TR 28-03

CRITERIA FOR VALIDATION, VERIFICATION, UNCERTAINTY OF MEASUREMENT AND QUALITY ASSURANCE IN MICROBIOLOGICAL AND MOLECULAR TESTING

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CONTENTS:

1. Purpose and Scope 3
2. Definitions and References 3
3. Method Validation and Method Verification 5
4. Quality Control & Quality Assurance 10
5. The Use of Media 11
6. The Use of Reference Materials and Reference Cultures 12
7. The Use of Polymerase Chain Reaction (PCR) Technology. 12
8. Veterinary Related Tests 13

Appendix 1: Calculation & Interpretation of Z-Scores 14
Appendix 2: Validation and Verification Parameters 15
Addendum 1: Amendment Record 16

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1. Purpose and Scope

The purpose of this document is to define the technical requirements for quality control, quality assurance, validation and verification of methods and uncertainty of measurement in microbiological and molecular testing laboratories. This document applies to laboratories that analyses microorganisms in products intended for human consumption (food & Water) and animal feeding, environmental samples in the area of food and feed production, handling and samples from the primary production stage.

This document contains supplementary conditions to meet 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.

SANAS acknowledges the use of extracts from the EuroChem Guide - Accreditation for Microbiological Laboratories (AML) 2013 (previously EA Guide EA-04/10: 2002) in this document. CABs are encouraged to refer to this document for more details.

2. Definitions and References

2.1 References

SANAS PM01	SANAS Policy Manual
SANAS A 01	References, Acronyms and Definitions
SANAS TR 57	Criteria for Validation and Quality Assurance in Veterinary Serology
SANAS R 80	Proficiency testing and other comparison programme requirements for testing and medical laboratories
Act No. 19 of 1982	Veterinary and para-veterinary Professions
ISO/IEC 11133	Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media.
ISO/IEC 13528	Statistical methods for use in proficiency testing by interlaboratory comparison
ISO/IEC 17025	General requirements for the competence of testing and calibration laboratories.
ISO/IEC 17043	Conformity assessment - General requirements for proficiency testing
ISO/IEC Guide 99	International vocabulary of metrology - Basic and general concepts and associated terms (VIM)
ISO Guide 30	Reference Materials – Selected Terms and Definitions
ISO 9000	Quality management systems – Fundamentals and vocabulary
ISO 13843	Water quality – Requirements for establishing performance characteristics of quantitative microbiological methods.
ISO 16140-1	Microbiology of the food chain – Method Validation – Part 1: Vocabulary
ISO 16140-2	Microbiology of the food chain – Method Validation – Part 2: Protocol for the Validation of alternative (propriety) methods against a reference method

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ISO 16140-3	Microbiology of the food chain – Method Validation – Part 3: Protocol for the Validation of alternative of reference methods and validated alternative methods in a single laboratory.
ISO 16140-4	Microbiology of the food chain — Method validation — Part 4: Protocol for method validation in a single laboratory.
ISO 16140-5	Microbiology of the food chain — Method validation — Part 5: Protocol for factorial interlaboratory validation for non-proprietary methods.
ISO 16140-6	Microbiology of the food chain — Method validation — Part 6: Protocol for the validation of alternative (proprietary) methods for microbiological confirmation and typing procedures.
AOAC INTERNATIONAL	Methods Committee Guidelines for validation of qualitative and quantitative food microbiological official methods of analysis. Journal of AOAC Int. Vol. 85, No 5, 2002
Eurachem Guide	The Fitness for Purpose of Analytical Methods. A laboratory guide to method validation and related topics.
ILAC G17:01/2021 ILAC	Guidelines for Measurement Uncertainty in Testing

2.2 Definitions

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

False positive rate: The proportion of positive results that are due to non-target organisms. Non-target organisms may be referred to as false positive.

Limit of detection: Applied to qualitative microbiological tests - The lowest number of microorganisms that can be detected, but in numbers that cannot be estimated accurately. The presence or absence of 1cfu in a fixed amount of sample can be investigated and proven in theory. In practice, this is very difficult to prove because of the presence of competing microorganisms as well as sample effects on the organisms. For example, it will be satisfactory to use test samples contaminated with 5 – 10 cfu to determine the limit of detection.

Reference culture: A reference culture is a microorganism preparation that is acquired from a culture type collection. Collective term for reference strains, reference stocks and working cultures

Reference materials: Material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process.

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.

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

Repeatability (within laboratory precision): Can be determined by investigating identical samples under the same conditions. Repeatability can also be assessed in those cases where samples are plated out in duplicate. Repeatability is the closeness of agreement between successive and independent results using the same test sample, test method under the same conditions e.g., analyst, equipment, laboratory, incubation time and temperature etc. The repeatability value is the value below which the difference between two test results under the same conditions may be expected to fall within 95% probability.

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

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. Selectivity for the purpose of this document can be divided into Sensitivity and Specificity.

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

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

Standard Method: Includes methods published in international, regional or national standards or a method which is widely adopted by a specific industry.

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.

Validation: establishment of the performance characteristics of a method and provision of objective evidence that the performance requirements of specified intended use are fulfilled.

Verification: demonstration that a validated method performs, in the users' hands, according to the method's specifications determined in the validation study and is fit for intended purposes.

3. Method Validation and Method Verification

It is an ISO/IEC 17025:2017 requirement that laboratories shall validate non-standard methods, laboratory-developed methods and standard methods that are used outside their intended scope or otherwise modified. This document presents the validation and verification requirements needed to demonstrate the proper operation of standard methods by a given laboratory.

3.1 Validation of methods

3.1.1 Method validation is the process of determining if a method is suitable for its intended purpose. Validation can be classified as primary validation and secondary validation according to its purpose. Method validation in this document refers to primary validation which is an exploratory process for establishing the operational limits and performance characteristics of a new, modified or otherwise inadequately characterized method.

3.1.2 Validation exercises for non-standard methods shall exceed the requirements presented below. The extent to which they exceed the requirements shall be justified by the laboratory concerned. The validation shall be as extensive as is necessary to meet needs of a given application or field of application i.e., matrices.

- Where validated methods are used outside their intended use, the validation of such test methods shall be fully demonstrated. The laboratory shall determine the influences and changes and where they are found to affect the original validation data, method validation shall be done as per ISO/IEC 17025:2017 clause 7.2.2.2, all influences of such changes shall be determined in a new method validation report.

- Performance characteristics of method validation shall be extensive as provided in Appendix 2 of this document.

Note:

- *ISO series 16140 provides additional information on food and feed analysis and ISO 13843 on water analysis.*

- *For other matrices refer to the applicable standard(s) in that field e.g., hydrobiology, toxicology etc.*

3.1.3 A validation plan/procedure shall be drafted prior to the initiation of validation experiments. As a minimum the validation plan/procedure shall include the following:

- Method title;
- Description of measurand(s);
- Description of matrices typically analysed;
- Brief description of the experimental approach used to investigate each parameter, including an acceptance criterion;
- Results of all analysts shall be included in the validation report e.g., raw data; and
- Validation records shall be updated at least once every 5 years (every accreditation cycle) using quality data generated within the accreditation cycle which may include proficiency testing, interlaboratory comparison results, quality control results etc.

3.1.4 Personnel responsible for the development, modification, and validation of methods shall be authorized by laboratory management.

3.2 Method Verification

3.2.1 It is an ISO/IEC 17025:2017 requirement that laboratories shall verify that they can properly perform methods before introducing them by ensuring that the laboratories can achieve the required performance.

Method verification, also referred to as secondary validation, is the process of gathering evidence that a laboratory can meet the specifications established in primary validation.

3.2.2 Verification of methods shall be demonstrated where it is found that the following has been affected:

- methods used by the laboratory has been revised by the issuing body. The supplemented information shall be demonstrated to ensure consistent application.
- validation reports of the standard methods from third parties (e.g., manufacturer methods approved and authorized by AFNOR, AOAC etc.) are available.
- changes that may adversely influence the results of the original validation (e.g., media, matrices, personnel etc.)
- Performance characteristics of method verification shall be extensive as provided in Appendix 2 of this document.

3.2.3 A verification plan/procedure shall be drafted prior to the initiation of verification experiments. As a minimum the verification plan/procedure shall include the following:

- Method title;
- Description of measurand(s);
- Description of matrices typically analysed;
- Brief description of the experimental approach used to investigate each parameter, including an acceptance criterion;
- Results of all analysts shall be included in the verification report e.g. raw data; and
- Verification records shall be updated at least once every 5 years (every accreditation cycle) using quality data generated within the accreditation cycle which may include proficiency testing, interlaboratory comparison results, quality control results etc.

3.2.4 Personnel responsible for the verification of methods shall be authorized by laboratory management.

3.3. Validation and Verification parameters:

3.3.1 The validation and verification parameters shall be selected for different types of methods in accordance with **Appendix 2.**-The list of parameters in Appendix 2 are the minimum requirements for both validation and verification.

3.3.2 The laboratory must select matrices including swabs, if applicable, that are similar to those routinely analyzed. The grouping / selection of these matrices must be justified by the laboratory. As a minimum matrix effect shall be included when determining reproducibility. Matrix effect may be any foreign substances that have potential influence on the sample, this may include matrix aging, microbial analyte stress, cell injury, and matrix-derived inhibition of analyte enrichment/growth. The results of uninoculated (unspiked) samples shall be clearly recorded.

3.3.3 Where a facility uses a commercial test kit in which the methodology and reagents are unchanged from the manufacturer's instructions, the kit does not need to be independently revalidated in the laboratory. Essentially the laboratory only needs to verify that their analysts using their equipment in their environment can apply the method obtaining the same outcomes as defined in the validation data provided in the standard method. Verification of methods by the laboratory must include statistical correlation with existing validated methods prior to use.

3.3.4 It must be noted however, that the documentation for standardised methods of analysis published by standardisation bodies and recognised technical organisations (e.g., ISO, AOAC), etc. varies.

- In some cases, there is no validation report as a basis for the method of analysis, or the performance characteristics are not – or only partially – validated. If this is the case, verification of the laboratory's ability to use the method of analysis is not directly possible then validation is necessary.
- Minor modifications to previously validated methods (e.g., use of a different non-selective or selective growth medium, changes in inoculation techniques, differences in details of sample dilutions as a consequence of expected counts or a slight change in a critical incubation temperature) should demonstrate that there are no significant changes to the expected outcome.

3.3.5 Swabs are a matrix group and shall be treated as such. Therefore, method validation and method verification shall be carried out using suitable methods for swab analysis. The presence of any inhibitory substances must be ruled out prior to testing. Where the laboratory has not enrolled on a formal PT Scheme for Swab Testing, the laboratory shall inform SANAS with suitable alternative for approval as per SANAS R80.

3.3.6 The acceptance criteria and number of replicates presented below shall be used, unless the laboratory is able to justify the use of alternative criteria and replicates:

i) Accuracy:

- The same data generated from method validation exercise shall be used to calculate accuracy.
- Additionally, data from inter-laboratory or Proficiency Testing exercise can be used.
- Acceptance criterion (z-score): $-2 > z < 2$ or any other acceptable criterion

ii) Robustness:

- The same data generated from method validation exercise shall be used to calculate robustness.
- Only applicable when changes are made to critical parameters in a standard method.
- Repeated a minimum of 7 times per matrix.

- Include all matrices as per scope of accreditation.
- Calculation: Any suitable statistical tool can be used to assess the statistical difference between the results obtained under the two conditions, if applicable.
- P-value > 5% / 0.05 or RSD ≤0,1

iii) Repeatability:

- The same data generated from method validation exercise shall be used to calculate repeatability.
- Same measurand tested under the same conditions of measurement a minimum of 7 times per matrices with low, medium and high counts. Where laboratories analyse different water matrices only, validation and verification should be based on expected ranges.
- Ideally these should be naturally contaminated samples. However, if naturally contaminated samples are not available then matrices may be spiked.
- Acceptance criterion: RSD ≤ 0.1 or CV % ≤ 10 %
- For statistical purpose, counts less than 30 cfu maybe excluded.

iv) Reproducibility:

- The same data generated from method validation exercise shall be used to calculate reproducibility.
- Same measurand tested under changed conditions within the laboratory a minimum of 7 times per matrices with low, medium and high counts. Where laboratories analyse different water matrices only, validation and verification should be based on expected ranges.
- Ideally these should be naturally contaminated samples. However, if naturally contaminated samples are not available then matrices may be spiked.
- Acceptance criterion: RSD ≤ 0.1 or CV % ≤ 10 %

v) Sensitivity and Specificity

- Natural samples shall be obtained with mixtures of target and non-target organisms. Ideally these should be naturally contaminated samples, a minimum of 10 typical and atypical colonies.
- Each sample matrix shall be prepared and tested as follows:
  - Uninoculated sample.
  - Sample inoculated with different known concentration of low, medium and high target organism.
  - Sample inoculated with a known concentration of high non-target organism.
  - Sample inoculated with a mixture of target and non-target organism.
- However, if naturally contaminated samples are not available then matrices may be spiked with mixtures of target and non-target reference cultures as stated above.
- The samples shall be analysed using the method being validated or verified.
- The typical and atypical colonies shall be counted. Typical and atypical colonies shall also be confirmed using relevant techniques such as: Vitek analysis, commercial identification kits (e.g., API strips), DNA sequencing or a biochemical test selected by the laboratory. The results shall then be classified as follows:
  - *True positive count* – Typical colonies which are confirmed as the target organism.
  - *True negative count* – Atypical colonies which are not the target organism.
  - *False positive count* – Typical colonies which are not confirmed as the target organism.
  - *False negative count* – Atypical colonies which are confirmed as the target organism.
- Calculate sensitivity and specificity as follows:

$$Senstivity = \frac{True\ positive\ count}{True\ positive\ count + False\ negative\ count}$$

$$Specificity = \frac{True\ negative\ count}{True\ negative\ count + False\ positive\ count}$$

- Acceptance criteria: Sensitivity and Specificity shall both exceed 90%.

vi) Limit of Detection

- LOD shall be done for both qualitative and quantitative test methods. However, a justification shall be provided if the LOD is not done. Criteria will be based on the test method.

vii) False positive rate for MPN methods

- MPN methods yield positive or negative reactions in tubes, vials, or wells. While it is possible to confirm that positive reactions contain target organisms it is difficult to classify negative reactions. This is because negative reactions may not contain microorganisms. As a result, sensitivity and specificity assessments cannot easily be made. Therefore, validations shall focus on determining the false positive rate.
- The false positive rate shall be determined as follows. Samples shall be obtained with mixtures of target and non-target organisms. Ideally these should be naturally contaminated samples. However, if naturally contaminated samples are not available then matrices may be spiked with mixtures of target and non-target reference cultures. Low concentrations of microorganisms shall be used to minimize the likelihood that multiple organisms are trapped in the same tube or well. The samples shall be analysed using the method being validated.
- Microorganisms shall be isolated from a minimum of ten positive reactions. The isolates shall be confirmed using relevant techniques such as: Vitek analysis, commercial identification kits (e.g., API strips), DNA sequencing or a biochemical test selected by the laboratory. The results shall then be classified as follows:

  True positive count – Positive reactions from which the target organism was isolated.

  False positive count - Positive reactions from which the target organism was not isolated.

- Calculate the false positive rate as follows:

$$False\ positive\ rate = \frac{False\ positive\ count}{False\ positive\ count + True\ positive\ count}$$

- Acceptance criterion: The false positive rate shall be less than 20%.

viii) Uncertainty of measurement:

- The uncertainty sources and associated control measures shall be documented in a validation and/or verification report for qualitative and quantitative methods.
- The laboratory must be able to demonstrate the application of uncertainty of measurement for quantitative methods.
- Results obtained from reproducibility evaluation shall be used to calculate final uncertainty of measurement.

Note:

- *Health Protection Agency, 2005. Uncertainty of Measurement in Testing, QSOP4. Issue no: 5, provides more information on Uncertainty of Measurement.*

  Health Protection Agency, 2005. Uncertainty of Measurement in Testing, QSOP4. Issue no: 5

3.4 Validation /Verification Report

3.4.1. Laboratories shall keep comprehensive records of method validation and/or verification, including the procedures used for validation and/or verification, the results obtained and a statement as to whether the method is fit for purpose.

Laboratories shall also continually check that a method of analysis meets the values for the performance characteristics documented in connection with validation/verification. This can be achieved by, for example, tracking the behaviour of internal quality control samples over time. Should the method no longer produce results consistent with the original validation/verification data, the method may be rendered unfit for its intended purpose.

3.4.2 Validation /Verification Reports shall be compiled and approved by technically competent person.

3.4.3 Reports shall include as a minimum the following information:

- Title;
- Introduction;
- Analysts involved;
- Matrices;
- Validation/Verification parameter(s) investigated;
- Acceptance criteria;
- Materials and methods;
- Results and discussion;
- Statement on fitness for purpose;
- Location of raw data; and
- References.

3.5. Validation and Verification Frequency

3.5.1 Validation or Verification records shall be updated at least once within the accreditation cycle as indicated under validation or verification of methods above.

3.5.2 As a minimum, revalidation/reverification shall cover specificity, sensitivity, repeatability, and reproducibility. Re-validation of standard and nonstandard methods can be referred to as verification of methods.

4. Quality Control & Quality Assurance

4.1 Proficiency testing

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

4.1.2 It is an accreditation requirement for laboratories to participate in proficiency testing relevant to their scope of accreditation. (Refer to SANAS R80 “Proficiency testing and other comparison programme requirements for testing, medical laboratories and blood transfusion services”).

4.2 Internal quality control

4.2.1 Internal quality control consists of all the procedures undertaken by a laboratory for the continuous evaluation of its work over and above proficiency testing.

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

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4.2.3 Method or in process quality control shall be prepared and processed as per method (starting from diluent), making use of reference materials and include the following: target organisms (positive control), non-target organisms (negative control) and blank (sterility). This type of quality control shall be performed daily when method is used.

4.2.4 Sterility testing may be combined but results shall be recorded separately to ensure evidence and traceability.

5. The Use of Media

5.1 In-house prepared media

5.1.1 The suitable performance of culture media, diluents and other suspension fluids prepared in-house shall be checked before use, with regard to:

- pH;
- Target organism (positive control);
- If applicable, Non-target organism (negative control); and
- Blank (sterility).

5.1.2 Media shall be tested using the same conditions that will be used during routine analysis.

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

5.1.4 Shelf life of prepared media under defined storage conditions shall be as per manufacturer's prescription or otherwise shall be determined.

5.1.5 If expired dehydrated or prepared culture media, reagents, diluents, and other suspension fluids are to be used then the onus is on the laboratory to demonstrate that their performance has not been compromised. For example, the suitability of the stored media shall be established by checking the media after defined storage times for their physical, chemical, and microbiological performance characteristics as per ISO 11133.

5.2 Ready to use media.

5.2.1 All media (diluents and other suspension fluids) procured ready to use or partially complete require verification before use which includes:

- Target organism (positive control);
- If applicable, non-target organism (negative control); and
- Blank (sterility).

5.2.2 Media shall be tested using the same conditions that will be used during routine analysis.

5.2.3 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;
- pH;
- Sterility check;
- Check of growth of target and non-target control organisms used (with their culture collection references) and acceptability criteria; and
- Physical checks and the acceptability criteria applied.

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5.2.4 Batches of media shall be identifiable and traceable to results. Each batch received shall be accompanied by evidence that it meets the quality specification. The user laboratory shall ensure that notification from the supplier regarding any changes to the quality specification will be received by the laboratory.

5.3 Traceability

Details of quality performance test results shall be recorded to ensure traceability to the corresponding media batch number. The following information shall be recorded, as a minimum:

- Medium batch no., 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; and
- Analyst signature and date.

5.4 Acceptance or rejection criteria

Media must perform to the purpose intended. Criteria for the acceptance/rejection of media shall be documented. All media which fail to support or suppress growth, or which do not demonstrate the required characteristics must be rejected and corrective actions conducted.

6. The Use of Reference Materials and Reference Cultures

6.1 Checks needed to maintain confidence in the status of reference materials and reference cultures shall be carried out according to defined procedures and schedules.

6.2 SANAS accredited laboratories shall have procedures for safe handling, transport, storage and use of reference materials and reference cultures in order to prevent contamination or deterioration and in order to protect their integrity.

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

6.4 No more than 5 transfers or passages shall be made from the traceable reference material. Repeated passage or serial culture increases the risk of contamination and can result in undesirable genotypic and/or phenotypic changes. It is the responsibility of the laboratory to verify the passages on reference material purchased (available on COA). Example: passage 4 may only be sub-cultured once (working culture). Growing a reference culture or reference stock culture from its preserved status (e.g., frozen or lyophilized) is not a passage or subculture.

7. The Use of Polymerase Chain Reaction (PCR) Technology.

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

7.1 Tests shall be executed in three separate laboratory environments (contained areas e.g., laminar flow or biosafety cabinets). 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 (PCR cabinet acceptable). The third should be used to house PCR thermocyclers and equipment used to manipulate PCR products. Amplification and manipulation of PCR products shall take place exclusively in the third environment.

7.2 Each of the environments indicated above shall have dedicated laboratory coats, consumables (e.g., microfuge tubes, pipette tips, gloves, disinfectants and marker pens) and equipment (e.g., pipettes, forceps, and thermometers, centrifuges, heating blocks, fridges and freezers).

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7.3 Filter pipette tips shall be used for all liquid manipulation. This is particularly important during nucleic acid extraction and addition of nucleic acids to PCR reactions.

7.4 Methods shall 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 thermocyclers.

8. Veterinary Related Tests

8.1 Laboratories performing veterinary related tests e.g., *Salmonella* in meat shall comply with all applicable statutes and regulations.

8.2 Laboratories performing veterinary serology related tests shall meet all the requirements set out in the SANAS TR 57. In addition, technical signatories authorized by laboratory management to review and authorize test reports must also meet all the requirements set out in the Veterinary and Para-Veterinary Act.

9. Transition Period

SANAS will apply a 4-month transition period, from the date of implementation of this document.

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Appendix 1: Calculation & Interpretation of Z-Scores

Z-scores:

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

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

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)

Interpretation of results:
z < 2 = Satisfactory
2<z<3 = Questionable
z>3 = Unsatisfactory

Questionable and Unsatisfactory results shall undergo corrective actions to determine root-cause and prevent reoccurrence.

Relative Standard Deviation (RSD):

The results obtained from method validation (Repeatability and Reproducibility) shall be used to determine the RSD

Recommended procedure for estimating Repeatability and Reproducibility Relative Standard Deviation (RSD):

- Perform at least 7 determinations at different times and dates using different analysts.
- For spiked samples, determine RSD at low, medium and high levels of microorganism contamination.

Use the following equation:

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

$(\log a_i - \log b_i) / x_i$ = 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.

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Appendix 2: Validation and Verification Parameters

Validation and Verification parameters relevant to quantitative methods.

Validation / Verification 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	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
LOD	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Sensitivity and Specificity	✗	✓	✗	✓	✗	✓	✗	✗	✓	✗
Linearity	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Robustness *	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Repeatability	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Reproducibility	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Uncertainty of Measurement	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

* Where applicable (changes made to Standard Methods)

Validation and Verification parameters relevant to qualitative methods.

Validation / Verification 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	
LOD	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗
Sensitivity and Specificity	✗	✓	✗	✓	✗	✓	✗	✓	✓	✓	✗
Robustness *	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Repeatability	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Reproducibility	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Uncertainty of Measurement	*To be identified and proven to be under specified conditions*										

***** Where applicable (changes made to Standard Methods)

ADDENDUM 1: Amendment Record

Proposed By:	Section	Change
STC	All	Requirements for Uncertainty of Measurement added. Requirements for re-validation of methods added The document now also applies to Molecular Testing The requirements of ISO/IEC 17025:2005 were changed to ISO/IEC 17025:2017 Extensive Changes to entire document
STC	Section 1 Section 2 Section 3 Section 5 Section 7	Give clarity on the scope and update citation. Updating definitions and adding more references Clarifying requirements of validation and verification Removing ATCC and replace it with traceable reference material. Giving clarity of what 'separate laboratory environments" meant

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