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

OIE Regional Workshop for OIE National Focal Points for Veterinary Products
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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

- Available and downloadable on the OIE website at:
Seven (7) Guidelines have been developed in complement of this standard:

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

An eight 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

Analytical specificity
- Analytical sensitivity

STAGE 1
- Analytical characteristics

STAGE 2
- Diagnostic characteristics

Diagnostic specificity
- Diagnostic sensitivity
- Cut-off determination

STAGE 3
- Reproducibility

Select collaborating labs
- Define evaluation panel

Reproducibility

Interpretation of test results
- Deployment to other labs

STAGE 4
- Implementation

Reference standards selected
- International recognition (OIE)

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

Validation Status Retention

Daily in-house QC
- Proficiency testing
1. Assay development pathway

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

[Diagram showing the pathway]
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:

Reproducibility

Assay Development Pathway

Definition of the intended purpose of the assay

Study design and protocol

Optimisation, Calibration to Standards

Reagents and controls

Preliminary considerations

ASSAY DEVELOPMENT PATHWAY

STAGE 1

Analytical characteristics

Analytical specificity

Analytical sensitivity

STAGE 2

Diagnostic characteristics

Diagnostic specificity

Diagnostic sensitivity

Cut-off determination

STAGE 3

Reproducibility

Select collaborating labs

Define evaluation panel

STAGE 4

Implementation

Cut-off determination

Interpretation of test results

Deployment to other labs

Monitor precision and accuracy

Assay-modifications and re-validation

Reference standards selected

ASSAY VALIDATION PATHWAY

Repeatability and preliminary Reproducibility

Candidate test compared with standard test method

Samples from reference animals or experimental animals (where used)

Provisional recognition

STAGE 1

ASSAY VALIDATION PATHWAY

STAGE 2

ASSAY VALIDATION PATHWAY

STAGE 3

ASSAY VALIDATION PATHWAY

STAGE 4

ASSAY VALIDATION PATHWAY

Validation Status Retention

Replacement of depleted reagents

Assay-modifications and re-validation

Comparability assessments

Monitored and maintenance of validation criteria

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)”.

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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

FAO/IAEA Animal Production and Health Laboratory
Agriculture and Biotechnology Laboratory
IAEA Laboratories
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Email: adama.diallo@iaea.org
Support - OIE Collaborating Centres

• Biotechnology-based Diagnosis of Infectious Diseases in Veterinary Medicine

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Thank you for your attention

Organisation Mondiale de la Santé Animale

World Organisation for Animal Health

Organización Mundial de Sanidad Animal