VALIDATION AND VERIFICATION OF DIAGNOSTIC TESTS IN VETERINARY MEDICINE

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**OIE list of tests for international trade**

<table>
<thead>
<tr>
<th>Chapter No.</th>
<th>Disease name</th>
<th>Prescribed tests</th>
<th>Alternative tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1.1.</td>
<td>Anthrax</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2.1.2.</td>
<td>Aujeszky’s disease</td>
<td>ELISA, VN</td>
<td>–</td>
</tr>
<tr>
<td>2.1.3.</td>
<td>Bluetongue</td>
<td>Agent id., ELISA, PCR</td>
<td>AGID, VN</td>
</tr>
<tr>
<td>2.1.4.</td>
<td>Echinococcosis/Hydatidosis</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2.1.5.</td>
<td>Foot and mouth disease</td>
<td>ELISA, VN</td>
<td>CF</td>
</tr>
<tr>
<td>2.1.6.</td>
<td>Heartwater</td>
<td>–</td>
<td>ELISA, IFA</td>
</tr>
<tr>
<td>2.1.7.</td>
<td>Japanese encephalitis</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2.1.8.</td>
<td>Leishmaniosis</td>
<td>–</td>
<td>Agent id.</td>
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<tr>
<td>2.1.9.</td>
<td>Leptospirosis</td>
<td>–</td>
<td>MAT</td>
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<tr>
<td>2.1.10.</td>
<td>New World screwworm (Cochliomyia hominivorax) and Old World screwworm (Chrysomya bezziana)</td>
<td>–</td>
<td>Agent id.</td>
</tr>
<tr>
<td>2.1.11.</td>
<td>Paratuberculosis (Johne’s disease)</td>
<td>–</td>
<td>DTH, ELISA</td>
</tr>
<tr>
<td>2.1.12.</td>
<td>Q fever</td>
<td>–</td>
<td>CF</td>
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<tr>
<td>2.1.13.</td>
<td>Rabies</td>
<td>ELISA, VN</td>
<td>–</td>
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<tr>
<td>2.1.14.</td>
<td>Rift Valley fever</td>
<td>VN</td>
<td>ELISA, HI</td>
</tr>
<tr>
<td>2.1.15.</td>
<td>Rinderpest</td>
<td>ELISA</td>
<td>VN</td>
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<tr>
<td>2.1.16.</td>
<td>Trichinellosis</td>
<td>Agent id.</td>
<td>ELISA</td>
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</tbody>
</table>

New OIE “prescribed tests” for international trade need to be validated according to the new OIE principles and methods.

“Alternative tests” are suitable for local settings and for import/export after bilateral agreement.
"Validation is a process that determines the **fitness of an assay or test**, which has been **properly developed, optimised and standardised**, for an **intended purpose**.”

OIE Manual of diagnostic tests and vaccines for terrestrial animals
Chapter 1.1.5 Principles and methods of validation of diagnostic assays for infectious diseases
OIE Reference or commercial laboratories are normally involved in assay development and validation.

Diagnostic laboratories normally start to use diagnostic test at advanced validation stages.

OIE Test development, validation and retention pathway:

1. **Preliminary considerations**
   - Definition of the intended purpose of the assay
   - Study design and protocol
   - Optimisation, Calibration to Standards

2. **Stage 1**
   - Analytical specificity
   - Analytical sensitivity
   - Diagnostic specificity
   - Diagnostic sensitivity
   - Cut-off determination
   - Candidate test compared with standard test method

3. **Stage 2**
   - Select collaborating labs
   - Define evaluation panel
   - Reproducibility
   - Samples from reference animals or experimental animals (where used)
   - Provisional recognition

4. **Stage 3**
   - Interpretation of test results
   - Deployment to other labs
   - Reproducibility
   - Assay designated as "validated for the original intended purpose(s)"

5. **Stage 4**
   - Replacement of depleted reagents
   - Assay-modifications and re-validation
   - Comparability assessments
   - Reference standards selected
   - Implementation
   - International recognition (OIE)

6. **Retention**
   - Monitoring and maintenance of validation criteria
   - Monitor precision and accuracy
   - Daily in-house QC
   - Proficiency testing
Summary

OIE parameters for assay development and validation

1) Definition of the intended purpose(s)
2) Optimisation
3) Standardization
4) Repeatability
5) Analytical sensitivity
6) Analytical specificity
7) Thresholds (cut-offs)
8) Diagnostic sensitivity
9) Diagnostic specificity
10) Reproducibility
Chapter 1.1.5. Principles and methods of validation of diagnostic assays for infectious diseases

1.1.4.1. Development and optimization of Ab detection tests
1.1.4.2. Development and optimization of Ag detection tests
1.1.4.3. Development and optimization of Nucleic Acid Detection tests (NAD)
1.1.4.4. Measurement of Uncertainty (MU)
1.1.4.5. Statistical approaches to validation
1.1.4.6. Comparability of assays after minor changes
1.1.4.7. Selection and use of reference panels

OIE & diagnostic test validation

Appendices

OIE validation template

Application form (validation template)
SOP for OIE Registration of diagnostic kits
Application Form for the Certification of Diagnostic Kits*

as validated fit for specific purposes

Use this form to submit an application to the OIE Procedure for registration of a diagnostic kit.

Before filling in this form and submitting an application, applicants should read “Standard Operating Procedure (SOP) for OIE Registration of Diagnostic Kits: Guide and Administrative Forms”.

OIE

- Validation
- Registration
- Certification

Register of diagnostic kits certified by the OIE as validated as fit for purpose

Fit for purpose means that the kit has to be validated to such a level to show that the kit's results can be interpreted to have a defined meaning in terms of diagnosis or another biological property being examined.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Name of the Diagnostic kit</th>
<th>Name of the Manufacturer</th>
<th>Contact</th>
<th>Type of kit</th>
<th>Purpose(s) validated</th>
<th>Date and Number of registration</th>
<th>Validation studies Abstract Sheet</th>
<th>Kit insert</th>
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</thead>
<tbody>
<tr>
<td>Avian Influenza</td>
<td>BioChek Avian Influenza Antibody test kit</td>
<td>BioChek UK Ltd</td>
<td><a href="mailto:info@biochek.com">info@biochek.com</a></td>
<td>ELISA</td>
<td>see Resolution No XXVII adopted in May 2008 by the World Assembly of the OIE Delegates</td>
<td>May 2008 Registration Number: 20080203</td>
<td>AS BioChek AI Antibody test kit</td>
<td>User’s manual</td>
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<tr>
<td>White spot disease</td>
<td>IQ 2000™ WSSV Detection and Prevention System</td>
<td>GeneReach Biotechnology Corp</td>
<td><a href="mailto:sales@genereach.com">sales@genereach.com</a></td>
<td>PCR</td>
<td>see Resolution No XXVII adopted in May 2008 by the World Assembly of the OIE Delegates</td>
<td>May 2008 Registration Number: 20080304</td>
<td>AS IQ 2000</td>
<td>User’s manual</td>
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<tr>
<td>Bovine spongiform encephalopathy</td>
<td>Pronicos AG - Check Western</td>
<td>Pronicos AG</td>
<td><a href="mailto:info@pronicos.com">info@pronicos.com</a></td>
<td>Western Blot</td>
<td>see Resolution No XXVII adopted in May 2008</td>
<td>May 2008 Registration Number: 20080102</td>
<td>AS Pronicos AG-Check WESTERN</td>
<td>User’s manual</td>
</tr>
</tbody>
</table>

Salmonellosis, PCR
Bovine Tuberculosis, ELISA
White Spot Disease, PCR
Validation Templates

- Validation template for Nucleic Acid Detection [MS Word Document (71.5 KB)]
- Validation template for Extension of an Existing Assay [MS Word Document (82.0 KB)]
- Validation template for Serological Assays [MS Word Document (76.5 KB)]
### SCAHLS approved tests

#### Approved 2014

<table>
<thead>
<tr>
<th>Species</th>
<th>Agent</th>
<th>Test name at time of approval</th>
<th>Principal at time of approval</th>
<th>Laboratory</th>
<th>Test methods</th>
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<tbody>
<tr>
<td>Equine</td>
<td>Hendra virus</td>
<td>Hendra Virus Soluble G IELISA</td>
<td>A Colling</td>
<td>AAHL</td>
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</table>

#### Approved 2012

<table>
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<th>Agent</th>
<th>Test name at time of approval</th>
<th>Principal at time of approval</th>
<th>Laboratory</th>
<th>Test methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine, ovine</td>
<td>Johnne’s disease</td>
<td>High Throughput-Johnne’s direct PCR test</td>
<td>I Marsh</td>
<td>EMAI</td>
<td><a href="#">Test methods</a></td>
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<tr>
<td>Bovine</td>
<td>Johnne’s disease</td>
<td>IDEXX Pourquier ELISA Antibody Screening Testkit</td>
<td>R Santangelo</td>
<td>IDEXX</td>
<td><a href="#">ANZSDP</a></td>
</tr>
<tr>
<td>Equine</td>
<td>Equine influenza</td>
<td>EI H3 TaqMan RT-PCR</td>
<td>A Colling</td>
<td>AAHL</td>
<td><a href="#">Test methods</a></td>
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</table>

#### Approved 2011

<table>
<thead>
<tr>
<th>Species</th>
<th>Agent</th>
<th>Test name at time of approval</th>
<th>Principal at time of approval</th>
<th>Laboratory</th>
<th>Test methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine</td>
<td>Foot-and-mouth disease</td>
<td>FMDV 3ABC C-ELISA</td>
<td>A Colling</td>
<td>AAHL</td>
<td><a href="#">Test methods</a></td>
</tr>
<tr>
<td>Chicken</td>
<td>Avian influenza</td>
<td>BioChek Avian Influenza Antibody Test Kit</td>
<td>Scolexia Pty Ltd</td>
<td>BioChek Smart Veterinary Diagnostics through Scolexia Pty Ltd</td>
<td><a href="#">Test methods</a></td>
</tr>
<tr>
<td>Bovine</td>
<td>Johnne’s disease</td>
<td>BJD Herd Environmental Culture Test</td>
<td>J Gwozdz</td>
<td>DPI Victoria</td>
<td><a href="#">Test methods</a></td>
</tr>
</tbody>
</table>

+ 24 tests...

http://www.scahls.org.au/LabTests/Pages/SCAHLS-approved-tests.aspx
Verification and Comparability
Validation and Verification of Diagnostic Tests in Veterinary Medicine; Axel Colling et al.

When laboratories introduce new methods that are “standard” (including kit methods that have been validated by the manufacturer, methods published in reputable journals, OIE methods, etc) the laboratory is not required to revalidate the method. Rather, laboratories are required to show that they can achieve the performance specifications of the manufacturer (i.e. verification), e.g. comparable precision, accuracy, linearity and regression analysis with the stated values for these parameters.

- the validity of the method needs to be monitored on a continuous basis with internal quality control (QC) and external quality assurance.

NATA Technical Note 17 Guidelines (Oct 2013)

Verification...

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Validation + Verification + Comparability assessments (Equivalence) *

START

Standard method ?, e.g. ANZSDP or validated by an accepted validation body ? such as AOAC, SCAHLS, OIE, etc.

Verification & confirmation of performance characteristics.

If changes are made or matrix differ full or comparative validation

Method peer reviewed paper?

Yes

No

Method modified?

Yes

No

Comparability Study

Is matrix within scope of method?

Yes

No

Method verification

Is matrix generically different?

Yes

No

Matrix verification

Full validation required

Yes

No

*Test validation and verification, NATA Technical Note Number 17, 2013 (modified)
Verification of existing (validated) assays (in-house validation)

Summary of parameters to be assessed

- Analytical Se
- Analytical Sp
- Initial estimates of precision

- Diagnostic Se
- Diagnostic Sp
- Cut-off (?)
- More robust estimates of precision
Development and validation of a 3ABC antibody ELISA in Australia for foot and mouth disease

Verification
- Comparative study: 3 overseas labs (relative DSe and DSp!!!)
- PT round: 6 state labs plus AAHL
  PT panel included 4 positive and 1 neg sample (*LEADDR)
  (2 positive samples were identical to assess precision)
- Testing of FMD free species

Results
- z-scores for within and between laboratory variation were < 3
- Qualitative results were 100% in agreement between participants (DSe and DSp ok)
- extended DSp estimates

*Laboratory Emergency Disease Diagnosis and Response
Conclusions and recommendations

Modern, science-based validation guidelines and templates and processes for registration and certification are available by OIE, SCAHLS and NATA.

There is a need to improve the quality of reporting of studies of diagnostic accuracy. Accurate and transparently reported test performance characteristics improve diagnostic quality and facilitate verification studies (Standards for Reporting Diagnostic Accuracy, STARD initiative).

The use of positive national calibrator control (NQC) samples is effective to measure changes in Se and detect tendencies of intra- and interlaboratory precision (LEADDR).

Collaborative studies, e.g. PT rounds provide useful information about test precision and accuracy in particular when no or limited validation information is available (LEADDR).

The ever changing repertoire of new and unique diagnostic reagents coupled with many novel assay platforms, represent continuous challenges about how to properly validate these assays, e.g. multiplex PCRs and Next Gene Sequencing technologies (NGS).
Thanks for your attention!