Ministry for Primary Industries
Manatū Ahu Matua

Guidance Document

A Guide for the Validation and Approval of New Marine Biotoxin Test Methods

10 April 2017

A guidance document issued by the Ministry for Primary Industries

New Zealand Government

Title

Guidance Document: Validation and Approval of New Biotoxin Test Methods

About this document

This guidance is to assist with the implementation and validation of new marine biotoxin test methods.

Document history

Version	Version Date	Section Changed	Change(s) Description
1	February 2002		
2	April 2017	All	New format and branding

Contact Details

Contact for further information:
Ministry for Primary Industries (MPI)
Regulation & Assurance Branch
Animal Products
PO Box 2526
Wellington 6140
Email: animal.products@mpi.govt.nz

Disclaimer

This guidance does not constitute, and should not be regarded as, legal advice. While every effort has been made to ensure the information in this guidance is accurate, the Ministry for Primary Industries does not accept any responsibility or liability whatsoever for any error of fact, omission, interpretation or opinion that may be present, however it may have occurred.

Copyright

Contents

1 Purpose

The shellfish research and analytical laboratories, MPI and the New Zealand Seafood Standards Council have developed these guidelines to assist with the implementation and validation of new marine biotoxin test methods.

As more marine biotoxins have been found in New Zealand in recent years, the test method regime developed in 1993/94 became inadequate. New methods are now being developed and this guideline has been prepared to assist in the acceptance of the new methods for regulatory use.

New methods of analysis must be rigorously tested and validated before they can be used for routine regulatory testing.

While this guideline was developed primarily to aid in the validation of new marine biotoxin test methods, it can be used, in conjunction with other guidance material, for the validation of other test methodologies such as microbiological test methods or gene probe technologies.

This Guideline has been developed in accordance with several robust internationally accepted validation guides (refer section 8).

2 Background

Researchers in New Zealand have worked at developing alternative test methodologies for marine biotoxins. This research has been driven on two main fronts:

- Firstly by the need to find methods that are "animal friendly" to satisfy an increasing demand for a halt to live animal bioassays.
- Secondly, as new and novel toxins are discovered there is an increasing need to develop a new test method regime to be used for monitoring and regulatory purposes that can address all the toxins.

Other possible reasons for introduction of new test methods could include requirements for improved turnaround times, individual toxin identification, confirmatory testing or other testing efficiencies.

In theory any work on new test methodologies should only be initiated once the need has been identified and agreed by all stakeholders. After agreement has been reached that a new test method is required the method must be rigorously tested before it can be used for routine regulatory testing. Researchers, industry representatives, regulators, and analysts need to have clear guidelines for the validation of new technologies or method improvements; these guidelines have been developed to aid in this.

Laboratories can, if desirable, use this guide in conjunction with other guidance material for the validation of other test methodologies such as microbiological test methods, gene probe technologies, bioassays and ELISA's etc.

This guide must be used for the introduction of new marine biotoxin test methodologies or for the introduction of improvements to currently accepted marine biotoxin test methods.

3 Definitions

IANZ
For laboratories to gain full approval to operate in the marine biotoxin monitoring programme both MPI approval and IANZ accreditation of the laboratory is required.

MPI
The Ministry for Primary Industries

NSSP
United States National Shellfish Sanitation Programme

USFDA
United States Federal Drug Administration

Technical Assessors
Persons who are experienced in test method validation in an area relevant to the method being validated. These persons should be external to the laboratory concerned. They should determine the validity of the validation data and assess if the data supports the use of the method. They should also ensure that the relevant validation data as laid out in this guide has been gathered.

4 Method Validation and Approval Guidelines

4.1 Type of Method

In 1981 a memorandum of understanding was formed between the USFDA and New Zealand. In order for New Zealand molluscan bivalve shellfish to be exported to the USA they must be harvested, processed and labelled in accordance with the NSSP.

Four types of method are proposed for NSSP analytical methods. These are summarised in Table 1. It is generally acknowledged that regulatory methods need to be of the highest standard. Type I methods described in the table below best fulfil this requirement.

However, it is also recognized that full collaborative studies required for Type I classification, will not be possible where few laboratories (possibly only one laboratory) use this method. Therefore, new methods are likely to be accepted as either Type III methods selected to fulfil on-going need, or Type IV methods selected to fulfil an immediate need.

Type I tests require a collaborative study with defined operational and statistical protocols. ISO 5725 could be used as a guide to assist in the design of collaborative studies.

Future participation in collaborative studies and/or appropriate proficiency testing schemes must occur when other laboratories adopt the new method(s). There must be a move by the laboratory involved towards establishing the new method as Type II or even Type I.

Table 1. Proposed four types of NSSP analytical Methods

Requirements	Type I	Type II	Type III	Type IV
(a) Described in a scientific or other peer reviewed professional publication	●	●	●	●
(b) Used successfully to detect or quantify	●	●	●	●
(c) Evaluated, at least in part, and the performance characteristics (at least for specific applications) have been reported	●	●	●	●
(d) Collaboratively studied and/or collaboratively tested	●			
(e) Long used as an acceptable method	●	●		

Requirements	Type I	Type II	Type III	Type IV
(f) Selected to fulfil a continuing need			●	
(g) Selected to fulfil an immediate need				●
(h) Designated for on-going review and assessment			●	●
Examples	PSP mouse bioassay, APHA MPN	Brevitoxin mouse bioassay	HPLC for domoic acid, DSP mouse bioassay	Direct plating + DNA probes for V.p.

4.2 Scope

This guide gives an overview of validation requirements without prescribing technical detail. It is aimed at laboratories needing to validate new methods but possibly working in isolation, with no immediate possibility of participation in collaborative studies.

Quality control and quality assurance needs to involve all steps of the analysis as an integral process, of which validation of the analytical methods used is only one, though important, step. Appendix 1 demonstrates the relationship of the validation process to an approved laboratory carrying out regulatory testing.

Method approval for routine regulatory testing will be partly based upon the validation work completed for a method and upon supporting documentation submitted. The approval process may assess any part of the analysis including; sample treatment, analysis and detection, data processing and storage, data interpretation and evaluation, and laboratory management practices. This process is summarised in Appendix 2.

The validation performance characteristics detailed in this guide must be considered when validating a new marine biotoxin test method. Development teams seeking approval for routine use of validated methods must follow the process outlined in this guide.

5 Method Validation and Approval Process

5.1 Validation Protocol and Method Validation

Those responsible for developing validation protocols and for carrying out method validation are referred to as Study Directors in this guide.

The evaluation checklist needs to classify the areas that are of “critical”, “key” or “other” importance in keeping the method under control.

The Study Director must develop a validation protocol and validate the method in accordance with the performance parameters and process detailed in this guide. The Study Director must also develop an evaluation checklist to be used by the MPI Laboratory Evaluation Officer for auditing the method when it is in routine use. The validation performance characteristics detailed in this guide must be considered when validating a new marine biotoxin test method. Development teams seeking approval for routine use of validated methods must follow the process outlined in this guide.

5.2 Peer Review (Technical Assessment)

5.2.1 Validation Protocol

It is recommended that method validation protocols be reviewed prior to validation work commencing so that any serious flaws in the protocol will be identified and remedied before validation work commences. It is the responsibility of the Study Director to obtain the services of a Technical Assessor. Technical Assessors are persons who are experienced in test method validation in an area relevant to the method being validated. A checklist for Technical Assessors to use for Protocol Review is contained in Appendix 3. The Study Director should respond to all comments and recommendations made by the Technical Assessor. The validation protocol can also be submitted to MPI for review prior to method validation commencing. The validation protocol review stage is optional with respect to both reviews by a Technical Assessor and by the MPI.

5.2.2 Validation Findings

The second phase of review occurs when the validation work has been completed.

A checklist for Method Application and Validation Review is contained in Appendix 4. This checklist can be used by the Study Director prior to application to use the method to ensure all aspects of the application have been addressed and by the Technical Assessor in reviewing the validation findings.

The Study Director must choose and obtain the services of an appropriately experienced Technical Assessor who must review the validation findings. The Technical Assessor must be independent of the laboratory involved. The Technical Assessor must determine the validity of the validation data and assess if the data supports the use of the method. The Technical Assessor must ensure that the relevant performance characteristics (section 3) have been defined. The Study Director must respond to all recommendations made by the Technical Assessor.

5.3 Approval

Applications to test for marine biotoxins using a new method must be submitted to MPI.

Applications need to contain:

- Details of the proposed method;
- Scope and rationale for using the method;
- Validation protocol for the new method;
- The validation findings for the proposed method;
- Peer review of the validation findings undertaken by the Technical Assessor;
- Any responses to the Technical Assessor made by the Study Director;
- Schematic diagram of the overall testing regime proposed by the lab;
- Details of back up testing arrangements should the proposed method fail e.g. equipment;
- Details of the units that the lab propose to report results in;
- Details of turnaround times, from sample receipt to issue of results, for samples that will be analysed by the new method once it is in regulatory use;
- Details of intention to participate in proficiency testing schemes or collaborative studies for the new method.

Suggested presentation and documentation of applications is detailed in section 4. The role of MPI is to form a recommendation as to whether a test method should be used as part of the regulatory testing programme for marine biotoxins. This recommendation will be based on the peer review undertaken by the Technical Assessor (section 2.2.2), advice received from the MPI Laboratory Evaluation Officer and any other consultants necessary.

The MPI will grant final approval for use of a new test method. Approval may be subject to conditions.

A checklist for the laboratory is contained in Appendix 5 to ensure that the correct documents have been submitted to the appropriate parties during the validation process.

5.4 Interim Approval

Situations may arise in which new test methods may be granted interim approval to be used for regulatory testing.

Interim approval to use a test method may be granted for a maximum of six months by the MPI.

To gain interim approval to use a new test method the following circumstances must apply:

- ***The method is needed to fulfil an immediate need or an on-going need;***
- ***Some validatory work has been undertaken for the method;***
- ***Full validation detailed in this guideline is being sought for the new method.***

Various performance characteristics such as accuracy, precision, measurement uncertainty, working and linear range, ruggedness and sensitivity must be considered when validating and determining the acceptability of any new method.

6 Method Validation Performance Characteristics

Determination of the following performance characteristics is generally considered to be necessary and is expected unless rationale is provided as to why a characteristic has not been assessed. References have been included for each performance characteristic described, consult these references for a more detailed description of the characteristic.

6.1 Quality Control Procedures

The Study Director must describe what performance characteristics are appropriate, how they will be measured and what is regarded as significant.

Specific controls must be developed to verify that the method remains in control during routine testing (quality control procedures).

Method validation studies help to determine the capability and limitations of the method that may be experienced in routine use while the method is in control.

The Eurachem Guide (2014) provides more detail on the sorts of quality control procedures that should be adopted to ensure the validity of results when the method is in routine use.

6.2 Accuracy – Trueness

The 'trueness' of a method reflects the degree of agreement of individual measurements with some true or reference value of the property being measured. This is often expressed as a percent recovery. For example, recoveries of 100 ± 10 % have frequently been discussed. Accuracy data is usually obtained using spiking trials. The mean and standard deviation of a series of replicated tests using spiked material should be obtained and compared with the characterised value for the reference material. If spiking trials are undertaken it is important that spike addition is made early in the analysis to ensure that extraction efficiency is included in the findings. In some cases where little or no reference material is available for spiking trials this parameter

will be difficult to assess. Accuracy may need to be determined for materials that have different matrices. For further guidance material on accuracy consult the Eurachem Guide (2014).

6.2.1 Specificity (selectivity)

This is the ability of the test method to respond only to the property being measured. Specificity needs to include both evidence of analyte identification and evidence of separation of the analyte from other interfering compounds. Matrix effects need to be considered. For further guidance material on specificity please consult the Eurachem Guide (2014).

6.2.2 Reference Materials

In analysing the performance characteristics described it is preferable to use certified reference materials. It is not possible to use certified reference materials for all analytes of interest. In cases where certified reference materials cannot be used reference materials can be used. Validity and stability of both reference materials and certified reference materials needs to be demonstrated. For definitions of certified reference materials and reference materials please refer to the Eurachem Guide (2014).

6.2.3 Comparability

When a new method is designed to replace an existing method then some comparative assessment needs to be made. Sometimes the use of certified reference materials can produce a matrix mismatch against true unknowns. In this situation comparison against existing methods (which are accurate by definition) is the preferred parameter. This usually involves running new and existing methods side-by-side and comparing results to determine if they are significantly different.

6.2.4 Recovery

Measurement of all the analyte present in the sample does not always occur when using a test method. The efficiency of the method in detecting all the analyte present needs to be assessed. Spiking trials using the analyte at various concentrations or alternatively recovery studies on certified reference materials if available may need to be undertaken. It must be understood that using a surrogate (spike or internal standard) to estimate recovery has problems. Some parts of the natural analyte are usually unrecoverable, unlike the free forms added during recovery estimates. Therefore recovery data may not reflect the real situation. However, if a surrogate cannot be recovered quantitatively, it does indicate a serious problem with the assay. For further guidance material on recovery refer to the Eurachem Guide (2014).

6.3 Precision

Precision is generally described in terms of repeatability and reproducibility. Repeatability and reproducibility are generally dependent on analyte concentration thus need to be determined at various analyte concentrations.

6.3.1 Repeatability

Repeatability needs to be calculated when validating a method and is a measure of agreement of replicate tests carried out on the same sample in the same laboratory by the same analyst. For further guidance material on repeatability studies please refer to the Eurachem Guide (2014).

6.3.2 Reproducibility

Reproducibility needs to be calculated when validating a method and is a measure of agreement between tests carried out in different laboratories. It is generally expected that within laboratory variations will be less than between laboratory variations. In single laboratory validation studies reproducibility could be a measure

of agreement between tests carried out on different days by different analysts. For further guidance material on reproducibility studies please refer to the Eurachem Guide (2014).

6.3.3 HORRAT Values

The calculated repeatability and reproducibility values for the method can be compared with existing methods and a comparison made. If there is no method with which to compare the precision parameters then theoretical repeatability and reproducibility values can be calculated from the Horwitz equation. HORRAT values give a measure of the acceptability of the precision characteristics of a method. The HORRAT value needs to be calculated when validating a method. For information on how to calculate HORRAT values refer to the Joint FAO/WHO Food Standards Programme, Report on the twenty-third session of the Codex Committee on the methods of analysis and sampling, Alinorm 01/23, Proposed Guidelines and Working Instructions to Aid the Implementation of the Criteria Approach to the Selection of Methods of Analysis for Codex Purposes.

6.3.4 Inter-laboratory Comparison

Where possible the results of inter-laboratory comparisons need to be provided. When inter- laboratory comparisons are not possible reproducibility will be the closeness of agreement between results obtained with the same method on replicate samples with different analysts and different equipment.

For further guidance on repeatability and reproducibility studies when inter-laboratory comparison is not possible please refer to the Guidelines for Single-Laboratory Validation of Analytical Methods for Trace-Level Concentrations of Organic Chemicals (Joint FAO/IAEA Expert Consultation, 1999).

6.4 Measurement Uncertainty

Measurement uncertainty is a combination of accuracy and precision data (it shows the total analytical error of a homogenous sample). Measurement uncertainty is a parameter (standard deviation or 95% confidence interval) that shows the range of values that are possible on the basis of the measurement result. Measurement uncertainty needs to be reported taking into account all components that are applicable in a given situation. It is preferable that measurement uncertainty is reported as a 95% confidence interval. For further guidance material on measurement uncertainty refer to NZS/ISO/IEC 17025 (2005).

6.5 Sensitivity

The sensitivity of the test method needs to be determined and can be described as the limit of reliable measurement. This means the limit at which a method can discriminate, with a high degree of confidence, between levels above and below some critical value near zero. Effectively sensitivity is the gradient of the response curve or the change in instrument response that corresponds to a change in analyte concentration. For further guidance on sensitivity testing please refer to the Eurachem guide (2014).

6.5.1 Limit of Detection

This is the smallest concentration at which the analyte can be identified. For validation purposes an indication of the level at which detection becomes problematic needs to be provided, usually defined when the analyte signal equals three times the background (noise). Limit of detection is matrix dependent so it will need to be established for different tissues and different organisms. The method must also be validated at the concentration range of interest (e.g. regulatory level), see working and linear range below. For further guidance material on the Limit of Detection please refer to the Guidelines for Single-Laboratory Validation of Analytical Methods for Trace-Level Concentrations of Organic Chemicals (Joint FAO/IAEA Expert Consultation, 1999).

6.5.2 Limit of Quantitation

This is the smallest concentration of the analyte that can be quantified with an acceptable level of precision and accuracy. At this concentration the analyte signal is sufficiently resolved from the noise to provide a meaningful measurement to be taken. The Limit of Quantitation is usually decided as ten times the background (noise). For validation purposes an indication of the level at which quantitation becomes problematic needs to be provided. For further guidance material on the Limit of Quantification please refer to the Guidelines for Single-Laboratory Validation of Analytical Methods for Trace-Level Concentrations of Organic Chemicals (Joint FAO/IAEA Expert Consultation, 1999).

6.6 Working and Linear Range

For quantitative methods it is necessary to determine the range of analyte concentrations or property values over which the method may be applied. Evaluation of the working and linear ranges will also be useful for planning what degree of calibration is required when using the method for routine analyses. The working and linear range needs to be evaluated for each matrix type used. For further guidance material on linear and working range refer to the Eurachem Guide (2014).

6.7 Ruggedness

Ruggedness is the ability of a particular method to withstand relatively minor changes in analytical technique, reagents, or environmental factors. All new methods designed for routine testing need to be deliberately challenged by introducing minor variations in the way a test is performed and then measuring the effect of these individual changes on the expected test result. Only tests that are suitably stable will be considered for routine testing. For further guidance material on ruggedness refer to the Eurachem Guide (2014).

6.8 Matrix

The type of tissue and type of organism being analysed may influence the test result significantly. Matrix effects will need to be considered when determining accuracy, specificity, precision and sensitivity.

New test methods must be validate against a 'representative' set of tissue types and organisms of interest

7 Application Presentation

7.1 Documented Method

The proposed new method must be documented and submitted for approval in a form that can be easily interpreted and followed.

The following format is suggested:

- Method title
- Method scope, including purpose of the method
- References to supporting literature, if appropriate
- Principle of the method
- Apparatus and reagents required
- Safety requirements
- Detailed procedural steps
- Calculation formulas

- Quality control steps

7.2 Validation Material

Copies of documented validation findings need to accompany any application to test using a new method.

In general this includes:

- Validation protocol
- A summary of validation data (results)
- Calculations carried out for the validation
- Interpretation of validation data
- Peer review undertaken by the Technical Assessor
- Any response to the Technical Assessor
- Conclusions
- Statement of suitability for desired purposed
- Supporting references

For further information on the documentation of validation findings refer to the NZS/ISO/IEC17025 (2005).

7.3 Supporting Documentation

The following supporting documentation must be included in the application for approval.

In general this includes:

- Cost comparison with existing methodology;
- Special technical skills required;
- Special equipment required;
- Any other special advantages;
- A checklist designed for the laboratory evaluation officer by the laboratory to use in the evaluation of the laboratory. This checklist should be based on current checklists for ASP, DSP, NSP and PSP;
- Schematic diagram of overall testing regime proposed by the lab;
- Details of back up testing arrangements should the proposed method fail;
- Details of the units that the lab propose to report results in;
- Details of expected turn-around times, from sample receipt to issue of results, for samples that will be analysed by the new method;
- Details of the intention to participate in proficiency testing schemes or collaborative studies for the new method.

7.4 Summary

All method, validation and supporting documentation will be stored by MPI.

A Method Application and Validation Study Checklist is contained in Appendix 4. This may be used as a guide for the Study Directors and Technical Assessors.

A Checklist for the Study Director is contained in Appendix 5. This contains a list of all validation processes required and may be used by the Study Director to keep track of required activities.

8 References

(1) Eurachem Guide, 2014. The Fitness for Purpose of Analytical Methods. A Laboratory Guide to Method Validation and Related Topics.

(2) The draft ISSC Process for Acceptance and Approval of Analytical Methods for the NSSP.

(3) Joint FAO/IAEA Expert Consultation, 1999. Guidelines for Single-laboratory validation of analytical methods for trace-level concentrations of organic chemicals.

(4) International Standard NZS/ISO/IEC 17025, 2005. General Criteria for accreditation: General requirements for the competence of testing and calibration laboratories.

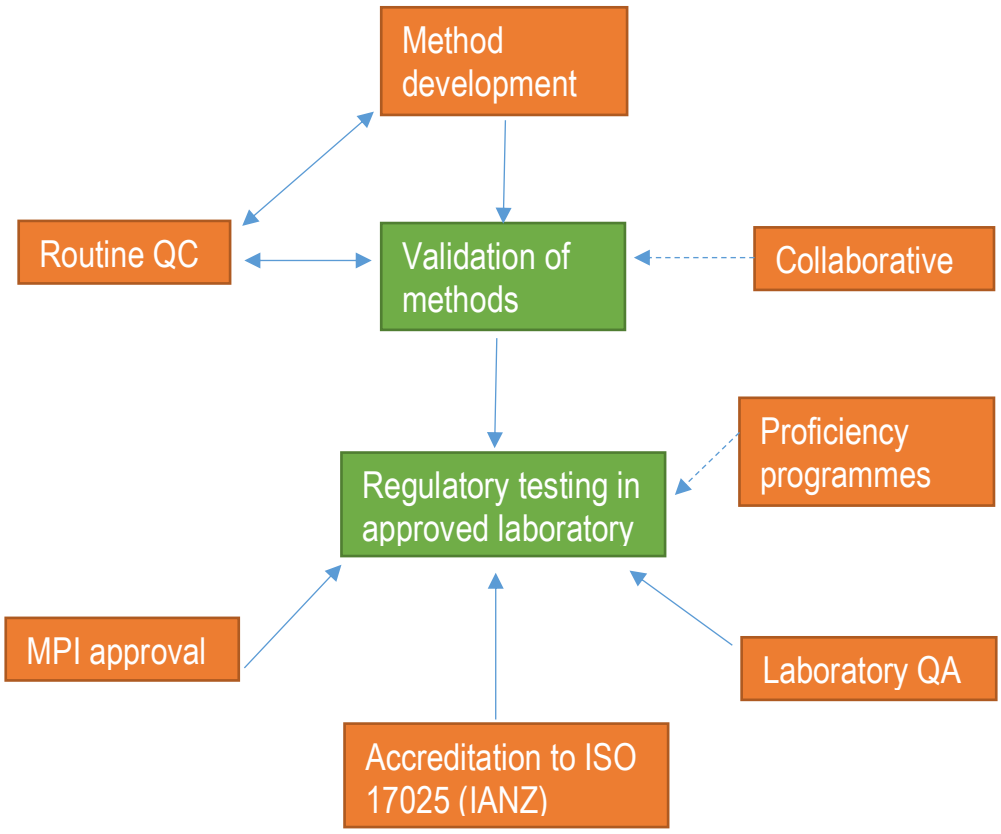
(5) ISO 5725 Accuracy (trueness and precision) of measurement methods and results.

(6) Joint FAO/WHO Food Standards Programme, Report on the twenty-third session of the Codex Committee on the methods of analysis and sampling, 26 February - 2 March 2001, Alinorm 01/23, Proposed Guidelines and Working Instructions to Aid the Implementation of the Criteria Approach to the Selection of Methods of Analysis for Codex Purposes.

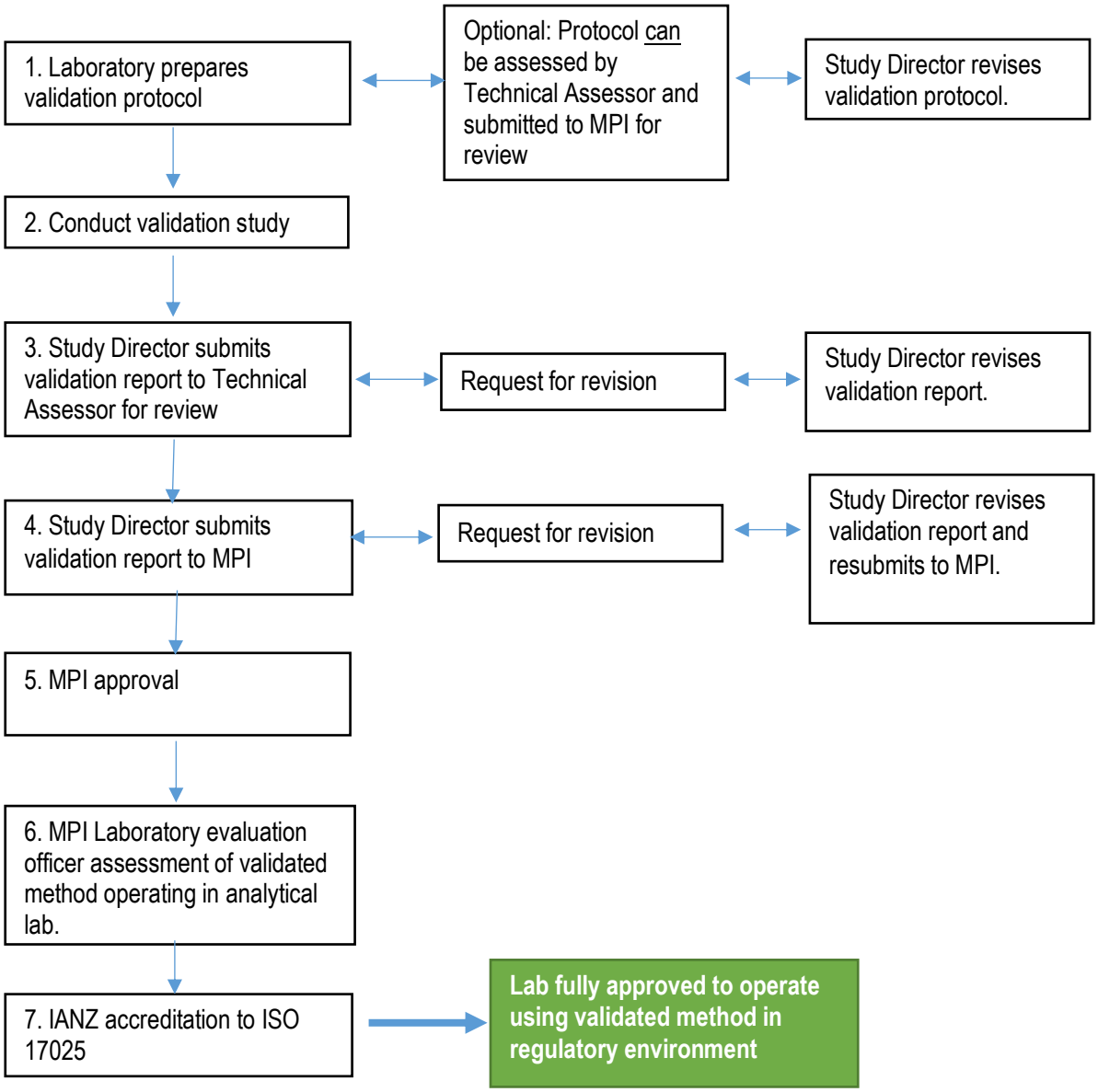
Appendix 1: Method Validation In Relation To Routine Testing

This figure demonstrates the relationship of the validation process to an approved laboratory carrying out regulatory testing.

This validation guideline document gives an overview of validation requirements without prescribing technical detail. References to suitable technical guidance documents are provided on page 13.



Appendix 2: Method Validation Process



Appendix 3: Validation Protocol Review Checklist

Name of New Method		
Name of the Study Director		**Date:**
Name of Technical Assessor		
Checklist		**Reviewer Comments**
A. Validation Material (1) Does the validation protocol include determination of the following? a) Accuracy b) Precision? c) Specificity? d) Measurement Uncertainty e) Sensitivity? f) Working and Linear range? g) Ruggedness? h) Matrix? (2) Does the protocol describe how data will be calculated, summarised and interpreted? (3) Suitability for purpose?		
Submitter signature:		**Date:**
Technical Assessor signature:		**Date:**
Submit validation protocol and reviewer comments to MPI		**Date:**
MPI Review Team (1) MPI (2) LEO (3) Consultant		
Accepted		**Date:**
Recommend further work		**Date:**
Approval to commence validation study. **MPI signature:**		**Date:**

Comments:

Appendix 4: Method Application and Validation Study Checklist

Name of New Method		
Name of the Study Director		**Date:**

Checklist	**Y/N**	**Submitter Comments**
A. Need for the New Method (1) Has the need for the new method been adequately stated? (2) Has the need been acknowledged by Industry? (3) Type of method? (Type I, II, III, or IV)		
B. Method Documented (1) Does the method include the following: a) Method title? b) Method scope? c) References? d) Principle? e) Apparatus? f) Reagents? g) Safety requirements? h) Procedural steps? i) Calculations? j) Quality control steps? (2) Is the method clear and easy to follow?		
A. Validation Material (1) Validation protocol? (2) Does the validation material include the following: a) Accuracy? b) Precision? c) Measurement uncertainty? d) Sensitivity? e) Working and Linear ranges? f) Ruggedness? g) Matrix effects? (3) Calculations for performance parameters included? (4) Peer review by Technical Assessor? (5) Responses to Technical Assessor? (6) Has data been summarised and interpreted? (7) Have appropriate conclusions been drawn? (8) Is there a statement of suitability for purpose? (9) Supporting references?		
Validation Study Checklist (continued)	**Y/N**	**Submitter Comments**

B. Other information (as appropriate) (1) Cost comparison with existing methodology? (2) Special technical skills required? (3) Special equipment required? (4) Any other special advantages? (5) Suitability for purpose? (6) Checklist for Laboratory Evaluation Officer? (7) Schematic diagram of overall testing regime? (8) Details of back-up testing arrangements? (9) Details of unit's laboratory propose to report in? (10) Details of turnaround times? (11) Details of intentions to participate in collaborative studies or proficiency testing schemes?		
Submitter signature:		**Date:**
Technical Assessor signature:		**Date:**
Submit validation report and draft method to MPI		**Date:**
MPI Review Team (1) MPI (2) LEO (3) Consultant		
Accepted		**Date:**
Recommend further work		**Date:**
MPI signature:		**Date:**

Comments:

Appendix 5: Checklist for the Validation Study Director

Name of New Method		
Name of the Study Director		**Date:**

Checklist	**Date**	**Submitter**
A. Validation Process followed:		
(1) Validation protocol prepared?		
(2) Validation protocol submitted to technical assessor (optional)?		
(3) Validation protocol revised?		
(4) Validation protocol submitted to MPI (optional)?		
(5) Validation protocol revised?		
(6) Validation study conducted?		
(7) Validation material submitted to technical assessor?		
(8) Validation material revised?		
(9) Validation material submitted to MPI?		
(10) Validation material revised?		
(11) Validated method approved by MPI?		
(12) Lab evaluation Officer Assessment?		
(13) Interim Approval Granted?		
(14) IANZ accreditation?		
(15) Laboratory and method approved by MPI?		