OIE Standard on principles and methods of validation of diagnostic assays for infectious diseases

Regional Seminar for OIE National Focal Points on Veterinary Laboratories
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OIE standards

**Terrestrial Animal Health Code** – mammals, birds and bees

**Aquatic Animal Health Code** – fish, molluscs and crustaceans

**Manual of Diagnostic Tests and Vaccines for Terrestrial Animals**

**Manual of Diagnostic Tests for Aquatic Animals**

*Codes and Manuals* available on the OIE website
Chapters 1.1.5. of the OIE Terrestrial Manual and 1.1.2. of the Aquatic Manual

• Identical chapter in both manuals because principles and methods are same

• Title: *Principles and methods of validation of diagnostic assays for infectious diseases*

• Included for the first time in the *Terrestrial Manual* in 2000 and in the *Aquatic Manual* in 2003
Chapters 1.1.5. of the OIE *Terrestrial Manual* and 1.1.2. of the *Aquatic Manual*

- Current version updated by an OIE *ad hoc* Group on validation of diagnostic tests and adopted by the World Assembly of Delegates in 2013

Chapters 1.1.5. of the OIE *Terrestrial Manual* and 1.1.2. of the *Aquatic Manual*

Seven (7) Guidelines have been developed in complement of this standard:

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<tbody>
<tr>
<td>Development and optimisation of antibody detection assay</td>
<td>Development and optimisation of antigen detection assay</td>
<td>Development and optimisation of nucleic acid detection assays</td>
<td>Measurement uncertainty</td>
<td>Statistical approaches to validation</td>
<td>Selection and use of reference samples and panels</td>
<td>Principles and methods for the validation Of diagnostic tests for infectious diseases applicable to wildlife</td>
</tr>
</tbody>
</table>
An eighth one in development:

- Comparability of assays after minor changes in a validated test method (under study)
Content of the Chapters

**Assay Development Pathway**
- Definition of the intended purpose of the assay
  - Study design and protocol
    - Optimisation, Calibration to Standards
    - Reagents and controls

**Assay Validation Pathway**
- Analytical specificity
- Analytical sensitivity
- Diagnostic specificity
- Diagnostic sensitivity
- Cut-off determination
- Select collaborating labs
- Define evaluation panel
- Reproducibility
- Interpretation of test results
- Deployment to other labs

**STAGE 1**
- Analytical characteristics
  - Candidate test compared with standard test method

**STAGE 2**
- Diagnostic characteristics
  - Samples from reference animals or experimental animals (where used)
  - Provisional recognition

**STAGE 3**
- Reproducibility
  - Assay designated as “validated for the original intended purpose(s)”

**STAGE 4**
- Implementation
  - Reference standards selected
  - International recognition (OIE)

**Validation Status Retention**
- Replacement of depleted reagents
- Assay-modifications and re-validation
- Comparability assessments

**Preliminary considerations**
- Monitor precision and accuracy
- Daily in-house QC
- Proficiency testing
Assay Development Pathway

- Definition of the intended purpose of the assay
- Study design and protocol
- Optimisation, Calibration to Standards
- Reagents and controls

STAGE 1
- Analytical specificity
- Analytical sensitivity
- Repeatability and preliminary Reproducibility
- Candidate test compared with standard test method

STAGE 2
- Diagnostic specificity
- Diagnostic sensitivity
- Cut-off determination
- Samples from reference animals or experimental animals (where used)
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STAGE 3
- Select collaborating labs
- Define evaluation panel
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Validation Status Retention

- Replacement of depleted reagents
- Assay-modifications and re-validation
- Comparability assessments
- Monitoring and maintenance of validation criteria
- Monitor precision and accuracy
- Daily in-house QC
- Proficiency testing
I. Assay development pathway

- **Assay Development Pathway**
  - Definition of the intended purpose of the assay
  - Preliminary considerations
  - Study design and protocol
  - Reagents and controls
  - Optimisation, Calibration to Standards
I. Assay development pathway

The most common purposes are to:

• Contribute to the demonstration of freedom from infection in a defined population (country/zone/compartment/herd)
• Certify freedom from infection or presence of the agent in individual animals or products for trade/movement
• Contribute to the eradication of diseases or elimination of infection from defined populations
• Confirm diagnosis of suspect or clinical cases
• Estimate prevalence of infection or exposure to facilitate risk analysis
• Determine immune status of individual animals or populations (post-vaccination)
I. Assay development pathway

Calibration of the assay to standards reagents:

- **International and national reference standards**
  
  OIE standards or other international reference standards. If no available, national reference standards becomes the standard of comparison.

- **In-house standard**
  
  Should be calibrated against an international or national standard.

- **Working standard**
  
  Calibrated against international, national or in-house standard and prepared in large quantities for routine use in each diagnostic run of the assay.
I. Assay development pathway

List of OIE approved international standard sera available on the OIE website:

Assay Development Pathway

- Definition of the intended purpose of the assay
- Study design and protocol
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Assay Validation Pathway

- Analytical specificity
- Analytical sensitivity
- Diagnostic specificity
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- Cut-off determination

- STAGE 1
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Validation Status Retention

- Replacement of depleted reagents
- Assay-modifications and re-validation
- Comparability assessments

- Monitoring and maintenance of validation criteria
- Monitor precision and accuracy
- Daily in-house QC
- Proficiency testing
II. Assay validation pathway

- Definition of the validation:

The validation of a diagnostic test is a process that determines the fitness of this test, which has been properly developed, optimised and standardised, for an intended purpose and for specific specimen(s) and specie(s).

It is an ongoing process.
II. Assay validation pathway

The OIE has defined a chronological validation pathway with 4 stages or steps:

- **Stage 1**: Analytical performance characteristics
- **Stage 2**: Diagnostic performance of the assay
- **Stage 3**: Reproducibility
- **Stage 4**: Programme implementation
II. Assay validation pathway

➢ **Stage 1: Analytical performance characteristics**

- **Analytical sensitivity:** smallest detectable amount of analyte that can be measured with a defined certainty
- **Analytical specificity:** Degree to which the assay distinguishes between the target analyte and other components in the sample matrix
- **Repeatability:** Level of agreement between replicates of a sample both within and between runs of the same test method in a given laboratory
II. Assay validation pathway

- **Stage 2: Diagnostic performance of the assay**
  - Selection of reference animals
  - **Diagnostic specificity**: Proportion of known uninfected reference animals that test negative in the assay
  - **Diagnostic sensitivity**: Proportion of known infected reference animals that test positive in the assay
  - Comparison with existing diagnostic test – Final Threshold determination
II. Assay validation pathway

Stage 3: Reproducibility

- **Definition**: ability of a test method to provide consistent results when applied to aliquots of the same samples tested at different laboratories

- Provides additional data for the estimation of the repeatability

- Provides additional data on the robustness if the test method has been developed as a diagnostic kit.
II. Assay validation pathway

➢ **Stage 4: Programme implementation**

- Extensive application of the test method in different laboratories,
- Interpretation of tests results, and
- International recognition
II. Assay validation pathway

➢ When a diagnostic test method is considered as validated?

• Different replies depending of the test methods, of the samples available and the status of the validation
II. Assay validation pathway

When a diagnostic test method is considered as validated?

STAGE 1
Analytical characteristics

STAGE 2
Diagnostic characteristics

STAGE 3
Reproducibility

STAGE 4
Implementation

- Adjunct tests or procedures can be considered as validated
- Provisional recognition
- Assay designated as “validated for the original intended purpose(s)”
II. Assay validation pathway

When a diagnostic test method is considered as validated?

STAGE 1

- **Adjunct tests or procedures:**
  Tests or procedures that are applied to an analyte that has been detected in a primary assay with the purpose to further characterise this analyte.

  Do not require the validation of the diagnostic perf.

  Example: VNT to type an isolated virus or molecular sequencing to confirm a real time PCR result.
II. Assay validation pathway

➤ When a diagnostic test method is considered as validated?

**STAGE 2**

- **Provisional recognition:**
  
  Situation where samples from the target population are scarce and animals difficult to access (e.g. wildlife)

  Prov. recogn. consists in stage 1 completed + preliminary estimates of DSp and DSe + preliminary estimates of reproducibility
II. Assay validation pathway

- When a diagnostic test method is considered as validated?

**STAGE 3**

- **Validated for the original intended purpose(s):**

  A diagnostic test method that has completed the first three stages of the validation pathway can be designated as “validated for the original intended purpose(s)”. 
Reproducibility

Assay Validation Pathway

Assay Development Pathway

Definition of the intended purpose of the assay → Study design and protocol → Reagents and controls → Repeatability and preliminary Reproducibility

Analytical specificity
Analytical sensitivity

Diagnostic specificity
Diagnostic sensitivity
Cut-off determination

Select collaborating labs
Define evaluation panel
Repeatability

Interpretation of test results
Deployment to other labs

Reference standards selected
International recognition (OIE)

Monitor precision and accuracy
Daily in-house QC
Proficiency testing

Validation Status Retention

Replacement of depleted reagents
Assay-modifications and re-validation
Comparability assessments

Monitoring and maintenance of validation criteria

Preliminary considerations

Optimisation, Calibration to Standards

Candidate test compared with standard test method

Samples from reference animals or experimental animals (where used)

Provisional recognition

Cut-off determination

STAGE 1
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International recognition (OIE)

OIE

STAGE 1
Analytical characteristics

STAGE 2
Diagnostic characteristics

STAGE 3
Reproducibility

STAGE 4
Implementation

OIE
III. Validation status retention

• Check and maintain the performance characteristics,
• Organisation of regular proficiency testing,
• Modifications (e.g. for new subtypes of existing pathogens) and enhancements (e.g. to improve assay efficiency or cost-effectiveness),
• Consideration for other purposes or other species,
• Etc.
Verification of existing assays (in-house validation)

1. A limited verification of both ASp and ASe using available reference materials, whether they be external and/or locally acquired from the target population.

2. A limited Stage 2 validation should be considered in the context of the intended application and target population before the assay is put into routine diagnostic use.
Support - OIE Collaborating Centres

- ELISA and Molecular Techniques in Animal Disease Diagnosis
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Agriculture and Biotechnology Laboratory
IAEA Laboratories
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Thank you for your attention

Organisation Mondiale de la Santé Animale

World Organisation for Animal Health

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