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REPORT OF THE MEETING OF THE OIE BIOLOGICAL STANDARDS COMMISSION

Paris, 28–30 January 2004

The OIE Biological Standards Commission met at the OIE Headquarters from 28 to 30 January 2004. Dr Bernard Vallat, Director General of the OIE, welcomed the Members of the Commission, Prof. Steven Edwards, President, Dr Beverly Schmitt, Vice-President and Dr Anatoly Golovko, Secretary General, the other participant, Dr Peter Wright, and Dr Adama Diallo, representing the OIE Collaborating Centre for ELISA[1] and Molecular Techniques in Animal Disease Diagnosis, IAEA[2], Vienna, Austria.

Dr Vallat outlined the Commission's activities for 2004, which includes evaluating the activities of Collaborating Centres and Reference Laboratories and advancing the Resolution on validation and certification of diagnostic assays for infectious animal diseases. Dr Vallat highlighted the proposal to 'twin' certain laboratories in the southern regions of Africa and other developing Member Countries across the globe with OIE Reference Laboratories in developed countries.

The Agenda and List of Participants are given at Appendices I and II, respectively.

1. OIE Reference Laboratories and Collaborating Centres

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

OIE Collaborating Centre for Veterinary Training, Epidemiology, Food Safety and Animal Welfare

The OIE Collaborating Centre for Epidemiology and Organisation of Veterinary Services in Developing Countries, Teramo, Italy, had requested that its title be changed to OIE Collaborating Centre for Veterinary Training, Epidemiology, Food Safety and Animal Welfare. The new activities will be incorporated into the remit of the existing Collaborating Centre. The Commission accepted this proposal.

OIE Collaborating Centre for New and Emerging Diseases

Following reassurances received from the Delegate of Australia, the Commission agreed to support the establishment of an OIE Collaborating Centre for New and Emerging Diseases at the Australian Animal Health Laboratory (AAHL) in Geelong. There is a remaining concern over the difficulty of shipping samples potentially containing foot and mouth disease virus to the Collaborating Centre.

1 ELISA: enzyme-linked immunosorbent assay
2 IAEA: International Atomic Energy Agency

The Commission recommends acceptance of the following new applications for OIE Reference Laboratory status:

OIE Reference Laboratory for the Application of PCR[3] *Methods for Diagnosis of Viral Diseases in Veterinary Medicine*

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Designated Reference Expert: Prof. Sándor Belak.

Brucellosis

Servicio Nacional de Sanidad y Calidad Agroalimentaria (SENASA) and the Instituto Nacional de Tecnología Agropecuaria (INTA), Av. Alexander Fleming 1653, 1640 Martínez, Pcia. de Buenos Aires, ARGENTINA
(This is a joint designation – the designated expert will send a joint annual report to the OIE covering activities at the SENASA and INTA laboratories.)
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Designated Reference Expert: Dr A.M. Nicola

1.2. Updating the list of Reference Laboratories

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

Contagious bovine pleuropneumonia

Dr F. Poumarat to replace Dr J.L. Martel at AFSSA[4] Lyon, France.

Highly pathogenic avian influenza and Newcastle disease

Dr Paul W. Selleck to replace Dr Tony Della-Porta at the AAHL, Geelong, Australia.

Brucellosis

Mrs Judith Stack to replace Dr Alastair MacMillan at the Veterinary Laboratories Agency (VLA), Weybridge, United Kingdom (UK).

Bovine spongiform encephalopathy and scrapie

Dr Danny Matthews to replace Dr Martin Jeffrey at the VLA, Weybridge, UK.

Bovine spongiform encephalopathy and scrapie

Dr Prof. A. Zurbriggen to replace Prof. M. Vandevelde at the Institute of Animal Neurology, University of Bern, Switzerland.

In follow-up to the last report, the Delegate of France confirmed that the Reference Expert at the proposed new Reference Laboratory for Trypanosomosis at CIRAD-EMVT[5], Montpellier, would be Dr Marc Desquesnes.

The Commission approved a request from the Federal Institute for Risk Assessment, Berlin, Germany, to be removed from the list of Reference Laboratories for brucellosis.

1.3. Annual Reference Laboratories report for 2003

Reports had been received from 117/123 Reference Laboratories and 11/11 Collaborating Centres for terrestrial animals. 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

3 PCR: polymerase chain reaction
4 AFSSA : Agence française de sécurité sanitaire des aliments
5 CIRAD-EMVT : Centre de coopération internationale en recherche agronomique pour le développement - Département d'élevage et de médecine vétérinaire

Member Countries and to all the Reference Laboratories and Collaborating Centres. The international activities relevant to the work of the OIE are summarised below:

Reference Laboratories

	General activities	Percentage of Laboratories carrying out these activities
1a)	Diagnostic tests performed	97%
1b)	Agent identification performed	80%
2	Production, testing and distribution of diagnostic reagents	77%
3	Research	81%
	Specific OIE activities	
1	International harmonisation/standardisation of methods	41%
2	Preparation and supply of international reference standards	40%
3	Collection, analysis and dissemination of epizootiological data	36%
4	Provision of consultant expertise	58%
5	Provision of scientific and technical training	52%
6	Organisation of international scientific meetings	14%
7	Participation in international scientific collaborative studies	56%
8	Presentations and publications	70%

Collaborating Centres

	General activities	Percentage of Collaborating Centres carrying out these activities
1	Activities as a centre of research, expertise, standardisation and dissemination of techniques within the sphere of competence	90%
2	International harmonisation of regulations	70%
3	Provision of consultant expertise	30%
	Specific OIE activities	
1	Provision of scientific and technical training	100%
2	Organisation of international scientific meetings	40%
3	Coordination of scientific and technical studies	70%
4	Publications/dissemination of information	70%

2. International standardisation of diagnostic tests and vaccines

2.1. OIE standardisation programmes for diagnostic tests

LIST A DISEASES

Highly pathogenic avian influenza (HPAI) – Coordinator: Dr B. Panigrahy, National Veterinary Services Laboratories, Ames, United States of America

The OIE Reference Laboratories for HPAI have jointly embarked on a programme to develop international standard sera for use in the AGID[6] test for this disease and to agree on a harmonised protocol.

LIST B DISEASES

Porcine brucellosis – Coordinator: Dr K. Nielsen, Canadian Food Inspection Agency, Nepean, Canada

Dr Nielsen has sent preliminary data on potential reference sera for porcine brucellosis.

6 AGID: agar gel immunodiffusion

Developing OIE International Diagnostic Standards for Bovine spongiform encephalopathy (*BSE*)

The Commission noted that the development of OIE prescribed International Diagnostic Standards for BSE is a difficult but important undertaking. The OIE Reference Laboratory in the UK has proposed to evaluate new assays using existing panels of tissues.

3. List of prescribed and alternative tests

3.1. Indirect ELISA for rinderpest diagnosis

The Commission received additional supporting documentation relative to the analytical and diagnostic performance characteristics of the indirect ELISA (I-ELISA) for the detection of bovine antibody to rinderpest virus. This assay uses a recombinant N protein as antigen. In addition to information from the developers of the assay, additional documentation was received from the Joint FAO[7]/IAEA Division of the IAEA. In the latter case, the diagnostic performance characteristics of several ELISAs were independently compared in defined groups of reference animals in Africa.

It was noted from the combined data sets that all ELISAs were capable of detecting bovine antibodies to rinderpest lineages I and II, as well as the tissue culture vaccine virus. However, it was noted that the efficiency of detection did vary between tests and affected diagnostic sensitivity estimates. The I-ELISA consistently demonstrated a high level of diagnostic sensitivity in these studies.

It was also noted that cross reactivity with peste des petits ruminants (PPR) virus was a potential issue for these ELISAs, irrespective of whether the assay targeted antibodies to the N or H antigens of the virus. The extent of cross reactivity was very much dependent on the diagnostic thresholds chosen. For the I-ELISA, the immunological specificity of the conjugate also had considerable affect on the cross reactivity detected. The broader the range of antibody isotypes detected, the more negative the influence on the diagnostic specificity of this assay. Estimates of diagnostic specificity for the I-ELISA using a broad specificity conjugate were shown to vary greatly depending on the population under test.

The Commission agrees that the I-ELISA could be used as a high sensitivity screening test. However, false positive rates will vary depending on the population under test. A test of high diagnostic specificity is recommended to confirm positive reactors in the I-ELISA.

The Commission recommends that this assay be referenced in the chapter on Rinderpest in the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (the *Terrestrial Manual*).

3.2. ELISA for caprine arthritis/encephalitis and maedi-visna (CAE/MV)

The OIE Reference Laboratory at AFSSA Sophia Antipolis had sent an agreed upon protocol for the ELISA for CAE/MV along with an offer from a French company to prepare standard sera. The Commission proposes to designate the ELISA as a prescribed test for trade for CAE/MV (see Appendix III for the proposed changes to the list of prescribed and alternative tests). The proposed protocol for the ELISA is given at Appendix IV. This text has been included in the draft chapter for the fifth edition of the *Terrestrial Manual.* If adopted by the International Committee, the marking 'prescribed test for international trade' will be added to the Web version of the *Terrestrial Manual.*

7 FAO: Food and Agriculture Organization of the United Nations

3.3 FPA[8] for determination of antibody to smooth *Brucella* spp. in sheep and goats

The Canadian Food Inspection Agency's Animal Diseases Research Institute in Nepean, Ontario, had sent a validation dossier in support of an application to designate the FPA as a prescribed test for the determination of antibody to smooth *Brucella* spp. in sheep and goats. The Commission will seek the advice of the other OIE Experts on this matter.

In 1998, the Commission designated the FPA for detection of bovine serum antibody to *Brucella abortus* as an alternative test for bovine brucellosis and not as prescribed test because at that time the equipment and reagents necessary to perform the test were not widely available. Given that since 1998 the test has become widely available, the Commission proposes that it be designated as a prescribed test for bovine brucellosis (see Appendix III).

4. Report of the Second Meeting of the Ad hoc Group on Nonstructural Protein Tests for Foot and Mouth Disease Diagnosis

The Ad hoc Group had agreed that the I-ELISA from the Pan American Foot-and-Mouth Disease Center, PAHO[9]/WHO[10] (PANAFTOSA) be accepted as a fully validated index test to be used for comparison purposes for other non-structural protein (NSP) tests for foot and mouth disease (FMD) diagnosis. The I-ELISA, used as a screening test, along with the EITB[11] western blot technique, which is used as a confirmatory assay, is currently described in the FMD chapter of the *Terrestrial Manual*. The Commission urges the Ad hoc Group to continue its work on developing reference standard sera for pigs and sheep, and to gather validation data on the NSP tests in these species. The need for further research on carrier states in relation to vaccination was also noted. The report of the Ad hoc Group meeting is given at Appendix V.

5. OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (*mammals, birds and bees*)

The Commission noted that the production schedule for the fifth edition of the *Terrestrial Manual* is on target. It is foreseen that the English version will be printed in late spring 2004. The French and Spanish versions are in preparation and will be available later (summer 2004). A teleconference was held between the Commission and the Consultant Editor, Dr James Pearson.

6. Validation and certification of diagnostic assays

The Commission noted the report of the Second OIE/FAO/IAEA Consultants meeting on 'OIE Validation and Certification of Diagnostic Assays for Infectious Animal Diseases' held in Vienna, Austria, from 9 to 12 December 2003. The proposed validation and registration scheme developed according to Resolution No. XXIX, adopted by the OIE International Committee in May 2003, will be presented to the OIE International Committee in May 2004 (see Appendix VI). The Commission will ask the OIE Collaborating Centre for ELISA and Molecular Techniques in Animal Disease Diagnosis, IAEA, Vienna, Austria, to carry out a study on methods of serum inactivation.

Guidelines to accompany the validation template and an internal SOP (standard operating procedure) will be developed by the OIE. The Commission considered that the proposed timeline for evaluations may be overoptimistic. This should be assessed by running some pilots on a limited range of tests.

8 FPA: Fluorescence polarisation assay
9 PAHO: Pan American Health Organization
10 WHO: World Health Organization
11 EITB: Enzyme-linked immuno-electrotransfer blot

7. Liaison with other Commissions and Groups

- **SCIENTIFIC COMMISSION FOR ANIMAL DISEASES**

7.1. OIE Expert Group on 'Atypical' Bovine Spongiform Encephalopathy (BSE) cases

The Commission reviewed the report of the meeting of the OIE Expert Group on 'Atypical' Bovine Spongiform Encephalopathy Cases (see Appendix VII) and stressed the need for further research on 'atypical' BSE cases. In particular there is a need for clarification of the most appropriate procedures to be used to identify agent 'strain' characteristics. This issue is also relevant to scrapie 'strains'.

- **TERRESTRIAL ANIMAL HEALTH STANDARDS COMMISSION**

7.2. Follow up on contagious bovine pleuropneumonia and rabies

The Commission noted that the OIE Terrestrial Animal Health Standards Commission had proposed changes to certain Articles in the *Terrestrial Animal Health Code* chapters on contagious bovine pleuropneumonia and rabies, as requested at the last meeting.

8. Any other business

8.1. Report of the Expert Surveillance Panel on Equine Influenza

The Commission received a detailed report from Dr J. Mumford (OIE Expert on Equine Influenza) with the interim recommendations of the equine influenza surveillance panel. The conclusion of the panel was that both the American and European lineage equine influenza A H3N8 strains used for vaccine preparation should be updated in the near future. However, this decision should be made with as much supporting evidence as possible. At present such evidence is sparse, particularly with respect to the identity of strains currently circulating on the American continent. It was therefore decided that more data would be sought within the next few weeks in order for a fully supported recommendation to be published in April.

Any further report from the panel will be reviewed by the Commission by correspondence in order to finalise any recommendation to the International Committee.

8.2. Conferences organised by IABs[12]

IABs is planning to hold a number of scientific conferences in collaboration with the OIE Collaborating Centre for Diagnosis of Animal Diseases and Vaccine Evaluation in the Americas, Ames, Iowa, USA. The Commission supports the proposed Conferences and suggests that it should participate actively in their organisation, namely on the Executive Committee or on the Steering Committee. Thus, in addition to Dr James Pearson representing the OIE, the Commission proposes that the Vice-President, Dr Beverly Schmitt, collaborate in the organisation of the conference entitled Marker Vaccines and Diagnostics, to be held in April 2005 in Ames. The Commission will review the programmes and other related issues during its meeting in September.

8.3. Joint WHO/FAO/OIE Expert Workshop on Non-human Antimicrobial Usage and Antimicrobial Resistance

The Biological Standards Commission reviewed the outcome of first Joint WHO/FAO/OIE Expert Workshop on Non-human Antimicrobial Usage and Antimicrobial Resistance, held in Geneva, Switzerland, 1–5 December 2003. The Commission agreed on the choice of experts proposed by the OIE to participate in the second Workshop, to be held in Oslo, Norway, 15–18 March 2004. The outcome of the Oslo Workshop will assist the OIE Ad hoc Group on Antimicrobial Resistance to proceed further in this important area.

12 IABs: International Association for Biologicals

8.4. Transport of pathogens

The Consultant Editor of the *Terrestrial Manual*, Dr James Pearson, agreed to prepare a document for submission to the United Nations Sub-Committee of Experts on the Transport of Dangerous Goods (UNSCETDG) detailing the OIE request to amend the list of infectious substances that are prohibited from being shipped as UN 3373 (Diagnostic specimens). The document will be considered by the UNSCETDG in July 2004. The OIE will also request observer status at these meeting. Dr Pearson has updated the chapter on sampling methods for the fifth edition of the *Terrestrial Manual* to reflect the current regulations governing transport of specimens to laboratories. These regulations will change in between editions of the *Terrestrial Manual* and readers are advised to consult the web site for the most recent version.

8.5. Training course on diagnosis of transmissible spongiform encephalopathies (TSEs), VLA Weybridge, November 2003

The OIE Reference Laboratory for TSEs at VLA Weybridge had run a training course for OIE Member Countries in November 2003, on diagnostic methods. The course itself had been highly successful, and the Commission noted the substantial set of course notes provided to participants. There were a few problems over travel arrangements for some participants, and the Commission requests the OIE both to facilitate this for any future courses and to review the possibility of financing participants from developing countries to attend.

8.6. OIE Fourth Strategic Plan 2005–2010

The Commission took note of the Fourth Strategic Plan.

8.7. WAVLD[13] proposal to establish a Diagnostic Laboratory Assessment and Evaluation Committee

The Commission supports a proposal from the WAVLD to establish a Diagnostic Laboratory Assessment and Evaluation Committee with the proviso that that any assessment and evaluation should be against the full *OIE Standard for Management and Technical Requirements for Laboratories Conducting Tests for Infectious Animal Diseases* and not against any subset of requirements. The Commission encourages the OIE to pursue this activity as it will significantly improve veterinary diagnostic laboratories world-wide.

8.8. Book on Rabies in Europe

The Commission had received a request from Dr Anthony Fooks, VLA Weybridge, for the OIE to publish a book entitled Rabies in Europe and the Mediterranean Basin that has been edited and prepared by Dr Fooks and his predecessor Dr Arthur King. The Commission reviewed the table of contents and recommends that the OIE sponsor its publication. Copies of the book could be given to all participants at the European Conference on Rabies, which will be held in Kiev, Ukraine, in December 2004 under the co-sponsorship of the OIE, WHO, the European Union and AFSSA.

8.9. International Veterinary Biosafety Advisory Group

The Commission discussed a proposal received from the International Veterinary Biosafety Advisory Group to prepare a manual on veterinary biosecurity under the aegis of the OIE and the FAO. The Commission approved the concept of developing such a document. There is a real need to establish standards for veterinary laboratory biosecurity. The Commission proposes that the OIE Director General convene an Ad hoc Group of experts in this field to address this issue and assist with drafting the manual. The draft manual should be submitted for scrutiny by the BSC before adoption.

13 WAVLD: World Association of Veterinary Laboratory Diagnosticians

8.10. Tests for equine arteritis antibody

A laboratory had reported that neutralisation tests for equine arteritis were giving a high proportion of cytotoxic sera that interfered with the standard virus neutralisation test. This appears to be associated with anticellular antibodies generated by certain equine vaccines. The OIE Reference Laboratory had advised that the problem could largely be overcome by using preformed cell monolayers in the test. This procedure is included in the draft chapter for the *Terrestrial Manual*

8.11. Dates of next Biological Standards Commission meeting

The next meeting of the Biological Standards Commission will be held from 31 August to 2 September 2004.

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.../Appendices

Appendix I

MEETING OF THE OIE BIOLOGICAL STANDARDS COMMISSION

Paris, 28–30 January 2004

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Agenda

1. OIE Reference Laboratories and Collaborating Centres
2. International standardisation of diagnostic tests and vaccines
3. List of prescribed and alternative tests
4. Report of the Second Meeting of the Ad hoc Group on Nonstructural Protein Tests for Foot and Mouth Disease Diagnosis
5. OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (*mammals, birds and bees*)
6. Specific procedures for OIE to validate and approve diagnostic tests
7. Liaison with the other Commissions
8. Any other business

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Appendix II

MEETING OF THE OIE BIOLOGICAL STANDARDS COMMISSION
Paris, 28–30 January 2004

List of participants

MEMBERS

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OIE *MANUAL OF DIAGNOSTIC TESTS AND VACCINES FOR TERRESTRIAL ANIMALS* (*MAMMALS, BIRDS AND BEES*)

Proposed changes to the List of prescribed and alternative tests

Ref. No.	Disease	Prescribed tests	Alternative tests
B103	Bovine brucellosis	BBAT, CF, ELISA, FPA	[FPA]
B153, B161	Contagious arthritis/encephalitis & maedi-visna	AGID, ELISA,	[ELISA]

AGID = Agar gel immunodiffusion
BBAT = Buffered *Brucella* antigen test
CF = Complement fixation
ELISA = Enzyme-linked immunosorbent assay
FPA = Fluorescence polarisation assay

Double underlined text = new proposal.
Reduced-size text between square brackets = proposed deletion.

STANDARD PROTOCOL FOR THE PROPOSED NEW PRESCRIBED TEST FOR CAPRINE ARTHRITIS/ENCEPHALITIS & MAEDI VISNA

General procedures

The enzyme-linked immunosorbent assay (ELISA) has a high sensitivity and a good specificity. It is easy to perform in laboratories that have the necessary equipment (a spectrophotometer) and reagents. The ELISA is convenient for large-scale screening and, particularly for veterinary diagnosis, as it is a reliable technique for demonstrating small ruminant lentiviruses (SRLVs) antibodies in sheep and goats. It requires a relatively pure antigen.

The production of antigens for the use in ELISAs has been described. Antigenic preparation must contain at least one of the major antigens of SRLVs, i.e. the envelop (gp135 = surface protein [SU] and gp44 = transmembrane protein [TM]) and the capsid (p25) (3). These antigens may be present in a whole-virus preparation or produced as recombinant proteins or synthetic peptides (1, 2, 4–6). Thus, recombinant *gag* gene product fused with glutathione S-transferase fusion protein antigen has been produced in *E. coli*. Recombinant antigens produced in *Escherichia coli* provide a consistent source of antigen for international distribution and standardisation. Recombinant antigens or synthetic peptides have been used in indirect ELISAs (I-ELISAs).

Specific monoclonal antibodies (MAb), which define surface protein epitopes, have been used in a competitive ELISA (C-ELISA) for SRLVs to capture surface envelop as antigen (1): C-ELISA overcomes the problem of antigen purity, as the specificity of this test depends only on the MAb used.

For I-ELISA, wells of the microplate are coated with antigen. Diluted serum samples are added to the wells and react to antigens bound to the solid support. Unbound material is removed by washing after a suitable incubation period. Conjugate (ex: horseradish-peroxidase-labelled anti-ruminant Ig) reacts with specific antibodies bound to the antigen. Unreacted conjugate is removed by washing after a suitable incubation period. Enzyme substrate is added. The rate of conversion of substrate is proportional to the amount of bound antibodies. The reaction is terminated after a suitable time and the amount of colour development is measured spectrophotometrically.

For C-ELISA, sample sera containing anti-SRLV antibodies inhibit binding of enzyme–labelled MAb to SRLV antigen coated on the plastic wells. Binding of the enzyme-labelled MAb conjugate is detected by the addition of enzyme substrate and quantified by subsequent colour product development. Strong colour development indicates little or no blockage of enzyme-labelled MAb binding and therefore the absence of SRLV antibodies in sample sera. In contrast, weak colour development due to the inhibition of the enzyme-labelled MAb binding to the antigen on the solid phase indicates the presence of SRLV antibodies in sample sera.

- **Materials and reagents**

Microtitre plates with 96 flat-bottomed wells, freshly coated or previously coated with SRLV antigen; microplate reader (spectrophotometer; 405, 450, 490 and 620 nm filters); 37°C humidified incubator; 1- 8- and 12-channel pipettes with disposable plastic tips; microplate shaker (optional); fridge; freezer.

Positive and negative control sera; conjugate (ex: ruminant anti-immunoglobulin labelled with peroxidase); tenfold concentration of diluent (ex: PBS/Tween); distilled water; 10X wash solution; substrate or chromogen (ex: ABTS [2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid)] or TMB [3,3',5,5'-tetramethylbenzidine]); stop solution (ex: detergent, sulfuric acid).

- **Indirect ELISA: test procedure**

i) Dilute the serum samples, including control sera, to the appropriated dilution (ex: 1/20) and distribute 0.1–0.2 ml per well (in duplicate if biphasic ELISA). Control sera are positive and negative sera provided by the manufacturer and an internal positive reference serum of the laboratory in order to compare the titres between different tests.

ii) Cover the plate with a lid and incubate at room temperature or 37°C for 30–90 minutes. Empty out the contents and wash three times in washing solution at room temperature.

iii) Add the appropriate dilution of freshly prepared conjugate to the wells (0.1 ml per well). Cover each plate and incubate as in step ii. Wash again three times.

iv) Add 0.1 ml of freshly prepared or ready-to-use chromogen substrate solution to each well (for example: ABTS in citrate phosphate buffer, pH 5.0, and 30% H_2O_2 solution [0.1 µl/ml]).

v) Shake the plate; after incubation, stop the reaction by adding stopping solution to each well (ex: 0.1 ml sulphuric acid).

vi) Read the absorbance of each well with the microplate reader at 405 nm (ABTS) or 450-620 nm (TMB). The absorbance values will be used to calculate the results.

Interpretation of the results

For commercial kits, interpretations and criteria of validation are provided with the kit.

For example: calculate the mean absorbance (Ab) of the sample serum and of the positive (Ab_{pos}) and negative (Ab_{neg}) control sera, and for each serum, calculate the percentage:

$$\frac{Ab - Ab_{neg}}{Ab_{pos} - Ab_{neg}} \times 100$$

Interpret the results as follows:
Ab <30% negative serum
Ab 30–40% doubtful serum
Ab >40% positive serum

- **Competitive ELISA: test procedure**

i) Add 0.05 ml of undiluted serum and positive/negative controls to antigen-coated plate.

ii) Incubate for 1 hour at room temperature.

iii) Empty the plate and wash plate three with wash solution

iv) Add 0.05 ml of diluted antibody-peroxidase conjugate to each well. Mix well and incubate for 30 minutes at room temperature.

v) After the 30-minute incubation, empty the plate and repeat the washing procedure described in step iv.

vi) Add 0.05 ml of substrate solution (ex: TMB) to each well. Mix and cover plate with aluminium foil. Incubate for 20 minutes at room temperature. Do not empty wells.

vii) Add 0.05 ml of stop solution to each well. Mix. Do not empty wells.

viii) Immediately after adding the stop solution, the plate should be read on a plate reader (620 nm).

ix) Interpretation of results:

Example: Calculation: 100- [(Sample OD x 100)/(Mean negative control O.D.)]= % inhibition.

If a test sample causes $\geq$ 35%, it is positive; if a test sample causes < 35% inhibition, it is negative.

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Appendix V

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September 2003

REPORT OF THE SECOND MEETING OF THE OIE AD HOC GROUP ON EVALUATION OF NONSTRUCTURAL PROTEIN TESTS FOR FOOT AND MOUTH DISEASE DIAGNOSIS

Paris, 17–19 September 2003

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A meeting of the OIE Ad hoc Group on Evaluation of Nonstructural Protein Tests for Foot and Mouth Disease Diagnosis was held at the OIE Headquarters in Paris from 17 to 19 September 2003. The meeting was chaired by Dr Peter Wright, who acted as rapporteur.

The List of Participants is given at Appendix I.

1. Background

The Ad hoc Group first met at OIE Headquarters in Paris from 2 to 4 October 2002. At this meeting, the Group conducted an in depth review of six nonstructural protein (NSP) enzyme immunoassays and examined available validation data. Diagnostic performance estimates in cattle were found to vary amongst these test methods. Data sets were independently generated and included naïve and vaccinated cattle which were subsequently infected with foot and mouth disease virus (FMDV). In the case of PANAFTOSA's test, a considerable number of data from field studies were also included during the validation process (performance characteristics, extension of validation criteria, evaluation of vaccine interference, etc). The disparity in results underscored the need to establish one test method as a fully validated index method. This method would then be used to develop and characterise reference standard sera for the calibration of all other assays, and to support the definition of minimum performance characteristics required for different purposes. In addition, a need was identified to develop panels of defined bovine sera that could be used to evaluate and compare the performance characteristics of the various test methods.

Standardisation and validation of an NSP system for cattle was considered to be the top priority. Once complete a similar exercise for sheep and then for pigs would then follow.

The indirect ELISA[1] (I-ELISA) from PANAFTOSA was selected as the best candidate for the index method since it has been used extensively in a number of South American laboratories and there are considerable data on its performance characteristics in cattle. This I-ELISA, used as a screening test, along with the EITB[2] western blot technique, which is used as a confirmatory assay, is currently described in the FMD chapter of the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (*Terrestrial Manual*).

At the end of the first meeting the Group agreed to work on the completion of a validation dossier for the I-ELISA/EITB (above) and to begin the selection and characterisation of candidate sera for the development of reference standard sera and evaluation panels.

2. Second meeting

The Ad hoc Group met for the second time at OIE Headquarters in Paris from 17 to 19 September 2003. The purpose of this meeting was; 1) to finalise the data and analyses required for the validation dossier, 2) to define and incorporate reference standard sera into the method protocol, and 3) to review progress on the establishment of evaluation panels. The Group also reviewed and revised the *Terrestrial Manual* chapter to reflect technical upgrades to the NSP tests.

1 ELISA: Enzyme-linked immmunosorbent assay
2 EITB: Enzyme-linked immuno-electrotransfer blot

3. Validation dossier

A preliminary draft of the validation dossier was examined. Data on analytical and diagnostic performance characteristics were examined and tabulated. In addition, repeatability and reproducibility data were expanded. A complete dossier will be finalised before the next meeting (January 2004) of the Biological Standards Commission.

4. Conventional versus high potency vaccines

Most of the available data with respect to induction of carrier states and seroconversion is based on conventional doses of vaccine. Work still needs to be done to determine whether or not the use of high potency vaccines will alter carrier states and diagnostic specificity (DSn) estimates in vaccinated animals.

5. Index test method

PANAFTOSA's I-ELISA/EITB system was reviewed for technical detail and upgrades with respect to incorporation of new reference standard reagents and internal quality control processes. A revised description of this method will appear in the 2004 edition of the *Terrestrial Manual*.

6. Reference standard sera

Dose–response curves of candidate sera were examined using the index method and other available assays and dilution ranges were selected for the strong and weak positive reference standards. The strong and weak standards will represent upper and lower points on the linear portion of the dose-response curve. Primary dilutions of the selected positive serum will be prepared in a negative serum (pool) that will also serve as the negative reference standard serum. Preparation and testing of bovine reference standard sera is underway.

7. Threshold/cut-off

In the current protocol, the positive or negative status of a test sample is interpreted relative to the reactivity of a cut-off reference serum that is included with every run. In essence, this serum represents a 'floating' cut-off because its run reactivity will vary within a range of values. To fall in line with OIE guidelines, it is recommended that the new strong positive reference standard sera be used to normalise both control and test sample data and express results in terms of per cent positivity. Once reference standard sera have been incorporated into the method, conversion to normalisation against the strong positive will take place.

8. Evaluation panels

Initial candidate sera have been identified for the evaluation panels. These sera are from experimental studies in cattle, sheep and pigs. They include non-vaccinated, infected animals, as well as, vaccinated animals that had been subsequently challenged. These sera will be characterised in the index test and stored for future reference and comparisons. Acquiring sera such as these and in sufficient volumes to be useful in evaluation panels will be a difficult challenge. The task of creating these panels, encompassing sera representing the various experimental and field scenarios has begun at PANAFTOSA, at Pirbright and at the OIE Collaborating Centre for ELISA and Molecular Techniques in Animal Disease Diagnosis, at the IAEA[3] in Vienna, Austria, and will require international cooperation for contributions of defined sera.

9. Fitness for purpose

The Group feels that the index ELISA as a screening test and the EITB as a confirmatory test have definite potential in the monitoring and recovery from FMD outbreaks in either vaccinated or naïve populations. Excellent progress has been made in the standardisation and validation of the I-ELISA and EITB for cattle. Sufficient data has been compiled to develop specific application, sampling and interpretation strategies, especially with respect to declaration of freedom.

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3 FAO/IAEA: Food and Agriculture Organization of the United Nations/International Atomic Energy Agency

10. Future work

Modifications to the index test with respect to the incorporation of new reference standard sera, internal control procedures and data expression should be monitored and repeatability and reproducibility data should be updated.

The characterisation and development of reference standard sera for sheep and pigs should be initiated.

Work still needs to be done to determine whether or not the use of high potency vaccines will alter carrier states and DSn estimates in vaccinated animals.

The development of evaluation panels should continue for all species. The composition, application and interpretation of these panels need to be defined with respect to comparative assessment of different test methods and inter-laboratory proficiency testing.

For the bovine system, the Group should seek additional expertise in the development of strategies for the implementation of these test methods. This would include various application scenarios from outbreak to surveillance.

It is proposed that the Ad hoc Group meet in years' time to review progress on the above and report to the OIE Biological Standards Commission.

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Appendix I

SECOND MEETING OF THE OIE AD HOC GROUP ON EVALUATION OF NONSTRUCTURAL PROTEIN TESTS FOR FOOT AND MOUTH DISEASE DIAGNOSIS

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Report of the Second FAO/IAEA/OIE Consultants Meeting on 'OIE Guidelines for Validation and Certification of Diagnostic Assays for Infectious Animal Diseases'

9–12 December 2003, Vienna, Austria

The main objectives of the Office International des Epizooties (OIE), the World Organisation for Animal Health, are:

- to collect, analyse and disseminate scientific veterinary information,
- to ensure transparency in the global animal disease and zoonosis situation,
- to provide expertise and encourage international solidarity in the control of animal diseases,
- to provide a better guarantee of the safety of food of animal origin and to promote animal welfare through a science-based approach,
- within its mandate under the WTO SPS Agreement, to safeguard world trade by publishing health standards
- to improve the legal framework and resources of national Veterinary Services

Until now, the OIE has considered animal disease testing mainly as it pertains to trade. Accordingly, it classifies animal disease diagnostic tests as prescribed or alternative tests. Prescribed tests are those that are required by the *Terrestrial Animal Health Code* for testing animals and animal products for international trade while alternative tests are those which could be used for import/export purposes upon bilateral agreement. These qualifications are given to the tests by the OIE International Committee upon recommendation of the Biological Standards Commission, formerly the Standards Commission. Some of the older tests have been granted the status of prescribed or alternative for historical reasons while newer tests were classified after analysis of a dossier. Unfortunately, in the current procedure of test classification by the OIE, there is no clear indication of the data requirements for the dossier to be submitted to the OIE for evaluation.

In the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (the *Terrestrial Manual*), diagnostic techniques with associated tests are described for each disease. Some tests are not classified as prescribed or alternative tests. But, unless indicated otherwise, all are presumed to be validated, i.e. capable of producing results that properly infer the infection/exposure status of animals. The purpose of implementation of the test, however, is not only to predict infection status of the animal for trade purposes. There are many other reasons for testing, including: seromonitoring, demonstration of freedom from infection, estimation of prevalence of infection for risk assessment, etc. Therefore, test validation should be a process that will demonstrate fitness of that test for a particular use. The current procedure in use by the OIE for the test qualification does not take into consideration all the different purposes for laboratory testing for animal diseases. Even for trade purposes, there is no guideline available that specifies what is required in a test dossier that is submitted to the OIE for evaluation. OIE has received requests from many Member Countries and also from commercial test manufacturers to provide clear guidelines and much broader recognition of diagnostic tests as fit for specific purposes, not only for trade.

Considering the necessity to improve the current system for the adoption of a test by the OIE, the OIE and the Joint FAO/IAEA Programme which operates the OIE Collaborating Centre for ELISA and Molecular Techniques in Animal Diseases Diagnosis convened a Consultant Meeting in November 2002 to discuss the assay validation process and the procedures needed to guide the certification of animal disease diagnostic tests by the OIE. This meeting identified six distinct purposes for which animal diagnostic tests are implemented. Its conclusions were adopted by a Resolution at the OIE General Session in May 2003: Resolution No. XXIX. This resolution indicates clearly the adoption of the fitness for specific purposes in the process of test validation and the necessity to write clear guidelines and provide a standard template to be used in preparing a dossier for test evaluation.

The Consultants Meeting, which was held in Vienna, Austria from 9 to 12 December 2003, was convened by the OIE, FAO and IAEA to address the issues detailed in the OIE Resolution. The principal expected output of this meeting is the production/establishment of a template that will guide the development of an assay validation dossier for submission to the OIE. The outcome would be that the use of assays validated according to fitness for purpose will give more confidence in animal disease management in general and also in animal and animal product trade specifically.

In this meeting, participating experts were from international organisations, public institutions, and importantly, also private companies. The conclusions and recommendations of the meeting are related to three main issues that were addressed by the experts:

1) The standard template to be used when validating and submitting a test to the OIE for evaluation,
2) The procedure used to evaluate the data submitted to the OIE, and
3) The establishment of reference material collections.

The recommendations from the experts are attached to this document as Appendices I, IIa, IIb and III, successively and have been sent to the Director General of the OIE for consideration.

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APPENDIX I

OIE Assay Validation Template

ITEM	DETAILS REQUESTED	EXAMPLE or DESCRIPTION
1. Background Information		
1.1. Test method	- Disease - Type of method - Target analyte - Species and specimen - Name of kit (if applicable)	
1.2. Intended purpose(s) of test	- Population freedom (declaration) - Animal freedom (trade) - Eradication/control - Investigation of clinical signs - Prevalence estimate (risk analysis) - Immune status	
1.3. Applicant	- Name and complete contact information - Job title within organisation - Type of organisation (i.e. commercial, institutional or governmental)	
1.4. Scientific contact	- Name and complete contact information - Job title within organisation	
1.5. Accreditation or certification status of laboratory	- OIE Quality Standard, ISO/IEC 17025, ISO/IEC 9000 series, GLP/GMP, etc.	
1.6. Intellectual property	- Confidentiality agreements, proprietary considerations	
2. Test method		
2.1. Protocol	- Test method protocol must include: - Introduction - Test method - Fitness for intended purpose(s) - Definitions - Equipment and instrumentation - Reagents - Chemicals - Biologicals - Preparation for test - Preparation of sample - Preparation of reagents - Preparation of equipment and instrumentation - Preparation of laboratory personnel - Performance of test - Test procedure - Interpretation of results - Test controls - Test results - References - Appendices	- See example of protocol (Appendix I)
2.2. Kit configuration (if commercial)	- Samples per kit - Production capacity (theoretical and actual)	

3. Validation – Stage I	**Analytical characteristics**	

3.1. Calibration	- Dose–Response curve
	  - Specify linear operating range
	- Calibration against reference reagents
	  - International, i.e. OIE, WHO, FAO, etc.
	  or
	  - In-house, i.e. selection of strong positive, weak positive and negative reference reagents from dose–response curve
		(Update glossary)

3.2. Repeatability	- Repeatability data
	  - Minimum of three in-house samples representing activity within linear range of assay. i.e. from strong positive to negative (as per Section 3.1 above)
	  - Within run – test each sample in quadruplicate
	  - Between run – minimum 20 runs (total), 2 or more operators, preferably on separate days (note – all runs must be independent of each other)
	  - Between serials – repeat above for each of 3 production batches (serials or lots) of kit, where applicable
	  Data should include means, SDs, UCL/LCLs on both raw and normalised test values
		(Update glossary)

3.3. Analytical specificity	- Cross-reactivity, near neighbour data
	  - Document cross-reactivity by comparing samples from animals infected with organisms with similar clinical presentations and organisms that are genetically closely related
	- Type/group specificity data
	  - Documentation affirming serotype or group specificity
		(Update glossary)

3.4. Analytical sensitivity	- Specify standard of comparison (i.e. currently accepted test method)
	- Comparison may include:
	  - End point titrations
	  - Earliest time of detection post-exposure
	  - Duration of detection post-exposure (if applicable)
		(Update glossary)

4. Validation - Stage II	**Diagnostic characteristics**	
4.1. Reference animals		

4.1.1. Negative reference animals
 	
(Note: negative refers to lack of exposure to or infection with the agent in question)
	- Complete description
	  - Age, sex, breed, etc.
	  - Immunological status
	  - Relatedness to intended target population
	  - Selection criteria including historical, epidemiological and/or clinical data
	  - Pathognomonic and/or surrogate tests used to define status of animals or prevalence within population
	  - Sampling plan and procedures	

4.1.2. Positive reference animals
 	
(Note: positive refers to known exposure to or infection with the agent in question)
	- Complete description
	  - Age, sex, breed, etc.
	  - Immunological status
	  - Relatedness to intended target population
	  - Selection criteria including historical, epidemiological and/or clinical data
	  - Pathognomonic and/or surrogate tests used to define status of animals or prevalence within population
	  - Sampling plan and procedures	

4.1.3. Experimental animals	- Complete description
	  - Age, sex, breed, etc.
	  - Immunological status
	  - Relatedness to intended target population
	- Exposure
	  - Inoculum – source, dose, etc
	  - Type of exposure – inoculation, aerosol, contact, etc.
	  - Sampling plan and procedures	

4.2. Threshold determination	- Complete description of method used
	  - empirical, ROC, mean ± SD, etc
	  - descriptive statistics, frequency distribution diagrams, etc	

4.3. Performance estimates	- Depending on available resources, one or all of the methods described below may be used to generate performance estimates - Irrespective of the method chosen, the standard method(s) of comparison should be run in parallel on all samples, i.e. the test methods in current use	

4.3.1. Diagnostic sensitivity and specificity estimates – with defined reference animals	- Conventional method using reference animals (see 4.1.1 and 4.1.2)
	  - Assuming a minimum sensitivity and specificity of 75% with an allowable error of ± 5% in the estimate at a level of confidence of 95%, number of reference animals required is 300 for each population
	  - Individual animals must be selected from negative and positive reference populations
	  - Include 2x2 table, calculations for diagnostic sensitivity and specificity including error and confidence
	  - Include same calculations for other tests if being compared to the test in question	

4.4.2. Diagnostic sensitivity and specificity estimates – without defined reference animals	- Complete description of model used
	  - Bayesian inference, latent class analysis, etc.
	  - Describe rationale, priors, supporting data
	  - Population selection criteria, including prevalence estimates
	- Other test methods in evaluated should also include the standard method of comparison
	- Using best available priors, choose test populations with appropriate prevalences and select animals in sufficient numbers to generate estimates of sensitivity and specificity with an allowable error of ± 5% at a level of confidence of 95%	

4.4.3. Agreement between tests	- Complete description of test methods in comparison
	  - Presumptive vs confirmatory tests
	  - Relatedness of analytes
	  - Potential biases
	- Complete description of samples tested
	  - Source of samples may include experimental animals sequentially sampled over time
	  - May also include animals or herds defined by reactivity in confirmatory tests or multiple presumptive tests and sampled over a period of time
	- Describe measures of agreement and explanations for results not in agreement	

5. Validation – Stage III	**Reproducibility**	

5.1. Laboratory selection	- Selection criteria for candidate laboratories
	  - Location, i.e. country
	  - Status, i.e. regional, national, provincial/ state
	  - Level of expertise, familiarity with technology
	  - Accreditation status
	- Number of laboratories included
	  - Minimum of three laboratories, should also include OIE Reference Laboratory, if possible	

5.2. Evaluation panel	- Description of test panel
	  - Selection criteria, number of samples (minimum of 20)
	  - Sample volume, allowable number of repeats
	  - Panel composition, i.e. number of replicates, range of analyte concentrations/reactivities
	  - Sample processing requirements, i.e. extractions, spiking, serial dilutions, preservatives, sterilisation
	  - Coding of unknown (blind) samples
	  - Frequency of testing	

5.4. Reproducibility	- Description of type of data/ interpretation
	  - Qualitative (categorical)
	  - Quantitative or semi-quantitative data
	  - Single dilution vs titration
	- Description of type of analysis
	  - Pre-determined limits, consensus, Youden plots
	- Descriptive statistics
	  - Include mean, SD, range of results
	  - Should include controls, as well as, blind samples
	  - Number and proportion of accepted/ rejected runs should be included	

6. Validation – Stage IV	**Current use in other laboratories**	

6.1. Laboratories	- List laboratories where this test method is in current use
	  - Location, i.e. Country
	  - Status, i.e. Regional, national, provincial/ state
	  - Accreditation status	

6.2. Test applications	- For each laboratory
	  - Indicate purpose of test, see Section 1.2
	  - Integration with other tests
	  - Status test, i.e. official test, supplementary, etc
	  - Throughput, i.e. daily, monthly, annual
	  - Turn-around-times	

6.3. International reference reagents	- List type and availability of international reference reagents
	  - Source
	  - Negative, weak/ strong positive reference reagents
	  - Other key biologicals, e.g. antigens, antibodies, etc	

6.4. Inter-laboratory testing programs	- Describe programs involving inter-laboratory comparisons using this test method - National, international - Describe eligibility and number of laboratories participating	
6.5. International recognition	- List internationally-recognised reference laboratory responsible for this test method and/or biologicals - List international standards containing this test method - List international programs employing this test method	

APPENDIX IIa

A Guide for Review of Assay Validation Dossiers Submitted to OIE to Achieve the Status of "Registered Assay"

1. Aim: To Produce a Register of Recognised Assays fit for one or more purpose(s) as outlined in the accompanying OIE Validation Template (Appendix I)

1.1. OIE member countries need assays that are known to be validated according to OIE criteria. The desire of countries is toward:

1.1.1. Improvement in quality of assays
1.1.2. Greater assurance that they correctly classify animal disease status
1.1.3. Enhanced confidence in assays

1.2. For test manufacturers, this process will provide:

1.2.1. Greater transparency and clarity of the validation process
1.2.2. Marketing advantages for test

2. This would be done through submission of a dossier of assay data and information compiled in response to the OIE Validation Template. Assays reviewed for validation would be primarily from the OIE List A/B diseases and others at the discretion of the Biological Standards Commission (See accompanying Appendix IIb that depicts a flow chart and time-line of the review process)

2.1. Who submits?

2.1.1. Any company, institution, academic laboratories, government – essentially anybody

2.2. What is submitted?

2.2.1. A dossier, based on the OIE Validation Template that specifies information required for review.
2.2.2. The purposes for which the test is deemed fit will be specifically stated by the submitter.

3. Evaluation of the dossier by a review panel

3.1. Review through use of the OIE Assay Validation Template containing specific, directed information pertaining to the validation of an assay. This would constitute a dossier.

3.2. Who would evaluate?

3.2.1. Panel of experts appointed by Biological Standards Commission (BSC) of the OIE.
3.2.1.1. From OIE Reference Laboratories and other known experts

3.3. Make-up of review panel

3.3.1. Chairperson and at least one other reviewer
3.3.2. Chairperson is contact point for submitter
3.3.3. Names of reviewers are available to submitters
3.3.4. Avoidance of conflict of interest is imperative
3.3.4.1. As part of the submission, the submitter has the right to specify those who should be precluded as reviewers prior to appointment of the review panel
3.3.4.2. Reviewers are required to list possible conflicts of interest

4. Administration and tracking of the review process

4.1. The OIE's responsibility

4.2. Submissions by electronic means; i.e. a paperless system

4.2.1. Submission on paper by exception only

4.3. Upon receipt by OIE, the review process will follow a strict time-line (see Appendix IIb)

5. Characteristics of the review

5.1. The review must be competent, thorough, transparent, fair, unbiased, reproducible, and free from conflicts of interest

5.2. It will be guided by a Standard Operating Procedure that will specify exactly what will be reviewed

5.3. The report will be confidential to the OIE and the submitter

5.4. A framework for reporting of the results needs to be developed to achieve consistency in the reporting process

6. Contingencies

6.1. During the review process:

6.1.1. If a conflict of opinions about the submission occurs between reviewers, the OIE, through the BSC, will resolve it

6.1.2. Reviewer's queries will be directed to the submitter via the chairperson of the review panel

6.1.3. Additional data from submitter will not be admissible during the review process except on specific request from the reviewer (s).

6.1.4. Right of appeal

6.1.4.1. Only if supported by evidence, including additional data.

6.1.4.2. The final decision is by the OIE (Director General/International Committee)

7. An assay that is approved by the OIE will be entered into the OIE Register of Validated Assays, which will designate the purpose(s) for which the assay is fit.

8. Change Control for assays previously recorded in the register

8.1. Manufacturer/laboratory must notify the OIE of changes that could affect assay performance

8.2. Supplementary validation data will be required

8.3. Batch (serials/lots) control must be specified in the original template (submission) and is not included in Change Control

9. Registration Renewal and Assessment of the assay post-recognition

9.1. Annually, the OIE will request a signature from the submitter specifying that the test is still viable and should be retained on the registry

9.2. Every 5th year, the OIE will use experts to assure that the assay remains within current state of the art for that particular disease/condition and purpose.

9.3. If external evidence is presented by whistleblowers that the assay is not performing properly, the OIE will review the evidence through experts

10. Registration of assays that currently exist in the list for A/B diseases including Prescribed and Alternate tests, as well as "Standard" tests (not kits) in widespread use.

10.1 These will be reviewed over time by OIE

11 Registration will be a fee-based system

11.1 Charging a fee for the registration of an assay is agreed by the consultants

11.1 It is anticipated that about 15 hours per reviewer will be required for an expert review of a submission

11.3. A consultancy fee, determined by the OIE will be paid to each reviewer

11.4. OIE will incur costs for administering the program that will have to be recovered

11.5. OIE will do a cost study which will be a basis for charges made to register a test

11.6. Considering that markets for most of animal disease assay kits are small, manufacturers suggested that registration fee be in an acceptable range. If the fee is high, it is likely that it would be a deterrent for submission: companies/institutions must see a positive cost/benefit ratio from registration.

11.7. Costs for use of Panels of Specimens to evaluate tests needs to be considered

12. Glossary

12.1. It was agreed that an extended glossary of terms be made available to submitters to aid in achieving characteristics under section 5.1 above

12.2. Terms to be added to glossary:

12.2.1. Review panel
12.2.2. Chairman of panel
12.2.3. Formulae to support definitions of DSe, DSp, Analytical Se, Analytical Sp, or at least cross-referenced
12.2.4. Register
12.2.5. Recognition (toward acceptance or register)
12.2.6. Variation

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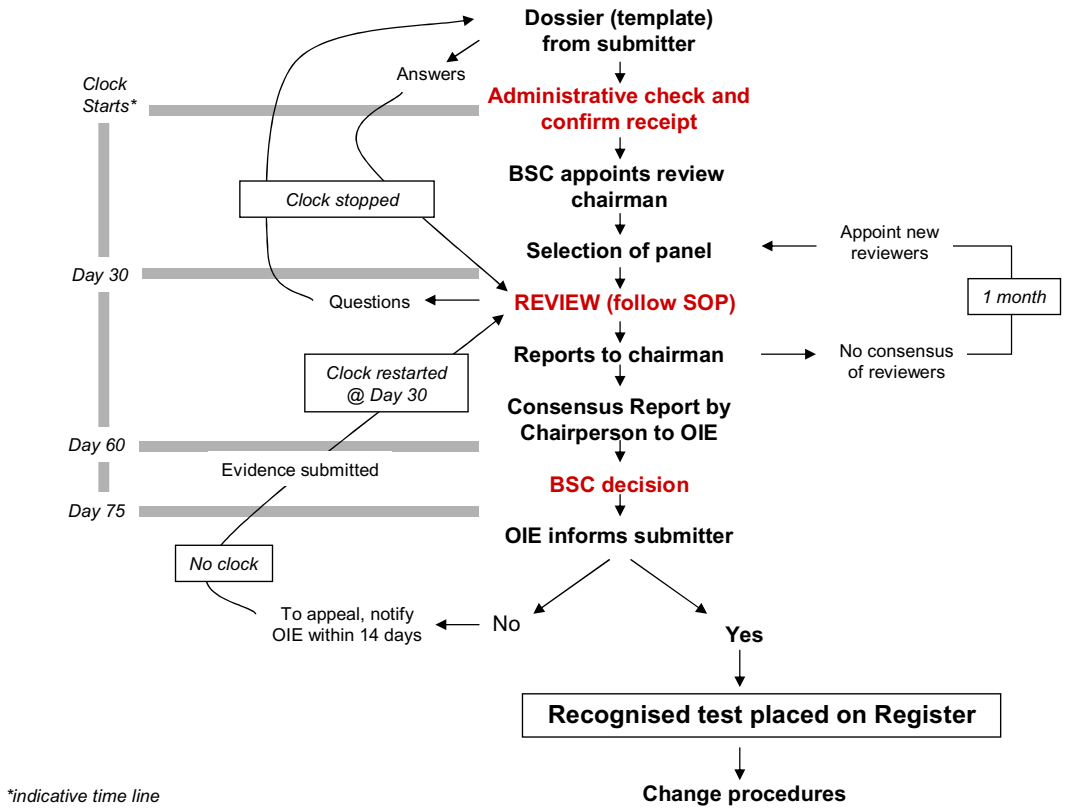
APPENDIX IIb:
Process For Registration of a Validated Diagnostic Assay



APPENDIX III

Reference Laboratories and Reference Standards Reagents

A. Antibody-based assays

I) OIE Reference standard antisera

These sera are provided for the 'calibration' of diagnostic assays in an attempt to standardise the analytical sensitivity of different assays and assay performance in different Laboratories. They will not be supplied in large volumes but will be used for the development of secondary standards. The selection and characteristics of the standard strong, weak and negative sera is already described in the booklet entitled *OIE Quality Standard and Guidelines for Veterinary Laboratories: Infectious Diseases*. The OIE reference standard sera selection will be based on performance characteristics in the prescribed test/s. The strong antiserum should be made up of a pool of positive sera, if possible collected from different geographical regions. There should be at least 3 litres of each serum. The weak positive serum can be prepared as a dilution of the strong positive serum but the dilution should be made in the negative serum. The negative serum should be adult animal serum and should be a pool. Commercially available pools of irradiated serum may be used after suitable screening for non-specific activity. Details of preparation, labelling and storage of the vial are described in the booklet mentioned above.

The reference standard sera should be irradiated so as to make them available to all countries without further inactivation procedures that may affect the performance of the sera. Where laboratories cannot undertake irradiation a central source should be sought to irradiate the bulk serum. The exact protocols will be determined based on expert advice regarding the irradiation process.

Recommendations:

i) OIE should take a co-ordination role in the production of reference standard sera that should include on-going activities by other Regional and International Agencies.

ii) Although sera for different diseases do not pose the same disease security problems, all OIE reference standards should be irradiated due to the risk of adventitious agents.

iii) To ensure that reference standard sera are free of infectious material, it is recommended in the OIE Quality Standard and Guidelines booklet to irradiate them at a dose of 25–30 kilo Gray. However, this dose seems to affect the performance of the sera. Therefore, as a matter of urgency, experiments to determine the optimal level of irradiation to allow inactivation of pathogens while maintaining the reactivity of the serum should be undertaken.

iv) OIE should undertake a full evaluation of alternative inactivation procedures that are widely accepted by other authorities

v) The Biological Standards Commission must prepare a list of disease priorities for the preparation of standard reference reagents.

vi) A full costing for the preparation of five reference standards should be calculated to facilitate negotiations for funding. Their selection should be based on the priority list prepared by the Biological Standards Commission. Their preparation, storage and distribution should be delegated to laboratories after an open-tender to allow participation of non-Reference Laboratories. It is appreciated that in some cases this may involve sub-contracting since third party laboratories may lack the resources to carry out the requisite testing.

vii) Test- manufacturers should pay for the resulting international reference reagents.

II) Assay Evaluation Panel

The aim of the Assay Evaluation Panel is to confirm the claims made by the Manufacturer. In no way can this be considered a complete re-validation panel but it should be selected to give an overall indication that the test performs at a similar level to that described in the Validation Dossier.

The evaluation of the assay by using the serum panel will decide whether the test is "fit for purpose" based on the Template and Dossier.

The assay evaluation serum panel will have to be designed on a disease-specific basis by a group of appropriate experts. It is envisaged that the following generic types of sera should be included **where appropriate**:

- Serial bleeds from infected animals
- Serotype/strain specific sera
- Group specificity
- Cross-reactive/ related organisms
- Different species/ breeds / ages
- Vaccinated/ multiple vaccinated
- Vaccinated/challenged
- Field sera

The Evaluation serum panels will be held in Reference Laboratories and Manufacturers may send kits for validation by Reference Centre staff or may visit the Reference Centre to carry out the validation.

Recommendations:

i) Group of experts in priority diseases should be identified to determine the composition of the assay evaluation serum panels

ii) OIE should seek funds for the preparation of these panels

iii) Manufacturers will be charged by the Reference Laboratories for test evaluation using the panels.

iv) Manufacturers should only be referred to the Reference Laboratories for Assay Evaluation Panel testing subject to a satisfactory evaluation of their Template and Dossier by the OIE reviewers (see Appendices IIa and IIb).

B. Non-Serological Methods

Recommendation:

When setting future priorities, the Biological Standards Commission should consider the identification of suitable reference standard reagents for use in non-serological methods. This may include convening a meeting of appropriate experts.

Appendix VII

Original: English
December 2003

OIE EXPERT GROUP ON EVALUATION OF 'ATYPICAL' BOVINE SPONGIFORM ENCEPHALOPATHY CASES

(Minutes of the meeting)

Paris, 4 December 2003

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The meeting of the Expert Group on Evaluation of 'Atypical' Bovine Spongiform Encephalopathy (BSE) Cases was held at OIE headquarters on 4 December 2003.

The Agenda and List of Participants are presented as Appendices I and II, respectively.

Dr Bernard Vallat, OIE Director General, welcomed the Expert Group and Prof. Vincenzo Caporale, President of the OIE Scientific Commission for Animal Diseases (Scientific Commission, formerly the Foot and Mouth Disease and Other Epizootics Commission), and thanked them for their participation.

Dr Vallat briefly explained the purpose of the meeting and indicated to the experts that the main topics to be discussed would be BSE case definition, revision of the BSE diagnosis procedures used among OIE Reference Laboratories, the need for close collaboration between OIE Reference Laboratories for BSE and national laboratories, the interpretation of the new data on the 'Atypical' BSE cases and the relevance of the results to BSE disease control, surveillance and international trade.

Prof. Caporale chaired the meeting and Dr Danny Matthews was designated as the rapporteur.

BSE case definition

The Expert Group recommended that a draft document prepared by the UK Reference Laboratory on case definition of BSE should be progressed, in consultation with other Reference Laboratories represented at the meeting. That document would then be submitted for consideration and possible adoption by the OIE Scientific Commission for Animal Diseases and the Terrestrial Animal Health Standards Commission.

Collaboration between OIE Reference Laboratories

The Expert Group recommended that existing links between OIE Reference Laboratories should be strengthened, with a view to ensuring the sharing of information and expertise, and for uniform application of knowledge around the world. Preliminary discussions on future collaborations, and possible joint meetings, have already been held.

The experts agreed on the need that National reference laboratories should consult OIE Reference Laboratories before significant findings that influence case definition of BSE, and which have potential implications with regard to the protection of animal and human health, and international trade, are published.

Interpretation of new data from Japan and Italy

The Expert Group reviewed the data on 'atypical' cases notified by Japan and Italy. The group did not believe that the data from respective countries identified a link between the Japanese and Italian cases. While acknowledging that the observations reported had not previously been described in BSE, further investigations already planned or in progress should clarify their significance. Therefore, results of such investigations should be awaited and interpreted before the existence of alternative phenotypes can be confirmed. This will require not only confirmation of

transmissibility, but also investigation into other factors that may influence pathological phenotype even though the infectious agent may be common. The group also stressed that even if the data did represent the existence of alternative phenotypes or strains of BSE, this did not necessarily mean that they were new. They may always have existed but remained unrecognised in the presence of an overwhelming epidemic presenting as a single phenotype, and especially in the absence of the application of current diagnostic procedures in the context of active surveillance.

Relevance of the results to disease control, surveillance and international trade

The Expert Group did not believe that the available evidence justified any changes in current disease control methodologies, or in measures taken to protect human health. There was no basis for suggesting that the risk to animal or human health had changed. Further investigations into the characterisation of the isolates would further inform that debate. Similarly there was no case for changes to international trade rules.

With respect to surveillance, further research into the outcome of positive test results is necessary, but the group recognised that scientific investigation was frequently compromised by the lack of brain material that is available from each animal. It recognised the practical constraints, especially in the abattoir, that make this difficult. Nevertheless, reliance solely on the brain stem prevents the recognition of pathological lesion of the nature identified in Italy where vacuolation and immunostaining patterns differed from those previously recognised for BSE. Therefore, wherever possible, efforts should be made to ensure access to the entire brain of positive animals.

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Appendices

Appendix I

OIE EXPERT GROUP ON 'ATYPICAL' BOVINE SPONGIFORM ENCEPHALOPATHY CASES
4 December 2003

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Agenda

1. Definition of a BSE case and basic standard procedure
2. Technologies and reagents
3. Expert opinion on the BSE 'atypical' cases reported in Japan and Italy

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Appendix II

OIE EXPERT GROUP ON 'ATYPICAL' BOVINE SPONGIFORM ENCEPHALOPATHY CASES

4 December 2003

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List of Participants

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