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## REPORT OF THE MEETING OF THE OIE STANDARDS COMMISSION

Paris, 29 January – 1 February 2002

The OIE Standards Commission met at the OIE Headquarters from 29 January to 1 February 2002.

Dr Bernard Vallat, Director General, sent his apologies for his absence – he was in Addis Ababa, Ethiopia, attending a meeting of the OIE Regional Representation for Africa. Prof. Marian Trusczyński read a letter from Dr Vallat in which he thanked the Commission for its excellent support. Dr Vallat also stated that the OIE will charge its various commissions to make decisions regarding animal welfare and food-borne diseases, a new disease categorisation system, and a method to review the BSE<sup>1</sup> status of Member Countries. Dr Vallat hoped the OIE Commissions would become more efficient and effective by their close cooperation. Dr James Pearson updated the Commission on staffing changes at the OIE Central Bureau.

The Agenda and List of Participants are given at [Appendices I](#) and [II](#), respectively.

### 1. OIE Reference Laboratories

#### 1.1. New applications for Collaborating Centre and Reference Laboratory status

The Commission determined that there is a need for an OIE Reference Laboratory for anthrax and will solicit nominations from the OIE Delegates by attaching a cover letter with this Standards Commission report.

#### 1.2. Updating the list of Reference Laboratories

The Commission approved a request by the National Veterinary Services Laboratories, United States of America (USA) to be removed from the list of Reference laboratories for rabbit haemorrhagic disease.

The Commission recommends delaying the approval of the Pan African Veterinary Vaccine Centre (PANVAC) as a Reference Laboratory for CBPP<sup>2</sup> until it is fully operational.

The OIE has been notified of the following changes to named experts at OIE Reference Laboratories. The Commission recommends their acceptance:

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1 BSE: Bovine spongiform encephalopathy

2 CBPP: Contagious bovine pleuropneumonia

#### *Foot and mouth disease*

Dr M.G. Mosienyane to replace Mr M. Proteau at the Botswana Vaccine Institute, Gaborone, Botswana.

#### *Contagious bovine pleuropneumonia*

Dr A. Pini to replace Dr F.G. Santini at the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale', Teramo, Italy.

#### *Bluetongue*

Dr P.S. Mellor to replace Dr J. Anderson at the Institute for Animal Health, Pirbright, United Kingdom (UK).

#### *Rabies*

Dr C.A. de Mattos to replace Dr J. Bingham at the Onderstepoort Veterinary Institute, South Africa.

### **1.3. Letter from USA re: Reference Laboratory reporting obligations**

The Standards Commission received recommended guidelines from the Delegate of the USA regarding the reporting obligations of OIE Reference Laboratories. The Commission feels these suggestions are welcome and can serve as a country's internal guidelines and be implemented by the Delegate if desired. It is also noted that OIE Reference Laboratories are nominated and their functions are supported by the Delegate. The Commission believes some of the concerns expressed were addressed by the changes that were made last year to require positive test result reporting by the Reference Laboratories.

### **1.4. Annual Reference Laboratories report for 2001**

Reports had been received from 106/116 Reference Laboratories and 7/8 Collaborating Centres. The Commission commented once again on the impressive range of activities by the Reference Laboratories towards the objectives of the OIE, and the continuing support provided by individual experts to the work of the Standards Commission. The full set of reports will be supplied to Member Countries and to all the Reference Laboratories and Collaborating Centres. The international activities relevant to the work of the OIE are summarised below:

<b>General activities</b>	<b>Percentage of Laboratories carrying out these activities</b>	<b>Percentage of Collaborating Centres carrying out these activities</b>
1a) Diagnostic tests performed	88%	14%
1b) Agent identification performed	83%	29%
2 Production, testing and distribution of diagnostic reagents	82%	29%
3 Research	84%	29%
<b>Specific OIE activities</b>		
1 International harmonisation/standardisation of methods	58%	57%
2 Preparation and supply of international reference standards	51%	29%
3 Collection, analysis and dissemination of epizootiological data	49%	43%
4 Provision of consultant expertise	49%	71%
5 Provision of scientific and technical training	56%	86%
6 Organisation of international scientific meetings	21%	100%
7 Participation in international scientific collaborative studies	55%	71%
8 Publications	81%	100%

## 2. International standardisation of diagnostic tests and vaccines

### 2.1. OIE standardisation programmes for diagnostic tests

#### LIST A DISEASES

*Contagious bovine pleuropneumonia – Coordinator Dr A. Pini*

Dr Pini reported that the candidate reference sera are being tested.

*Classical swine fever – Coordinator Dr S. Edwards*

Dr Steve Edwards provided validation data for a strong positive, a weak positive and a negative reference antisera for use in the virus neutralisation test for classical swine fever. The Commission reviewed the data and recommended that these sera be designated as OIE-approved International Reference Sera for classical swine fever. They are available from the OIE Reference Laboratory at VLA, Weybridge, UK.

#### LIST B DISEASES

*Enzootic bovine leukosis (PCR<sup>3</sup>) – Co-ordinator Dr L. Renström*

Dr Renström reported that no progress has been made on standardising the PCR or on preparing reference sera for enzootic bovine leukosis; she was encouraged to continue to take the project forward.

*Equine influenza – Coordinator Dr J. Mumford*

The Commission agreed that the September meeting of the Expert Surveillance Panel on Equine Influenza should meet at the OIE Headquarters. The Commission recommended that the Panel meet immediately prior to the Standards Commission meeting so that they can provide their report and recommendation on appropriate viral strains for the vaccine to the Commission.

### 2.2. Validation/standardisation of tests for foot and mouth disease

Dr Kris De Clercq, Chairman of the EUFMD<sup>4</sup> Research Group, met with the Commission to discuss the development of additional FMD reference standards. It was noted that there is a need for reference sera to additional subtypes of FMD. In addition, the use of differential FMD ELISAs<sup>5</sup> requires reference sera from vaccinated animals. It is also apparent that there is a need for PCR reference reagents.

The Commission strongly supports the concept that additional reference sera for FMD serological testing are needed due to the high risk of FMD infection globally. The highest priority was given to generating reference sera for the NSP<sup>6</sup> ELISA. Standard reference antigen and protocols should be used to generate these sera. The Commission also agreed that a panel of reference sera was needed to a wider range of FMD subtypes. In addition, existing weak positive reference sera need to be evaluated in the NSP ELISA in order to assess the need to standardise the cut off point of the test. Species-specific sera are needed for the indirect ELISA as well as sera from vaccinated animals. The Commission agreed that new reference sera should be tested by FMD reference laboratories and additional laboratories doing FMD serology. The OIE will contact IAEA<sup>7</sup> to see if their laboratories can generate sera for the NSP ELISA and from vaccinated animals. The Commission will ask the author of the chapter on FMD in the *Manual of Standards for Diagnostic Tests and Vaccines* (the

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3 PCR: Polymerase chain reaction

4 EUFMD: European Commission for the Control of Foot and Mouth Disease

5 ELISA: Enzyme-linked immunosorbent assays

6 NSP: Nonstructural protein

7 IAEA: International Atomic Energy Agency

*Manual*) to add a section regarding the purity of vaccines for NSP. The Commission will refer the question concerning the performance of current weak positive reference sera in the solid phase ELISA test for FMD to the Pirbright laboratory.

### **3. List of prescribed and alternative tests**

#### **3.1. Review of definition of ‘prescribed test’**

The Commission recommends that tests suitable for determining freedom from disease for the four diseases for which the OIE has official ‘disease free’ recognition procedures – FMD, Rinderpest, CBPP and BSE – be described as such in the *Manual* as they may or not be designated also as prescribed tests for international trade.

#### **3.2. Solid-phase ELISA for FMD**

The Commission proposes that the solid-phase structural protein ELISA be designated a prescribed test. The protocol for the test that is in the draft FMD chapter of the 2004 edition of the *Manual* was reviewed and found acceptable with minor changes to be accepted as the standard for the test. ([Appendix III](#)).

#### **3.3. ELISA for rabies serology**

The Commission proposes that the ELISA be designated a prescribed test ([Appendix IV](#)). The Commission did ask for clarification of some points in the validation dossier. The acceptance of this test will require making a small change in the chapter on rabies in the *International Animal Health Code* (the *Code*). The suggested change is as follows: Article 2.2.5.5 point 4) were subjected not less than 3 months and not more than 24 months prior to shipment to an antibody test as described in the *Manual* with a positive result equivalent to at least 0.5 IU/ml of serum [neutralising antibody titration test, and that their serum contained at least 0.5 IU/ml].

#### **3.4. Nonstructural protein tests for FMD**

The Commission discussed the NSP tests for FMD. The Commission recommends that OIE reference sera be developed including vaccine-positives from infected and non-infected animals. The Commission reiterated the discussion from its last meeting that the 3ABC test is appropriate for use in determining FMD status in vaccinated animals on a herd basis. The Commission approved the NSP procedures, including the ELISA, that have been submitted for publication in the 2004 edition of the *Manual* ([Appendix V](#)). In the light of decisions by the FMD and Other Epizootics Commission, the Standards Commission has modified statements regarding use of the NSP assays from its September 2001 meeting:

The NSP ELISA can be used for recovery of free status in countries originally ‘free without vaccination’ in a serosurveillance programme to determine the absence of infection in remaining vaccinated population.

The NSP ELISA can be used in a serological survey in ‘an FMD free country or zone where vaccination is practised’ to regain disease-free status.

#### **3.5. Bovine anaplasmosis (from September 2001)**

The Commission consulted with experts regarding the complement fixation test for bovine anaplasmosis and a recommendation was received to keep the CF as alternative test in the *Manual*.

#### **3.6. Other proposed changes to the list of prescribed and alternative tests**

The Commission recommends that the ELISA be approved as a prescribed test for porcine brucellosis and that the BBAT<sup>8</sup> be moved to alternative test status. It also recommends that the PCR test for enzootic bovine leukosis be listed as an alternative test ([Appendix VI](#) contains all the proposed changes to the list of prescribed tests).

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8 BBAT: Buffered *Brucella* antigen test

#### **4. Questionnaire on bovine tuberculosis**

The Commission discussed the input from the questionnaire sent to Delegates regarding the use and manufacture of tuberculin in their countries. The Commission will send a questionnaire to manufacturers of tuberculin regarding protocols used for production and testing. The OIE Reference Laboratory at the Veterinary Laboratories Agency, Weybridge, UK, is evaluating various aspects of the manufacture and standardisation of tuberculins and will keep the Commission informed.

#### **5. OIE *Manual of Standards of Diagnostic Tests and Vaccines***

##### **5.1. Feedback from Member Countries on the fourth edition of the *Manual***

The Commission welcomed Dr Anthony Cullen to its discussion regarding feedback from Member Countries on the fourth edition of the *Manual*. The Commission will put new, pertinent information, including protocols for new prescribed tests, on its planned Web site in order to keep the *Manual* up-to-date. There are plans to publish the 2000 edition of the *Manual* in French.

##### **5.2. The fifth edition of the *Manual***

The Commission discussed the need for authors for several new chapters on bacterial diseases. A list of potential authors was generated and they will be contacted. The Commission reviewed the list of chapters, authors and reviewers.

#### **6. Preparation of booklet on guidelines**

The Commission has finalised the contents of the booklet on guidelines. It will include: the OIE Standard for Management and Technical Requirements for Laboratories Conducting Tests for Infectious Animal Diseases, OIE Guidelines for the Validation of Diagnostic Assays for Infectious Diseases, OIE Guidelines for International Reference Standards for Antibody Tests, and OIE Guidelines for Laboratory Proficiency Testing.

##### **6.1. Reworking of assay validation paper from the *Review* for the proposed booklet**

The Commission accepted the revised version of OIE Validation of Diagnostic Assays for Infectious Diseases ([Appendix VII](#)).

##### **6.2. OIE Standard – Copyright issue**

The OIE has agreed to pay ISO<sup>9</sup> the royalties associated with the publication of the OIE Standard. This will be included in the sale cost. A statement should be put in the foreword strongly encouraging laboratories doing testing for international trade to meet ISO 17025 standard.

#### **7. Liaison with other Commissions**

- **CODE COMMISSION**

##### **7.1. Proposal to remove atrophic rhinitis of swine from List B**

The Commission will consult with experts in the field regarding issues of trade and atrophic rhinitis.

##### **7.2. Ovine pulmonary adenomatosis**

The Code Commission accepted the recommendation of the Standards Commission not to include ovine pulmonary adenomatosis in the *Code* at this time due to the lack of suitable diagnostic tests.

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9 ISO: International Organization for Standardization

- **FISH DISEASES COMMISSION**

### **7.3. Regulations governing packing and posting of infectious material**

The Commission concurs that IATA<sup>10</sup> UN602 regulations must be complied with as specified in the *Code, Aquatic Animal Health Code* and the *Manual*.

### **7.4. Cost recovery for reagents, standards, testing, etc., for Reference Laboratories**

The Commission proposed to change the OIE Reference Laboratory mandate to include a statement regarding their ability to charge for their services and the reporting requirements for notifiable aquatic animal diseases (see [Appendix VIII](#)).

## **8. Follow-up from the General Session in May 2001**

### **8.1. Resolution No. XXV from General Session, paragraph 2a, on Antimicrobial resistance**

The Commission reviewed the report of the OIE Ad hoc Group on Antimicrobial Resistance that was published in the *Scientific and Technical Review*. It commended the Ad hoc Group on the quality of the report. The Commission will arrange to have the report reformatted so that it can be submitted to the Member Countries and the International Committee for approval as an OIE Guideline recognised by the SPS Agreement<sup>11</sup>.

### **8.2. Resolution No. XX from General Session on emerging diseases**

The Commission discussed ways to provide Member Countries with information on diagnostic procedures for emerging diseases. It was decided that the Standards Commission's Web page would be used for this purpose and the Members would assist the Central Bureau in identifying emerging diseases that should be addressed.

The Commission also recommends that if an emerging disease is suspected samples should be submitted to the national reference laboratory. If the national reference laboratory cannot deal with the emerging disease in question, the Chief Veterinary Officer should seek assistance from one of the OIE Reference Laboratories or Collaborating Centres that have expertise in handling diagnosis of emerging diseases for that particular species.

## **9. Any other business**

### **9.1. The International Symposium on the Quality Control of Equine Influenza Vaccines**

This meeting was sponsored by the European Directorate for the Quality of Medicines and the OIE; it was held in Budapest, Hungary 10–11 December 2001. Changes needed to provide better European and OIE Standards for the evaluation of efficacy of vaccines were discussed and the Chapter in the *Manual* will be changed accordingly. In addition, it was recommended that the OIE Reference Laboratories improve their surveillance and reporting procedures to facilitate the rapid adaptation of appropriate new strains into the vaccines.

### **9.2. Multisystemic wasting disease of swine**

The Commission notes the economic importance of PMWS<sup>12</sup>. However, the Commission would not recommend establishing trade Standards for PMWS due to its reported multifactorial nature.

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<sup>10</sup> IATA: International Air Transport Association

<sup>11</sup> SPS: Agreement on Sanitary and Phytosanitary measures of the World Trade Organization

<sup>12</sup> PMWS: Post-weaning multisystemic wasting syndrome

### 9.3. Standardisation of molecular assays

The Commission recognises the need for standardisation of molecular assays and will ask an expert to attend its next meeting in September to discuss this important issue. An expert has also been invited to contribute a chapter on validation of molecular assays to the fifth edition of the *Manual*.

### 9.4. Advances in prion testing research

The Commission took note of progress being made in this important area.

### 9.5. Standards Commission's Web page

The Commission discussed potential design issues for its new Web page. It was agreed that the site would be used to provide new information that will eventually be included in the *Manual* after gaining the approval of the International Committee, such as techniques for emerging diseases. It will also include links to the *Manual*, the list of Reference Laboratories, available OIE-approved International Reference Reagents, and reports of its meetings.

### 9.6. Pre-Proposal for a CGIAR<sup>13</sup> Challenge Program

The Commission discussed with Dr Vallat potential areas of research in the Challenge Program. The conclusion was that the animal disease that has the greatest impact on trade for developing countries throughout the world is FMD. Almost all underdeveloped countries are infected and have had their trade severely restricted due to this disease. As the overall aim of the programme is the alleviation of poverty, it was agreed that Africa should be a major focus for attention. The second most important disease is therefore African swine fever. This disease has recently spread to more countries in Africa and is having a significant effect on trade. Control is difficult and expensive, as there is no vaccine. Two other animal diseases have a high priority. They are Rift Valley fever, which has had a major impact on trade in Africa, and Newcastle disease, which has resulted in some restrictions on trade and has had a large impact on production of inexpensive animal protein for the developing world.

The following are the research priorities for these diseases:

- Development and implementation of rapid, robust and inexpensive diagnostic tests so that effective control programmes can be implemented,
- Development of improved and inexpensive vaccines, including vaccines that do not require a cold chain,
- Development of a surveillance infrastructure that will allow a country to substantiate the presence of disease free zones.

The Commission envisioned that a research facility in the developed world would form a partnership with a facility in the developing world to carry out this research and implement it in the developing country. A primary goal of this research should be capacity building of the facility in the developing world so that the results are sustainable and a market that is opened can be maintained. The OIE and FAO<sup>14</sup> Reference Laboratories and the OIE Collaborating Centres have the capability to address these diseases, particularly in partnership with laboratories in developing countries, and should be a major contributor to this research.

A resolution will be submitted to the International Committee following a consultation to be held in March 2002 at the OIE headquarters with donors and centres of the CGIAR.

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13 CGIAR: Consultative Group on International Agricultural Research

14 FAO: Food and Agriculture Organization of the United Nations

### **9.7. Joint OIE/WAVLD<sup>15</sup> Symposium in Thailand 2003**

The Commission supported the proposed focus on companion diagnostic kits for use with marker vaccines. It was proposed that the speakers for the following areas be contacted: FMD, classical swine fever, herpesviruses, brucellosis, a general overview of strategies for use of marker vaccines and the potential for marker vaccines in other diseases.

### **9.8. OIE Bluetongue meeting**

The Third International Conference on Bluetongue, African Horse Sickness and Related Orbiviruses is being planned for the autumn of 2002 or spring of 2003 and will be held in Italy.

### **9.9. Dates of next Standards Commission meetings**

The following dates for future meetings were established: 25–27 September 2002 and early in January 2003.

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.../Appendices

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<sup>15</sup> WAVLD: World Association of Veterinary Laboratory Diagnosticians





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Appendix I

## MEETING OF THE OIE STANDARDS COMMISSION

Paris, 29 January – 1 February 2002

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### Agenda

1. OIE Reference Laboratories
  2. International standardisation of diagnostic tests and vaccines
  3. List of prescribed and alternative tests
  4. Questionnaire on bovine tuberculosis
  5. OIE *Manual of Standards for Diagnostic Tests and Vaccines*
  6. Preparation of booklet on guidelines
  7. Liaison with the other Commissions
  8. Follow-up from the General Session in May 2001
  9. Any other business
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**MEETING OF THE OIE STANDARDS COMMISSION**  
**Paris, 29 January – 1 February 2002**

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## PROTOCOL FOR THE PROPOSED NEW PRESCRIBED METHOD FOR FOOT AND MOUTH DISEASE SEROLOGY

### Solid-phase competitive enzyme-linked immunosorbent assay

Rabbit antiserum to the 146S antigen of one of the seven types of FMD virus is used as the trapping antibody at a predetermined<sup>16</sup> optimal concentration in carbonate/bicarbonate buffer, pH 9.6.

Antigens are prepared by inactivating viruses propagated in cell culture with ethyleneimine using the procedures described for vaccine manufacture. The final dilution chosen is that which, after addition of an equal volume of diluent, gives an absorbance on the upper part of the linear region of the titration curve (optical density approximately 1.5). Phosphate buffered saline (PBS) containing 0.05% Tween 20 and phenol red indicator is used as a diluent (PBST).

Guinea-pig antisera, prepared by inoculating guinea-pigs with 146S antigen of one of the seven serotypes and preblocked with normal bovine serum, is used as the detecting antibody. Predetermined optimal concentrations are prepared in PBS containing 0.05% Tween 20, and 5% dried, nonfat skimmed milk (PBSTM).

Rabbit (or sheep) anti-guinea-pig immunoglobulin conjugated to horseradish peroxidase and preblocked with NBS is used at a predetermined optimum concentration in PBSTM.

Test sera are diluted in PBST.

Collaborative studies have shown this test to be more specific and as sensitive as the liquid-phase blocking ELISA (1).

#### • Test procedure

- i) ELISA plates are coated with 50 µl/well rabbit anti-FMD virus antigen, diluted in carbonate/bicarbonate buffer, pH 9.6, and left overnight in a humid chamber at 4°C.
- ii) The ELISA plates are washed five times with PBS.
- iii) Add 50 µl of the FMD virus antigen diluted in blocking buffer to each well of the ELISA plates, cover, and place on an orbital shaker at 37°C for 1 hour, with continuous shaking.
- iv) After washing five times with PBS, add 40 µl of blocking buffer to each well, followed by 10 µl of test sera (or control sera), giving an initial serum dilution of 1/5.
- v) Immediately add 50 µl of guinea-pig anti-FMD virus antisera diluted in blocking buffer, giving a final serum dilution of 1/10.
- vi) Cover the plates and incubate on an orbital shaker at 37°C for 1 hour.
- vii) After washing five times with PBS, add 50 µl of anti-guinea-pig Ig conjugate diluted in blocking buffer; cover and incubate for 1 hour at 37°C on an orbital shaker.
- viii) After washing five times with PBS, add 50 µl of orthophenylene diamine containing 0.05% H<sub>2</sub>O<sub>2</sub> (30%, w/v) to each well.
- ix) The reaction is stopped after 10 minutes by the addition of 50 µl of 2 M sulphuric acid. The plates are read at 492 nm on a spectrophotometer linked to a microcomputer.
- x) *Controls*: on each plate two wells are used for conjugate control (no guinea-pig serum), four wells each for strong and weak positive sera, two wells for negative sera, and four wells for 0% competition (no test sera).

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<sup>16</sup> A checkerboard titration of the rabbit-trapping antiserum, the guinea-pig antiserum and the anti-guinea-pig antiserum is performed. Before using the antigen-trapping ELISA or the liquid-phase blocking ELISA, each of these reagents is titrated, one against another, keeping the third reagent at a fixed concentration. In this way the optimal dilutions (for positive colour and low background colour) can be determined. These 'predetermined' dilutions are then used for all future tests using these particular batches of reagents.

### Appendix III (contd)

- xi) *Interpretation of the results:* a percentage of inhibition is calculated for each well, either visually or using a suitable computer program ( $100 - [\text{optical density of each test or control value}/\text{mean optical density of the 0\% competition}] \times 100\%$ ), representing the competition between the test sera and the guinea-pig anti-FMD virus antisera for the FMD virus antigen on the ELISA plate. Greater than 60% inhibition is positive. (N.B. blocking buffer: 0.05% [w/v] Tween 20, 10% [v/v] normal bovine serum, 5% [v/v] normal rabbit serum.)

### **Reference**

1. MACKAY D.K.J., BULUT A.N., RENDLE T., DAVIDSON F. & FERRIS N.P. (2001). A solid-phase competition ELISA for measuring antibody to foot-and-mouth disease virus. *J. Virol. Methods*, 97 (1–2), 33–48.
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## PROTOCOL FOR THE PROPOSED NEW PRESCRIBED ENZYME-LINKED IMMUNOSORBENT ASSAY FOR RABIES SEROLOGY

### Enzyme-linked immunosorbent assay

This indirect enzyme-linked immunosorbent assay (ELISA) allows a quantitative detection of rabies antibodies in individual dog and cat serum samples. A minimum of 0.5 International Units (IU) per ml rabies antibodies is required to protect against rabies infection, according to the World Health Organization recommendations (WHO 1992. Expert Committee on Rabies, Eighth Report. World Health Organization, Geneva, Technical Report Series No. 824).

The reaction is composed of three steps:

1. Each serum sample is placed in a well sensitised with inactivated rabies viral antigens. Antibodies present in the sample bind to the viral antigens coated at the bottom of the well.
2. After a wash step, Protein A/peroxidase conjugate is added. It fixes to the previously captured immunoglobulins (antibodies), forming a complex: (rabies Ag)–(Ab anti-rabies)–(Protein A/peroxidase).
3. Excess conjugate is eliminated by a wash step. The enzyme linked to the complex is revealed by the addition of a substrate that is transformed into a coloured product. After stopping the reaction, the optical densities are measured.

#### • Preparation of antigen

Rabies virus strain G52 (Pasteur derivative) is grown in low passage NIL2 cells or hamster embryo cell line. The virus harvest is clarified to eliminate cell debris by gel filtration and the virus suspension is inactivated by betapropiolactone .

#### • Reagents<sup>17</sup>

Microplate containing six 16-well strips sensitised with rabies antigens. Use in the 4 weeks after opening the sachet, which must be closed after each use;

Conjugate (CJ): Protein A/peroxidase (10 × concentrated). Dilute ten times in the conjugate diluent (CD) and use within 24 hours following dilution;

Buffered peroxidase substrate (PS); 3,3', 5,5'-Tetramethylbenzidine;

Negative control serum (N), Specific pathogen free sera diluted in Stabilzyme, a commercial stabiliser provided by Surmodics Inc., MN 55344-3523 USA;

Positive control serum (P), hyperimmune sera from vaccinated dogs diluted in Stabilzyme a commercial stabiliser provided by Surmodics Inc., MN 55344-3523 USA;

Sample diluent (SD), PBS buffer, pH = 7.8, including 0.28% w/v caseine, 0.055 % v/v X100 Triton; 0.55% w/v PEG, 0.056% w/v SDS, 1% w/v, PVP, 0.42% w/v Tetronic and 1% v/v heat-inactivated bovine serum;

Wash solution (W), Tris/NaCl buffer, pH = 7.5, including 1% Tween 20;

Conjugate diluent (CD), Tris buffer, pH = 8;

Stop solution (S), 4 N sulphuric acid solution.

Diluted reagents should be stored at 5°C ± 3°C. Place all reagents at laboratory temperature for at least 1 hour prior to use.

#### • Samples

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17 Available from Synbiotics Europe S.A.S., 2 rue Alexander Fleming, 69367 Lyon Cedex 07, France.

#### Appendix IV (contd)

The reaction is performed on heat-inactivated (30 minutes at 56°C) individual serum diluted at 1/100. Testing the appropriate set of dilutions of the OIE International Standard Rabies Serum containing 6.7 IU/ml is necessary (available from the OIE Reference Laboratory for Rabies, Nancy, France).

Serum samples should be stored at 5°C ± 3°C. For prolonged storage, the samples should be frozen at -20°C.

- **Preliminary predilution steps**

Strictly comply with the procedure indicated below. Use negative and positive controls in duplicate for each test run and/or for every plate.

- i) Carefully set up the distribution and identification of controls and samples using the plans below.
- ii) Prepare the sera to be tested. Dilutions are performed in the sample diluent (SD) as follows: the samples are first prediluted at 1/10 in a blank microplate (10 µl of sample in 90 µl of SD).
- iii) For serum titration, a set of six dilutions of the OIE Standard Serum should be performed either in tubes or a blank microplate, starting with the initial dilution 1/10, then 1/30, 1/100, 1/300, 1/1000 to the final dilution 1/3000. This set of dilutions of the OIE Standard Serum should be included in each test run and/or in microplates with an initial dilution of 1/10, 1/30, 1/100, 1/300, 1/1000 and 1/3000.

The following scheme to prepare the appropriate set of dilutions is recommended:

OIE dilution	Preparation
1/10	10 µl of OIE International Standard Rabies serum + 90 µl of sample diluent
1/30	10 µl of OIE International Standard Rabies serum + 290 µl of sample diluent
1/100	10 µl of the 1/10 dilution + 90 µl of sample diluent
1/300	10 µl of the 1/30 dilution + 90 µl of sample diluent
1/1000	10 µl of the 1/100 dilution + 90 µl of sample diluent
1/3000	10 µl of the 1/300 dilution + 90 µl of sample diluent

This range of dilution of the OIE Standard Serum should be present in every plate.

- **Test procedure**

- i) *Control distribution*: dispense 90 µl of sample diluent, and add 10 µl of the negative control into wells A1 and A2, and 10 µl of the positive control to wells B1 and B2.
- ii) *Distribution of samples and OIE Standard Serum dilutions*: dispense 90 µl of sample diluent, add 10 µl of either 1/10 sample predilution or each OIE serum dilution from 1/10 to 1/3000 into the test wells and mix thoroughly.

Samples and OIE Standard Serum dilutions should be tested in duplicate. The following distribution plans (reporting final testing dilutions) are recommended:



**Antibody quantification (final dilution)**

	1	2	3	4
A	N 1/10	N 1/10	S1 1/100	S1 1/100
B	P 1/10	P 1/10	S2 1/100	S2 1/100
C	OIE 1/100	OIE 1/100	S3 1/100	S3 1/100
D	OIE 1/300	OIE 1/300	S4 1/100	S4 1/100
E	OIE1/1000	OIE1/1000	S5 1/100	S5 1/100
F	OIE 1/3000	OIE 1/3000	S6 1/100	S6 1/100
G	OIE 1/10000	OIE 1/10000	S7 1/100	S7 1/100
H	OIE 1/30000	OIE 1/30000	S8 1/100	S8 1/100

Strips should always be placed on the frame so that both washer and reader can be used. Cover the wells with adhesive film, cut to the necessary length by the number of strips used. Mix by gently shaking the plate manually or by using a plate agitator.

iii) Incubate samples for 1 hour  $\pm$  5 minutes at 37°C  $\pm$  3°C.

iv) *Reagent dilution:*

*Wash buffer:* dilute the concentrated washing solution (W) 1/10 in distilled or demineralised water.

*Conjugate:* dilute the concentrate (CJ) 1/10 in the conjugate diluent (CD); 2 ml is needed for one strip, i.e. 20  $\mu$ l of CJ in 1.88 ml of CD.

v) Carefully remove the adhesive film and wash four times.

vi) Add 100  $\mu$ l of diluted conjugate to all the wells and cover with a new piece of adhesive film.

vii) Incubate conjugate for 1 hour  $\pm$  5 minutes at 37°C  $\pm$  3°C.

viii) Carefully remove the adhesive film and wash four times.

ix) Add 100  $\mu$ l of buffered peroxidase substrate (PS) per well. Do not cover with adhesive film at this stage. Mix by shaking the plate gently manually or use a plate agitator to ensure correct homogenisation.

x) Incubate for 30  $\pm$  5 minutes at laboratory temperature (20°C  $\pm$  5°C), shielded from light.

xi) Add 50  $\mu$ l of stop solution (S) per well. Mix by gently shaking the plate manually or by using a plate agitator. Make sure that no bubbles occur in the wells. Carefully wipe the bottom of the wells.

xii) Measure the optical density (OD) bichromatically at 450 and 630 nm or monochromatically at 450 nm (in the yellow band).

• **Antibody quantification: expression and interpretation of results**

Titre calculation using the regression curve

i) Calculate the average OD value for each sample tested and each OIE serum dilution.

ii) Calculate the natural logarithm (*ln*) value for each average OD and the *ln* value of the rabies Ab concentration for each OIE dilution (from 6.7 to 0.0223 IU/ml, without taking into account the 1/100 testing dilution factor).

#### Appendix IV (contd)

- iii) Plot the  $\ln$  (OD) (Y-axis) as a function of the  $\ln$  (rabies Ab concentration) (X-axis) in order to draw the reference curve for the OIE standard serum.
- iv) Using all individual results obtained for the OIE standard serum dilutions, perform a linear regression between  $\ln$  rabies Ab concentrations (expressed in ELISA Units (EU)/ml) and  $\ln$  (OD), to establish the corresponding mathematic model:

$$\ln \text{ rabies Ab concentration (EU/ml)} = a + b \times \ln \text{ OD}$$

- v) For each tested sample, calculate the average OD value and then the rabies antibody concentration of the sample expressed as 'equivalent units per ml' (eu/ml), from the established model:

$$\text{Sample Rabies Ab concentration (eu/ml)} = e (a + b \times \ln \text{ OD})$$

- **Test validation**

The results of each test run (or for each plate) are valid:

- if the optical density obtained with the positive control (OD P) is greater than or equal to 0.300, and
- if the optical density obtained with the negative control (OD N) is less than  $0.50 \times \text{OD P}$ .
- if the correlation coefficient between  $\ln$  ODs and  $\ln$  rabies Ab concentrations for the OIE standard serum is greater than 0.95.

- **Examples**

*Positive control:*

$$\text{OD well B}_1 = 0.610 \quad \text{OD Well B}_2 = 0.690 \quad \Rightarrow \quad \text{OD P} = 0.650$$

*Negative control:*

$$\text{OD well A}_1 = 0.190 \quad \text{OD well A}_2 = 0.210 \quad \Rightarrow \quad \text{OD N} = 0.200$$

$$\text{Sample 1:} \quad \text{OD well 1} = 1.790 \quad \text{OD well 2} = 1.750 \quad \Rightarrow \quad \text{OD} = 1.770$$

$$\text{Sample 2:} \quad \text{OD well 1} = 0.350 \quad \text{OD well 2} = 0.390 \quad \Rightarrow \quad \text{OD} = 0.370$$

*Test validation*

$\text{OD P} = 0.650 > 0.300$  and  $\text{OD N} = 0.200 < 0.50 \times 0.650 = 0.325$ , therefore the test is valid.

- **Results and interpretation (quantitative antibody titration)**

If the calculated titre is  $\geq 0.6$ , the animal is considered as protected.

If the calculated titre is  $< 0.6$ , the animal is considered as potentially unprotected. As the ELISA is intended as a screening test, a confirmatory fluorescent antibody virus neutralisation (FAVN) test may be carried out.

## PROTOCOL FOR NONSTRUCTURAL PROTEINS METHODOLOGY FOR DETERMINING FOOT AND MOUTH DISEASE STATUS IN VACCINATED ANIMALS ON A HERD BASIS

### Nonstructural protein antibody tests

Antibodies to expressed, recombinant FMD virus non-structural (NS) proteins can be measured by ELISA or immunoblotting. An MAb trapping (MAT) ELISA for detecting antibody to 3ABC (2) and blocking ELISAs for detecting antibody to 3AB or 3ABC (5) have been shown to be sensitive, specific and reliable in a number of laboratories. The simultaneous detection of antibody to several NS proteins in a single test by ELISA (3, 5) or by enzyme-linked immuno-electrotransfer blot (EITB), a type of Western blot (1), is useful for confirmation of animals positive for antibody to 3AB or 3ABC. There are currently no internationally recognised standards for antibody to FMD virus NS proteins, but an application for these tests is described in detail below.

- **Indirect enzyme-linked immunosorbent assay**

#### *Preparation of recombinant antigens*

- The five bioengineered FMD virus NS proteins 3A, 3B, 2C, 3D and 3ABC are expressed in *Escherichia coli* C600 by thermo-induction. The 3D polypeptide is expressed in its complete form (4), whereas the rest of the proteins are obtained as fusions to the N-terminal part of the MS-2 polymerase gene (6).
- The expressed polymerase is purified over phosphocellulose, followed by poly(U) Sepharose columns. The fused proteins 3A, 3B, 2C and 3ABC are purified by sequential extraction of the bacterial extracts with increasing concentrations of urea. The 7M fraction containing the fusion proteins is further purified on a preparative 10% SDS/PAGE (sodium dodecyl sulphate/polyacrylamide gel electrophoresis). The fusion protein band is excised from the gel and electroeluted (4).
- A mixture containing 20 ng/ml of each one of the purified recombinant polypeptides is separated on 12.5% SDS/PAGE and electrophoretically transferred to nitrocellulose (4).

- **Test procedure**

- Microplates are coated overnight at 4°C with 1 µg/ml of the fusion antigen 3ABC in carbonate/bicarbonate buffer, pH 9.6 (100 µl per well). Antigen 3ABC was expressed and purified as indicated for the EITB tests (4).
- The plates are washed six times with PBS, pH 7.2, supplemented with 0.05% Tween 20 (PBST).
- Test sera (100 µl per well) are added in a 1/20 dilution in blocking buffer consisting of PBS, 0.05% Tween 20, 5% nonfat dry milk, 10% equine sera and 0.1% *E. coli* lysate. Each plate includes a set of reference standards as above.
- The plates are incubated for 30 minutes at 37°C and washed six times in PBST.
- Horseshradish-peroxidase-conjugated rabbit anti-species IgG is diluted optimally in the blocking buffer, added at 100 µl per well and the plates are incubated for 30 minutes at 37°C.
- After six washings, each well is filled with 100 µl of 3,3', 5,5'-tetramethylbenzidine plus 0.004% (w/v) H<sub>2</sub>O<sub>2</sub> in phosphate/citrate buffer, pH 5.5.
- The reaction is stopped after 15 minutes of incubation at room temperature by adding 100 µl of 0.5 M H<sub>2</sub>SO<sub>4</sub>. Absorbance is read at 450 nm and at 620 nm for background correction.

- **Interpreting the results**

For the test system to be valid the following performance criteria are applied: the absorbance of negative controls should be <0.10 after correction for absorbance of blank wells. The cut-off serum, obtained as described in the EITB test, should give absorbance values of 0.15–0.40. Results are expressed as an index derived by dividing the absorbance value of the serum tested by that of the cut-off control. The ratio of the weak positive/cut-off controls should be 2.5 with a coefficient of variation <20%. Test sera with ratios >0.8 are considered to be suspect or positive and are retested by EITB. Coated plates and secondary standards are available from the PANAFTOSA<sup>18</sup> on request.

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**OIE MANUAL OF STANDARDS FOR DIAGNOSTIC TESTS AND VACCINES**

**Proposed changes to the List of prescribed and alternative tests**

Ref. No.	Disease	Prescribed tests	Alternative tests
A010	Foot and mouth disease	ELISA*, VN	CF
B058	Rabies	<u>ELISA</u> , VN	–
B108	Enzootic bovine leucosis	AGID, ELISA	<u>PCR</u>
B253	Porcine brucellosis	[BBAT] <u>ELISA</u>	<u>BBAT [ELISA], FPA</u>

\* Please refer to *Manual* chapters to verify which method is prescribed.

AGID = Agar gel immunodiffusion  
BBAT = Buffered *Brucella* antigen test  
CF = Complement fixation  
ELISA = Enzyme-linked immunosorbent assay  
FPA = Fluorescence polarisation assay  
PCR = Polymerase chain reaction  
VN = Virus neutralisation

Double underlined text = new proposal.

Reduced-size text between square brackets = proposed deletion.





## OIE GUIDELINES FOR THE VALIDATION OF DIAGNOSTIC ASSAYS FOR INFECTIOUS DISEASES

### 1. Introduction

#### 1.1. Purpose

This document provides guidelines for the validation of test methods (assays) for infectious diseases and is an adjunct to Chapter I.1.3, Principles of Validation of Diagnostic Assays for Infectious Diseases, *OIE Manual of Standards for Diagnostic Tests and Vaccines*, 2000 and Jacobson R.H. (1998). Validation of serological assays for diagnosis of infectious diseases. *Rev. sci. tech. Off. int. Epiz.*, 17 (2), 469–486.

#### 1.2. Scope

These guidelines are intended for use by OIE Member Countries for determining and verifying the performance characteristics of assays, in general, and for ‘validation’ of assays developed in-house as referred to in the OIE Standard for Management and Technical Requirements for Laboratories Conducting Tests for Infectious Animal Diseases.

#### 1.3. Validated Assay

A validated assay consistently provides test results that identify animals as positive or negative for a particular analyte (e.g. antibody or antigen) or reaction (e.g. induration at a skin test site) which, by inference, accurately predicts the infection status of the animal with a predetermined degree of statistical certainty.

#### 1.4. Stages of Assay Development and Validation

Development and validation of an assay is an incremental process consisting of at least five stages. It is important to understand all of the stages of development, as the initial stages may heavily influence the capacity of the assay to provide accurate and reliable results.

These stages include:

- a) Feasibility studies,
- b) Development and standardisation,
- c) Characterisation of assay performance,
- d) Validity of assay results: predictive value,
- e) Maintenance and extension of validation criteria.

### 2. Feasibility studies

#### 2.1. Appropriateness

Diagnostic assays should be developed only after feasibility studies have taken place. First and foremost, new assays should address specific diagnostic applications (i.e. import/export, surveillance, disease control, etc).

Analytical sensitivity (defined below) with respect to the type and concentration of analyte or level of reaction to be detected and analytical specificity with respect to the organism in question should be appropriate for the intended application.

The application itself should delineate the minimum acceptable requirements for diagnostic sensitivity and specificity.

Host factors should be considered with respect to the intended target species and should include the effects of age, sex, breed, nutritional status, pregnancy and immunological responsiveness.

Protocol design with respect to integration into diagnostic routine, as well as, reagent and test sample requirements, quality control, repeatability and data expression should be appropriate.

## **2.2. Cost and availability**

The cost and availability of specialised laboratory equipment and service, of chemicals and labware, including plasticware and of biological reagents, including monoclonal antibodies and recombinant antigens should not be limiting factors.

## **3. Development and standardisation**

### **3.1. Standardisation of protocol parameters and optimal reagent concentrations**

All physical/chemical parameters of the assay should be standardised and defined in a written protocol. Critical control points should be identified.

All biological reagents (e.g. antigens, antibodies, controls, enzyme/ substrate systems, etc), as well as, storage conditions and preparation for use should be described or referenced in the protocol. This should also include procedures for titration of reagents and for calibration against international reference standards where applicable.

Detailed descriptions of acceptance/rejection criteria for assay runs (i.e. based on internal controls) and for individual test sample results should also be included. In addition, descriptions of data normalisation and expression, as well as, data interpretation should be detailed.

### **3.2. Repeatability estimates**

Preliminary estimates of repeatability should be established. Agreement between replicates both within and between runs should be compatible with the inherent variability of the particular type of assay. Excessive variability should be investigated and corrected before proceeding any further.

### **3.3. Analytical sensitivity and specificity**

Analytical sensitivity represents the smallest amount of analyte or the least reaction detectable. Determining analytical sensitivity in absolute terms requires the use of purified analytes. In complex biological systems such as antigen-antibody interactions, this is often not possible. Indirect measures of analytical sensitivity may be derived, for example, by end point titration of reference standards.

Analytical specificity may be assessed by testing panels of samples derived from animals which have experienced infections with related organisms. The lower the level of cross-reactivity, the greater the level of analytical specificity. Depending on the intended application of the assay, the appropriate level of analytical specificity may be species, group or sub-group specific.

## **4. Determination of Assay Performance Characteristics**

### **4.1. Estimates of diagnostic sensitivity and specificity**

Estimates of diagnostic sensitivity and specificity are the basis for calculations of other parameters from which are made inferences about test results. Therefore, it is imperative that these estimates are as accurate as possible.

Ideally, these estimates should be derived from testing a series of samples from reference animals of known history and infection status. However, it is often difficult to assemble panels of samples from known infected animals. Proof of infection requires isolation of the organism or pathognomonic histopathological criteria. In some cases, it may be necessary to immunise or experimentally infect a group of animals and collect serial samples during the development of the immune response or the infection. It may also be difficult to assemble panels of samples from known uninfected animals. This is especially true in areas where the disease is endemic. In some instances, it may be necessary to test uninfected groups of animals far removed from the target population.



Diagnostic sensitivity (Sn) is the proportion of known infected animals that test positive in the assay. Infected animals that test negative are considered to exhibit false negative results.

Diagnostic specificity (Sp) is the proportion of known uninfected animals that test negative in the assay. Uninfected animals that test positive are considered to exhibit false positive results.

The number of reference samples required to determine estimates of Sn and Sp can be calculated. To do this, a reasonable prediction of both Sn and Sp must be used. An allowable error for the estimates for both Sn and Sp must be chosen. Lastly, the desired confidence in the estimate must be factored into the equation (normally 95%).

However, no formula can account for the numerous host/organism factors which can affect the outcome of the test. A general rule of thumb is to test no fewer than 300 infected animals and no fewer than 1000 uninfected animals to determine estimates of Sn and Sp, respectively.

#### 4.2. Selection of positive/negative cut-off

In order to calculate estimates of diagnostic sensitivity and specificity, test results need to be classified as either positive or negative. Irrespective of assay type, (e.g. qualitative, semi-quantitative or quantitative) positive/negative cut-off criteria must be unequivocally defined.

Numerous methods have been used to establish cut-off points. No one method is infallible and in many cases, it may be appropriate to choose more than one cut-off for further investigation and confirmation.

#### 4.3. Calculation of diagnostic sensitivity and specificity

Given that the appropriate panels of samples from reference infected and uninfected animals have been assembled and tested and a cut-off has been chosen, estimates of diagnostic sensitivity and specificity can be calculated.

Test Result:	Infection Status:	
	Infected	Uninfected
Positive	TP	FP
Negative	FN	TN

$$\text{Diagnostic sensitivity} = \text{TP} / (\text{TP} + \text{FN})$$

$$\text{Diagnostic specificity} = \text{TN} / (\text{TN} + \text{FP})$$

Where 'TP' represents true positive, 'TN' represents true negative, 'FP' represents false positive and 'FN' represents false negative according to test results compared to infection status.

To compare the diagnostic sensitivity and specificity estimates of any one assay to another, it must be done only after testing the very same infected and uninfected reference samples. Otherwise, the comparison is invalid.

#### 4.4. Other standards of comparison

Frequently, new assays are compared to an existing standard assay. Often this standard is the assay which is accepted as having the greatest diagnostic sensitivity and/or specificity of all of the tests in current use. A new assay may be compared to an existing standard in terms of 'relative' sensitivity and specificity. However, a critical assumption is made that the results of the standard assay are an accurate reflection of the true infection status of the animal.

Test Result:	Standard Assay Result:	
	Positive	Negative
Positive	TP	FP
Negative	FN	TN

$$\text{Relative sensitivity} = \text{TP} / (\text{TP} + \text{FN})$$

$$\text{Relative specificity} = \text{TN} / (\text{TN} + \text{FP})$$

Where ‘TP’ represents true positive, ‘TN’ represents true negative, ‘FP’ represents false positive and ‘FN’ represents false negative according to test results of the standard assay of comparison.

The problem with this type of comparison is that it is difficult to explain disagreement without doing extensive follow-up on these animals to determine their true infection status. Another way to look at this data is to calculate total agreement as  $(\text{TP} + \text{TN}) / (\text{TP} + \text{FN} + \text{TN} + \text{FP})$ , but again it is difficult to explain any disagreement.

To reduce the bias introduced by the inherent FP and FN rates of the standard assay in the above comparison, it would be better to use a battery of tests to define the reactivity of reference samples.

#### 4.5. Repeatability and reproducibility

Precision is a measure of the dispersion of results for a repeatedly tested sample. Accuracy, on the other hand, is a measure of the agreement between a test value and the expected value for a reference standard of known titre or concentration.

Repeatability should be determined within a given laboratory. The degree of variability should be determined for replicates of the controls both within each run and between runs of the assay. Upper and lower control limits should be established for each of the positive and negative controls as a measure of assay precision. These limits will determine whether or not a particular run is in control or should be rejected.

If one of the positive controls also represents a working standard, then each assay run also becomes a measure of accuracy.

Test samples should also be examined for agreement between replicates. Excessive variability between replicates, especially around the cut-off point will adversely affect the ability to make a diagnostic decision concerning infection status.

Reproducibility is determined when a panel of samples of defined reactivity is tested by several laboratories using identical assay protocols and reagents. The extent to which the collective results for each sample deviate from the expected value is an indicator of assay reproducibility and provides measures of precision and accuracy between laboratories.

In some cases, it may not be appropriate to predetermine the expected result but rather to statistically establish an upper and lower limit of acceptable activity based on a consensus of results from the participating laboratories. This is especially important when developing international standards.

### 5. Validity of assay results: predictive value

The predictive value of a positive test (PV+) is the proportion of positive results in the assay which correctly identify infected animals.

The predictive value of a negative test (PV-) is the proportion of negative results in the assay which correctly identify uninfected animals.

The predictive values of an assay are dependent on both the estimates of diagnostic sensitivity and specificity and the prevalence of disease in the target population.

The prevalence of disease in the target population has a dramatic effect on PV's if the estimates of diagnostic sensitivity and specificity remain constant. Diagnostic results cannot be interpreted at face value alone without knowledge of disease prevalence. Therefore, the validity of assay results is not simply a function of its performance characteristics.

## **6. Maintenance and enhancement of validation criteria**

A validated assay requires constant monitoring and maintenance to ensure its reliability. Internal quality control data should be monitored continually as a measure precision and accuracy within the laboratory.

A panel of samples representing the full range of reactivities anticipated in the target population should be used to assess all new batches of reagents to ensure uniform production quality.

Modifications to production protocols or assay parameters will require assessment to determine whether there has been any change in the performance characteristics of the assay. Minor modifications which improve repeatability and reproducibility without affecting the analytical performance of the assay may not require a full reassessment of diagnostic sensitivity or specificity.

Any major modification to the assay such as the introduction of a totally new production protocol or reagent will require a complete assessment of assay performance characteristics and comparison with the original protocol. It may not necessarily require comparisons with other assays unless the analytical sensitivity or specificity has been radically altered.

As data is generated from the testing of field samples, estimates of diagnostic sensitivity and specificity should be updated. The greater the number of samples used to generate these figures, the greater the confidence in the estimates.

Declines in disease prevalence, seasonal trends, emergence of related organisms or changes in vaccination practices may require that diagnostic performance characteristics be re-evaluated with respect to the appropriateness of the assay for its intended application.





## REFERENCE LABORATORIES

### MANDATE

Reference Laboratories of the Office International des Epizooties shall have as their principal mandate:

- to function as a centre of expertise and standardisation for a designated disease(s) or topics [of techniques relevant to their field of specialisation];
- to store and distribute biological reference products and any other reagents used in the diagnosis and control of the designated disease(s) or topics [animal diseases of Lists A and B];
- to develop new procedures for diagnosis and control of the designated disease(s) or topics [these diseases];
- to gather, process, analyse and disseminate epizootiological data relevant to their speciality;
- to place expert consultants at the disposal of the Office International des Epizooties.

They may also contribute to:

- provision of scientific and technical training for personnel from Member Countries of the Office;
- provision of diagnostic testing facilities to Member Countries:

In the case of positive results for diseases that are reportable to OIE, the Reference Laboratory should immediately inform the OIE Delegate of the Member Country from which the samples originated;

- organisation of scientific meetings on behalf of the Office;
- coordination of scientific and technical studies in collaboration with other laboratories or organisations;
- publication and dissemination of any information in their sphere of competence which may be useful to Member Countries of the Office.

OIE Reference Laboratories might charge for the services that they provide.

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## REFERENCE LABORATORIES

### INTERNAL RULES

#### *Article 1*

Applications for the title of Reference Laboratory of the Office International des Epizooties shall be submitted to the Director General by the Delegate of the Member Country to which the laboratory belongs or by the corresponding Regional Commission.

#### *Article 2*

Applications received shall be presented [to the Standards Commission] by the Director General, after consultation with the Standards Commission or the Fish Diseases Commission, as appropriate, to the Administrative Commission at its annual meetings [on the occasion of the Commission's meetings]. Applications shall be selected solely on the basis of scientific and technical competence of the candidate establishment.

#### *Article 3*

Applications endorsed by the Commission shall be presented to the Committee for approval.

#### *Article 4*

The Director General shall notify approved laboratories of their designation as an 'OIE Reference Laboratory'.

#### *Article 5*

This notification shall confer on the laboratory the right to use the title 'OIE Reference Laboratory' and the OIE emblem on all documents issued by the laboratory in its official capacity, and the right of the designated specialist within the laboratory to use the title of OIE Expert.

#### *Article 6*

OIE Experts exercise their function within the 'rules applicable to OIE Experts'.

#### *Article 7*

The rights conferred by Article 5 upon a laboratory and an expert require full compliance with the Mandate of an OIE Reference Laboratory, within the limits of facilities available, and provision of a brief report of activities at the end of each calendar year of their mandate. This report will be distributed to all Member Countries.

#### *Article 8*

The designation shall be valid for four years, at the end of which the Director General may propose to the Committee that it be renewed. Either party may revoke the designation at any time.

#### *Article 9*

Any major change within the Laboratory which may impair its competence (particularly the retirement of a designated expert) shall be reported immediately to the Director General of the Office.

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