CRITERIA FOR VALIDATION AND QUALITY ASSURANCE IN MICROBIOLOGICAL TESTING

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1. PURPOSE AND SCOPE

This document defines the technical requirements for quality control, quality assurance and the validation of methods in microbiological testing laboratories.

This document contains supplementary requirements for meeting the requirements of ISO/IEC 17025:2017. Wherever doubt may exist, ISO/IEC 17025:2017 remains the authoritative document in establishing the competence of a laboratory to produce valid measurements.

2. DEFINITIONS

2.1 **Accuracy:** Closeness of the agreement between a test result and the accepted reference value.

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

2.3 **Limit of detection (LOD):** The lowest number of microorganisms that can be detected, but in numbers that cannot be estimated.

2.4 **Linearity:** Ability of a method to obtain test results proportional to the concentration of the analyte.

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

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

2.7 **Precision:** The degree of agreement between individual test results when a method is applied repeatedly to more than one sampling from a homogenized sample. Precision is usually expressed as relative standard deviation. Precision is a measure of either the degree of repeatability or reproducibility.

2.8 **Reference cultures:** Collective term for reference strain, reference stocks and working cultures.

2.9 **Reference strains:** Microorganisms defined at least to the genus and species level, catalogued and described according to its characteristics and preferably stating its origin. Normally obtained from a recognized national or international collection.

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

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

2.12 **Reference stocks:** A set of separate identical cultures obtained by a single sub-culture from the reference strain.

2.13 **Relative trueness:** The degree of correspondence of the results of the method under evaluation to those obtained using a recognized reference method.

2.14 **Robustness:** A measure of an analytical procedure's capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

2.15 **Trueness:** The closeness of agreement between the average value obtained from a large series of test results and an accepted reference value. *Trueness* is equivalent to an absence of "***bias***", which is the difference between the expectation of the test results and an accepted reference value and is a measure of total systematic, but not random, error.

It is very difficult to determine the trueness of a microbiological method, especially on a naturally contaminated sample. The most appropriate way to determine trueness is to conduct tests within several laboratories and then determining the mean of the group result. Trueness can therefore be determined by the use of Certified Reference Materials or artificially contaminated samples. These tests can also be performed in a single laboratory using different analysts. The methods must be able to detect or recover organisms at the correct concentrations.

2.16 **Repeatability:** Closeness of the agreement between the results of successive measurements of the same measurand under the same conditions of measurement.

2.17 **Reproducibility:** Closeness of the agreement between the results of measurements of the same measurand carried out under changed conditions of measurement.

2.18 **Selectivity:** The ability of a method to determine accurately and specifically the analyte of interest in the presence of other components in a sample matrix under the stated conditions of the test.

2.19 **Sensitivity:** The fraction of the total number of positive cultures or colonies correctly assigned in the presumptive inspection.

2.20 **Specificity:** The fraction of the total number of negative cultures or colonies correctly assigned in the presumptive inspection.

2.21 **Uncertainty of Measurement:** Parameter associated with the result of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the measurand.

2.22 **Validation:** Confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled.

2.23 **Verification:** Confirmation, through the provision of objective evidence, that specified requirements have been fulfilled.

2.24 **Working culture:** A primary sub-culture from a reference stock.

2.25 **Sample Matrix:** Everything that is present in the typical **sample** except for the analytes of interest

3. BACKGROUND

A valid measurement may be assured when (ILAC G9):

- validated methods and appropriate equipment are used
- qualified and competent staff undertake the work
- comparability with measurements made in other laboratories is assured (traceability and measurement uncertainty)
- independent evidence of performance is available (Proficiency Testing)
- well defined Quality Control and Quality Assurance procedures are employed, preferably involving third party accreditation
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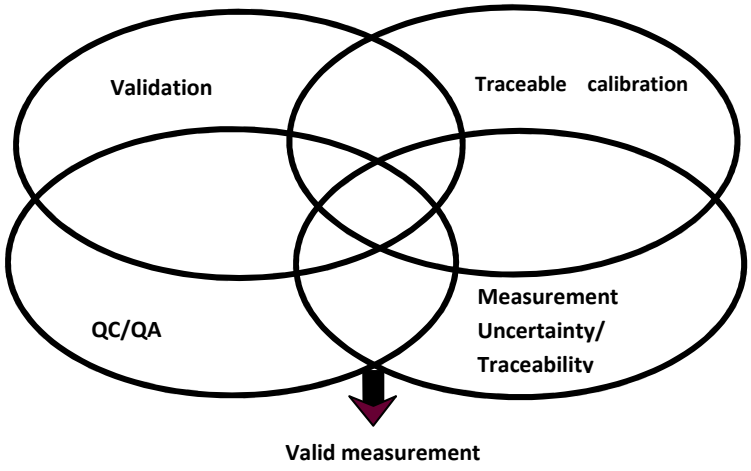
Figure 1*:* Overlap between functions associated with Measurement Traceability and Analytical Quality



4. METHODS

4.1 Microbiological analyses can be divided into two groups:

- **Qualitative analyses**, a method of analysis, which demonstrates either the presence or absence of a specific microorganism in a certain amount of test sample.
- **Quantitative analyses**, a method of analysis which determines the amount of microorganisms present in a certain amount of sample either directly (enumeration obtaining colony forming units) or indirectly (Most Probable Number indices, absorbance, impedance).

4.2 Within these two groups, there exist three types of methods.

4.2.1 Standard Methods

When using a standard test method, the laboratory must demonstrate its competence to meet the performance characteristics of the method. This criterion is satisfied by method verification.

4.2.2 Rapid Methods

Rapid methods such as immunological, molecular biological or instrumental can be used as equivalent to certain standard methods.
Test kits - When the manufacturer of the test kits supplies validation data, the laboratory will only perform secondary validation (verification). If however, no validation data is available for a specific kit, primary validation must be performed. Evidence that the manufacturer of the kits operates to a quality assurance program is desirable.

Laboratories shall retain validation data on commercial test systems (kits) used in the laboratory. This validation data may be obtained through collaborative testing or from validation data submitted by the manufacturers that has been subjected to a third party evaluation (e.g. AOAC).

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

4.2.3 Non-standard methods

The term refers to cases where standard methods have been modified or used outside of their scope of application and cases where the method is laboratory-developed.

Performing primary validation on a non-standard method requires a validation protocol to be established that indicates fitness for purpose by comparison with a standard method, pre-collaborative studies or inter-laboratory collaborative studies.

Primary validation is not required:

- When a non-standard method has already been validated by a national or international organization
- For a method that is validated and accepted by specific industries e.g. Dairy industry, and published in a recognized scientific journal.

If a modified version of a method is required to meet the same specification as the original method, then comparisons should be carried out using replicates to ensure that this is the case. Experimental design and analysis of results must be statistically valid.

4.3 Validation of Methods

All methods submitted by microbiological laboratories for accreditation, must be validated.

The term 'validation' refers to the process that is followed to demonstrate with the provision of objective evidence, that a specific method is suitable for the intended purpose.

The extent of the validation must reflect where possible actual test conditions. For example, this can be achieved by using a naturally contaminated sample or a product contaminated with a known level of microorganisms.

When validation is complete, the laboratory needs to verify on a regular basis that the documented performance can be met e.g. by the use of spiked samples or reference materials incorporating relevant matrices.

4.3.1 Primary validation

Laboratory-developed methods, standard methods that have been modified in such a way that the final result could be influenced (incubation temperature and time, alternative media), standard methods used outside its intended scope as well as rapid methods must undergo primary validation**.**

A limited number of laboratories develop and implement "new" microbiological methods. Therefore not many laboratories have to carry out a primary validation on methods.

4.3.2 Secondary validation (verification)

When a laboratory implements a standard method, which has been developed, and validated elsewhere, only secondary validation (verification) shall apply.

Verification refers to the process where the applicability of the method to all products under test as well as staff competence in the method is established.

The process should include objective evidence that the laboratory is competent to perform the method in accordance with the characteristics that have been published and accepted.

Verification can be accomplished by internal and external quality controls in the lab, inter and intra-laboratory comparative testing as well as participation in proficiency test schemes where available.

4.4 Acceptable methods for the validation of qualitative and quantitative analyses

4.4.1 **Qualitative analyses**

Qualitative microbiological test methods, such as where the result is expressed in terms of detected/not detected and confirmation and identification procedures, should be validated by determining, where appropriate, the specificity, relative trueness, positive deviation, negative deviation, limit of detection, matrix effect, repeatability and reproducibility.

The validation should demonstrate the applicability of the specific method to various types of samples e.g. foods, water and pharmaceuticals.

Preferably naturally contaminated samples should be used (e.g. food poisoning samples) but as these are not always readily obtainable, spiked samples are used more commonly.

Product contamination should be conducted with a pure culture of one strain.
Microorganisms used for verification must be checked for purity.

5. INOCULATION

5.1 If it is known that the organisms in certain types of samples are stressed (e.g. processed foods), the contaminating organisms should also be stressed before inoculation.

5.2 Raw, unprocessed samples must be inoculated with unstressed organisms.

5.3 Each sample type is divided into 3 portions. One sample serves as a negative control; one sample is inoculated with a low sample concentration and one sample with a high sample concentration. The only acceptance requirement for the verification of qualitative methods is to achieve a proportion of approximately 50% between positive and negative results on the same set of samples. This requirement is referred to as fractional recovery.

5.4 A low inoculation level is set at the lowest detection level of the method, e.g. 1 – 5 cfu/25g. The high inoculation level is set at 10 – 50 cfu/25g.

5.5 Inoculums levels that lead to only positive or negative results is of no use in the determination of the lowest detection limit and therefore does not satisfy the validation requirements.

5.6 To demonstrate specificity and sensitivity, a test sample should be inoculated with strains of the specific microorganism under test as well as strains that are considered as potentially competitive.

5.7 If the un-inoculated control sample test positive for the test organisms, the test is invalid.

5.8 Control samples are not included when the verification tests are performed on naturally contaminated samples.

6. QUANTITATIVE ANALYSES

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

6.2 For different types of samples, prepare high, medium and low levels of contamination as well as an un-inoculated control sample. The lowest level should fall at approximately the limit of detection, the medium and high levels one and two log levels higher respectively. Results from counts obtained should be converted to log values and plotted. When using a consensus value, outliers must be removed via statistical analysis.

7. QUALITY CONTROL & QUALITY ASSURANCE

7.1 Proficiency testing

Proficiency testing in this document refers to inter-laboratory comparisons, blind test samples analyzed by the laboratory and proficiency testing schemes.

It is an accreditation requirement for laboratories to participate in proficiency testing relevant to their scope of accreditation. (Refer to SADCAS TR 08 “Proficiency testing and other comparison programme requirements for Testing and Medical laboratories”).

The type and extent of proficiency testing selected by a laboratory must address the risk involved in producing results that are not reliable.

Preference should be given to proficiency testing which use similar sample matrices to samples that are tested by the laboratory.

7.2 Internal Quality Control

Internal quality control consists of all the procedures undertaken by a laboratory for the continuous evaluation of its work.

The main objective is to ensure the consistency of results day-to-day and their conformity with defined criteria.

A program of periodic checks is necessary to demonstrate that variability (i.e. between analysts and between equipment and materials etc.) is under control. All tests included in the laboratory's scope of accreditation need to be covered by the quality control program.

The program may involve:

- the use of spiked samples
- the use of reference materials (including Proficiency Testing Scheme materials)
- replicate testing
- replicate evaluation of test results
- Intra-laboratory comparisons

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

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

7.3 **The use of media**

7.3.1 In-house prepared media

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

- Recovery or survival maintenance of target organisms,
- Inhibition or suppression of non-target organisms,
- Biochemical (differential and diagnostic) properties,
- Physical properties (e.g. pH, volume and sterility).

Raw materials (both commercial dehydrated formulations and individual constituents) should be stored under appropriate conditions, e.g. cool, dry and dark places. All containers, especially those for dehydrated media, should be sealed tightly. Dehydrated media that are caked or cracked or show a color change should not be used. Distilled deionised, or reverse osmosis produced water, free from bactericidal, inhibitory or interfering substances, should be used for preparation unless the test method specifies otherwise.

Shelf life of prepared media under defined storage conditions shall be determined and verified.

7.4 All media (diluents and other suspension fluids) procured ready to use or partially complete require validating before use. Evaluation of performance in recovery or survival of target

organisms and the inhibition or suppression of non-target organisms needs to be fully quantitative. Attributes (e.g. physical and biochemical properties) should be evaluated using objective criteria.

7.4.1.1 As part of the validation, the user laboratory needs to have adequate knowledge of the manufacturer's quality specifications, which include at least the following:

- Name of the media and list of components, including any supplements
- Shelf life and the acceptability criteria applied
- Storage conditions
- Sample regime /rate
- Sterility checks
- Check for growth of target and non-target control organisms used (with their culture collection references) and acceptability criteria
- Physical checks and the acceptability criteria applied
- Proof that the media supports growth of very low counts of organism as specified by certain tests

7.4.1.2 Batches of media should be identifiable. Each one received should be accompanied by evidence that it meets the quality specification. The user laboratory should ensure that notification from the supplier regarding any changes to the quality specification will be received by the laboratory.

7.4.1.3 Where the manufacturer of media procured ready to use or partially complete is covered by a recognized quality system (e.g. ISO 9000-series registered), checks by the user laboratory of conformance of supplies with the specification defined through initial validation may be applied in accordance with the expectation of consistency. In other circumstances, adequate checks would be necessary on every batch received.

7.4.2 Sterility and contamination checks

Checks for sterility and contamination should be performed on all batches of media. These checks are performed by incubating a sample of the media at temperatures and times that would allow the growth of micro-organisms. The incubation should also be performed at the same temperature at which the media will be used. Incubation periods should not be less than 48h (at the temperature where the media will be used). It is advisable to perform growth promotion checks on diluents used, on a regular basis.

7.4.3 Performance Testing

Growth support and recovery tests must be performed on media batches prior to release. The performance testing should include both positive and negative controls. Media should preferably be tested under conditions as close to those in which they will be used.

Details of quality performance test results should be recorded to ensure traceability to the corresponding media batch number. The following information should be recorded:

- Medium batch number, preparation date, date medium was tested
- Sterility evaluation, temperature and time incubated, presence or absence of growth
- Performance assessment (positive and negative controls)
- General comments about acceptance/rejection of the batch
- Analyst signature and date.

7.4.4 **Acceptance or rejection criteria**

Media must perform to the purpose intended. Criteria for the acceptance/rejection of media should be detailed in a procedure. All media which fail to support or suppress growth or which do not demonstrate the required characteristics must be rejected. Under normal circumstances, a laboratory shall not use any expired media.

In circumstances where a laboratory has to use expired media, it is required to demonstrate that proper quality control measures are in place to prove that the media supports growth prior to testing being carried out and that the use of expired media is negotiated with the customer. The use of expired media must take into consideration the risk involved in producing unreliable results and the related application of the results by the customer.

8. THE USE OF REFERENCE MATERIALS AND REFERENCE CULTURES

8.1 Reference materials shall, where possible, be traceable to SI units of measurement, or to certified reference materials. Internal reference materials shall be checked as far as is technically and economically practicable**.**

8.2 Checks needed to maintain confidence in the calibration status of reference, primary, transfer or working standards and reference materials shall be carried out according to defined procedures and schedules**.**

8.3 SADCAS accredited laboratories shall have procedures for safe handling, transport, storage, processing, maintenance and use of reference standards and reference materials in order to prevent contamination or deterioration and in order to protect their integrity**.**

Quality assurance in microbiology laboratories cannot be performed without properly stored, processed and maintained reference cultures**.**

8.4 Reference materials and reference cultures (ISO/IEC 17025:2017 clause 6.4.1 – see Note 1).

8.4.1 Reference Materials

Reference Materials and Certified Reference Materials (see definition in Appendix A) provide essential traceability in measurements and are used, for example;

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

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

8.4.2 Reference cultures

Reference cultures are required for establishing acceptable performance of media (including test kits), for validating methods and for assessing/evaluating on-going performance. Traceability is necessary, for example, when establishing media performance for test kit and method validations. To demonstrate traceability, laboratories must use reference strains of microorganisms obtained directly from a recognized national or international culture collection, where these exist. Alternatively, commercial derivatives for which all relevant properties have been shown by the laboratory to be equivalent at the point of use may be used.

Following the guidance in ISO 11133-1, reference strains may be sub-cultured once to provide reference stocks. Purity and biochemical checks should be made in parallel as appropriate. It is recommended to store reference stocks in aliquots either deep-frozen or lyophilised. Working cultures for routine use should be primary subcultures from the reference stock. If reference stocks have been thawed, they must not be re-frozen and re-used.

Working stocks should not be sub-cultured unless it is required and defined by a standard method or laboratories can provide documentary evidence that there has been no change in any relevant property. Working stocks shall not be sub-cultured to replace reference stocks. Commercial derivatives of reference strains may only be used as working cultures.

- A **reference culture** is a microorganism preparation that is obtained from a culture type collection such as ATCC.
- A **reference stock culture** is a microorganism preparation derived from a reference culture.
- **Working stock cultures** is growth derived from a reference stock culture.
- A **subculture** is the transfer of established microorganism growth on media to fresh media. Growing a reference culture or stock culture from its preserved state (e.g. freeze dried) is not a subculture.

8.6. **Subculture and maintenance**

Incorrect storage and repeated sub-culturing of a culture can lead to alterations and mutations. These alterations occur when a microorganism fails to produce the known and predictable characteristics for which it was selected.

Sub-culturing freeze-dried cultures**:**

Option 1:

- For freeze dried cultures aseptically open the ampoule/vial containing the culture.
- Suspend in a non-selective broth medium and immediately streak from the broth onto the surface of a non-selective agar plate such a way as to obtain single colonies.
- This plate can now be labeled “primary growth”.
- Incubate at the designated temperature.
- After incubation, single colonies are transferred to a non-selective agar medium again in such a way as to obtain single colonies. The Petri dish is labeled “Week 1”.
- After incubation the culture is stored at 4^{o} C – 8^{o}C, the growth is used for quality control purposes for a period of seven days.
- After a period of seven days streak out from the “Week 1” plate onto non-selective agar medium labeling “Week 2”. Carry on in this way for a period of 4 weeks (28 days).
- After the 4 week cycle, a new freeze-dried culture is opened.

Option 2:

Another option is to open a freeze dried culture and then store the culture on cryobeads. A new bead can then be cultured every four weeks.

Further guidance can be obtained from ILAC-G9:2005 - ‘The selection and use of reference materials’**.**

9. THE USE OF POLYMERASE CHAIN REACTION (PCR) TECHNOLOGY

The following points provide guidance on the most essential precautions required to prevent contamination when using Polymerase Chain Reaction technology.

9.1 Ideally tests should be executed in three separate laboratory environments. The first should be used exclusively for the storage and manipulation of core PCR reagents, template material should not be manipulated in this environment. The second should be used for the extraction of nucleic acids and addition of nucleic acids to PCR reactions. The third should be used to house PCR thermo-cyclers and equipment used to manipulate PCR products. Amplification and manipulation of PCR products should take place exclusively in the third environment.

9.2 Each of the environments indicated in 9.1 should have dedicated laboratory coats and disposable gloves.

9.3 Each of the environments indicated in 9.1 should have dedicated equipment (e.g. pipettes, forceps, thermometers, centrifuges, heating blocks, fridges and freezers).

9.4 Where possible each of the areas indicated in 9.1 should have dedicated consumables (e.g. microfuge tubes, pipette tips, gloves, disinfectants and marker pens).

9.5 Ideally filter pipette tips should be used for all liquid manipulation. This is particularly important during nucleic acid extraction and addition of nucleic acids to PCR reactions.

9.6 Where possible methods should ensure that there is a unidirectional flow of activities and personnel from the environment used to prepare PCR master mixtures to the nucleic acid extraction area and then the area where PCR reactions are run on thermo-cyclers.

10. GUIDELINES FOR ASSESSORS (PALCAN: 8.6)

10.1 How are test methods selected by the laboratory?

10.2 Is the laboratory knowledgeable about validation and do they have access to relevant documents?

10.3 Does the laboratory have procedures for assuring the quality of test results generated by test methods used for routine/ad hoc/non-routine testing?

10.4 Does the laboratory have procedures for method validation?

10.5 Who is assigned responsibility for validations? Is the staff trained in conducting validations and evaluating of raw validation data?

10.6 Is there a separation in the technical records between method development and validation?

10.7 Is the validation documentation complete, including the raw data?

10.8 Is there evidence that the method has been successfully transferred to routine use?

10.9 Is there a process to review performance data generated for methods in routine use to demonstrate to client's ongoing fitness for purpose?

10.10 Is the method declared fit for purpose according to the laboratory's acceptance criteria?

10.11 What is the basis of choosing the laboratory's acceptance criteria?

11. RE-VALIDATION/RE-VERIFICATION

The laboratory is expected to continually prove that the validation/verification is still current through the quality control procedures that include the method's performance characteristics/parameters. Partial or full re-validation/re-verification may be considered when:

- new instrument is introduced;
- new samples with new compounds or new matrices are introduced *(Refer to Huber: 8.3);*
- a new location with different environmental conditions is used[p1] [m2]*(Refer to Huber: 8.3);*
- new chemicals and/or reference standards are used *(Refer to Huber: 8.3);*
- modifications are implemented due to analytical problems *(Refer to Huber: 8.3);*
- a review of quality control indicates an established method is changing with time;
- scheduled as per laboratory procedures;
- in the case of the method performance criteria falling outside the acceptance criteria.

Note: In the case where a new analyst is appointed to perform analysis, the laboratory is expected to ensure that the analyst is competent and meets the method's/relevant procedure's performance criteria.

12. REFERENCES

- B. Magnusson and U. Ornemark (eds.) Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics, (2nd ed. 2014). ISBN 978-91-87461-59-0. www.eurachem.org.
- ISO/IEC 17025 - General Requirements for the competence of testing and calibration laboratories. SADCAS TR 26-03 – Criteria for validation of methods used by Chemical laboratories in the Coal, Oil, Petroleum, Metals and Minerals, Food, Pharmaceuticals, water and related industries.
- Ludwig Huber, Validation and Qualification in Analytical Laboratories, second edition. Ludwig Huber Agilent Technologies. Waldbronn, Germany. http://www.labcompliance.com
- PALCAN Guidance for the Validation of Test Methods. Can-P-1629. November 2006. Standards Council of Canada.
- Skoog, D.A. & West, D.M. Fundamentals of Analytical Chemistry, 99th Edition, 2014.
- SLR Ellison and A Williams (eds.) Eurachem/CITAC Guide: Quantifying Uncertainty in Analytical Measurement, (3rd ed. 2012). Available from www.eurachem.org.
- International Vocabulary of Metrology - Basic Concepts and Associated Terms (VIM), (2nd ed. 2008) by Working Group 2 of the Joint Committee for Guides in Metrology.
- GUM: Guide to the Expression of Uncertainty in Measurement. ISO, Geneva (1993).
- (ISBN 92-67-10188-9) (Reprinted 1995: Reissued as ISO Guide 98-3 (2008), also available from http://www.bipm.org as JCGM 100:2008). http://www.bipm.org as JCGM 100:2008)

- VAM Projects 3.2.1 Development and Harmonization of Measurement Uncertainty Principles Part (d) Protocol for Uncertainty evaluation from Validation data. V J Barwick and S L R Ellison. January 2000 LGC/VAM/1998/088.

APPENDIX A - CALCULATION & INTERPRETATION OF Z-SCORES and RELATIVE STANDARD DEVIATION

For each individual result, a z-score is calculated as follows:

$$Z = \frac{(x - X)}{esd}$$

Where: z = the standard score

X = the assigned value, the best estimate of the "true" value

x = the reported value

esd = estimate of variation (target value for standard deviation)

z < 2 = Satisfactory

2<z<3 = Questionable

z>3 = Unsatisfactory

Recommended procedure for estimating within laboratory intermediate precision Relative Standard Deviation (RSD):

- Perform at least 15 determinations at different times and dates using different analysts.
- Determine RSD at low, medium and high levels of microorganism contamination.

Use the following equation:

$$RSD = \sqrt{\sum_{i=1}^{i=n} \frac{[(\log a_i - \log b_i / x_i)]^2}{2p}}$$

$(\log a_i - \log b_i) / x_i$ = Difference between duplicate log results

p = number of duplicate determinations

Personal repeatability can be calculated using the same equation, where

p = amount of tests performed by the specific analyst.

An RSD value of greater than 0,1 indicates a problem.

APPENDIX B - EXAMPLE OF AN APPROACH TO METHOD VALIDATION.

1. One of the first tasks is to decide how extensive the validation exercises should be. For standard methods ISO/IEC 17025:2017 clause 7.2.1.5 states that *"The laboratory shall verify that it can properly perform methods before introducing them by ensuring that it*[p3] *can achieve the required performance."* For novel methods ISO/IEC 17025:2017 clause 7.2.2.1 states that *"The laboratory shall validate non-standard methods, laboratory-developed methods and standard methods used outside their intended scope or otherwise modified. The validation shall be as extensive as is necessary to meet the needs of the given application or field of application."*

   Some form of validation is required for both standard and novel methods. When a standard method is being implemented the work required to demonstrate *"proper operation"* will obviously be less extensive than when an entirely novel method is validated. However, emphasizing distinctions between primary and secondary validations can be distracting. Instead it is suggested that the scale of validation exercises should be adjusted so that laboratory managers are confident they have objective evidence showing their methods are fit for purpose. For a standard method this may involve sourcing a copy of the original primary validation report and supplementing it with evidence the method operates within the specifications of the original validation. For novel methods the validation may result in an extensive series of reports where each validation parameter is investigated in detail.

2. Early in method implementation a standard operating procedure should be prepared. At a minimum the procedure should include the following:

   - A definition of the measurand
   - A description of the purpose of the method (e.g. to demonstrate compliance with a national standard)
   - A list of the matrices that will be analyzed
   - A list of consumables that will be used
   - A list of equipment that will be used and
   - A Step-by-step instruction on how the method will be executed.

3. Uncertainty sources should then be identified and efforts should be made to minimize their impact. This is important since the results of validation experiments are only relevant if they are executed in a controlled environment. Appendix C provides an overview of some uncertainty sources that may be considered during this process. The uncertainty sources and associated control measures should be documented in a short report.

4. Validation parameters that are relevant to the method should be identified. Appendix D provides guidance on the relationship between various microbiology methods and validation parameters.

5. A validation plan should then be drafted. This can be conveniently achieved using a tabulated format such as that presented in Appendix E.

6. The validation plan should include a description of the experiments to be executed. These descriptions may include details of samples to be used, the experimental approach and the acceptance criterion. Guidance on validation parameters and their relevance to methods in microbiology is provided in Appendix F.

7. The experiments described in the validation plan should then be executed.

8. Validation reports should be drafted. Reports may consider individual validation parameters or groups of parameters as deemed appropriate. Ideally reports should include the following headings:

   - title;
   - introduction;
   - validation parameter(s) investigated;
   - acceptance criteria;
   - materials and methods;
   - results and discussion;
   - statement on fitness for purpose;
   - location of raw data;
   - references

   Guidance on validation reporting is provided in Appendix G.

9. Once evidence is in place indicating that method performs within relevant acceptance criteria a final memorandum may be issued indicating that the method is fit for its intended purpose.

APPENDIX C - EXAMPLES OF UNCERTAINTY SOURCES FOR MICROBIOLOGY METHODS

Note: The table below was adapted from Health Protection Agency (2005). Uncertainty of Measurement in testing. National Standard Method QSOP 4 Issue 5.

Source of uncertainty	
Technical competence	All stages of processing a sample, operation of equipment and qualitative or quantitative reading of tests
	Variation between and within members of staff
Sample	Homogeneity of original sample source
	Test portion used in the subsample analysis
	Precision and accuracy of balance or volumetric equipment
	Non-uniform distribution of micro-organisms between subsamples or test portions
	Time, transport and storage conditions between sampling and testing
Homogenization of sample	Degree of heterogeneity of suspensions made from the sample
	Clumping of micro-organisms
	Uneven distribution of micro-organisms
	Insufficient mixing
Dilutions	Accuracy of pre- measured volumes or weights of dilution fluids
	Volume of dilution fluid used
	Degree of mixing at each dilution step
	Number of steps in a serial dilution
	Precision, accuracy and appropriate use of diluting equipment
	Pipette volume used
	Micro-organisms adhering to pipettes
Media and reagents	Quality of raw materials
	Accurate weighing of materials
	Water quality including pH and conductivity
	Personal error in preparation and use of culture media (including appropriate Temperature when adding supplements)
	Heat processing and control
	Adequate mixing
	Degree of dryness of solid media
	Performance of media and reagents such as selectivity and sensitivity
	Shelf life
Inoculation of media	Volume of inoculum
	Equipment used in dispensing, spreading and filtering
	Temperature
	Humidity
	Atmospheric conditions
Reading and interpretation of results	Recognition of target colonies
	Number of colonies counted
	Dilutions chosen for counting (one dilution or more than one dilution)
	Proportion of colonies confirmed
	Properties of media especially when using automated counters

APPENDIX D - RELATIONSHIP BETWEEN VALIDATION PARAMETERS AND METHODS IN MICROBIOLOGY.

Table 1 - Validation parameters relevant to quantitative methods.

Validation parameters	Quantitative methods.									
	Solid media						Liquid medium		Real-time PCR	Microscope slide counts
	Pour plate		Spread plate		Membrane filtration		Multiple tube/well fermentation			
	Non-selective media	Selective media	Non-selective media	Selective media	Non-selective media	Selective media	Non-selective media	Selective media	N/A	N/A
Accuracy	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Selectivity	✗	✓	✗	✓	✗	✓	✗	✓	✓	✓
Detection limit	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Linearity	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Robustness	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Repeatability	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Reproducibility	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Uncertainty of Measurement	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Table 2 - Validation parameters relevant to qualitative methods.

Validation parameters	Qualitative methods with or without pre-enrichment.										
	Solid medium						Liquid medium		End point or Real-time PCR	Microscope Presence/ Absence	Taxonomic identification of strains and isolates
	Pour plate		Spread plate		Membrane filtration						
	Non-selective media	Selective media	Non-selective media	Selective media	Non-selective media	Selective media	Non-selective media	Selective media	N/A	N/A	
Accuracy	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✓
Selectivity	✗	✓	✗	✓	✗	✓	✗	✓	✓	✓	✗
Detection limit	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗
Linearity	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗

Validation parameters	Qualitative methods with or without pre-enrichment.										
	Solid medium						Liquid medium		End point or Real-time PCR	Microscope Presence/ Absence	
	Pour plate		Spread plate		Membrane filtration						
	Non-selective media	Selective media	Non-selective media	Selective media	Non-selective media	Selective media	Non-selective media	Selective media	N/A	N/A	Taxonomic identification of strains and isolates
Robustness	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Repeatability	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Reproducibility	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Uncertainty of Measurement	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗

APPENDIX E - EXAMPLE OF A TABULATED FORMAT FOR DRAFTING VALIDATION PLANS.

Method title	
Description of measurand(s)	
Description of matrices typically analyzed	

Validation parameter	**Brief description of experimental approach used to investigate the parameter.**
Accuracy	Description of samples: Description of Experimental approach: Acceptance criterion:
Selectivity	Description of samples: Description of Experimental approach: Acceptance criterion:
Limit of detection (LOD):	Description of samples: Description of Experimental approach: Acceptance criterion:
Robustness	Description of samples: Description of Experimental approach: Acceptance criterion:
Repeatability	Description of samples: Description of Experimental approach: Acceptance criterion:
Reproducibility	Description of samples: Description of Experimental approach: Acceptance criterion:
Uncertainty of Measurement	Description of samples: Description of Experimental approach: Acceptance criterion:

APPENDIX F - VALIDATION PARAMETERS CONSIDERED IN THE CONTEXT OF METHODS IN MICROBIOLOGY

ISO/IEC 17025:2017 refers but does not limit the evaluation to the following parameters: Measurement range, Accuracy, Bias, Selectivity, limit of detection, Limit of Quantification, Linearity, Robustness, Repeatability, Reproducibility, Uncertainty of Measurement, Cross-sensitivity against interference from the matrix. It follows that these parameters should be considered when planning validation experiments. The points below provide information on how validation parameters could be interpreted for methods in microbiology.

Parameter	Parameter considered in the context microbiology methods	Hypothetical examples to illustrate the practical investigation of validation parameters.
Accuracy: closeness of the agreement between a test result and the accepted reference value	For microbiology methods there are obvious challenges associated with determining the true number of organisms in a sample. Therefore the best estimate of the true value will often have to be used. This could be derived from: 1) A certificate of analysis for a quantified reference materials, 2) The consensus value of a proficiency testing scheme or 3) The result of an alternative reference method. Ultimately the selection of a true value will require an element of judgment on the part of the laboratory. Once the accepted reference value has been selected accuracy can be expressed using the formula below. $Accuracy = \frac{Number\ of\ target\ organsisms}{Accepted\ reference\ val}$ When drafting validation reports it would be ideal to document any bias observed. Bias may be considered to be the systematic measurement error or its estimate, with respect to a reference quantity value. (VIM-3rd edition, ISO international vocabulary of basic and general terms in metrology).	Lyophilized units of a certified reference material are used to spike *E. coli* into ten drinking water samples. The certificate of analysis for the reference material indicates that each lyophilized unit contains 100 *E. coli* organisms. If an average of 95 *E. coli* /100mL were detected in the ten samples accuracy could be expressed as follows. The average number of *E. coli* detected was 95 cfu/100mL The best estimate of the true value is 100cfu/100mL. $Accuracy = \frac{95}{100}\ x\ 100 = 95\%$

Parameter	Parameter considered in the context microbiology methods	Hypothetical examples to illustrate the practical investigation of validation parameters.
Selectivity: The ability of a method to determine accurately and specifically the analyte of interest in the presence of other components in a sample matrix under the stated conditions of the test. Eurachem Guide (1998). The Fitness for Purpose of Analytical Methods. A laboratory guide to method validation and related topics. Copyright LGC (Teddington) Ltd 1998	For microbiology methods the ability of a method to determine accurately and specifically the analyte of interest might best be expressed using the concepts of sensitivity and specificity below. Sensitivity is the Proportion of positive targets (colonies, tubes, wells) correctly assigned by the method. $Senstivity = \frac{True\ positive\ coun}{True\ positive\ count + False\ ne}$ Specificity is the proportion of negative targets (colonies, tubes, wells) correctly assigned by the method. $Specificity = \frac{True\ negative\ cou}{True\ negative\ count + False\ p}$	A method using membrane filtration and selective chromogenic media is used to quantify *E. coli* in ten 100mL surface water samples. A total of 100 colonies were counted. Seventy of the colonies had a presumptive *E. coli* phenotype. Of these 60 were confirmed to be *E. coli* using biochemical tests (true positives) while 10 were found to be of another species (false positives). Thirty colonies did not have a typical *E. coli* phenotype. Of these 25 were found to be of another species (true negatives) while 5 were shown to be *E. coli* using biochemical tests (false negatives). From the example above the true positive count was 60 and the false negative count was 5. Therefore *Sensitivity* = 60 / (60 +5) = 0.92 From example above the true negative count was 25 and the false positive count was10. Therefore *Specificity* = 25 / (25+10) = 0.71
Limit of detection (LOD): The lowest number of microorganisms that can be detected, but in numbers that cannot be estimated. EA Guide EA-04/10: 2002, Accreditation in microbiological laboratories	Attempts to determine the LOD for microbiology methods are complicated by the difficulties associated with preparing low concentrations of target organisms. The points assume that microorganisms in a perfectly mixed matrix have a Poisson distribution. • *"Random uncertainty increases rapidly as the colony count decreases...."* • *"In the count range below about ten, which happens to be of considerable public health*	A commercial lyophilized reference material is obtained with an average count of 30 *E. coli* per unit. The material is used to spike ten 100mL water samples. *E. coli* is then enumerated in the samples using a multiple tube fermentation assay. If *E. coli* is detected in each of the ten samples it can be stated that the method has demonstrated the ability to detect the target organism at a concentration of 30 *E. coli* per 100mL. It may also be stated that the actual detection limit may be lower but cannot be investigated due to practical constraints around the accurate preparation of spiked samples with concentrations of *E. coli* below 30 organisms per sample.

Parameter	Parameter considered in the context microbiology methods	Hypothetical examples to illustrate the practical investigation of validation parameters.
	interest, single measurements are so imprecise that they can hardly be characterized as better than semi-quantitative." • *"At very low particle concentrations all microbiological methods, MPN and colony count included, become essentially P/A methods."* • *"Colony numbers such as 20, 25 or 30 have been traditionally considered the lowest statistically reliable counts."* Given the challenges associated with the preparation of low numbers of microorganisms, experiments which attempt to demonstrate an LOD below 30 organisms may not generate statistically significant results.	
Linearity: Ability of a method to obtain test results proportional to the concentration of the analyte. Eurachem Guide (1998). The Fitness for Purpose of Analytical Methods. A laboratory guide to method validation and related topics. Copyright LGC (Teddington) Ltd 1998	When executing methods to assess linearity it may be appropriate to simultaneously define the upper working limit of the method. Essentially this would be the highest concentration of target organisms in a sample which falls within the methods linear range.	A lyophilized and quantified reference material was used to spike six 100mL drinking water samples. The amount of reference material added was adjusted so that the concentrations of *E. coli* spanned a range consistent with the intended purpose of the method. *E. coli* was enumerated in each sample. These results were plotted against the amount of reference material spiked into each sample. A commercial spreadsheet program was used to assign a linear trend line to the data set. A random distribution about the trend line confirmed the linearity of the method. A systematic trend of data points away from the trend line would have indicated a departure from linearity.
Robustness: A measure of an analytical procedure's capacity to remain unaffected by small, but deliberate variations in method parameters	Despite efforts to execute methods consistently there will always be slight variations in the test conditions. In the context of microbiology two of the most important parameters are incubation time and incubation temperature. Others include slight differences in the concentration of	A method is being validated for the identification of *E. coli* in untreated water samples using membrane filtration technology and a commercially available media. The manufacturer of the media suggests that incubation should proceed for a period of between 20 and 24 hours. An experiment to demonstrate the robustness of incubation time may be executed as follows.

Parameter	Parameter considered in the context microbiology methods	Hypothetical examples to illustrate the practical investigation of validation parameters.
and provides an indication of its reliability during normal usage. Eurachem Guide (1998). The Fitness for Purpose of Analytical Methods. A laboratory guide to method validation and related topics. Copyright LGC (Teddington) Ltd 1998	media prepared, age of the media used and sample holding time. For any method a degree of judgment is required to identify those experimental parameters which could influence the results. Once key experimental variables have been identified the extent to which they are expected to vary should be defined (e.g. incubation time could vary between 20 and 24 hours). Validation experiments should then be conducted to examine the impact of the variable. The concept is best illustrated with an incubation time example.	• Thirty raw water samples are collected. • Each sample is split so that there are two equivalent sets. • The first set is processed and incubated for 20 hours. • The second set is processed and incubated for 24 hours. • A statistical test such as a Student t-test can be used to compare for the two incubation times. • The method may be considered robust if there is no significant difference in the results for the two incubation times.
Repeatability: Closeness of the agreement between the results of successive measurements of the same measurand under the same conditions of measurement. [VIM: 1993 ISO International vocabulary of basic and general terms in metrology]	Repeatability gives an indication of the degree of variation in results that can be expected when one analyst executes a method over a short space of time using the same consumables, media and equipment. It is important to recognize that for microbiology methods repeatability will be a component of reproducibility. Therefore if the reproducibility has been thoroughly examined and deemed to be acceptable, there may be little value in estimating the repeatability of the method separately.	Repeatability can be estimated using the same approach as that provided for reproducibility below. However, variation in the experimental conditions should be minimized as far as possible. The same analyst should execute all the work using one set of consumables and instruments over a short space of time.
Reproducibility: Closeness of the agreement between the results of measurements of the same measurand carried out under changed conditions of	Reproducibility gives an indication of the degree of variation in results that can be expected when different analysts execute a method at different times using different batches of consumables, media and equipment.	Example of a hypothetical experiment to determine the relative standard deviation of reproducibility (*RSDR*) for a quantitative method using split samples. This example was adapted from the Health Protection Agency (2005) National Standard Method QSOP 4 Issue 5 Appendix A. It is recommended that the original reference be consulted for greater detail on the methodology and equations used.

<table>
<tr><th>Parameter</th><th>Parameter considered in the context microbiology methods</th><th>Hypothetical examples to illustrate the practical investigation of validation parameters.</th></tr>
<tr><td>measurement. [VIM: 1993 ISO International vocabulary of basic and general terms in metrology]</td><td>Due to the labile nature of microorganisms the same samples cannot typically be stored over a long time period of time and analyzed using different instruments, analysts and consumables.
This can be partly overcome by expressing reproducibility as a Relative Standard Deviation (RSD) derived from the results for split samples. The concept is illustrated in the example.</td><td>The experiment considers a method used to enumerate E. coli in water samples. It runs over four days. On each day a single water sample is collected and split into two aliquots. The aliquots are analyzed separately with as much variation in the analytical conditions as permitted by the method (e.g. different analysts, different consumables and different equipment). Common logarithms (log_{10}) are taken for the counts. Relative Standard Deviation RSD_R is determined for each paired count. A hypothetical data set is presented below.

Results for split samples
<table><tr><th rowspan="2">Day</th><th rowspan="2">Sample</th><th colspan="2">E. coli cfu/100mL</th></tr><tr><th>Split Result 1</th><th>Split Result 2</th></tr><tr><td>1</td><td>1</td><td>1089.00</td><td>1211.00</td></tr><tr><td>2</td><td>2</td><td>122000.00</td><td>142000.00</td></tr><tr><td>3</td><td>3</td><td>32500.00</td><td>29000.00</td></tr><tr><td>4</td><td>4</td><td>28000.00</td><td>35020.00</td></tr></table>
Common logarithms (log_{10}) are taken from the data above. Relative Standard Deviations are then calculated for each data pair.
<table><tr><th rowspan="2">Day</th><th rowspan="2">Sample</th><th colspan="2">Log_{10} values for counts</th><th rowspan="2">(RSD_R)</th></tr><tr><th>Split 1</th><th>Split 2</th></tr><tr><td>1</td><td>1</td><td>3.037028</td><td>3.083144</td><td>0.010656</td></tr><tr><td>2</td><td>2</td><td>5.086360</td><td>5.152288</td><td>0.009106</td></tr><tr><td>3</td><td>3</td><td>4.511883</td><td>4.462398</td><td>0.007798</td></tr><tr><td>4</td><td>4</td><td>4.447158</td><td>4.544316</td><td>0.015281</td></tr></table>
An estimate of the combined reproducibility RSD_{RC} is obtained by determining the quadratic mean of the relative standard deviations (RSD_R) for each pair. An RSD_{RC} estimate of 0.011 would be derived using the data in the tables above.</td></tr>
<tr><td><u>Uncertainty of Measurement</u>: Parameter associated with the result of a measurement that</td><td>EA Guide EA-04/10: 2002, Accreditation in microbiological laboratories makes to key points which place uncertainty estimates in microbiology in context.</td><td>This example was adapted from the Health Protection Agency (2005) National Standard Method QSOP 4 Issue 5 Appendix A. It illustrates how an estimate of Uncertainty of Measurement (UM) can be derived from the reproducibility data presented in the example above. It uses a</td></tr>
</table>

Parameter	Parameter considered in the context microbiology methods	Hypothetical examples to illustrate the practical investigation of validation parameters.
characterizes the dispersion of the values that could reasonably be attributed to the measurand Eurachem Guide (1998). The Fitness for Purpose of Analytical Methods. A laboratory guide to method validation and related topics. Copyright LGC (Teddington) Ltd 1998	EA Guide EA-04/10: 2002, clause 5.2 states: **"***Microbiological tests generally come into the category of those that preclude the rigorous, metrologically and statistically valid calculation of uncertainty of Measurement. It is generally appropriate to base the estimate of uncertainty on repeatability and reproducibility data alone, but ideally including bias (e.g. from proficiency testing scheme results).*" EA Guide EA-04/10: 2002, clause 5.4 states: *"The concept of uncertainty cannot be applied directly to qualitative test results such as those from detection tests or the determination of attributes for identification. Nevertheless, individual sources of variability, e.g. consistency of reagent performance and analyst interpretation, should be identified and demonstrated to be under control."* The example provided illustrates how an estimate of uncertainty can be derived from reproducibility data.	hypothetical scenario where a result has been obtained for *E. coli* in water of 6.76 x 10^4 cfu/100mL. The following formula is used to determine uncertainty for a given result. • Upper UM estimate = $\log_{10}$(result) + k x RSD_{RC} • Lower UM estimate = $\log_{10}$(result) - k x RSD_{RC} The following data are substituted into the UM equation. • The result is 6.76 x 10^4 cfu/100mL. • The RSD_{RC} from the reproducibility example above was 0.011. • A coverage factor (k) of 2 is selected (See Health Protection Agency (2005) for further guidance on selection of coverage factors). • Upper UM estimate = $\log_{10}(6.76 \times 10^4)$ + 2 x 0.011 = 4.8519 • Lower UM estimate = $\log_{10}(6.76 \times 10^4)$ - 2 x 0.011 = 4.8079 The result above provides an uncertainty estimate on the $\log_{10}$ scale. If a result is required on the natural count scale then the antilog of these two values should be determined as follows • Upper UM estimate = $10^{4.8519}$ = 7.11 x 10^4 cfu/100mL • Lower UM estimate = $10^{4.8079}$ = 6.43 x 10^4 cfu/100mL

APPENDIX G - VALIDATION/VERIFICATION PLAN – SAMPLE TEMPLATE

Method title :	
Description of measurand(s) :	
Performance criteria of verification/validation :	
	1. Repeatability 2. Intermediate precision 3. Reproducibility 4. Bias/trueness 5. Uncertainty of Measurement 6. Accuracy 7. Selectivity/sensibility/specificity 8. Linearity 9. Robustness 10. Limit of determination (LOD) 11. Limit of quantification(LOQ) 12. Negative deviation 13. Positive deviation

Indicate by means of an X whichever is applicable

1. Description of the method

Method title:	
Analyte/Measurand:	
Principle of the method or general principle of the techniques :	
Primary sample type (specify the matrix: water, food, etc.):	
Type of container, additives (specify the type of container: tube/additive/bottle/media, swab etc.):	
Pretreatment of the sample: methods of pre-treatment of the sample (centrifugation, dilution, acidification, alkalinization, extraction etc.):	
Units: mode of expression of the result (cfu/ml, g, ratio etc.	
Interpretation criteria (reference intervals, origin definition criteria, threshold values etc...)	
Laboratory Code/ Sample ID (if it exists) :	
Equipment used :make, model, reference, number of identification etc.	
Reagent used: reference, vendor, expiration date :	
Calibration material(references) : metrological connection	

2. Implementation of the method

Qualified and recognized competent operator(s) having performed the verification/validation of method : identity of the operator	
Validation procedure/instruction/operating procedure : reference and version of the procedure used	
Reference and version of the procedure used :	
Study period : specify dates from : xx/xx/xx to xx/xx/xx, specify if previous results are repeated	
Date of first use : specify xx/xx/xx	

3. Risk control/critical points

5M	**Critical points**	**Elements to be considered**	**Means of competence (staff training , experimental verification…)/documents(procedure, instruction, recording,…) with references to the QMS of the laboratory**
Material(samples)	Identity	Training and information of staff	Laboratory identification procedure
	Container type	Container training sampling instructions	Instructions criteria for acceptance/refusal
	Nature and volume of the sample	Check on receipt	
	Time and temperature before analytical treatment	Logistics management (conditions of transport)	
	Pretreatment: centrifugation, dilution…	Centrifugation, dilution conditions…	Centrifugation, dilution criteria
	Interferences	Training of samplers, inspection on r receipt	Staff training instruction
Material(reagents)	Storage and conditions of use	Pipe metrology (mapping and temperature monitoring)	Metrology traceability
	Inventory management	acceptance upon receipt of reagents	Including on each delivery
	Reconstitution of reagents, standards, controls	Controls pipette metrology	Instruction of reconstitution

5M	Critical points	Elements to be considered	Means of competence (staff training , experimental verification…)/documents(procedure, instruction, recording,…) with references to the QMS of the laboratory
Material(equipment)	Water quality	Measurement of resistivity/sterility	Traceability of verifications
	Drifts monitoring	Periodicity of maintenances Control of equipment (metrological monitoring, connection, ...)	Maintenance records Metrological traceability, CIQ / EEQ
	Contamination	Respect of supplier's operating conditions	Bibliography and / or registration of the on-site test
	On-board computer Setup,	Setup, calibration, connections, archiving of data,	Recordings of test games
Method	Limits of the method (detection, quantification, linearity, interferences, ...)	Limit of detection, limit of quantification, linearity, interferences, ... Sensitivity, specificity	
	Uncertainties of measurement	Calculation of measurement uncertainties (not quantifiable for qualitative methods)	

5M	Critical points	Elements to be mastered	Means of mastery(staff training , experimental verification…)/documents (procedure, instruction, records,…) with references to the QMS of the laboratory
Environmental conditions	Conditions of storage and use of reagents (temperature, humidity…)		
	Environmental requirements for equipment or operator	Environmental requirements for equipment or operator Environmental conditions (static and / or dynamic over time)	Environment for reading results Equipment requirements /user manual Records of environmental conditions
Personnel (staff)	Competence and maintenance of staff competence	Training and assessment of staff skills, training plan. Availability of staff to ensure compliance with the procedure (e.g. subjective reading tests).	Records of staff skills, Traceability of the of the workstations

5. Evaluation performances of the method

Validation parameter	**Brief description of experimental approach used to investigate the parameter**
Accuracy	Description of samples
	Description of experimental approach
	Acceptance criterion
	Results
	conclusion
Selectivity	Description of samples
	Description of experimental approach
	Acceptance criterion
	Results
	Conclusion
Limit of detection(LOD)	Description of samples
	Description of experimental approach
	Acceptance criterion
	Results
	Conclusion
Limit of quantification(LOQ)	Description of samples
	Description of experimental approach
	Acceptance criterion
	Results
	Conclusion
Robustness	Description of samples
	Description of experimental approach
	Acceptance criterion
	Results
	Conclusion
Repeatability	Description of samples
	Description of experimental approach
	Acceptance criterion
	Results
	Conclusion
Intermediate precision	Description of samples
	Description of experimental approach
	Acceptance criterion
	Results
	Conclusion
Reproducibility	Description of samples
	Description of experimental approach
	Acceptance criterion
	Results
	Conclusion
Uncertainty of Measurement	Description of samples
	Description of experimental approach
	Acceptance criterion
	Results
	Conclusion

6. Conclusion/statement on fitness for purpose

7. Technical Manager/Analyst signature and date

APPENDIX H – AMENDMENT RECORD

Revision status	Change			Approved by	Effective Date
	Page No.	Clause	Description of change		
Issue 1	-	-	-	SADCAS CEO	2018-03-24