Biological Testing ISO/IEC 17025 Application Document

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Table of Contents

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This document provides interpretative criteria and recommendations for the application of ISO/IEC 17025 in the field of Biological Testing for both applicant and accredited facilities.

Applicant and accredited facilities must also comply with the ISO/IEC 17025 Standard Application Document and any field annexes, policies and/or technical circulars (refer to *NATA Procedures for accreditation).*

The following annexes apply in the field of Biological Testing:

- Biological Testing Annex A: Accreditation of Facilities for Testing of Water Samples for Cryptosporidium and Giardia
- Biological Testing Annex B: Accreditation for on-site abattoir facilities and contract testing facilities approved by DAFF to test carcass hygiene samples and export meat samples
- Biological Testing Annex C: Accreditation of seed testing facilities
- Biological Testing Annex D: Accreditation of facilities testing for genetically modified organisms (GMO)
- Biological Testing Annex E: Accreditation of facilities for biological tests on water
- Biological Testing Annex F: Plant Health Diagnostic Testing
- Biological Testing Annex G: Media Preparation and Quality Control
- Biological Testing Annex H: Maintenance of Microbiological Reference Culture Collections (MRCC)

The clause numbers in this document follow those of ISO/IEC 17025 but since not all clauses require interpretation the numbering may not be consecutive.

5 Technical requirements

5.2 Personnel

5.2.1 Staff competence and technical control

Facilities must be under the control of an appropriately qualified and experienced staff member.

All facility staff must have education and experience appropriate to their level of responsibility and the complexity of testing they conduct.

Evidence of the qualifications and experience used in the approval of staff to hold positions of responsibility will be requested as part of the assessment process.

Where staff are expected to work in areas, or at times other than those in which they would normally work, (e.g. when relieving other staff or working on a weekend) a program of regular refresher training must be established and records retained. Staff who work only ‘out-of-hours’ must have regular contact with routine staff and in particular supervisory staff. As a guide, one day per month spent in the facility during normal working hours would be appropriate. The time allocated should, however, be sufficient for the staff member to update all skills required for the out-of-hours service. Records of the above must be available to the assessment team and be sufficiently detailed to demonstrate compliance.

Facility personnel may be asked in the course of an assessment to demonstrate or explain the principles or steps involved in test procedures for which accreditation is held or is being sought.

Any testing away from the base facility (such as in field or mobile testing facilities) must be under adequate technical control.

Staff with colour vision impairment may have difficulty performing some tests. Colour vision is, therefore, one of the issues that facility management must consider, when determining the suitability of staff to perform specific tests.

Staff authorised to release test results

1. Facilities must document a policy and procedure for the approval of staff to release test results for work covered by the scope of accreditation.
2. Staff releasing results must be approved on the basis of their demonstrated ability to evaluate the validity of test results. This may be demonstrated by a combination of academic qualifications and practical experience for the testing.
    - Academic qualifications must include:
        - a degree in a subject relevant to the testing concerned and a minimum of 2 years practical experience.
        - a diploma or certificate IV in a subject relevant to the testing concerned and a minimum of 5 years practical experience.
        - no tertiary qualifications and a minimum of 10 years practical experience.
    - Practical experience must include:
        - sound knowledge of the principles of the core competencies related to the testing for which approval has been authorised which must include participation in proficiency testing and or internal staff assurance programs
        - sound understanding of quality control data including:
            - results of method controls run in-conjunction with testing
            - results of quality control checks on consumables
        - awareness of the status of equipment checks and calibrations
        - understanding of the requirements for sample acceptance applied to samples under test
        - understanding of the principles and application of measurement uncertainty
        - understanding of the NATA requirements for the content and issue of test reports including the use of the NATA endorsement

Records of the staff approved to release test results and the information on which this approval was made must be maintained.

5.3 Accommodation and environmental conditions

Requirements for laboratory accommodation are determined by the nature of the work undertaken. Accommodation must be appropriate to the nature of the

testing undertaken and must not compromise the integrity of samples or the results generated.

Specific requirements for facilities carrying out molecular testing including analysis of genetically modified organisms (GMO) are detailed in Biological Testing Annex D: Accreditation of facilities testing for genetically modified organisms (GMO)

5.4 Test and calibration methods and method validation

5.4.1 General

Facilities must maintain an up-to-date table of all test methods (in use) covered by the scope of accreditation. A copy of this table will be used by NATA at the initial assessment, reassessment and surveillance visit to track the assessment of individual methods. The current copy will be provided to the facility before each reassessment and surveillance visit for revision as required.

Facilities may use international, national, industry sourced, peer reviewed validated and published methods and in-house or customer nominated methods. In all cases, the facility must ensure that each particular method is adequate for its intended purpose.

A standard method is defined as a method written by a body that has authority to write standards. Standard methods must be followed without variation for it to be referenced as a standard method on the scope of accreditation. The date of publication is not required, where standard methods are used, the current version must be used unless a legal or regulatory requirement requires the use of a superseded or withdrawn version.

Each batch of samples tested must include additional samples to which the target organism (positive control) and example(s) of non target organism(s) (negative control) have been added.

Documentation must refer to the method source and acknowledge any modifications and/or additions. Standard methods must be verified for their intended scope on introduction into the laboratory. Modifications to standard methods must be substantiated by technical justifications and verification or validation data as applicable. Refer to Technical Note 17.

Technical justification may include an independent study or article which supports the modification made.

Methods must be documented in a manner that provides clear instructions to an operator and does not allow for difference in interpretation of procedural steps.

Guidance to the documentation of methods is set out in AS 2929 *Test Methods – Guide to the format, style and content.*

Changes to methods will not be considered at surveillance visits unless prior notification has been given by the facility and a technical assessor is present to review the method.

5.4.2 Selection of methods and application of methods

Method Validation and Verification

Specific guidance can be found in NATA Technical Note 17: Guidelines for the validation and verification of quantitative and qualitative test methods, and specifically Appendix 1. Method Validation and verification decision tree.

In addition the following guidance is provided.

Two analytical procedures that differ in any detail could be considered different methods. However, some differences may not be sufficient to affect the test results. These differences can be categorised as minor modifications. It is impossible to differentiate what would be regarded as minor modifications to a method. Some examples might be:

- differences in details of sample dilutions as a consequence of expected counts or a slight change in a non-critical incubation temperature (e.g. 35℃ rather than 37℃ for coliform determinations);
- use of a different non-selective growth medium (e.g. Trypticase Soy Agar rather than Brain Heart Infusion Agar for purification of colonies prior to the performance of confirmation tests).

Equally, it is impossible to differentiate what constitutes a major modification i.e. one that will affect the test results. Some examples might be:

- differences in the formulation of the selective/differential medium (e.g. addition of an alternative antibiotic);
- different antibiotic concentration to the base medium than that specified;
- a change to a critical incubation temperature or time (e.g. 3 days rather than 5 days incubation for yeast and mould determinations);
- different confirmation procedure (e.g. use of an alternative suite of biochemical tests other than those specified).

As matrices are difficult to define and categorise this aspect requires additional consideration. Standard methods and methods validated by validation bodies state in their scope the matrices with which the method is applicable.

- If a standard method is applied to a matrix slightly different to that specified in the method, than this may (but not always) constitute a minor modification to the method (e.g. recreational water with a method validated for use for drinking water or yoghurt with a method validated for sour cream).
- If the matrix is significantly different to that specified in the method, then this constitutes a fundamental modification that the method should be regarded as a new method (e.g. fruit juice by a method validated for use with meats or soil with a method validated for use for water).

If a facility uses a rapid method where validation has been approved/registered by one of the peer accepted organisations (that undertakes professional validation of new methods), verification is sufficient provided the manufacturer's procedures are followed implicitly and the method is used for the matrices that have undergone validation. A minor modification of the procedure will in this instance constitute a requirement for comparative validation.

Training module

The term training module refers to the action required to be taken when a new version of a method for which a facility is currently accredited is introduced. Training modules require staff to be advised of the changes in the new version of the method and for this to be recorded in staff training records. A training module takes into consideration the competency of the facility staff to perform the technique and familiarity with the target organism(s).

Method Verification

When accreditation is first sought for a standard method verification data must be generated. (Where a new version of a standard method is introduced a training module is required. Refer to Technical Note 17).

Verification is the process of demonstrating the performance criteria included in the method can be met by the facility prior to introducing them for routine use.

The facility must demonstrate that the proposed method can be applied and interpreted competently by setting up positive and negative samples for the target determination(s).

Procedures used for the verification of standard methods must be documented and records of any verifications made must be retained. These will be reviewed at assessments.

Matrix Verification

When a facility needs to apply a standard method or peer reviewed method to matrices which are generically different from the scope of what the method was originally intended for, it is necessary to verify this method is appropriate for the new matrix.

Consideration must be given to the properties of the new matrix before a validation protocol is undertaken. For example, the nature of the physical properties of the matrix or the presence of a biostatic agent, which may affect the recovery of the determinant, must be considered and appropriate action taken.

The records for verification must include at least the following:

- Documented test method;
- A minimum of 5 test results – including positive and negative samples;
- Seeding protocol of the target organisms employed;
- Media quality control ;
- Reference culture records relating to the test method;
- External proficiency results (where offered for this test parameter);
- Internal quality assurance results;
- Summary of outcome and suitability of method for intended use (including implications of test results).

5.4.4 Non standard methods

Methods that require validation may include:

- Modified standard methods (e.g. major changes to media, incubation conditions, and techniques of confirmation);
- Alternative/new methods (usually rapid methods) for which validation data is not available (e.g. instrumental, biochemical, impedimetric, immunological, nucleic acid, turbidimetric methods);
- Client in-house methods.

5.4.5.2 Validation of methods

All non-standard methods that have no formal endorsement or published peer reviewed validation data must be validated before use.

The performance of any method is usually affected by many factors. Consequently the design of the validation study will vary depending on the purpose, scope and application of the method. Therefore it is not possible to define minimal or optimal requirements of validation that would suit all situations.

Validation studies can be divided into comparative and full validations. See Technical Note 17

Comparative validation

Comparative validation aims to demonstrate equivalent performance between two (or more) methods. There is no single test of establishing method equivalence or numerical acceptance criteria for it. Generally, a method with the greatest sensitivity or highest recovery for the target microorganism is the best.

AS/NZS 4659 *Guide to Determining the equivalence of Food Microbiology Test Methods* is an example of a validation protocol that can be used for comparative validation. This standard provides guidance on the validation of qualitative, quantitative, confirmation and antibiotic tests and constitutes comparative validation.

Validation of water methods follows the same principles as food methods, with water being regarded as another matrix (e.g. potable waters, environmental waters, recreational and treated waters). Hence the principles included in AS/NZS 4659 can also be used for validation of changes in water microbiology methods.

However, AS/NZS 4659 might not be suitable for all possible comparative scenarios and alternative protocols on validation should be evaluated for suitability. Numerous protocols, written by standard bodies, are available.

Guidelines for validation of some pharmaceutical methods are documented in compendial texts (e.g. BP, USP, AOAC or APHA).

The report on the results of the comparative validation study must address the following issues:

a) Detailed validation rationale and reason(s) why the validation study is required (e.g. to assess the equivalence of the new method or modification with the standard method currently used).

b) The reason that this method has been selected in preference to others.

c) A statement of the implications of test results including a risk assessment.

d) Target organism(s).

e) Specification of the matrix(ces) under examination and the characteristics that define it (them).

f) The non-standard method precisely defined by reference to a publication, manufacturer's instructions or by deviation from the published method.

g) The reference/standard method defined precisely.

h) Detail of the steps of the method compared (i.e. confirmation only, qualitative method or quantitative method).

i) Explanation of the type and the number of samples chosen.

j) Statement of whether duplicate analyses were performed for quantitative tests.

k) If performed, specification of the seeding protocol including seed levels, organisms used and justification.

l) The raw data and QC analyses for all samples must be provided to allow for an independent review to verify each determination and calculation performed.

m) Specification of the starting and finishing dates of the tests.

n) For quantitative validations: Analysis of results using a t-test or other appropriate statistical test.

o) For qualitative validations: Analysis of operating characteristics of the method (e.g. sensitivity, specificity, presumptive false positive and presumptive false negative).

p) Discussion of study results based on statistical analysis and predetermined acceptance criteria.

q) The conclusions drawn from the study based on the data analysis, discussion and a statement indicating whether the study objectives were achieved and whether the method is fit for the intended purpose.

Validation studies can be supported by additional technical studies sourced externally from the facility.

Primary validation

For situations where comparative validation is not applicable (e.g. use of a matrix significantly different to those specified in the scope of the method or use of an alternative isolation or detection principle), primary validation must be undertaken prior to introducing the method. In such cases validation becomes an exploratory process with the aim of establishing operational limits and performance characteristics of the alternative, new or otherwise inadequately characterised method. It should result in numerical and descriptive specifications for the performance of the method. See NATA Technical Note 17: *Guidelines for the Validation and Verification of Quantitative and Qualitative Test Methods.*

Guidance on the approaches that can be taken in primary validation are available in protocols such as those used by AOAC (stage 3) and other peer recognised method validation bodies.

Records of validation must be kept as long as the method is retained and as long as necessary to ensure adequate traceability of raw data and results. Validation data must be kept and be available for reference and audit purposes.

5.4.6.2 Estimation of measurement uncertainty (MU)

All laboratories undertaking quantitative determinations, including the most probable number technique, are required to establish the MU associated with measurand. MU is defined as:

"A parameter associated with the result of a measurement, that characterises the dispersion of the values that could reasonably be attributed to the measurand".

Where results of tests are not numerical (e.g. pass/fail, positive/negative, detected/not detected or other qualitative data) estimates of uncertainty or other variability estimates will not be required at this stage. This should not however preclude the facility from developing an understanding of the components that contribute significantly to the variability of the results.

In estimating the measurement uncertainty, the facility needs to consider those components under its control. For example, if the facility is not involved in the taking of the sample then it does not have to estimate the measurement uncertainty of this process. It should however be clear what components have been included in the uncertainty estimation.

Quantitative tests include those where a numerical value is reported as a qualitative result, such as ELISA assays with a 'cut off' value, where the numerical result is reported as detected or not detected.

Prior to assessment, facilities will be requested to provide the following as part of their pre-assessment information:

- a plan or schedule detailing the progress made towards completing measurement uncertainty estimations for quantitative test methods;
- the procedure for estimating measurement uncertainty;
- an example of a completed estimation using the documented procedure;
- sample matrix MU estimations where matrices are significantly different.

In cases where a quantitative test consistently returns results at the limit of detection e.g. <1/10mLs the result is to be treated as a qualitative result and MU is therefore not required to be reported.

A measurement uncertainty must be made for new methods requested to be added to the scope of accreditation. Accreditation will not be granted until an acceptable MU estimation has been reviewed by NATA.

For further details refer to ISO/TS 19036 *Microbiology of food and animal feeding stuffs – Guidelines for the estimation of measurement uncertainty for quantitative determinations.*

Additional information is available on the NATA website as well as the ILAC (www.ilac.org) and APLAC (www.aplac.org) websites.

5.6 Measurement traceability

Microbiological culture collection management

Refer to Biological Testing Annex H – Maintenance of Microbiological Reference Culture Collections.

5.7 Sampling

Specimen collection

Where specimen collection is outside the control of the facility, the collectors must be informed of the facility's collection requirements. For example:

- containers/tubes required for each test;
- amount of specimen required;
- 'order of draw' for multi-sampling vacuum tubes;
- labelling requirements;
- specimen storage requirements (e.g. room temperature vs refrigeration);
- specimen transport requirements;
- requirements with respect to request forms;
- provision of relevant clinical information.

These requirements must be documented.

In general, specimen containers should not be pre-labelled.

Consumables provided by the facility or used in the facility, in particular tubes containing additives, must be monitored for expiry dates.

5.8 Handling of test and calibration items

5.8.1 Sample reception

Documented reception procedures must be available which cover:

a) criteria for acceptance/rejection of unsuitable specimens (e.g. containers leaking or broken, specimens collected into wrong containers, specimens unsuitable for the examination requested, inadequately-labeled specimen containers);

b) action to be taken in the event that an unsuitable specimen is received;

c) procedures for handling urgent specimens;

The date, and if relevant, the time of receipt of specimens at the facility, must be recorded.

Specimens and associated records (worksheets, slides etc.) must be uniquely identified during all stages of testing, for example, by using a traceable numbering system.

Transport of specimens

Sample containers must be leak-proof and impervious to contamination during transport. When temperature or other environmental tolerances are specified in test methods, these must be provided to the submitter and must be satisfied during transport and storage.

5.8.2 Sample identification

Identification labels must be secure and legible. Samples must be labelled on the body of the container and the lid. Labelling only on caps and lids is not acceptable because of the risk of wrongly replacing lids.

5.9 Assuring the quality of test results

Quality control data (both internal and external) shall be documented in such a way that it is readily accessible for troubleshooting and following up on possible errors and trends.

5.9.1 Proficiency testing (external)

Facilities must participate in proficiency testing programs relevant to their scope of accreditation.

The primary function of Proficiency Testing (PT) is to supplement the internal quality control procedures of facilities by providing an external evaluation of their testing capability.

The results from participation in proficiency testing are used to complement NATA's assessment activities. Facilities can use the results from their participation in relevant PT programs to demonstrate competence in performing the tests for which they hold or seek NATA accreditation.

Participation

Where a biological testing specific proficiency testing programs is available and applicable to the testing conducted by the facility, participation is mandatory. Participation in at least two rounds of relevant programs shall be undertaken annually.

PT programs applicable to testing falling under the field of Biological Testing are currently available to cover the following testing areas:

- Microbiology of environmental and potable waters;
- *Legionella* in water;
- Carcass Hygiene
- Pathogens in food;
- Non pathogens in food;
- Phycology;
- *Cryptosporidium* and *Giardia*.

When no PT program is available to meet requirements, the facility must investigate alternative means for assuring the quality of test results generated (as highlighted in section 5.9.1 of ISO/IEC 17025:2005), for example, sample exchange programs with other laboratories, replicate testing, etc.

Known PT providers are currently listed on the NATA website www.nata.com.au under Accreditation Guidance and Information > Proficiency Testing (PT) Guidance and Information.

Facilities should consider the accreditation status of PT providers and are advised to choose accredited providers, where possible.

Provision of PT programs where gaps exist

Where gaps are identified in proficiency testing coverage, the Biological Testing Accreditation Advisory Committee (BTAAC) may scope the need for a new program highlighting where deficiencies currently exist. Such requests for new PT providers are posted on the NATA website in an effort to increase coverage in necessary areas. A current or new PT provider may wish to include such testing in

their available services. Where more than one provider indicates interest and meets the identified features of PT programs, NATA does not recommend one program in favour of another.

Performance

A facility's performance in proficiency testing will be assessed on-site during assessments and surveillance visits.

Evidence of review of returned results and any corrective action taken in response to outliers will also be reviewed by the NATA assessment team. In preparation for this review, information on the performance and any follow up required in proficiency testing programs will be requested to be submitted prior to assessment visits.

NATA may contemporaneously review a facility's PT performance at the time of release of the results by the PT provider in specific cases e.g. during the investigation of a complaint.

Confidentiality

As with NATA's assessment activities, all information received regarding a facility's participation in a proficiency testing program is treated as confidential. This information may be disclosed to relevant NATA staff members, assessors, assessment observers and NATA committee members. All have signed confidentiality agreements. It may also be disclosed to agencies to which NATA has a legal obligation or with whom NATA has a formal agreement.

5.9.1 Internal quality control

The program for monitoring the reliability of results established based on the implementation and documentation of a comprehensive internal quality control program. This includes:

- maintenance, propagation and use of positive and negative controls;
- media quality control (see clause 4.6);
- instrument calibration, maintenance and use;
- staff training and competency performance evaluation;
- checking of calculations and results.

Positive and negative controls must be run for each method in parallel with each batch of samples. The availability of manufacturers' kit controls does not preclude the use of positive and negative controls.

The level of the inoculum of positive and negative controls must be between 10-100 cells to replicate low level contamination and the limit of detection of the tests.

Criteria for rejecting suspect results must be based on non-compliance with the predetermined criteria as defined in the internal quality control program (e.g. incubator temperature out of the range for duration or part of the test; negative control samples analysed concurrently with test samples found to contain the determinant organism; insufficient dilution of perishable samples; concurrently evaluated media found deficient; unusual/confounding biochemical results; significant difference in duplicate results etc.).

5.10 Reporting of results

5.10.5 Opinions and interpretations

Facilities can include expressions of opinion and interpretation of test data on test reports for testing covered by the scope of accreditation where the opinion or interpretation is based on the data reported and is technically valid. Such opinion must be demonstrated to be professionally valid and be traceable to authoritative references*. Any opinions or interpretations offered by the organisation will be reviewed as part of the assessment of the related testing.

Alternatively facilities may include comments and/or interpretation of results in a separate document that is clearly linked; (i.e. by report number) to the report on which the opinion is based.

Organisations engaged in testing performed on human specimens may not include any opinions or interpretations on test reports for the purposes of diagnosis, treatment or monitoring of a patient. Where opinions or interpretations are to be reported, accreditation against ISO 15189 in the field of Medical Testing is to be sought.

Note: * Authoritative references include guidelines and standards set by government bodies such as the NEPC and NHRMC.

References

This section lists publications referenced in this document. The year of publication is not included as it is expected that only current versions of the references shall be used.

Standards

APHA	*Standard methods for examination of water and waste water.* APHA-AWWA-WPCF (American Public Health Association, American Water Works Association, Water Pollution Control Federation), Washington DC, USA.
AS 2929	*Test methods-Guide to the format, style and content*
AS/NZS 4659	(Parts 1-4) – *Guide to determining the equivalence of food microbiology test methods.*
ISO 11133	*Microbiology of food and animal feeding stuffs -- Guidelines on preparation and production of culture medium*
Part 1	*General guidelines on quality assurance for the preparation of culture media in the laboratory*
Part 2	Practical *guidelines on performance testing of culture media*
ISO 19036	*ISO technical specification – Microbiology of food and animal feed stuffs – Guide on estimation of measurement uncertainty for quantitative determinations.*

NATA Publications

NATA Policy Circular 2	Proficiency Testing policy
NATA Technical Note 17	Guidelines for the Validation and Verification of Quantitative and Qualitative Test Methods
Biological Testing Annex A:	Accreditation of Facilities for Testing of Water Samples for Cryptosporidium and Giardia
Biological Testing Annex B:	Accreditation for on-site abattoir facilities and contract testing facilities approved by DAFF to test carcass hygiene samples and export meat samples
Biological Testing Annex C:	Accreditation of seed testing facilities
Biological Testing Annex D:	Accreditation of facilities testing for genetically modified organisms (GMO)
Biological Testing Annex E:	Accreditation of facilities for biological tests on water
Biological Testing Annex F:	Plant Health Diagnostic Testing
Biological Testing Annex G:	Media Preparation and Quality Control
Biological Testing Annex H:	Maintenance of Microbiological Reference Culture Collections (MRCC)

Other references

ASM *Guidelines for Assuring Quality of Food and Water Microbiological Culture Media*

Amendment Table

The table below provides a summary of changes made to the document with this issue.

Section or Clause	Amendment
AD introduction and references	List of related Annexes included