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
Maputo, Republic of Mozambique
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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*

- Two chapters similar on diagnostic test validation covering all the types of tests and terrestrial and aquatic animals
- Title: *Principles and methods of validation of diagnostic assays for infectious diseases*
- Provides principles and methods for diagnostic test validation
- 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 are in development:

- Development and optimisation of antibody detection assays
- Development and optimisation of antigen detection tests
- Development and optimisation of nucleic acid detection tests
- Measurement of Uncertainty
- Statistical approaches to validation (including Latent Class Models)
- Equivalency (Method comparability)
- Selection and use of reference panels
Content of the Chapters

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

Reproducibility
- Reagents and controls

Assay Validation Pathway
- Analytical specificity
- Analytical sensitivity

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

Diagnostic specificity
Diagnostic sensitivity
Cut-off determination

STAGE 2
- Diagnostic characteristics
- Provisional recognition

Select collaborating labs
Define evaluation panel

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

Repeatability and preliminary Reproducibility

STAGE 4
- Implementation
- Reference standards selected

Assay-modifications and re-validation

Implementation
- International recognition (OIE)

Validation Status Retention
- Replacement of depleted reagents
- Monitor precision and accuracy
- Daily in-house QC

Comparability assessments
- Proficiency testing

STAGE 1
- Analytical characteristics

Study design and protocol
Reproducibility

Assay Development Pathway

- Preliminary considerations
  - 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

STAGE 2
- Diagnostic specificity
- Diagnostic sensitivity
- Cut-off determination

STAGE 3
- Reproducibility
- Candidate test compared with standard test method
- Samples from reference animals or experimental animals (where used)
- Provisional recognition

STAGE 4
- Interpretation of test results
- Deployment to other labs

Assay Validation Pathway

- Select collaborating labs
- Define evaluation panel
- Reproducibility

Validation Status Retention

- Reference standards selected
- International recognition (OIE)
- Monitor precision and accuracy
- Daily in-house QC
- Proficiency testing

STAGE 1
- Analytical characteristics

STAGE 2
- Diagnostic characteristics

STAGE 3
- Reproducibility

STAGE 4
- Implementation

Preliminary considerations

- Defined evaluation panel
- Analytical specificity
- Diagnostic specificity
- Repeatability and preliminary Reproducibility
- Provisional recognition
- Assay designated as “validated for the original intended purpose(s)”
I. Assay development pathway

Definition of the intended purpose(s),
Design of the test method,
Selection of the reference materials,
Calibration, optimisation and standardisation,
Robustness,
Etc.
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:

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 data on the ruggedness 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?

Assay Validation Pathway

- **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?
  - **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 performance.

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?

• **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?

• 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

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
Reproducibility

STAGE 4
Implementation

Assay designated as “validated for the original intended purpose(s)”

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

Optimisation, Calibration to Standards

Assay development path

Preliminary considerations

Study design and protocol

Reagents and controls

Repeatability and preliminary Reproducibility

Candidate test compared with standard test method

Samples from reference animals or experimental animals (where used)

Provisional recognition

Reference standards selected

International recognition (OIE)

Definition of the intended purpose of the assay
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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Support - OIE Collaborating Centres

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Thank you for your attention