from (i) and (ii) are obtained, a decision needs to be made on any action to be taken, if necessary, i.e. reduce load sizes, or alter load compositions to ensure heat-up times and lag times are kept to a minimum.

Where significant heat-up time differences within loads of different bottle sizes are found, laboratories would be expected to adjust load contents to include only bottles with comparable times, e.g. if 500 ml bottles of agar are mixed with 9 ml diluent tubes, then if you time the load for 500 ml bottles, the tubes may be heated for too long.

It is considered appropriate that load validations are conducted on the actual typical loads used by the laboratory rather than any arbitrary loading specified in the British Standard. This more realistically reflects what the autoclave is being used for. Nevertheless, if an autoclave complies in all respects to the specific requirements of the British Standard this would be accepted.

Where autoclaves are fitted with mobile "wander" probes which can be inserted into a particular bottle of media to monitor temperature of a typical load (rather than the chamber or drain temperature), then "lag times" has a different meaning altogether, i.e. it would be the difference in time between the slowest and fastest bottle to reach 121$^{o}$C. Validation processes need to demonstrate that this is not excessive and define where in the load this bottle should be.

### (c) Destruction (or Kill) Cycles

Again, validations need to be done to establish temperature profiles within autoclave bags, pipette canisters and the like. The emphasis is not so much on "over-cooking" but rather ensuring that sterilisation temperatures are being reached for the appropriate time, e.g. 121$^{o}$C for 30 minutes.

Any lag times between the contents and the chamber are expected to be added on to the cycle. Thus, for example, if a load of autoclave bags containing petri dishes for destruction takes an extra 25 minutes to come up to 121$^{o}$C relative to the chamber, then 25 minutes is added to the cycle. For the plates to be at 121$^{o}$C for 30 minutes, the chamber needs to be at 121$^{o}$C for 55 minutes, and the probe in the chamber needs to demonstrate this.

### (d) Temperature Monitoring

Once the cycle parameters for each load type are established the laboratory needs to demonstrate these are maintained each time a load is put through. This necessitates a monitoring probe within the chamber, requiring calibration at 121$^{o}$C. A recording device should preferably be attached.

# Appendix 7

## Control of Reference Organisms

Cultures of micro-organisms with defined characteristics are required for most microbiological tests performed by IANZ-accredited laboratories. For example, reference or control organisms are used in a wide range of determinations; test organisms are needed for micro-bioassays and biocide effectiveness tests; and organisms with known properties may be used in proficiency testing. In order to enhance the traceability requirements in microbiological determinations (Section 5) and to obtain valid results, these organisms need to be of high quality. A well maintained culture collection is an essential element of good laboratory practice.

### (a) Source of Reference Organisms

Authenticated organisms are normally obtainable in New Zealand from *The New Zealand Reference Culture Collection* (ESR Kenepuru Science Centre) in a freeze-dried form with instructions for reconstitution.

### (b) Verification of Reference Organisms

Reference organisms 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 and the capability of the individual 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 has the capability to conduct such checks.

### (c) Maintenance Guidelines

Micro-organisms 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.

A wide variety of techniques have been used for the preservation of micro-organisms. The objective of preservation methods is to maintain the viability and genetic stability of the culture by reducing the organism's metabolic rate, thereby extending the period between sub-cultures. Most preservation methods achieve a reduction in metabolic rate by withholding nutrients, water and oxygen, by reducing the storage temperature or by a combination of these.

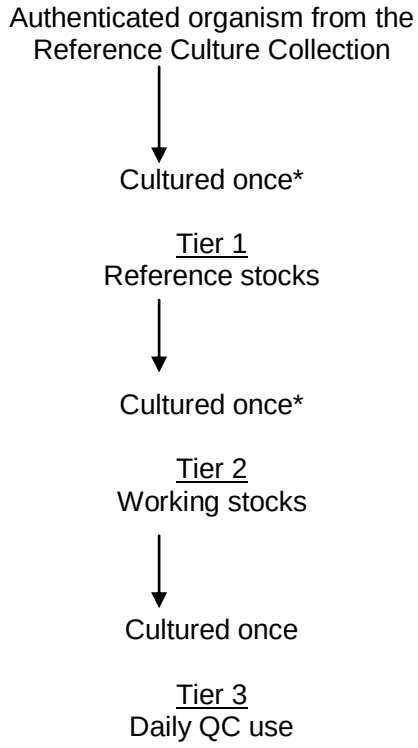
There is no universal method of preservation that is successful for all micro-organisms. Taxonomic groups of micro-organisms respond differently to different preservation methods. The preservation methods used reflect the different biological properties of the various groups of micro-organisms such as the bacteria, viruses, fungi, yeasts, algae and protozoa. In general, the most successful methods in terms of longevity and genetic stability employ freezing or desiccation.

Where a laboratory has the facilities, cryogenic or lyophilisation procedures can be utilised.

Various microbiological method texts (18 & 19) provide recommendations for the maintenance of a reference culture collection. The following guidelines are not intended to supersede these but to provide guidance to laboratories on the general principles involved. They are generally applicable to most organisms in common use, but there are exceptions e.g. Clostridia, which are required to be maintained by alternative processes.

Reference organisms from a recognised culture collection are used to provide reference stocks (Tier 1). Tier 2 is obtained by sub-culturing from Tier 1, and Tier 3 is obtained by sub-culturing from Tier 2. At no time should Tier 2 or Tier 3 be used to re-establish Tier 1 (see diagram below).

The underlying principle is that at no time should any culture used in the laboratory be more than three sub-cultures from the reference organism sourced from a recognised culture collection.




** Purity checks and biochemical tests as appropriate*

In the majority of laboratories, one or other of the following two techniques is used:

(i) 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 an 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 pipette. 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.

(ii) 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.

Generally, selective media containing carbohydrates, etc. should not be used in the maintenance of control organisms. Storage of reference organisms must be appropriately segregated from test samples.

### (d) General

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

(i) Details of the organisms held in the laboratory, their source and identification, and the purposes for which they are used
(ii) Procedures for the verification of identity and purity of each organism
(iii) Details on the maintenance programme 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 8

# Media, Standards and Reagent Quality Control

## 1 Standards and Reagent Quality Control

Details of the preparation of all types of standard and/or reagent consumables must be recorded e.g. in a logbook, and must include results of standardisation, verification, etc. together with the date of preparation and the identity of the person who prepared them. Each container of prepared standard and/or reagent solution must be labelled as appropriate, with the date of preparation, the factor or concentration, the name or initials of the person who prepared the solution and an expiry date or be traceable to readily available preparation records containing this information. Each batch of commercially prepared consumables must be verified before use and records must be kept of these checks e.g. pH buffers, standardised solutions, biochemical test kits, rapid test kits, etc.

## 2 Purchasing and Preparing Microbiological Media

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

### 2.1 Media Prepared In-house (generally from dehydrated stocks)

#### 2.1.1 Purchasing

Laboratories must purchase media, which has the formulation specified by the test method. Details of these specifications, the supplier(s), expected appearance, expected pH, and methods of preparation need to be documented in the laboratory's manuals.

Records documenting batch number, date received, date opened, and results of visual inspection need to be kept for both dehydrated media and raw materials (as appropriate). The date received and the date opened may also be written on the containers. The date approved for use can be added when evaluation of performance has been completed.

When purchasing, the manufacturers' recommended shelf-life (expiry dates) as well as storage conditions required for individual items need to be considered. Annual turnover of stock is advisable and ordering of appropriate sized containers may assist overall product preservation.

#### 2.1.2 Storage (dehydrated)

It is important that culture media in dehydrated form are prevented from taking up additional moisture from their environment during storage. The higher the moisture content the greater the possibility of degradation of the various constituents of the medium.

Dehydrated media needs to be stored in a cool, dark, minimal humidity environment e.g. not near autoclaves. Prolonged opening of a container is best avoided and careful replacement of the closure will ensure maximum possible life. Dehydrated media that are caked or cracked or show colour change should not be used.

Given the right conditions, most dehydrated media will remain in good condition for several years. However, a few products that contain ingredients of high sensitivity are less stable, especially if the moisture level is allowed to rise. Dehydrated media are generally labelled with expiry dates and should not be kept or used beyond these dates.

#### 2.1.3 Supplements and additives

Supplements and additives (where used) need to be stored appropriately, e.g. under refrigeration where this is required. Light sensitive chemicals need to be stored in the dark.

### 2.1.4 Reagent water

For the preparation of media, distilled water, deionised water or water processed by reverse osmosis is generally suitable. Regular assessment of the water quality for specific chemical parameters and biological evaluation is needed and procedures are described in Appendix 9. Only water that has been tested and found to be free from bactericidal or inhibitory compounds is to be used for preparation of culture media, reagents and diluents.

### 2.1.5 Glassware

Glassware washing procedures need to ensure there are no toxic residues left from detergents, disinfectants, reagents etc. Recommendations for the evaluation for inhibitory residues are also given in Appendix 9.

### 2.1.6 Preparation

Records must be kept of all aspects of each batch of medium prepared. A batch sheet needs to include the following information:

(a) Date
(b) Medium name, manufacturers batch number, quantity used and volume prepared
(c) Laboratory batch number
(d) Operators signature
(e) Sterilisation time and temperature, and any control results
(f) Post-sterilisation pH. Laboratories should also check and record the pre-sterilisation pH along with any pH adjustments made to ensure the post-sterilisation pH conforms to specifications
(g) General comments (appearance, sterility, volume, etc).

These batch sheets could also contain the quality performance test results using reference organisms, where these checks are conducted by the laboratory prior to releasing the media for use.

When pouring plates, the correct temperature needs to be employed since an incorrect agar temperature will result in alterations to the final water content of the medium through excessive evaporation and media shrinkage. For pour plate methodologies, excessive temperatures will also result in thermal shock to the sample under test.

Where additives or supplements are required to be added after sterilisation, these need to be added at the correct media temperature to avoid any degradation of the additive.

Where a medium is prepared from basic ingredients, the batch number of each ingredient should be noted, so that when a new batch of any ingredient is used, the new complete medium can be evaluated for this difference.

### 2.1.7 Sterilisation

Records must be kept of all sterilisation loads. Required times and temperature of sterilisation will depend on the volumes of media dispensed, the types of media, and the performance of the autoclave being used (see Appendix 6). Appropriate chemical or biological indicators can be used to monitor autoclaving efficiency in addition to (but not as a substitute for) temperature monitoring.

### 2.1.8 Volumes

When the volumes of the prepared media are critical, e.g. diluents, checks of volume after sterilisation need to be made and recorded.

Generally, the accepted limits are within ± 2% of the target volume.

### 2.1.9 Appearance

Any pertinent comments relating to the appearance of the media during preparation should be recorded.

### 2.1.10 Labelling

All prepared media needs to be labelled with the date of preparation, date of expiry and the media code. This provides traceability to the preparation records.

### 2.1.11 Storage

Many test methodologies specify the acceptable lifetime of prepared media, and laboratories will be required to adhere to these expiry periods. In the absence of these, prepared media shelf life guidelines are detailed below, with a note that these are guidelines only as specific media may have a much shorter shelf life. The formulation and packaging of the media however, will decide the media's basic susceptibility to deterioration during storage. The presence of an antibiotic of only moderate stability will severely limit the useful life of media. The preservation of an agar media in a Petri dish will drastically shorten the potential storage period compared with the same media stored in an effectively sealed glass bottle.

The optimum storage temperature for the majority of prepared media is about 4°C. The useful life of media will shorten as the storage temperature rises above the optimum.

Most liquid media will keep for several months at 4°C, but some have a tendency to form deposits, especially those made up at double strength. Media containing dye may fade, especially if exposed to light.

Solid media will keep for several months if stored in an airtight container. Agar gel is normally very stable, but in media where the pH is low (below 5.0), softening of the gel may take place during sterilisation, subsequent storage, or re-melting.

Storage of agar plates presents two main problems - contamination and dehydration. The length of time that plates can be kept before use will depend on the ability to avoid contamination and minimise loss of moisture. Moisture loss may be minimised by wrapping plates in plastic bags during storage at 4°C. It is important that plates of culture media not be exposed to sunlight as this may affect the performance due to the formation of peroxides.

**Recommended Holding Times for Prepared Media *(Reference 16)***

| **Medium** | **Holding Time** |
|---|---|
| Agar or broth in loose-cap tubes at 4°C | 1 week |
| Agar or broth in tightly closed screw-cap tubes at 4°C | 3 months |
| Poured agar plates with loose-fitting covers in sealed plastic bags at 4°C | 2 weeks |

Laboratory validated expiry dates may be appropriate if the validation has been performed in accordance with recognised published methodology, however, the expiry dates stated in reference methods will always take precedence.

## 2.2 Pre-prepared Purchased Media

Many laboratories now choose to purchase their microbiological media in a ready-to-use form from media suppliers. Laboratories will still be required to demonstrate that the requirements outlined in 2.1 above are being met. In particular, laboratories which purchase pre-prepared media will need to implement the following:

### 2.2.1 Purchasing

Laboratories must purchase media which has the formulation specified by the test method. Details of these specifications, the supplier(s), expected appearance, and expected pH need to be documented in the laboratory's manuals.

A record or log of each batch of pre-prepared media receipted into the laboratory needs to be maintained, detailing:

(a) Media type and amount
(b) Manufacturer's batch number (of the prepared batch)
(c) Date received
(d) Condition/appearance on receipt (such as packaging integrity, labelling, medium appearance, contamination, leakage, etc.)
(e) Manufacture date (see below under Storage and Expiry Dates)
(f) The laboratories own expiry date (based on the manufacture date)
(g) Availability of performance evaluation certificates (see 3.2 below)
(h) An acceptance/rejection decision from an authorised laboratory staff member.

### 2.2.2 Storage and Expiry Dates

As discussed above, many test methodologies specify the acceptable lifetimes of a prepared media and laboratories will be required to adhere to these expiry periods. Often pre-prepared media suppliers allocate expiry dates to the batches of media supplied. Where these differ from particular method specifications, the method requirements take precedence. Any specified expiry periods apply from the date of manufacture and laboratories need to ensure this information is provided from the supplier (see 2.2.1 Purchasing above). Laboratories then allocate their own expiry date (from the date of manufacture) in accordance with their own test specification.

Many test method specifications place reasonably restrictive expiry periods on certain media, so laboratories should detail manufacturing date requirements in their purchasing specification to ensure adequate time is allowed for transportation and use before the expiry date.

Where there is no specified expiry period, laboratories should adopt the time and temperature requirements recommended in the table above.

Laboratories could opt to use the expiry dates assigned by the media manufacturer where the media manufacturer's testing laboratory is accredited for shelf life testing, and this must be able to be appropriately demonstrated from the laboratory's scope of accredited testing.

## 3 Evaluation of Media Performance

The ability of media to support the growth of the target organism or to selectively isolate the target organism in the presence of other fauna in an appropriate manner is one of the key method assumptions made in the validation of a microbiological method. In terms of result traceability and contribution to the measurement uncertainty, it is critical that media performance are tightly controlled and monitored.

To ascertain what media validation is required the laboratory should look first to their predominant reference methodology i.e. ISO, FDA, or MIMM etc. Where these are not clear in their requirements, the laboratory should use the guidelines below.

### 3.1 Media Prepared In-house

Where a medium is prepared from basic ingredients, the batch number of each ingredient should be noted, so that whenever a new batch of any ingredient is used, the completed medium can be evaluated for this difference.

Each batch of dehydrated media purchased needs to be evaluated prior to use. It is usual that each batch of media prepared (from the dehydrated batch) is checked for sterility by incubating a "blank", and checked using a positive control organism (as a method control) each time the test is performed. The use of a negative control organism as well is recommended and in many cases, it is a requirement of the reference method.

Prior to this routine use of dehydrated media, laboratories generally need to evaluate the performance of the manufacturer's batch of the dehydrated media as related to the use of media i.e. if used selectively and

not for enumeration then qualitative evaluation may be all that is needed. The table below provides guidance if the requirements are not already stated in the reference methodology.

**Recommended Validation Guidelines for Media Evaluation**

| Media Type: / Media Use: | **Selective Media** | **Non–selective Media** |
|---|---|---|
| Enumeration and/or Enrichment | Quantitative evaluation with positive control organisms | Quantitative evaluation with positive control organisms |
| Non enumerative eg. confirmation, presence / absence | Qualitative evaluation with positive and negative control organisms | Qualitative evaluation with positive control organisms |

Laboratories should establish whether the control organisms used for media evaluation need to be actively growing cultures or in a stationary phase and ensure this is included in the media evaluation procedures. The requirements may be dependent on the media being evaluated.

### 3.1.1 Qualitative Evaluation

For all media, the laboratory should check in a qualitative manner the ability of the media to support the growth of the target organism(s) in a typical manner, using a reference culture of the target organism(s) i.e. the positive media control. If quantitative evaluation has been carried out, this may already have been covered.

For non-enumerative media (selective), the laboratory should check the ability of the media to inhibit the growth of typical non-target organism(s) using suitable reference organism(s) i.e. the media negative control(s).

The dilution of the target organism should be such that 25-250 CFU/plate is achieved and for the non-target organism such that 250-2500 CFU/plate is achieved. The target organisms need to grow in the presence of many more non-target organisms, hence the ten-fold difference.

Laboratories need to ensure that plating / streaking techniques allow for isolation of individual colonies. This is important to allow for the above checks to assess the suitability of the growth, colony morphology, biochemical reactions, etc. under the defined incubation time and temperature conditions in which the media are to be used.

### 3.1.2 Quantitative Evaluation

For media used for enumerative and/or enrichment purposes, the laboratory needs to evaluate in a quantitative manner the ability of the batch of media to support, recover, or promote the growth of the target organism(s).

The following procedures are recommended for the assessment of different media types:

(a) Evaluation of Broths
To evaluate a broth's ability to support the growth of low numbers of the appropriate organisms, 1 ml of a suspension known to contain a count of 10-20 cfu/ml of the relevant positive reference culture is added to at least 10 tubes of the broth. Incubation is according to normal requirements.

The demonstration of growth in at least 90 % of the broth tubes confirms the acceptability of the batch. Counts (by inoculation of 1 ml on an appropriate non-selective agar) at each dilution should be determined and recorded to confirm the target inoculum is correct.

(b) Evaluation of Solid Media

Either:

(i) Comparison of selective media (used for enumeration) against non-selective media.

Suspensions of the relevant positive reference organism are prepared and successive dilutions performed until a dilution with an appropriate low number of organisms is obtained i.e.

- Where the selective medium to be evaluated is normally used as a pour plate, 1 ml of a culture suspension of approximately 100 organisms/ml is prepared which is inoculated into the pour plate. At the same time, pour plates of a non-selective agar are inoculated. At least five sets of plates for each medium are prepared.
- Where a medium to be evaluated is normally used as a streak or spread plate, 0.1 ml of a culture suspension of approximately 1000 organisms/ml is spread on the agar surface of the plate. At the same time, plates of a non-selective agar are inoculated. At least five sets of plates for each medium are prepared.

In both instances, both sets of plates are incubated in accordance with the requirements of the selective medium. Counts at all dilutions tested should be recorded.

As most selective media will not demonstrate 100% recovery when compared with the above non-selective media, acceptable minimum recovery limits need to be established. Laboratories need to establish acceptable recoveries based on the data obtained.

And/or:

(ii) Comparison of a new batch of media against an approved batch of the same media.

Suspensions of the relevant positive reference organism are prepared and inoculated (as above) into both the new batch of media to be evaluated and a batch of the same media previously found to exhibit acceptable performance.

After normal incubation, the two batches of media are compared for bacterial colony size and appearance. If colonies on the new batch of media appear atypical or smaller than the colonies on the acceptable batch of media, inhibition is occurring. The colonies on each set of plates are counted. Counts at all dilutions tested should be recorded.

Colony counts on the new batch of medium should not be less than ~90% of the counts obtained on the approved batch of medium to be acceptable.

Where this methodology is used, laboratories should calculate the cumulative recovery ratio over successive batches so that any downward drift in media performance can be monitored.

And/or:

(iii) Comparison of a new batch of media against an approved batch of media during product testing.

The use of pure cultures may not be satisfactory to test a medium for suitability. For example, variations in the concentration of a selective ingredient might be detected only by comparing recoveries in genuine samples.

At least 5 separate product samples need to be tested in duplicate using both the new batch of media and the approved batch of media.

After incubation in accordance with normal procedures, the five sets of plates for each media batch are counted and the bacterial population per g or ml for each sample is calculated and

compared. As the counts may vary markedly between samples, the results should be log transformed and the five sets of duplicate results compared. The recovery of the batch under evaluation should not be less than ~90% of the recovery of the reference batch.

If the results using the new lot of media are significantly greater than those of the accepted batch of media, the new batch is more stimulatory.

If the results are significantly less, the new lot of medium is more inhibitory.

Bacterial colony size and appearance differences between the two batches of media also need to be considered.

(c) Evaluation of Diluents

When the volumes are critical, check of volumes after sterilisation need to be made and recorded. Diluents also need to be checked for inhibitory effects. This should be carried out in accordance with recognised methods.

### 3.2 Pre-Prepared Purchased Media

As with media prepared in-house, laboratories need to ensure the pre-prepared media they purchase are also subject to the evaluation requirements detailed in 3.1 above, namely:

(a) Selective media undergo a qualitative evaluation, and/or
(b) Media used for enumerative and/or enrichment purposes undergo quantitative evaluation.

An important distinction with pre-prepared media is that the required evaluation will normally be on each manufacturer's batch of the prepared media, rather than the batch of dehydrated media from which it is prepared. This is because the manufacturer's preparation processes are not generally subject to the level of control and assessment as those in an accredited laboratory that makes its own media i.e. the requirements detailed Section 2.1 above and in Appendices 6 and 9 for example.

The majority of prepared media suppliers will provide test certificates with the results of these evaluations for each batch supplied. This is accepted with the following comments:

(a) The test certificate must be from a laboratory accredited to perform media evaluation i.e. an IANZ-endorsed test certificate
(b) In their purchasing specification, laboratories should specify what level of evaluation they require of the media being purchased e.g. qualitative evaluation, quantitative evaluation, or both; and ensure an appropriate certificate is provided for each batch (see Purchasing under 2.2.1 above)
(c) A laboratory accredited for the evaluation of media is accredited only for the conduct and reporting of the evaluation testing, and not for making judgements on the quality of the media. The purchasing laboratories need to evaluate the results on the test certificate for themselves and decide whether the particular batch meets or otherwise their own internal specifications (see Purchasing under 2.2.1 above). This includes not only any evaluation results but also other parameters that may be reported such as pH, volumes, and the like.

# Appendix 9

## Glassware and Reagent Grade Water Evaluation

### 1 Detergent Residues

Modern detergents are very effective for cleaning laboratory glassware. Some however, are highly bactericidal and care needs to be taken during rinsing procedures to ensure that all traces of the detergents are removed.

Testing for detergent residues on glassware needs to be performed at least annually or when there is a change in washing procedures or a change in detergent. The appropriate reference test should be followed e.g. References 16, 17.

A possible alternative is as follows:

(a) Wash and rinse six glass petri dishes (or similar) according to usual laboratory practices and call these Group A
(b) Wash six more glass petri dishes as in (a) above, but rinse with 12 successive portions of distilled or deionised water and call these Group B
(c) Rinse six further glass petri dishes with detergent wash water (in use concentration), dry without further rinsing and call this Group C
(d) Sterilise the items comprising Groups A, B and C by usual procedures
(e) Add not more than 1mL of suspension of *E. coli* or *Enterobacter aerogenes* known to contain 50-150 organisms per ml to each of the dishes comprising Groups A, B and C
(f) Add an appropriate amount of non-selective agar to each of the dishes and incubate according to normal procedures
(g) Count the number of colonies in each group of dishes
(h) Difference in bacterial counts of less than 15% among all groups indicates the detergent has no toxic or inhibitory effect.

Differences in bacterial counts of 15% or more between Groups A and B demonstrate that inhibitory residues are left on glassware after the normal washing procedure.

Difference of less than 15% between Groups A and B and greater then 15% between Groups A and C indicate that the detergent used has inhibitory properties which are eliminated during routine washing.

### 2 Reagent - Grade Water Evaluation

Only water that has been treated to free it from traces of dissolved metals, bactericidal and inhibitory compounds is to be used to prepare culture media, reagents and dilution blanks. Day to day checks on the conductivity of purified reagent water are expected to be carried out, with the generally accepted limits being <2 μS/cm for microbiology laboratories (Reference 16). Checks on pH (5.5 – 7.5) and total aerobic plate count (<1000 cfu/ml) should also be conducted routinely.

Testing of reagent-grade water for inhibitory (or growth promoting) properties needs to be performed at least annually, or when there has been a change to or maintenance of the water purification system. More frequent evaluation may also be necessary where other quality control indicators suggest changes. The appropriate reference test should be followed e.g. References 16, 17.

Alternatively, the following procedure may be used:

(a) Preparation of dilution water for testing:
Add 1.25ml of stock phosphate buffer solution and 5ml of stock magnesium sulphate solution to reagent water and make up to 1 litre. Dispense appropriately and autoclave in accordance with normal procedures
    (i) Stock phosphate solution: 34g $KH_2PO_4$/500 ml. Adjust pH to 7.2 and make to 1 litre volume
    (ii) Stock magnesium sulphate solution: 50 g $MgSO_4.7H_2O$ in 1 litre.

(b) Preparation of bacterial suspension:
Prepare a suspension of *E. coli* or *Enterobacter aerogenes* so that cell densities of approximately 80 cells/ml are achieved when the suspension is added to the diluent prepared in a. above. Cell densities less than 30 cells/ml can result in inconsistent ratios; while above 100 cells/ml can result in decreased sensitivity to the nutrients in the diluent

(c) Assessment of dilution water toxicity:
(i) Plate out 1ml of the inoculated diluent immediately into a petri dish with non-selective agar. Perform in quintuplicate
(ii) Allow the inoculated diluent to stand at room temperature for 60 minutes and repeat the procedure detailed in (i) above
(iii) Incubate the two sets of plates in accordance with normal procedures
(iv) Count the colonies on the plates at each of the 0 minute and 60 minute inoculations, and calculate the % change in population as follows:

$$\frac{\text{count @ 60min - count @ 0min}}{\text{count @ 0min}} \times 100\%$$

To be considered acceptable, the % change in population should not exceed 15%.