Organisation Mondiale de la Santé Animale
World Organisation for Animal Health
Organización Mundial de Sanidad Animal

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REPORT OF THE MEETING OF THE OIE BIOLOGICAL STANDARDS COMMISSION

Paris, 17–19 September 2003

The OIE Biological Standards Commission (in brief, Laboratories Commission) met at the OIE Headquarters from 17 to 19 September 2003. Dr Bernard Vallat, Director General of the OIE, welcomed the newly elected Members of the Commission, Prof. Steven Edwards, President, Dr Beverly Schmitt, Vice-President and Dr Anatoly Golovko, Secretary General. Dr Vallat reminded the Members of the new Terms of Reference for the Laboratories Commission and outlined the main direction of its activities. He also informed the Commission of a new programme from the World Bank called 'ALIVE' (African Livestock), which may provide the opportunity for the OIE to assist laboratories in some developing countries.

Prof. Edwards thanked Dr Vallat for his support to the work of the Commission and affirmed the commitment both of the Members and of the other participants to take the work forward. He intended that the Commission should make more use of the Web Site to ensure that timely information on new tests and standards is made available to Member Countries. The Commission also has an important initiative underway on the development of a register of tests validated as 'fit for purpose'.

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 Training of Official Veterinarians

The Commission received an application for an OIE Collaborating Centre for Training of Official Veterinarians. It acknowledged the value of and the need for a Collaborating Centre in this field, and referred the application to the Terrestrial Animal Health Standards Commission (Code Commission) for review of its technical contents, before submission to the Regional Commission and the Administrative Commission.

OIE Collaborating Centre for New and Emerging Diseases

An application had been received for the establishment of an OIE Collaborating Centre for New and Emerging Diseases. The Commission supports the proposal in principle, but requested clarification of certain aspects of the application before recommending it to the Administrative Commission. The views of the appropriate Regional Commission will also be sought.

OFFICE INTERNATIONAL DES EPIZOOTIES
12, rue de prony
75017 paris france
tél. 33 (0)1 44 15 18 88
fax 33 (0)1 42 67 09 87
www.oie.int oie@oie.int

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

Hendra and Nipah virus diseases

CSIRO[1] Australia Animal Health Laboratory (AAHL), Geelong, Victoria 3220, Australia.
Tel.: (+61.3) 52.27.50.00, Fax: (+61.3) 52.27.55.55; CSIRO Web Site: www.csiro.au/li
Designated Reference Expert: Dr Peter Daniels.

Highly pathogenic avian influenza

Hokkaido University Graded School of veterinary medicine, Department of Disease Control,
Kita-18, Nishi-9, Kita-Ku, Supporo, 060-0818, Japan.
Tel.: (+81-11) 706.52.07, Fax: (+81-11) 706.52.73, E-mail: kida@vetmed.hokudai.ac.jp.
Designated Reference Expert: Dr Hiroshi Kido.

Trypanosomosis

CIRAD-EMVT[2], Programme Santé animale TA 30/G Campus international de Baillarguet,
34398 Montpellier Cedex 5, France. Tel.: +33 (0)4 67.59.37.12, Fax: +33 (0)4 67.59.37.98,
E-mail: emmanuel.camus@cirad.fr or marc.desquesnes@cirad.fr
Designated Reference Expert: to be confirmed by the Delegate of France.from the two experts proposed

Contagious bovine pleuropneumonia (CBPP)

CIRAD-EMVT Montpellier, 34398 Montpellier Cedex 5, France.
Tel.: 33 (0)4 67.61.58.00, Fax: 33 (0)4 67.59.37.95, E-mail: francois.thiaucourt@cirad.fr
This will be a joint designation linked to the existing OIE Reference Laboratory at AFSSA[3] Lyon. (AFSSA and CIRAD-EMVT will send a joint annual report to the OIE.)
Designated Reference Expert from CIRAD: Dr François Thiaucourt.

Control of Veterinary Medicinal Products in Sub-Saharan Africa

Ecole Inter-Etats de Science et Médecine Vétérinaire (EISMV), BP 5077, Dakar, Sénégal
Tel: (+221) 865.10.08, Fax: (221) 825.42.83, E-mail: faabiola@refer.sn
Designated Reference Expert: Dr François Abiola.

Bee diseases

The Commission noted that the two existing OIE Reference Laboratories for bee diseases are located in Europe, and that it would be useful to receive nominations from other Regions.

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:

Foot and mouth disease and vesicular stomatitis

Dr Ingrid Bergmann to replace Dr Rossana Allende at the Centro Panamericano de Fiebre Aftosa, Brazil.

Sheep pox and goat pox

Dr Hamid Reza Varshovi to replace Dr M. Hessami at RAZI Vaccine and Serum Research Institute, Iran.

African swine fever

Dr David Paton to replace Dr Philip J. Wilkinson at the Institute for Animal Health, Pirbright, United Kingdom.

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1 CSIRO: Commonwealth Scientific and Industrial Research Organisation
2 CIRAD-EMVT : Centre de coopération internationale en recherche agronomique pour le développement - Département d'élevage et de médecine vétérinaire
3 AFSSA : Agence française de sécurité sanitaire des aliments

Rabies

Dr Thomas Müller to replace Dr James H. Cox at the Federal Research Centre for Virus Diseases of Animals, Wusterhausen, Germany.

Paratuberculosis

Dr Jacek Gwozdz to replace Dr Robin Condron at the Victorian Institute of Animal Science, Victoria, Australia.

Infectious bovine rhinotracheitis/infectious pustular vulvovaginitis (IBR/IPV)

Dr Johannes A. Kramps to replace Prof. J.T. van Oirschot at the CIDC[4], Lelystad, the Netherlands.

2. International standardisation of diagnostic tests and vaccines

2.1. OIE standardisation programmes for diagnostic tests

LIST A DISEASES

Foot and mouth disease (FMD) – Coordinator: Dr D. Paton, Institute of Animal Health, Pirbright, United Kingdom

Drs D. Paton and J. Anderson had confirmed that the OIE Reference sera for strain O Manisa had been tested with satisfactory results in the solid-phase ELISA[5].

Peste des petits ruminants (PPR) – Coordinator: Dr G. Libeau, CIRAD-EMVT Montpellier, France

Dr Libeau reported that a new weak positive reference serum was being prepared. Once irradiated, this will be sent to the other OIE Reference Laboratories for PPR for analysis.

Contagious bovine pleuropneumonia (CBPP) – Coordinator: Dr A. Pini, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale', Teramo, Italy

Dr Pini provided data sheets for the international standard sera he had prepared for the CFT[6] and the ELISA for CBPP diagnosis. The Commission accepted these sera, which will be added to the list of OIE-approved International Standard Sera. The sera will be available as two lots: one non-irradiated lot suitable for the CFT and one irradiated lot suitable for the ELISA.

Highly pathogenic avian influenza (HPAI) – Coordinator: Dr B. Panigrahy, National Veterinary Services Laboratories, 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[7] test for this disease.

LIST B DISEASES

Rabies – Coordinator: Dr F. Cliquet, AFSSA Nancy, France

The Delegate of Mauritius has kindly supplied the negative canine serum to Dr Cliquet. The Reference Laboratory is working on the preparation of a weak positive serum.

Enzootic bovine leukosis – Coordinator: Dr L Renström

Dr Renström informed the Commission that good progress had been made between the OIE Reference Laboratories on the evaluation of different PCR[8] protocols, and one of these will be selected for further validation studies. The OIE International Strong Positive Reference Serum (E4) had now run out. A candidate replacement performed well in ELISA but was less effective as a control for AGID. Studies are continuing.

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4 CIDC: Central Institute for Animal Disease Control
5 ELISA: enzyme-linked immunosorbent assay
6 CFT: complement fixation test
7 AGID: agar gel immunodiffusion
8 PCR: polymerase chain reaction

Caprine and ovine brucellosis – Coordinator: Dr A.P. MacMillan, VLA Weybridge, United Kingdom

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

Dr MacMillan has sent a progress report from the network of OIE Reference Laboratories preparing sera for caprine, ovine and porcine brucellosis.

3. List of prescribed and alternative tests

3.1. Indirect ELISA for rinderpest diagnosis

For this item, the Commission was joined by Dr Joseph Sarr, ISRA[9], to review the validation data for the N-protein indirect ELISA for rinderpest antibodies. This shows promise as a screening test for use in surveillance programmes. It was noted that the assay cross-reacts with PPR. Further data were requested to define quantitative estimates for the analytical and diagnostic sensitivity and specificity.

3.2. Competitive ELISA for equine piroplasmosis

The Commission reviewed the validation dossier on the competitive ELISA for equine piroplasmosis and recommended that it be adopted as a prescribed test for international trade and that the CFT, the current prescribed test, be moved to the list of alternative tests (see Appendix III for the proposed changes to the list of prescribed and alternative tests). The proposed protocol for the competitive ELISA for equine piroplasmosis 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.*

3.3. Competitive ELISA for CBPP diagnosis

The Commission received a report entitled Final Research Co-ordination Meeting for the FAO/IAEA[10] Co-ordinated Research Programme on the "Monitoring of Contagious Bovine Pleuropneumonia in Africa Using Enzyme Immunoassays". In light of this report and of a validation dossier received in 1999 that the Commission had reviewed previously, the Commission recommended that the competitive ELISA be adopted as a prescribed test for international trade (see Appendix III). If adopted by the International Committee, the marking 'prescribed test for international trade' will be added to the Web version of the *Terrestrial Manual.* The Code Commission will be requested to modify certain Articles in the *Terrestrial Animal Health Code* (*Terrestrial Code*) in line with this change.

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

The OIE Reference Laboratory in Sophia Antipolis has recommended that the Commission should consider ELISA as a suitable prescribed test for CAE/MV. The Commission is awaiting further advice on the specific protocol from Sophia Antipolis in consultation with the other OIE Reference Laboratory in the United States of America.

4. Cartagena Protocol – biological diversity

The Intergovernmental Committee for the Cartagena Protocol on Biosafety (ICCP) has invited the OIE to be a partner in the implementation of the Convention on Biological Diversity. The Commission noted the increasing importance of genetically modified organisms in the development of veterinary diagnostic tests and vaccines. Given the role of OIE in setting international standards in this area, it was recommended that the OIE should have a voice in this Convention.

9 ISRA: Institut Sénégalais de Recherches Agricoles

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

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

For this section of the agenda the Commission was joined by the Consultant Editor, Dr James E. Pearson.

The Commission discussed the remaining Member Country comments on chapters for the fifth edition of the *Terrestrial Manual* and advised the Consultant Editor on how to insert them. The Laboratories Commission very much appreciates the helpful comments received from experts in Member Countries. The *Terrestrial Manual* was adopted by the International Committee in May and is scheduled to be published in the first half of 2004. The Commission considered the proposed title of the *Terrestrial Manual*, and recommended that the words 'mammals birds and bees' should be included in parenthesis in order to clarify its scope; the new title is therefore, the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (mammals, birds and bees*). The fifth edition will be published in English, French and Spanish. Any changes adopted by the International Committee between editions will be added to the Web Site on an annual basis, so that the updated version of the *Manual* will in future be the one on the OIE Web Site.

6. Validation and certification of diagnostic assays

6.1. Second OIE/FAO/IAEA Consultants Meeting on 'OIE Validation and Certification of Diagnostic Assays for Infectious Animal Diseases' – Specific procedures for OIE to validate and approve diagnostic tests, organised by the OIE Collaborating Centre for ELISA and Molecular Techniques in Animal Disease Diagnosis, IAEA, Vienna, Austria

The Commission considered Resolution No. XXIX 'OIE Procedure for Validation and Certification of Diagnostic Assays (Test Methods) for Infectious Animal Diseases', adopted by the International Committee during the General Session in May, and requested the OIE Collaborating Centre for ELISA and Molecular Techniques in Animal Disease Diagnosis, IAEA, Vienna, Austria, to proceed with a Second OIE/FAO/IAEA Consultants meeting on 'OIE Validation and Certification of Diagnostic Assays for Infectious Animal Diseases'. Dr Adama Diallo confirmed that this was being arranged for December. It will include participation from OIE and from private sector diagnostic companies. The Commission prepared a draft format for submissions to the OIE register for validated diagnostic assays. This is at Appendix V and will be included in the documents for the Second Consultants Meeting for further comment.

6.2. Evaluation of confirmatory tests for bovine spongiform encephalopathy

The OIE Reference Laboratory for bovine spongiform encephalopathy (BSE) at VLA[11] Weybridge had written to the OIE concerning the provision of an evaluation service for new tests (including kits) for BSE using a standard panel of samples of known provenance. The Commission supported this concept, including the possibility to charge for the service, but recommended that it should be under the responsibility of OIE Reference Laboratories only. Tests and kits that had a satisfactory performance in the evaluation could be entered onto an OIE Register, which will be created once that is activated.

7. Liaison with other Commissions and Groups

- **SCIENTIFIC COMMISSION FOR ANIMAL DISEASES**

7.1. Report of the meeting of the Bureau of the Commission

The Commission reviewed the report of the meeting of the Bureau of the Scientific Commission for Animal Diseases. The Commission looks forward to collaborating with the Scientific Commission and facilitating liaison between that Commission and the OIE network of Reference Laboratories.

- **AQUATIC ANIMAL HEALTH STANDARDS COMMISSION**

7.2. Regulations governing packing and posting of infectious material

This topic is addressed in more detail in Section 8.2 of this report. It was noted that none of the examples of pathogens classed as 'dangerous goods' under UN codes 2814 or 2900 were related to diseases of aquatic animals. Under the proposed new regulations therefore aquatic

11 VLA: Veterinary Laboratories Agency

animal diagnostic samples should be shipped under the less stringent requirements of 'diagnostic samples' (UN 3373). However, any pathogen that has been amplified or propagated to generate a high concentration must be shipped under UN codes 2814 or 2900. Cultures intended for diagnostic tests and subcultures may be shipped as 'diagnostic specimens'.

- **TERRESTRIAL ANIMAL HEALTH STANDARDS COMMISSION**

7.3. Rabies and contagious bovine pleuropneumonia

For this agenda item, the Commission was joined by Dr Alejandro Thiermann, President of the OIE Code Commission.

The Commission discussed a proposed change to point 4 of Article 2.2.5.5. of the *Terrestrial Code* chapter on rabies regarding the antibody tests.

The Commission also proposed changes to certain Articles in the *Terrestrial Code* chapter on CBPP (see point 3.3 of this report).

Both of these proposed changes will be discussed at the next meeting of the Code Commission and, if approved by that Commission, will be presented as Appendices to that report for consideration by Member Countries.

- **AD HOC GROUP ON EVALUATION OF NON-STRUCTURAL PROTEIN TESTS FOR FMD DIAGNOSIS**

The Ad hoc Group was meeting in parallel with the Laboratories Commission, and opportunity was taken to exchange views and explain to the Group the importance of agreeing on an international standard protocol with an acceptable level of validation. The report of the Ad hoc Group meeting will be discussed at the next meeting of the Laboratories Commission.

8. Any other business

8.1. Follow-up from the General Session

The Commission reviewed relevant parts of the Final Report of the General Session and noted the suggestion of a need for Collaborating Centres for Animal Welfare. The Commission would welcome applications from Member Countries.

The query from the Delegate of India concerning possible risks from the importation of avian influenza vaccines from countries facing outbreaks of HPAI was considered in the light of advice from OIE Experts. Vaccines for HPAI are usually embryonated egg-derived, and should be prepared in eggs from specific pathogen free flocks that have been intensively monitored for infectious agents and have not been vaccinated (as recommended in the *Terrestrial Manual* chapter I.1.7). Thus, provided the manufacturer can provide guarantees on this point there should be very little risk from importing such vaccines, even from infected countries, and even less so from countries that had regained freedom following an outbreak.

8.2. Transport of pathogens; Centers for Disease Control conference on transport of infectious substances

For this agenda item, the Commission was joined by Dr J.E. Pearson, who had attended a meeting entitled 'Infectious Substances – Transport by Air After January 2005', held by the Centers for Diseases Control and Prevention (CDC), United States of America. The ICAO[12] Dangerous Goods Panel is reviewing the IATA[13] regulations with a view to facilitating the shipment of diagnostic specimens to laboratories. New regulations were adopted in January 2003, which allow diagnostic specimens that do not contain pathogens considered to be 'dangerous goods' under UN 2418 (human infections) or UN 2900 (animal infections) to be shipped under the less stringent requirements of UN 3373. Modifications to these regulations are being considered and will be effective from January

12 ICAO: International Civil Aviation Organization
13 IATA: International Air Transport Association

2005. The IATA regulations contain a list of pathogens that had been drawn up on advice from WHO[14]: these pathogens must be shipped under UN 2418 or 2900 guidelines. The Commission welcomed the facilitation of the shipping of diagnostic specimens, but was concerned that the classification of animal pathogens under UN 2418 and 2900 should have been determined with advice from the OIE. Accordingly the Commission recommended that the OIE should write to ICAO and to WHO suggesting a number of changes to the proposed lists in line with the assessed risks. The text of the relevant section of the *Terrestrial Manual* chapter on sampling will be updated to reflect the new regulations.

8.3. Letter from Peru re: the use of tests for detecting mammalian and avian protein in fish meal products

The Commission had received a letter from Peru on the use of tests for detecting mammalian and avian protein in fish meal products. There appears to be some doubt as to the specificity of such tests, with a high rate of false positive results being reported. The Commission will consult experts on this subject to ascertain whether the tests are sufficiently well validated to consider developing OIE Standards.

8.4. United States Code of Federal Regulations

The Commission had been alerted to the fact that the United States Code of Federal Regulations does not recommend the use of OIE-approved International Standard Sera for standardisation of vaccines for equine influenza. The Commission emphasised the importance of using OIE Standard Sera and requested the Central Bureau to contact the United States of America.

8.5. Report of the mission to the OIE Collaborating Centre for Diagnosis of Animal Diseases and Vaccine Evaluation in the Americas, Ames, United States of America

Dr Alejandro Schudel, Head of the OIE Scientific and Technical Department, and Prof. Steven Edwards, President of the OIE Laboratories Commission, reported on their mission to the OIE Collaborating Centre for Diagnosis of Animal Diseases and Vaccine Evaluation in the Americas,

Ames, United States of America. They also visited the OIE Reference Laboratories and Experts located in the National Veterinary Services Laboratory and the National Animal Diseases Center in Ames. They were assisted by Dr Beverly Schmitt, Vice-President of the Laboratories Commission, and Dr J.E. Pearson, Consultant Editor of the *Terrestrial Manual.* Dr Schudel and Prof. Edwards were greatly encouraged by the attitude of the staff at the Collaborating Centre and the Reference Laboratories, the very strong profile of the OIE in this important complex of veterinary institutes, and the support offered to OIE Member Countries.

8.6. Dates of next Biological Standards Commission meeting

The next meeting of the Biological Standards Commission will be held from 28 to 30 January 2004.

.../Appendices

14 WHO: World Health Organization

Appendix I

MEETING OF THE OIE BIOLOGICAL STANDARDS COMMISSION

Paris, 17–19 September 2003

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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. Cartagena Protocol – biological diversity
5. OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (*mammals, birds and bees*)
6. Second FAO/IAEA Consults Meeting on 'OIE Validation and Certification of Diagnostic Assays for Infectious Animal Diseases' – Specific procedures for OIE to validate and approve diagnostic tests, organised by the OIE Collaborating Centre for ELISA and Molecular Techniques in Animal Disease Diagnosis, IAEA, Vienna, Austria
7. Liaison with the other Commissions
8. Any other business

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Appendix II

MEETING OF THE OIE BIOLOGICAL STANDARDS COMMISSION
Paris, 17 - 19 September 2003

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List of participants

MEMBERS

Prof. Steven Edwards *(President)*
VLA Weybridge
New Haw, Addlestone
Surrey KT15 3NB
UNITED KINGDOM
Tel.: (44-1932) 34.11.11
Fax: (44-1932) 34.70.46
Email: s.edwards@vla.defra.gsi.gov.uk

Dr Beverly Schmitt
(Vice-President)
National Veterinary Services
Laboratories, Diagnostic Virology
Laboratory, P.O. Box 844, Ames
IA 50010
UNITED STATES OF AMERICA
Tel.: (1-515) 663.75.51
Fax: (1-515) 663.73.48
Email:
beverly.j.schmitt@aphis.usda.gov

Dr Anatoly Golovko
(Secretary General)
State Scientific-Control Institute
Biotechnology and
Microorganisms Strains,
30 Donezkaya St., Kiev 03151
UKRAINE
Tel.: (380-44) 243.83.31
Fax: (380-44) 243.70.65
Email: golovko@biocontrol.kiev.ua

OTHER PARTICIPANT

Dr Peter Wright
Canadian Food Inspection Agency, National Centre for
Foreign Animal Disease, 1015 Arlington Street
Winnipeg, Manitoba R3E 3M4
CANADA
Tel.: (1-204) 789.20.09
Fax: (1-204) 789.20.38
Email: pwright@ inspection.gc.ca

OIE COLLABORATING CENTRE

Dr Adama Diallo
FAO/IAEA Centre for ELISA and Molecular Techniques
in Animal Disease Diagnosis International Atomic
Energy Agency, Wagramerstrasse 5
P.O. Box 100, A-1400 Vienna
AUSTRIA
Tel.: (43-1) 2600.28355
Fax: (43-1) 2600.28222
Email: a.diallo@iaea.org

OIE CENTRAL BUREAU

Dr Bernard Vallat
Director General
12 rue de Prony
75017 Paris
FRANCE
Tel.: (33-1) 44.15.18.88
Fax: (33-1) 42.67.09.87
Email: a.schudel@oie.int

Dr Alejandro Schudel
Head, Scientific and Technical Dept
Email: a.schudel@oie.int

Dr Alejandro Thiermann
President, Terrestrial Animal Health Standards
Commission

Dr Dewan Sibartie
Deputy Head, Scientific & Technical Dept
d.sibartie@oie.int

Ms Sara Linnane
Scientific Editor, Scientific and Technical Dept
Email: s.linnane@oie.int

CONSULTANT EDITOR

Dr James E. Pearson
4016 Phoenix
Ames, Iowa 50014
UNITED STATES OF AMERICA
Email: jpearson34@aol.com

GUEST PARTICIPANT

Dr Joesph Sarr
Institute Senegalais de Recherches Agricoles (ISRA),
Laboratoire National de l'Elevage et de Recherches
Vétérinaires (Virologie), BP 2057, Dakar, SENEGAL
Tel.: (211) 832.40.81
Email: josarr@refer.sn

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

Proposed changes to the List of prescribed and alternative tests

Ref. No.	Disease	Prescribed tests	Alternative tests
A060	Contagious bovine pleuropneumonia	CF, ELISA	[ELISA]
B207	Equine piroplasmosis	[CF], ELISA, IFA	CF

CF = Complement fixation
ELISA = Enzyme-linked immunosorbent assay
IFA = Indirect fluorescent antibody

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

PROTOCOL FOR THE PROPOSED NEW PRESCRIBED TEST FOR EQUINE PIROPLASMOSIS

Enzyme-linked immunosorbent assay

The production of recombinant antigens for the use in enzyme-linked immunosorbent assays (ELISAs) has been described. The recombinant *Theileria equi* merozoite protein (EMA-1) has been produced in *Escherichia coli* (1) and in insect cells by baculovirus (2). In addition, recombinant *T. equi* Be 82 gene product fused with glutathione S-transferase fusion protein antigen has also been produced in *E. coli* (3). Recombinant *Babesia cabelli* rhoptry-associated protein antigen has been produced in *E. coli* (4, 5). Recombinant antigens produced in *Escherichia coli* or by baculovirus have the obvious advantage of removing the need to infect horses for antigen production, and they provide a consistent source of antigen for international distribution and standardisation. Recombinant antigens have been used in the indirect ELISA (4) and the competitive inhibition ELISA (C-ELISA) (6).

The recombinant *T. equi* merozoite protein (EMA-1) and a specific monoclonal antibody (MAb), which defines this merozoite surface protein epitope, has been used in a C-ELISA for *T. equi* (1). This C-ELISA overcomes the problem of antigen purity, as the specificity of this test depends only on the MAb used. A 94% correlation was shown between the C-ELISA and the complement fixation test (CFT) in detecting antibodies to *T. equi*. Sera that gave discrepant results were evaluated for their ability to immunoprecipitate ^{35}S-methionine-labelled *in-vitro* translated products of *T. equi* merozoite mRNA. Samples that were C-ELISA positive and CFT negative clearly precipitated multiple *T. equi* proteins. However, immunoprecipitation results with serum samples that were C-ELISA negative and CFT positive were inconclusive (7). Limited data at this stage would suggest that the C-ELISA is specific for *T. equi* (7). This C-ELISA for *T. equi* was also recently validated in Morocco and Israel, giving a concordance of 91% and 95.7% with the indirect fluorescent antibody (IFA) test, respectively (8, 9).

A similar C-ELISA has been developed using the recombinant *B. caballi* rhoptry-associated protein 1 (RAP-1) and an MAb reactive with a peptide epitope of a 60 kDa *B. caballi* antigen (5). The results of 302 serum samples tested with this C-ELISA and the CFT showed a 73% concordance. Of the 72 samples that were CFT negative and C-ELISA positive, 48 (67%) were shown to be positive by IFA tests, while four of the five samples that tested CFT positive and C-ELISA negative were positive by the IFA tests (5).

A test protocol for an equine piroplasmosis C-ELISA has been described and used for additional validation studies (6, 10). The apparent specificity of the *B. equi* and *B. caballi* C-ELISAs lay between 99.2% and 99.5% using sera from 1000 horses presumed to be piroplasmosis free. Apparent diagnostic sensitivity of both tests applied to over 1000 foreign-origin horses of unknown infection status resulted in net detection of 1.1% (*B. equi*) and 1.3% (*B. caballi*) more seropositive animals than the CFT, as confirmed by independent dual-observer IFA testing. Eight experimentally infected horses (four for *B. equi*, four for *B. caballi*) were serially tested from 4 to 90 days post-exposure. Both C-ELISA procedures were again found to more sensitive than the CFT for the detection of infected animals; the results were confirmed by IFA testing. Seroconversion was detected by C-ELISA as soon as or sooner than by CFT. Both tests were highly reproducible well-to-well, plate-to-plate, and day-to-day, with overall variances of ± 1.2% and ±1.6% for the *B. equi* and *B. caballi* tests, respectively.

An example of a C-ELISA protocol is given below.

- **Solutions**

Antigen coating buffer: prepare the volume of antigen coating buffer required by using the following amounts of ingredients per litre: 2.93 g sodium bicarbonate; 1.59 g sodium carbonate; sufficient ultra-pure water to dissolve, and make up to 1 litre with ultra-pure water. Adjust to pH 9.6.

C-ELISA wash (high salt diluent): prepare the volume of C-ELISA wash required by using the following amounts of ingredients per litre: 29.5 g sodium chloride; 0.22 g sodium phosphate monobasic; 1.19 g sodium phosphate dibasic; 2.0 ml Tween 20; sufficient ultra-pure water to dissolve, and make up to 1 litre with ultra-pure water. Mix well. Adjust pH to 7.4. Sterilise by autoclaving at 121°C.

- **Antigen production**

Frozen transformed *E. coli* is inoculated at a 1/10,000 dilution into any standard non-selective bacterial growth broth (e.g. Luria broth) containing added carbenicillin (100 µg/ml) and isopropyl-thiogalactoside (IPTG, 1 mM). Cultures are incubated on an orbital shaker set at 200 rpm at 37°C overnight. Cells grown overnight are harvested by centrifugation (5000 ***g*** for 10 minutes), washed in 50 mM Tris/HCl and 5 mM ethylene diamine tetra-acetic acid (EDTA) buffer, pH 8.0, and harvested again as before. (Antigen is available from the National Veterinary Services Laboratories, P.O. Box 844, Ames, Iowa 50010, USA.)

Cells are resuspended to 10% of the original volume in the Tris/EDTA buffer to which 1 mg/ml of lysozyme has been added, and incubated on ice for 20 minutes. At that time Nonidet P-40 detergent (NP-40) is added to a final 1% (v/v) concentration, vortexed, and incubated on ice for 10 minutes. The material is next sonicated four times for 30 seconds each time at 100 watts, on ice, allowing 2 minutes between sonications for the material to remain cool. The sonicate is centrifuged at 10,000 ***g*** for 20 minutes. The resulting supernatant is dispensed in 0.5 ml aliquots in microcentrifuge tubes and may then be stored at –70°C for several years.

- **Test procedure**

i) Microtitration plates are prepared by coating the wells with 50 µl of either *B. equi* antigen or *B. caballi* antigen diluted in antigen-coating buffer. The dilution used is determined by standard serological titration techniques. The plate is sealed with sealing tape, stored overnight at 4°C, and frozen at –70°C.

ii) The biotin-labelled anti-murine IgG is diluted in sterile water as directed by the manufacturer, stored at 4°C, and further diluted at the time of use in C-ELISA wash to a concentration of 1/220, with 2% (v/v) normal equine serum added. The avidin–alkaline phosphatase enzyme conjugate is diluted 1/43 (v/v) in C-ELISA wash, and the chromogenic enzyme substrate is mixed according to manufacturer's instructions.

iii) Plates are thawed at room temperature, the coating solution is decanted, and the plates are washed twice with C-ELISA wash.

iv) Undiluted equine sera (50 µl/well) is added to wells. Serum should not be heat-treated. Each serum is tested in duplicate wells. Plates are incubated at 37°C for 40 minutes in a humidified chamber.

v) All wells then receive 50 µl/well of diluted anti-*B. equi* or anti-*B. caballi* monoclonal murine ascites. (MAb is available from the National Veterinary Services Laboratories, P.O. Box 844, Ames, Iowa 50010, USA.) Plates are incubated for 30 minutes at 37°C in a humidified chamber, then washed four times in C-ELISA wash.

vi) Diluted biotinylated anti-murine IgG is added (50 µl/well) to wells. Plates are incubated for 20 minutes at 37°C in a humid chamber, then washed four times in C-ELISA wash.

vii) Avidin–alkaline phosphatase conjugate is added (50 µl/well) to all wells. Plates are incubated, covered for 15 minutes at room temperature, then washed four times in C-ELISA wash.

viii) Chromogenic enzyme substrate (50 µl/well) is added to wells, and plates are incubated with shaking at room temperature during colour development.

ix) The colour development is stopped by adding 50 µl EDTA stop solution (2.5% [w/v] solution of EDTA in ultra-pure water) to all wells when the negative serum control wells have an optical density of 0.2–0.7 at 590 nm wavelength (OD_{590}).

x) The plates are read at 590 nm. The average OD_{590} is calculated for the duplicate wells for all sera. For a valid test, the positive control average OD_{590} cannot exceed 30% of the negative serum control average OD_{590}, and the coefficients of variation of the negative and positive control sera cannot exceed 10%.

xi) If the sample average OD_{590} is less than or equal to the positive control OD_{590}, the unknown is considered positive. If the sample average OD_{590} is greater than the positive control OD_{590}, the unknown is considered negative. A sample may be declared indeterminate or suspect if the average OD_{590} is very close to that of the positive control.

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Draft OIE Registration Form for Validated Diagnostic Assays

1. Name of the diagnostic assay

2. Classification

(For OIE use only, and according with the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* [*mammals, birds and bees*])

3. Applicant information:

3.1. Name of laboratory:

3.2. Address (street, city, code, country, tel./fax numbers, e-mail, Web Site):

3.3. Country:

3.4. Responsible (person):

3.5. Has the laboratory been accredited (Y/N)

To what standard has it been accredited

4. Manufacturer (if applicable):

4.1. Name:

4.2. Address (street, city, code, country, tel./fax numbers, e-mail, Web Site):

4.3. Country:

4.4. Responsible technical person:

5. Distributor (if applicable):

5.1. Name:

5.2. Address (street, city, code, country, tel./fax numbers, Web Site)**:**

5.3. Country:

5.4. Responsible technical person:

6. Application of the assay

7. Use – complete description of the protocol

Should include:

7.1. Biological and/or chemical constitution of the reagents

Antigens/antiserums/other reagents: identification, amount/titre; concentration in International Units (IUs), inactivants, stabilisers, conservants; emulsifiers, diluents, substrates, others

7.2. Method of obtaining the reagents included in the assay

Brief description of the origin and characteristics of the reagents

7.3. Animal species in which the assay will be used

a) Controls

Negative/uninfected animals

Strongly positive animals

Weakly positive animals

b) Reference standards

7.4. Complete and detailed procedures for the instrumentation of the assay (if applicable):

a) Sample conditions

b) Maximum length of time for the reagents to be used

c) Laboratory conditions under which the assay is to be performed

d) Interpretation of results

7.5. Storage, conditions and half-life of reagents

7.6. General precautions

a) Adequate storage and use

b) Disposal of used reagents

c) Risk for public or animal health during usage

7.7. Control on the final product (for commercial laboratories only):

7.7.1. Quality and purity

a) Biological tests

b) Physical-chemical tests

7.7.2. Safety

a) Describe the test used for the different reagents

7.7.3. Inactivation and/or modification

a) Inactivation

b) Modification (method)

8. Validation

8.1. Complete description of the validation process and results according to the OIE *Terrestrial Manual* chapter

- Level achieved
- Species
- Requirements for master seeds and ingredients of animal origin
- Sensitivity (analytical and diagnostic)
- Specificity (analytical and diagnostic)
- Stability
- Repeatability
- Suitability
- Predictive values
- Source of the samples
- Study design
- Study reports
- Published papers, technical reports or OIE Reference Laboratories specific report
- Other/s

9. Interpretation of the assay

a) Limitations on its use

b) Precautions

10. Scientific papers and reports related to the product

a) Discovery

b) Application

c) Modifications

d) Practical use

11. Date and signature of the person responsible for the application

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