## CRITERIA FOR VALIDATION AND QUALITY ASSURANCE IN VETERINARY SEROLOGY

<table>
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<td>Date of Approval:</td>
<td>2014-06-20</td>
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<tr>
<td>Date of Implementation:</td>
<td>2014-06-20</td>
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1. **Purpose and Scope**

This document provides the managerial and technical accreditation requirements of a Veterinary Serology Laboratory, and shall be used in conjunction with the requirements listed in the ISO/IEC 17025:2005 standard and the reference documents mentioned below.

It applies to all SANAS accredited Veterinary Laboratories. The "clauses" mentioned in this document refer to the corresponding clauses in the ISO/IEC 17025:2005 standard.

2. **References, Definitions and Acronyms**

2.1 References

SANAS PM SANAS Policy Manual
SANAS A01 References, Acronyms and Definitions
SANAS R 80 Proficiency Testing and other comparison programme requirements for testing laboratories

2.2 Acronyms

ASn Analytical sensitivity
ASp Analytical specificity
DSn Diagnostic sensitivity
DSp Diagnostic specificity

3. **General List of Requirements**

All assessments are done in accordance with the relevant ISO/IEC Standards and SANAS accreditation requirements. SANAS documents are available on the SANAS website at www.sanas.co.za.

4. **Serology Scope**

(Refer to SANAS P34 “SANAS Scopes of Accreditation in Veterinary, Pharmaceutical, Blood Transfusion and Good Laboratory Practice (GLP) Facilities”)

The scope of activities of the laboratory shall indicate which services are provided on site. These may include but are not limited to:

a) Agar gel immunodiffusion tests.
b) Complement fixation tests.
c) Enzyme linked immunosorbent assays.
d) Haemagglutination inhibition tests.
e) Indirect fluorescent antibody tests.
f) Microscopic agglutination tests.
g) Rose bengal plate tests.
h) Serum agglutination tests.
i) Serum neutralisation tests.
j) Latex agglutination tests.
k) Specialised tests (specific tests to be listed).
l) Other (free text to be used to describe tests).
5. Technical Requirements

5.1 General (Clause 5.4.1)

It is imperative to ensure that each serological test is validated before it is used for the diagnosis of infectious diseases, whether standard or non-standard, because even highly reproducible test results produced by competent laboratories may still misclassify animals as to their infection status due to an improper assay validation process.

It is the responsibility of the laboratory to ensure that test results are derived from a validated assay.

5.2 Selection of methods (Clause 5.4.2)

The intended purpose of the selected assay must be specified and may include but not be limited to the following:

- Demonstration of freedom from infection in a defined population (country/zone/compartments/herd):
  - Freedom with and/or without vaccination;
  - Re-establishment of freedom after outbreaks.
- Certification of freedom from infection of agent in individual animals or products for trade or movement purposes;
- Eradication of infection from defined populations;
- Confirmatory diagnosis of suspect or clinical cases;
- Estimation of prevalence of infection or exposure to facilitate risk analyses (surveys, herd health status, disease control measures);
- Determination of immune status of individual animals or populations (post-vaccination).

5.3 Validation of methods (Clauses 5.4.5 and 5.4.6)

5.3.1 The relevant chapter in the latest available edition of the OIE Terrestrial Manual should be read for detailed guidance on validation.

5.3.2 An assay is valid only to the extent that test results are valid. A validated assay consistently provides test results that, when applied to target populations, identify animals as positive or negative for an analyte (e.g. antibody or antigen) and, by inference, accurately predicts the infection status of animals with a predetermined degree of statistical certainty (positive and negative predictive values).

5.3.3 In this definition, the diagnostic sensitivity (DSn) and diagnostic specificity (DSp) are performance characteristics of the assay for a given target population. They provide, along with evidence-based data on prevalence of infection in the population being tested, a high degree of confidence in the predictive values of positive and negative test results.

5.3.4 The laboratories shall indicate the following:

- The purpose of the test (e.g. screening test whereby DSn is set high with associated lower DSp or confirmation test with high DSp associated with lower DSn);
- The validation criteria tested (repeatability, ASn, ASp, thresholds or cut-offs, DSn, DSp, reproducibility and robustness) and how they have been affirmed or fulfilled;
• The sources of critical reagents whether obtained commercially as ready to use kits or made in-house;
• The types of data generated (i.e. quantitative, semi-quantitative, qualitative); and
• The types of statistical methods used to analyse the data.

5.3.5 **Laboratory-developed and non-standard methods:**

The laboratories shall follow all the stages of the assay validation pathway and retain the validation status of validated tests as prescribed in OIE Manual. They include:

5.3.5.1 **Stage 1: Analytical characteristics**

Field samples obtained from infected animals or experimentally exposed animals are used to determine the ASp and ASn or lower limit of detection (LOD). The candidate test can also be compared with standard test method. Test repeatability (minimum of three samples tested in triplicate) and preliminary reproducibility (inter-laboratory test comparison) should be determined.

5.3.5.2 **Stage 2: Diagnostic characteristics**

Sera from known infected (and/or vaccinated) and uninfected reference animals and serum standards of known status shall be used. The test cut-off/threshold shall be determined. The DSn and DSp shall be calculated. The number of reference samples to be used for calculating DSn and DSp must be within statistically defined limits depending on diagnostic performance targets.

5.3.5.3 **Stage 3: Reproducibility**

Aliquots or replicates of the same specimens are analysed for one or more variables by participating laboratories to determine reproducibility and ruggedness of the test.

5.3.5.4 **Stage 4: Implementation**

Reference standards should be selected for measurement traceability and accurate interpretation of test results. The test shall fulfil OIE requirements for fitness for intended use to achieve international recognition and deployment to other laboratories.

5.3.6 **Standard methods:**

The power of an assay to correctly classify animals in the target population as infected or uninfected is dependent upon how well the reference animal populations used to validate the assay represent the populations to which the assay will be applied.

5.3.6.1 **Validated commercial kits:**

The laboratories shall check the validation data/report of the manufacturer of the kit to determine if reference sera from animal populations targeted by the test were included in the validation process for tests developed under different geographical conditions. If they were not, the laboratories shall ensure that validation under local conditions is done.

5.3.6.2 **Validated commercial kits used with in-house prepared reagents:**

When a validated commercial kit is used, but some of the buffers are prepared in-house, such buffers should be prepared as per manufacturer’s instructions.
5.3.6.3 Replacement of reagents:

When replacing existing reagent of an assay the new reagent should be verified through parallel testing with the existing one before depletion. The reagents should be traceable to SI Units by calibrating them against standard/reference materials or preparing them following an International Standard or a protocol published in a peer-reviewed journal.

5.3.7 Monitoring and maintenance of validation criteria

The validation status of validated assays shall be retained by means of internal and external monitoring procedures, including the use of certified reference materials (primary or secondary), and plotting of test results on the control chart, revalidation of the assay when it has been modified, amplified or used outside of its intended scope, proficiency testing (comparability assessments), monitoring of precision and accuracy of the test, establishing the proportional relationship between new and old reagents (i.e. multiple parallel runs) before replacement of the latter.

When necessary, the number of reference sera should be expanded and be used to update estimates of DSn and DSp for the population targeted by the assay.

5.4 Assuring the quality of test results (QA) (Clause 5.9)

5.4.1 Internal quality control (IQC)

5.4.1.1 All points addressed in ISO/IEC 17025 clause 5.9 should be followed for IQC.

5.4.1.2 The laboratories shall document procedures for selecting and preparing quality control materials and calculating control limits and target means, as applicable.

5.4.1.3 Decisions shall be made regarding the number of control materials to be used per run of samples and what control rules should be used to accept or reject the test.

5.4.1.4 Assays shall be monitored by plotting sequentially the results of the serum controls on a Control chart to determine if they are in control or out of control (i.e. the expected mean and the control limits at the mean ± a defined standard deviation (commonly ± 2SD) is used).

5.4.1.5 The use of certified reference materials and/or retesting of samples kept in the laboratories under defined optimal storage conditions should also form part of the internal monitoring procedures to assure the quality of test results.

5.4.2 External quality control (EQC)/Proficiency Testing (PT)

5.4.2.1 External quality assurance/PT procedure shall be undertaken in accordance with SANAS R 80 “Proficiency Testing and other comparison programme requirements for testing laboratories”, to provide unbiased external measure of the accuracy of laboratory procedures.
ADDENDUM 1: Amendment Record

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<thead>
<tr>
<th>Proposed By</th>
<th>Section</th>
<th>Change</th>
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<tbody>
<tr>
<td>FM</td>
<td></td>
<td>Changed from Technical Guidance (TG57) to Technical Requirements (TR57) document</td>
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<tr>
<td>sm</td>
<td>Heading</td>
<td>Changed from: Criteria for the Accreditation of Veterinary Serology</td>
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<tr>
<td>STC</td>
<td>1, Par. 1</td>
<td>Added: “and the reference documents mentioned below”</td>
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<tr>
<td>STC &amp; QM</td>
<td>2.1</td>
<td>Included reference to OIE manual and SANAS R80</td>
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<tr>
<td>STC</td>
<td>2.2</td>
<td>Included Acronyms</td>
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<tr>
<td>QM</td>
<td>3, 2nd sentence</td>
<td>Replaced: “SANAS documents are available from SANAS and are made available on receipt of the application for Accreditation. Additional copies of SANAS documentation may be purchased from the office”</td>
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<tr>
<td>STC</td>
<td>4</td>
<td>Serology Scope heading replaced: “3.1 Quality System; Subcontracting of Tests and Handling of Test Items (Clauses 4.2, 4.5 &amp; 5.8)”</td>
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<tr>
<td>STC</td>
<td>5</td>
<td>Added entire section 5</td>
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