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REPORT OF THE MEETING OF THE OIE BIOLOGICAL STANDARDS COMMISSION

Paris, 23–25 January 2007

The OIE Biological Standards Commission met at the OIE Headquarters from 23 to 25 January 2007. Dr Gideon Brückner, Head, OIE Scientific and Technical Department, speaking on behalf of Bernard Vallat, Director General of the OIE, welcomed the Members of the Commission, Professor Steven Edwards, President, Dr Beverly Schmitt, Vice-President and Dr Mehdi El Harrak, Secretary General as well as the other expert participants, Dr Adama Diallo, representative of the OIE Collaborating Centre for ELISA[1] and molecular techniques in animal disease diagnosis, IAEA[2], Vienna, Austria, and Dr Peter Wright, President of the OIE *ad hoc* Group on Nonstructural Protein Tests for Foot and Mouth Disease Diagnosis. Dr Santanu K. Bandhopadhyay and Dr Vladimir Drygin, the other members of the Commission were invited but could not attend the meeting.

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:

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

OIE Collaborating Centre for Wildlife Disease Surveillance and Monitoring, Epidemiology and Management
Canadian Cooperative Wildlife Health Centre (CCWHC), CANADA
Tel.: (+1-800) 567.20.33; ccwhc@usask.ca

OIE Collaborating Centre for Animal Welfare Science and Bioethical Analysis
Animal Welfare Science and Bioethics Centre (AWSBC) at Massey University, NEW ZEALAND
Tel.: (+6) 350.48.07; d.j.mellor@massey.ac.nz

OIE Collaborating Centre for Epidemiology, Training and Control of Emerging Avian Diseases
Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Padova, ITALY
Tel.: (+39-049) 808.42.79; dirsan@izsvenezie.it

OIE Collaborating Centre for Veterinary Services Capacity Building
Partner organisations: Center for Animal Health and Food Safety (University of Minnesota); Michigan State University; and the Centers for Epidemiology and Animal Health (USDA, APHIS, VS), UNITED STATES OF AMERICA.
Tel.: (+1-612) 624.67.72; huest001@umn.edu

1 ELISA: immunoenzymatic method
2 IAEA: International Atomic Energy Agency

OIE Collaborating Centre for Biotechnology-based Diagnosis of Infectious Diseases in Veterinary Medicine
The OIE Collaborating Centre for Application of Polymerase Chain Reaction Methods for Diagnosis of Viral Diseases in Veterinary Medicine, Uppsala, Sweden, had requested that its title be changed to OIE Collaborating Centre for Biotechnology-based Diagnosis of Infectious Diseases in Veterinary Medicine. The Commission accepted this proposal.

OIE Reference Laboratory for Turkey rhinotracheitis
AFSSA[3] Ploufragan, Unité de virologie, immunologie et parasitologie aviaires et cunicoles, BP 53, 22440 Ploufragan, FRANCE
Tel: (+33-[0]2) 96.01.62.22 Fax: (+33-[0]2) 96.01.62.63; n.eterradossi@ploufragan.afssa.fr
Designated Reference Expert: Dr Nicolas Eterradossi

OIE Reference Laboratory for Porcine reproductive and respiratory syndrome
National Veterinary Research Institute, Department of Swine Diseases, Partyzantow str. 57, 24-100 Pulawy, POLAND
Tel.: (+48-81) 886.30.51; Fax: (+48-81) 886.25.95; stadejek@piwet.pulawy.pl
Designated Reference Expert: Dr Tomasz Stadejek

OIE Reference Laboratory for Enzootic abortion of ewes (*ovine chlamydiosis*)
Institute for Veterinary Pathology (IVPZ), Vetsuisse Faculty, University of Zurich, Winterhurerstrasse 268, CH-8057, Zurich, SWITZERLAND
Tel.: (+41-44) 635.8551; Fax: (+41-44) 635.8934; apos@vetpath.unizh.ch
Designated Reference Expert: Dr Nicole Borel

OIE Reference Laboratory for Salmonellosis
National Reference Laboratory for Salmonella, Istituto Zooprofi lattico Sperimentale delle Venezie, Viale dell'Università, 10, 35020 Legnaro (PD) ITALY
Tel: (+39-049) 8084.296; Fax: (+39-049) 8830.268; e-mail: aricci@izsvenezie.it
Designated Reference Expert: Dr Antonia Ricci

OIE Reference Laboratories for Scrapie and Chronic wasting disease
Canadian Food Inspection Agency, Ottawa Laboratory – Fallowfield, CANADA
Tel.: (+1-613) 228.6698; Fax: (+1-613) 228.6669; balachandrana@inspection.gc.ca
Designated Reference Expert: Dr Aru Balachandran

OIE Reference Laboratories for Equine piroplasmosis and Bovine babesiosis
National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, JAPAN
Tel: (+81-155) 49.5642; Fax: (+81-155) 49.5643; protozoa@obihiro.ac.jp
Designated Reference Expert: Prof. Ikuo Igarashi

OIE Reference Laboratory for Surra
National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, JAPAN
Tel: (+81-155) 49.5647; Fax: (+81-155) 49.5643; protozoa@obihiro.ac.jp
Designated Reference Expert: Prof. Noboru Inoue

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:

African horse sickness
Dr Concepción Gómez-Tejedor Ortiz to replace Dr M.A. Jimenez Clavero & Dr Consuelo Rubio at the Laboratorio de Sanidad y Producción Animal de Madrid, Algete, Spain.

Brucellosis
Dr Heinrich Neubauer, to replace Dr Konrad Sachse at Friedrich-Loeffler-Institut, Jena, Germany.

3 AFSSA : Agence française de sécurité sanitaire des aliments

Brucellosis
Dr Massimo Scacchia to replace Dr Donatella Nannini at Istituto Zooprofilattico Sperimentale, Teramo, Italy.

Salmonellosis
Dr Cornelius Poppe to replace Dr Anne Muckle at Health Canada, Population and Public Health Branch, Laboratory for Foodborne Zoonoses, Guelph, Canada

Sheep pox and goat pox and Lumpy skin disease
Dr Eeva Tuppurainen to replace Dr Philip Mellor at the Institute for Animal, Pirbright, United Kingdom.

1.3. Follow-up to the First International Conference of OIE Reference Laboratories and Collaborating Centres, Brazil, December 2006

Prof. Edwards had asked a number of participants for their views on the Conference in Florianopolis so that lessons could be learned for future conferences. The participants agreed unanimously that it had been a successful, useful and productive meeting that afforded a chance for experts to meet face-to-face to discuss, network, build future collaboration or exchange expertise, etc. A number of experts however, expressed their discontent with the logistical arrangements (travel) and some believed that the location was too remote. The Conference was focused on the role and activities of reference laboratories and not enough consideration was given to issues relative to collaborating centres.

The Commission agreed that future conferences should endeavour to find a balance between scientific sessions and broader, organisational issues. Time should be set aside on the programme for meetings of specialists on specific diseases, collaborating centre directors, and for specialised technical workshops on relevant topics. A list of registrants and their specialty should be provided (this would improve networking and impromptu meetings) and the conference badge should include the species and disease for which the person is an expert. One participant had suggested that future conferences be linked to the WAVLD[4]. The Commission believed that this was a very good suggestion; the 1-day OIE Biotechnology Seminar that is held in conjunction with the WAVLD could be replaced by a meeting of OIE Reference Laboratories and Collaborating Centres. The Commission recommended that the OIE should explore the possibility with WAVLD organisers in the context of the WAVLD meeting to be held in Madrid, Spain in 2009.

1.4. The OIE twinning concept: guidance for applications

The Commission reviewed the status of the twinning concept. A number of OIE Reference Laboratories and some laboratories in developing and in-transition countries had expressed an interest in twinning and they had been encouraged to draft project proposals. The Commission believes that projects would need to be accompanied by a detailed financial plan and that the OIE Financial Department should develop a suitable template. The template should request details of all aspects of the twinning project (travel, subsistence, staff time, equipment, reagents, etc.) and the OIE could indicate upon receipt of the dossier for which aspects it would try to find funding. It is also important to stress to applicant laboratories that the purpose of twinning is to assist them to become OIE Reference Laboratories engaged in international activities and that it is not only for national capacity building. Project proposals should, therefore, detail the laboratory's needs specific to becoming recognised by the OIE. In the near future, an expert would be appointed to OFFLU[5] charged with twinning avian influenza laboratories. This development should give a renewed impetus to the concept of twinning.

1.5. Annual Reference Laboratories/Collaborating Centre reports for 2006

Reports had been received from 131/132 Reference Laboratories and 19/19 Collaborating Centres for terrestrial animals. The Commission commented on the enthusiastic support given to OIE by the Reference Laboratories and Collaborating Centres, and the great value to the Biological Standards Commission of the expert advice they provide. The full set of reports will be supplied to Member

4 WAVLD: World Association of Veterinary Laboratory Diagnosticians
5 OFFLU: OIE/FAO Network on Avian Influenza

Countries and to all the Reference Laboratories and Collaborating Centres on a CD-ROM as well as in printed format for those Member Countries that prefer to receive a hard copy. The international activities relevant to the work of the OIE are summarised in the table:

Reference Laboratories

	General activities	Percentage of Laboratories carrying out these activities
1	Test(s) in use/or available for the specified disease	99%
2	Production and distribution of diagnostic reagents	86%
	Specific OIE activities	
3	International harmonisation/standardisation of methods	71%
4	Preparation and supply of international reference standards	49%
5	Research and development of new procedures	88%
6	Collection, analysis and dissemination of epizootiological data	68%
7	Provision of consultant expertise	76%
8	Provision of scientific and technical training	65%
9	Provision of diagnostic testing facilities	55%
10	Organisation of international scientific meetings	29%
11	Participation in international scientific collaborative studies	64%
12	Presentations and publications	86%

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	100%
2	Proposal or development of any procedure that will facilitate harmonisation of international regulations applicable to the surveillance and control of animal diseases, food safety and animal welfare	77%
3	Placement of expert consultants at the disposal of the OIE	83%
	Specific OIE activities	
4	Provision of scientific and technical training within to personnel from OIE Member Countries	83%
5	Organisation of scientific meetings on behalf of the Office	55%
6	Coordination of scientific and technical studies in collaboration with other laboratories or organisations	72%
7	Publication and dissemination of any information that may be useful to OIE Member Countries	88%

2. International standardisation of diagnostic tests and vaccines

2.1. OIE standardisation programmes for diagnostic tests

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

As Dr Cliquet cannot secure a reliable and ample source of naïve dog sera, she has reached an impasse in the project to prepare a weak positive serum. The Commission will explore possibilities with the other Reference Laboratories.

Enzootic bovine leukosis (EBL) standard reference serum – Coordinator: Dr Dagmar Beier, Friedrich Loeffler Institute, Wusterhausen, Germany

Dr Beier had sent a data sheet for the newly adopted OIE international standard serum E5 for EBL diagnostics.

Caprine and ovine brucellosis – Coordinator Mrs J. Stack, VLA Weybridge, UK.

Mrs Stack had submitted a report on a meeting of the OIE Reference Laboratories for Brucellosis, held during the Reference Laboratories Conference in Florianopolis. The project to develop standard sera had met with a number of problems and consensus results had not been reached for a number of reasons such as not enough replicate testing had been performed in each of the laboratories participating in the ring test, many of the laboratories were reporting results from tests that they did not routinely use, etc. The report included a work plan aimed to address the problems and progress the project. This was endorsed by the Commission.

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

The indirect ELISA kits had been tested by a number of Reference Laboratories and the porcine serum seemed to be working well. As a last step, the porcine serum will be sent out along with the small ruminant serum (OIE reference serum for *Brucella melitensis*) by VLA Weybridge to the Reference Laboratories for testing by their in-house tests.

2.2. Tests for dourine

Dr Louis Touratier was invited by the Commission to give further information on the discrepancy reported between the CFT[6] results obtained by the OIE Reference Laboratory and a German laboratory for two exported Russian mares. It is so far unclear whether the problem relates to the strain of parasite used for antigen preparation, or to some other aspect of the methodology. The Commission endorsed Dr Touratier's proposals to try and resolve this through interlaboratory comparisons. In particular there is a need to obtain new isolates of *Trypanosoma equiperdum* representative of currently circulating strains, to progress work on parasite species definition and differentiation between *T. equiperdum* and *T. evansi,* and to validate alternative serological methods such as ELISA.

2.3. Tuberculin production

Dr Amelia Bernardelli from the OIE Reference Laboratory for Tuberculosis in Argentina had submitted a report on a satellite meeting of reference experts held during the Florianopolis conference. She indicated that there are a number of unresolved issues related to tuberculin production and standardisation. This is important because of the global importance of tuberculosis and its potential impact on international trade. The Commission considered that making progress on this topic would be challenging, but agreed the proposal that an *ad hoc* Group should be convened to address the problems related to the production of tuberculin. The Group will be asked to consider what are the barriers to international harmonisation and standardisation of tuberculin production taking into account regulatory and scientific factors, and to recommend a work programme that OIE Reference Laboratories could take forward.

3. List of prescribed and alternative tests

3.1. Proposal for rabies ELISA to be a prescribed test for international trade/movement of dogs and cats

At the General Session in May 2007, the OIE International Committee will be asked to adopt a kit for inclusion on the OIE register (see item 6.1). In view of this, the Commission recommends that the rabies ELISA be adopted as a prescribed test for international trade in dogs and cats (see Appendix III) with the proviso that a kit be used that has been recognised on the OIE Register as fit for that purpose. It was noted that the virus neutralisation (VN) methods described in the *Terrestrial Manual* remain also as prescribed tests.

6 CFT: Complement fixation test

4. Expert, *Ad hoc* and Working Groups

4.1. Report of the Fifth Meeting of the *ad hoc* Group on Evaluation of Nonstructural Protein (NSP) Tests for Foot and Mouth Disease Diagnosis

The *ad hoc* Group met for the fifth time. The purpose of this meeting was to further review the validation data for sheep and pigs, to review evaluation panels and to develop guidelines on the use and interpretation of NSP assays. The Commission endorsed the report, which can be found at Appendix IV.

4.2. Report of the Meeting of the *ad hoc* Group on Antimicrobial Resistance

The Commission noted the ongoing work of the *ad hoc* Group on Antimicrobial Resistance. The draft list of veterinary critically important antimicrobials had been presented to the OIE International Committee during the General Session in May 2006 and had stimulated debate and discussion. The Group was asked to consider the comments of OIE Member Countries taking into account Resolution XXXIII that was adopted. Other areas for the Group to consider were the use of antimicrobials in aquatic animals following the recent expert consultation on antimicrobial use in aquaculture and antimicrobial resistance, and the Codex Task Force on Antimicrobial Resistance. The Report can be found at Appendix V.

4.3. Report of the Meeting of the OIE *ad hoc* Group on Biotechnology

The Commission accepted the report of the meeting of the *ad hoc* Group on Biotechnology. The Report can be found at Appendix VI.

4.4. Report of the Meeting 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 conclusions and recommendations of the Equine Influenza Surveillance Panel. The Report can be found at Appendix VII. Dr Mumford reported that the number of virus isolates received by OIE Reference Laboratories was low. The Commission agreed with the Panel that there is a need for more Reference Laboratories for this disease in other parts of the world, possibly initially via twinning arrangements. It also proposed to ask the Reference Laboratory in the United States of America to contact State laboratories in North America with the request to provide more isolates. The Expert Panel questioned the role of licensing authorities in allowing the continued use of unreliable methods of measuring antigenic content. The Commission also endorsed the Panel's proposal for a meeting, under the auspices of the OIE, of vaccine manufacturers and licensing authorities.

4.5. Update on *ad hoc* Group on Biosecurity

Dr Beverly Schmitt reported on the status of the 'Veterinary Biosafety Facility Construction Handbook'. This is now in its final draft form and had been widely distributed to experts around the world. For this reason, the situation regarding formal publication and future collaboration by the OIE remains uncertain.

4.6. Report of the Meeting of the *ad hoc* Group on vaccination strategies for avian influenza

The Commission noted the report that had been produced under the auspices of the Scientific Commission.

5. Progress on the new edition of the OIE Quality Standard and Guidelines

Work on the second edition of the booklet "OIE Quality Standard and Guidelines for Veterinary Laboratories" had progressed well. The updated guidelines on "Validation of Diagnostic Assays for Infectious Diseases" and on "Laboratory Proficiency Testing" and a new guideline on "Validation and Quality Control of Polymerase Chain Reaction Methods used for the Diagnosis of Infectious Diseases" are given at Appendices VIII, IX and X, respectively. The remaining guideline on "International Reference Antibody Standards for Antibody Assays" was appended to the report of the September 2006

meeting. Texts and references in the OIE Quality Standard have been updated in line with new editions of the ISO/IEC[7] standards, and the glossary has been reviewed. It is proposed to publish the second edition of the booklet later this year.

6. OIE Register of diagnostic tests

6.1. First kit proposed for inclusion in the OIE register "Platelia Rabies II", Bio-Rad.

Following a report from the expert evaluation panel, the Commission approved the "Platelia Rabies II", Bio-Rad kit for inclusion in the OIE register as fit for purpose for the determination of immune status post-vaccination in individual dogs or cats (for regulation of international movement or trade), and in fox populations (for monitoring wildlife vaccination programmes). This will be proposed for adoption by the International Committee at the General Session in May this year.

In the light of experience carrying out this evaluation, one of the experts had offered to provide some suggestions for improving the current dossier to make it more user-friendly for applicants. He will also provide some suggestions for adapting the dossier to applications for PCR kits.

6.2. Future applications

The Commission agreed that applications for rapid tests for TSEs[8] should use a modified validation template developed by the *ad hoc* Group on TSE tests, and the *ad hoc* Group will be asked to carry out the evaluations. Proposals will be submitted to the Commission and then to the OIE International Committee.

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

For this agenda item, the Commission was joined by the Consultant Editor, Dr James Pearson.

Member Countries had now been sent 96 draft chapters and any comments received were reviewed and the chapters were modified where necessary. Work on the last batch of draft chapters is in progress and it is planned to send this batch to Member Countries in March. This final batch will include the remaining updated chapters and some chapters that were originally adopted in 2005 but that have been further updated since then. Prof. Edwards will propose the sixth edition of the *Terrestrial Manual* for adoption by the International Committee in May this year with the proviso that the Commission will address any pending comments during its autumn meeting. Publication of the sixth edition is scheduled for early 2008.

The Commission noted that some Member Countries use lapinised vaccines for the control of foot and mouth disease. It was agreed that the chapter should include a statement that lapinised vaccines do not comply with OIE standards and should not be used.

8. Review of the OIE web pages related to the Commission

The Commission reviewed its OIE web pages and suggested some improvements.

9. Liaison with other Commissions and Groups

9.1. Scientific Commission for Animal Diseases

The Commission endorsed the view of the Scientific Commission in its latest report that it is very important to validate tests for use in wildlife species and encourages OIE Reference Laboratories to do so.

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7 ISO/IEC: International Organization for Standardization/International Electrotechnical Commission

8 TSEs: Transmissible spongiform encephalopathies

9.2. Terrestrial Animal Health Standards Commission

Despite the recommendations of the Biological Standards Commission at its September 2006 meeting, the Code Commission had not decided to alter the 60-day waiting period after vaccination with inactivated vaccine for bluetongue. The Commission felt it would be helpful to clarify that its earlier advice referred only to inactivated vaccines, which are now available for some serotypes.

Expert advice had now been sought regarding border disease transmission through semen, and the Code Commission was recommended to consider reinstating in the *Code* the requirement to test small ruminant semen donors for this disease.

Based on advice from one of the disease experts, the Commission advised the Code Commission that tests for paratuberculosis continue to have serious limitations, and it would therefore be premature to consider radical revision of the *Code* chapter.

9.3. Aquatic Animal Health Standards Commission

The Aquatic Animals Commission had provided the names of two experts who would be asked to review the list of critically important antimicrobials from an aquatic animal health perspective. The finalised list will then be submitted for adoption by the International Committee in May.

10. Any other business

10.1. Report of the Steering Committee of the OIE/FAO FMD Reference Laboratory Network meeting held in Brazil

The Commission noted the report of this meeting.

10.2. OIE Biotechnology Seminar

The Commission finalised the draft programme and a proposed list of speakers for the OIE Biotechnology Seminar to be held in conjunction with the next WAVLD Symposium in Melbourne, Australia, 11–14 November 2007. The Commission agreed to the suggested title of the seminar: "Applications of biotechnology to the diagnosis and pathology of animal diseases". These speakers would be contacted shortly.

10.3. OIE Registry of experts

The Commission was pleased to note that OIE is preparing an online template to create a database of individual experts who could assist the OIE. This would supplement rather than replace the existing network of Reference Laboratory-based experts.

10.4. Report of the "Consultants meeting; Standards, referencing and validation", IAEA, Vienna, Austria, 21–24 November 2006

The Commission noted the draft report, conclusions and recommendations of the above-named meeting. A number of recommendations had also been made by the AEFRV[9], including a request that the fee be reduced and that the template be made more flexible.

10.5. Dates of next Biological Standards Commission meeting

The next meetings are planned for 25–27 September 2007 and 22-24 January 2008.

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.../Appendices

9 AEFRV: Association Européenne des Fabricants de Réactifs Vétérinaires

Appendix I

MEETING OF THE OIE BIOLOGICAL STANDARDS COMMISSION

Paris, 23–25 January 2007

Agenda

1. OIE Reference Laboratories and Collaborating Centres
2. International Standardisation of Diagnostic Tests and Vaccines
3. List of Prescribed and Alternative Tests
4. Expert, Ad hoc and Working Groups
5. New edition of the OIE Quality Standards and Guidelines
6. OIE Register of diagnostic tests
7. *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*
8. Review of the OIE web pages related to the Commission
9. Liaison with other Commissions
10. Any Other Business

MEETING OF THE OIE BIOLOGICAL STANDARDS COMMISSION
Paris, 23–25 January 2007

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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

Disease	Prescribed tests	Alternative tests
Rabies	ELISA, VN	[~~ELISA~~]

ELISA = Enzyme-linked immunosorbent assay
VN = Virus neutralisation

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

Proposed text to be added to the *Terrestrial Manual* chapter:

An ELISA may be used as a prescribed test so long as the kit used is listed on the OIE Register as fit for purpose for international trade. A typical protocol for such a test is given below.

a) Enzyme-linked immunosorbent assay (ELISA) (a prescribed test for international trade)

The assay is an immuno-enzymatic technique for the detection of rabies virus antiglycoprotein antibodies. This assay can be carried out on the serum of several species of animals – dog, cat and fox. The test is based on the use of a solid-phase enzyme immunoassay technique referred to as an indirect ELISA. A microplate is coated with rabies glycoprotein extracted from the inactivated and purified virus membrane. The enzymatic conjugate consists of a protein A from *Staphylococcus aureus* coupled with peroxidase. Positive controls are calibrated against the OIE positive reference standard serum by the user laboratory to obtain a 0.5 IU/ml (called 'OIE 0.5') and a 4 IU/ml (called 'OIE 4') controls that allow the qualitative or quantitative determination of anti-rabies antibody titre in the serum[1].

The test comprises the following reaction steps:

i) The unknown sera as well as the calibrated positive controls or the quantification standards are distributed in the glycoprotein coated wells of the microplates. During incubation of one hour at 37°C, anti-rabies antibodies present in the sample bind to the glycoprotein coated to the microplate wells. After incubation, unbound antibodies and other serum proteins are removed by washings.

ii) The conjugate (protein A labelled with peroxidase) is added to the microplate wells. During a second incubation of 1 hour at 37°C, the labelled protein A binds to the anti-rabies-antibody-antigen complexes attached to the microplate wells. The unbound conjugate is removed by washings.

iii) The presence of immune complex is demonstrated by the addition of a mixture of a chromogen with a peroxidase substrate (1 part 0.25% tetramethylbenzidine with 10 parts 0.015% hydrogen peroxide in citric acid/sodium acetate buffer and 4% DMSO). This initiates a colour development reaction.

1 The OIE Reference Standard (= Standard Serum of Dog Origin) is available from: AFSSA-LERPAS - Laboratoire d'études sur la rage et la pathologie des animaux sauvages, Domaine de Pixérécourt, BP 9, 54220 Malzéville, France; Tel: (+ 33-[0]3) 83.29.89.50; Fax: (+33-[0]3) 83.29.89.56.

iv) After 30 minuntes' incubation at room temperature, the enzymatic reaction is stopped by addition of a solution H_2SO_4 1N. The optical density reading obtained with a spectrophotometer set at 450–620 nm is proportional to the amount of anti-rabies antibodies present in the samples. A standard curve is constructed using the quantification standards (S1 to S6), obtained by serial dilutions of the OIE 4 calibrated positive controls.

The optical density values for the unknown samples are compared with the positive controls. Sera titres in quantification tests are obtained after a direct reading on the standard curve and are expressed as Equivalent units per ml (EU/ml), unit equivalent to the international units defined by virus neutralisation (VN).

It is recommended to use only the primary reference standard available from the OIE Reference Laboratory titrated in IU by VN. This lyophilised primary reference standard is to be reconstituted following the recommendations of the OIE Reference Laboratory, using distilled water.

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Appendix IV

Original: English
January 2007

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

Paris, 22–23 January 2007

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The fifth meeting of the OIE *ad hoc* Group on Evaluation of Nonstructural Protein (NSP) Tests for Foot and Mouth Disease (FMD) Diagnosis was held at the OIE Headquarters in Paris from 22 to 23 January 2007.

Dr Gideon Brückner, Head of the OIE Scientific and Technical Department, welcomed the members on behalf of the OIE Director General, Dr Bernard Vallat, and explained the importance of the application of NSP tests by OIE Member Countries carrying out surveillance for FMD.

The meeting was chaired by Dr Peter Wright, and Dr Rich Jacobson acted as rapporteur. The Agenda and the list of participants are presented as Appendices I and II, respectively.

1. Background

1.1. First meeting

The *ad hoc* Group first met at the OIE Headquarters in Paris from 2 to 4 October 2002. At this meeting, the Group conducted a review of current NSP enzyme immunoassays and examined available validation data. Diagnostic performance estimates were based on relatively few experimental animals and were found to vary widely amongst these test methods. 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.

The indirect enzyme-linked immunosorbent assay (iELISA) from Panaftosa was selected as the best candidate for the index method. This iELISA, along with the enzyme-linked immunoelectrotransfer blot (EITB) Western Blot technique, had been described in the FMD chapter of the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (*Terrestrial Manual* [2000 edition]).

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 follow.

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

1.2. Second meeting

The *ad hoc* Group met for the second time at the OIE Headquarters in Paris from 17 to 19 September 2003. A preliminary draft of the validation dossier was examined. Data on analytical and diagnostic performance characteristics were examined and tabulated. The iELISA and EITB were reviewed for technical detail and upgrades with respect to incorporation of new reference standard reagents and internal quality control processes. Revised descriptions of these methods were incorporated into the 2004 edition of the *Terrestrial Manual*.

Dose–response curves of candidate sera were examined and dilution ranges were selected for the strong and weak positive reference standards. Final preparation and testing of strong and weak positive and negative bovine reference standard sera was then to be undertaken.

Initial candidate sera were identified for the evaluation panels. Sera had been obtained from experimental studies in cattle and include non-vaccinated infected animals, as well as vaccinated animals that had been subsequently challenged. These sera were to be characterised in the index test and stored for future reference and comparisons. Similar types of sera from sheep and pigs were being sought. Additional sera from all species will be added to the bank as they become available.

The Group felt that sufficient data had now been compiled to begin development of specific application, sampling and interpretation strategies, especially with respect to declaration of freedom.

1.3. Third meeting

The *ad hoc* Group met for the third time at the OIE Headquarters in Paris from 6 to 8 September 2004. An NSP validation dossier for the proposed index test was reviewed against the requirements of the new, prototype OIE validation and certification template. This template now serves as the basis for the new OIE register of diagnostic tests and may be found at www.oie.int – *Certification of diagnostic assays*. The index method, as applied to cattle, was shown to satisfy all of the first three of four stages of assay validation, which include the analytical, diagnostic and reproducibility parameters of the template.

Data sheets were reviewed for the negative, weak positive and strong positive candidate reference standard sera (bovine) prepared by Panaftosa. These were forwarded to the OIE Biological Standards Commission with a recommendation that they be approved. Progress on the establishment of bovine evaluation panels was reviewed. A number of candidate sera from Pirbright and Panaftosa had been identified and were being circulated amongst the OIE Reference Laboratories for characterisation in the various NSP tests.

Preliminary data, derived from an NSP ELISA workshop held in Brescia, Italy (3–15 May 2004), were shared with the *ad hoc* Group. Sera from three species: cattle (2415), sheep (693) and pigs (721) were tested in six different NSP tests. Although analysis of the data was still ongoing at the time of writing of the third report, data on the diagnostic specificity and sensitivity in cattle for the index iELISA has confirmed its choice as the OIE index method.

Considerable discussion took place relative to application of both SP and NSP tests in either vaccinated or non-vaccinated populations. Based on diagnostic specificity and diagnostic sensitivity data, the index iELISA was considered to be a suitable screening test when used in combination with confirmatory tests, such as the EITB. With proper sampling strategies, these tests would be appropriate for: a) declaration of population freedom, b) surveillance programmes, c) prevalence surveys, and d) outbreak management, especially recovery.

1.4. Fourth meeting

The *ad hoc* Group met for the fourth time in January 2006. The purpose of the meeting was to: a) review the status of the NSP tests for sheep and pigs, b) review the results obtained in comparative studies carried out by concerted actions, and c) review validation data in sheep and pigs according to the OIE validation criteria. Dr Kris De Clercq, on behalf of Dr Emiliana Brocchi, gave an account of the analysis of the Brescia NSP workshop data with respect to sheep and pigs, and Dr David Paton presented the results of a study on vaccinated/infected Hong Kong pigs, as well as a presentation on the NSP sensitivity panel that Pirbright have been developing. Dr Ingrid Bergmann presented validation dossiers based on the OIE template for sheep and pigs, as well as data on an evaluation panel prepared by Panaftosa for NSP tests in cattle. In addition, invited colleagues from Thailand and Taipei China shared their experiences in the application of a number of NSP tests under field conditions.

1.5. Fifth and current meeting

In January 2007, the *ad hoc* Group met for the fifth time. The purpose of this meeting was to: a) further review the validation data for sheep and pigs, b) review evaluation (sensitivity) panels for sheep and pigs as had been done for cattle, and c) to develop guidelines on the fitness for purpose and interpretation of NSP assays. Because of the many, varied, and very specific purposes for which FMD-NSP assays may be required, the *ad hoc* Group considered it essential that these specific and unique purposes be outlined within the context of the broadly defined purposes that validated assays may fulfil (as listed in 1.4 above). The *ad hoc* Group also found it necessary to discuss the pressing issue of the OIE Validation/Certification process as it relates to NSP-FMD tests, and made recommendations accordingly.

2. Review the status of NSP tests for sheep and pigs

2.1. Validation data for pigs

While there are sufficient data to achieve estimates of diagnostic specificity in naïve sheep and pigs and, to a lesser extent, in non-vaccinated and infected animals, it was concluded that data were lacking from vaccinated and infected (including carrier) sheep and from experimentally vaccinated and infected pigs. The question remained whether or not vaccinated pigs can become subclinically infected and if so, whether or not such animals produce NSP antibodies.

In a study at the Institute for Animal Health, Pirbright, two groups of O1 Manisa vaccinated pigs were exposed to direct contact challenge with O1 UKG 2001 FMDV-infected pigs for 9 hours at 10 and 29 days after vaccination. The pigs were monitored thereafter for 30 days. Although vaccination could not provide complete clinical or virological protection, it reduced virus excretion in infected pigs.

Serology: NSP seroconversion was evaluated using three commercial tests suited to pigs, i.e. Cedi (*Ceditest® FMDV-NS* [Cedi Diagnostics B.V., Lelystad, the Netherlands]), Bommeli (*CHEKIT-FMD-3ABC* [Bommeli Diagnostics, Bern, Switzerland]) and UBI (*UBI® FMDV NS ELISA* [United Biomedical Inc., New York, USA]). Of eight vaccinated pigs challenged on the 10^{th} day post-vaccination, three remained subclinically infected while five became clinically infected for FMD. All of these pigs were positive on the UBI assay through the 30-day post-challenge period, while five pigs (two subclinically and three clinically infected) were similarly detected by Cedi and only two (both clinical) on the Bommeli assay. For the group of eight pigs challenged 29 days post-vaccination, six remained subclinically infected while two became clinically infected. The UBI test detected both clinical animals throughout the trial, while Cedi and Bommeli detected one each over the duration of the study. Among the six subclinically infected animals, the UBI test remained positive for one animal over the course of the experiment, while the other two tests failed to do so for any of the animals. Two pigs were transiently positive on Cedi, as were two on UBI, but none of the tests was positive on the

Bommeli assay[1]. The conclusion from this study is that vaccinated pigs subjected to contact exposure with FMDV-infected pigs can become subclinically infected, accompanied by seroconversion. But the detection of NSP antibodies is sometimes incomplete and transient, based on the three different commercial ELISAs that were evaluated. The samples from this study will be sent to Panaftosa and Brescia for testing on their assays.

2.2. Validation data for sheep

Dr Paton reported that vaccination of sheep followed by aerosol challenge is going to be assessed by his group in an experiment on sheep to be conducted during 2007. Additional data are being collected from vaccinated sheep that have been exposed to FMDV in a study in Jordan. Dr Kris De Clercq indicated that another study is being planned on sheep that were vaccinated in the Lebanon after an outbreak of FMD, in which clinical signs were commonly observed. All of these data will become available during 2007.

Preliminary indications are that the performance of the tests in sheep is similar to that in cattle. The convenience of checking these results in the test from Panaftosa and in the in-house test from the Laboratory at Brescia (which give equivalent results) was discussed. The data will then be made available to the OIE Biological Standards Commission.

A checklist comparing stages of validation for cattle, sheep and pigs is updated from the last meeting, and given at Appendix III.

3. Calibration sera for sheep and pigs

3.1. Swine

A discussion on what constitutes appropriate calibration sera resulted in these conclusions:

a) It was determined that calibration reference sera are useful as primary international reference sera. Secondary standards for daily use in assays lend a quality assurance component to the assay and help to harmonise the assay with a standard that is available to all laboratories and companies that have FMD NSP assays. Reference laboratories, in particular, need to use such standards, and not revert to in-house standards as a matter of convenience. Only with harmonised assays will the international diagnostic community be well served.

b) The relative merits of pre-preparing weak positive sera at a given dilution of a strong positive serum were discussed compared with the supply of strong positive and negative serum so that dilutions can be made locally.

c) For each species (cattle, pigs and sheep), three sera – a negative, a weak positive, and a strong positive – are required as standards for non-vaccinated/infected animals, and three analogous sera are required as standards for vaccinated/infected animals.

d) For theoretical and practical reasons, it is best to prepare a weak positive standard by diluting a strong positive in negative serum.

e) So, for each species, a total of five reference sera are needed. These consist of:

- a negative serum,
- one strong positive serum from a non-vaccinated/infected animal,
- one strong positive serum from a vaccinated/infected animal,

1 Reference: PARIDA S., FLEMING L., OH Y., HAMBLIN P.A., MAHAPATRA M., GLOSTER J., DOEL C., DASH P., TAKAMATSU H.-H. & PATON D.J. (2006). Evaluation of emergency FMD vaccine in pigs following direct contact challenge. Oral presentation and submitted as full paper for the Proceedings of the open session of the research group of the standing technical committee of the European Commission for the control of Foot and mouth Disease, 16– 20 October 2006, Paphos, Cyprus.

- one weak positive prepared by diluting the strong positive serum from a non-vaccinated/infected animal in a negative serum,
- and one weak positive serum prepared by diluting the strong positive from a vaccinated/ infected animal in a negative serum.

3.1.1. These reference standards will be prepared in Dr Bergmann's laboratory at Panaftosa (from non-vaccinated/infected animals) and Dr Paton's laboratory at Pirbright (from vaccinated/infected animals). Their use is for test calibration and preparation of secondary standards. Data sheets for the negative and strong positive sera were provided by Dr Bergmann for both pigs and sheep. For pigs, a strong positive serum from an infected non-vaccinated animal, and a negative serum from a non-vaccinated animal have been selected. The equivalent standard for strong positive vaccinated/infected needs to be similarly prepared.

3.1.2. Based on titrations of the sera from infected pigs, the weak positive should be made by a dilution of 1/480 of the strong positive in negative serum (i.e. a 1/24 dilution of the strong positive in negative serum, and a working dilution of 1/20, giving a final dilution of 1/480). The strong positive should be diluted 1/160 to assure that it remains below the upper limit of the assay (1/8 dilution in negative serum at a working dilution of 1/20 = 1/160 final dilution).

3.2. Sheep

The same rationale for sheep was followed for preparation of the ovine weak positive and strong positive as supplied by Panaftosa. The strong positive was from a non-vaccinated infected sheep. Based on the titration data, a weak positive will be prepared by diluting the strong positive in negative serum 1/24, and then subjected to a working dilution of 1/20, resulting in a final dilution of 1/480. The strong positive will be diluted 1/5 to assure adherence to the linear part of the dose response curve. At a working dilution of 1/20, the final dilution is 1/100.

3.2.1. A similar set of standards will be prepared from a vaccinated/infected sheep that is strongly positive.

3.3. Reference Standards

Once the standards have been prepared at Panaftosa and Pirbright, they will be assessed with the index test and with other available commercial kits and the in-house Brescia's test.

4. Evaluation panels of pig and sheep sera analogous to the bovine serum panel (described in last year's report)

Evaluation panels consisting of 20 to 30 sera for each species need to be collected and characterised. Panaftosa has about 10 sera for sheep and six from pigs that could be included in panels. It is expected that a pig and a sheep serum panel will be assembled following the studies at and made available from Pirbright, with experimentally vaccinated/challenged animals and sera collected in the field from Hong Kong and Jordan. Such panels are very useful for serial release testing, equivalency studies, technical development studies, proficiency testing and sensitivity-establishment testing. Negative sera are also required for analytical specificity testing.

5. FMD NSP assays fitness for various purposes

5.1. A follow-up workshop took place in Belgium from 24 to 25 January 2007 and dealt with the West European situation. The purpose of the meeting for the 12 invited countries, all of which have dense livestock populations, is to devise plans for testing, during management of and recovery from an outbreak. **Internationally validated assays that are fit for specific purposes are necessary in such outbreak situations**. Two additional meetings are planned for the same purpose, one for countries in south-east Europe, and another for north-east European countries.

5.2. The *ad hoc* Group determined last year that NSP assays are fit for applications that include; a) declaration of population freedom, b) surveillance programmes, c) prevalence surveys, and d) outbreak management, especially recovery. However, there was concern that more detail may be needed to explain the test system attributes that would be applied for the many and varied purposes for which FMD testing is required. So, a first attempt was made to develop some guidelines toward that end. A table that extends and further defines OIE Fitness for Purpose Criteria to FMD-NSP test attributes is attached as Appendix IV. It should be emphasised that the Group did not consider the table to be all-inclusive or fully definitive. Rather, it is a first attempt that deserves further consideration, clarification, and definition and needs to be discussed in a joint effort between laboratory and epidemiology groups (meeting preferably as an *ad hoc* Group).

5.3. **Scientific Papers:** Three papers, directly related to efforts of several of our Ad hoc Group have been or are to be published. They are:

BROCCHI E., BERGMAN I.E., PATON D.J., DE CLERCQ K. & GREINER M. *et al.* (2006). Comparative evaluation of six ELISAs for the detection of antibodies to the non-structural proteins of foot-and-mouth-disease virus. *Vaccine*, **24**, 6966–6979.

PATON D.J., DE CLERCQ K., GREINER M., BROCCHI E. & BERGMAN I.E. *et al.* (2006). Application of non-structural protein antibody tests in substantiating freedom from foot-and-mouth disease virus infection after emergency vaccination of cattle. *Vaccine*, **24**, 6503–6512.

PARIDA S., FLEMING L., OH Y., HAMBLIN P.A., MAHAPATRA M., GLOSTER J., DOEL C., DASH P., TAKAMATSU H.-H. & PATON D.J. (2006). Evaluation of Emergency FMD vaccine in pigs following direct contact challenge. Oral presentation and submitted as full paper for the Proceedings of the open session of the research group of the standing technical committee of the European Commission for the control of Foot and mouth Disease, 16–20 October 2006, Paphos, Cyprus.

6. Recommendations and future activities

6.1. Although there are some loose ends on the pig and sheep evaluation panels that will be completed this year, the Group felt that as the data would be shared with the interested parties, another meeting of this *ad hoc* Group was not warranted.

6.2. The *ad hoc* Group achieved, early on, agreement with respect to choosing a representative index method. The performance characteristics of this test method were thoroughly established using the OIE certification template as a guide. Using this index method, standard reference sera for all three host species of interest have been prepared, as well as evaluation panels for these species. Data were also reviewed on the performance of other in-house and commercial test methods in a number of experimental and field trials. In some cases, data from actual field applications of test kits were presented to the *ad hoc* Group.

As may be seen in a number of comparative studies, such as those listed above (see item 5.3), the diagnostic performance characteristics of individual test methods do vary considerably. Therefore, the choice of test method must be considered carefully for a particular field application. The *ad hoc* Group cannot recommend specific test methods or kits for specific applications. However, it can recommend those test characteristics or attributes (i.e. DSp and DSe) that would best suit a particular application and this it has attempted to do (see item 5.2), to be further discussed in the context of a joint laboratory/epidemiology *ad hoc* Group.

In the future, it is hoped that new test kits coming on the market will have made use, during their development and standardisation, of the reference sera and evaluation panels that have been prepared. In addition, it is recommended that developers of kits make use of the OIE test certification process so that validation data may be properly assessed against intended purposes (fitness).

6.3. In the time that has passed since the first meeting of the *ad hoc* Group has met, the OIE has made progress in establishing a process for the validation of assays. However, in reviewing validation data for NSP tests, it has become apparent that there is a need for the development of clear guidelines for both i) the design of test evaluations and ii) the analysis of validation data. It is recommended that the OIE considers establishing another *ad hoc* Group with a mission to develop such guidelines. By establishing guidelines and/or best practices, the relevance and acceptance of the OIE certification process and registry by Member Countries should be enhanced.

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…/Appendices

Appendix I

REPORT OF THE FIFTH MEETING OF THE OIE *AD HOC* GROUP ON EVALUATION OF NONSTRUCTURAL PROTEINS TESTS FOR FOOT AND MOUTH DISEASE DIAGNOSIS

Paris, 22–23 January 2007

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Agenda

1. Review progress made on NSP tests since the previous meeting

2 Validation data for sheep and pigs

3 Evaluation panels

4 Fitness for purpose

5. Prepare future work plan

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Appendix II

REPORT OF THE FOURTH MEETING OF THE OIE *AD HOC* GROUP ON EVALUATION OF NONSTRUCTURAL PROTEINS TESTS FOR FOOT AND MOUTH DISEASE DIAGNOSIS

Paris, 22–23January 2007

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Appendix III

Validation Template Check List - NCPanaftosa-screening ELISA

	ELEMENT	Cattle	Sheep	Pigs
1	**Background Information**			
1.1	Test method	Complete	Complete	Complete
1.2	Intended purpose(s) of test	Complete	Complete	Complete
1.3	Applicant	Complete	Complete	Complete
1.4	Scientific contact	Complete	Complete	Complete
1.5	Accreditation or certification status of laboratory	Complete	Complete	Complete
1.6	Intellectual property	Complete	Complete	Complete
2	**Test Method**			
2.1	Protocol	Complete	Complete	Complete
2.2	Kit configuration (if Commercial)	Complete	Complete	Complete
3	**Validation – Stage I**			
3.1	Calibration	Complete	Complete	Complete
3.2	Repeatability	Complete	Complete	Complete
3.3	Analytical specificity	Complete	Complete	Complete
3.4	Analytical sensitivity	Complete	Complete	Complete
4	**Validation - Stage II**			
4.1	Reference Animals			
4.1.1	Negative reference animals	Complete	Complete	Complete
4.1.2	Positive reference animals	Complete	Partially Complete (Infected, +/- vaccinated + carriers in process - 07)	Partially Complete (Infected, +/- vaccinated in process - 07)
4.1.3	Experimental animals	Complete	Partially Complete (Infected +/- vaccinated + Carriers in process - 07)	Partially Complete (Infected, +/- vaccinated in process - 07)
4.2	Threshold determination	Complete	Complete	Complete
4.3	Performance Estimates			
4.3.1	Dx Se and Sp estimates – with defined reference animals	Complete	Complete	Complete
4.3.2	Dx Se and Sp estimates – without defined reference animals	Not applicable	Not applicable	Not applicable
4.3.3	Agreement between tests	Complete	Complete	Complete
5	**Validation – Stage III**			
5.1	Laboratory selection	Complete	Pending	Pending
5.2	Evaluation panel	Complete	Pending	Pending
5.3	Reproducibility	Complete	Pending	Pending
6	**Validation – Stage IV**			
6.1	Laboratories	Complete	Complete	Complete
6.2	Test applications	Complete	Complete	Complete
6.3	International reference reagents	Complete	Pending	Pending
6.4	Inter-laboratory testing programmes	Complete	None	None
6.5	International recognition	Complete	Pending	Pending

Appendix IV

Fitness for Purpose - Extensions of OIE Criteria to FMD-NSP Test System Attributes				
Purposes for Testing with FMD-NSP as part of a testing regiment	Epidemiological Unit			
	Animal	Herd	Region	
1) Prevalence estimate in non-free region by way of a survey			X	Well characterised DSe and DSp
-> non-vaccinated				DSe and DSp is less critical
-> vaccinated				High DSe and DSp is advantageous
2) Confirmation of previous exposure	X	X		Known kinetics of seroconversion
-> non-vaccinated				High Dse* and Absolute DSp is advantageous
-> vaccinated				High Dse* and Absolute DSp is advantageous
3) Disease management and surveillance	X	X	X	
-> infection status (vac +/-)				See Purpose 4; high DSp less critical
-> prevalence (vac +/-)				See Purpose 1
4) Certifying freedom from infection		X	X	Requires very high DSp & high Dse (very low prevalence expected).
a) Free with vaccination				
				DSp by paired testing/profiling (need quantitative results) &
-> virus circulation				confirmatory test
-> testing non-vaccinated sentinels				See Purpose 2, non-vaccinated
b) Free without vaccination (after emergency vaccination)				
				DSp by paired testing/profiling (need quantitative results) &
-> virus circulation				confirmatory test
-> testing non-vaccinated sentinels				See Purpose 2, non-vaccinated
-> recovered animals; carriers				Absolute DSp by confirmatory tests
c) Free without vaccination (no emergency vaccination)				
-> virus circulation				See Purpose 2, non-vaccinated
-> recovered animals; carriers				See Purpose 2, non-vaccinated
5) Vaccine purity testing		X		Should be assessed using same test to be used in the field

* Particularly if sentinels (sheep) and carriers are going to be tested

Note: The qualitative descriptions of DSe and DSp attributes are meant as a consideration when choosing one test (kit) over another or a combination of tests for a particular purpose (application).

Appendix V

Original: English
September 2006

REPORT OF THE MEETING OF THE OIE *AD HOC* GROUP ON ANTIMICROBIAL RESISTANCE

Paris, 26–28 September 2006

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A meeting of the OIE *ad hoc* Group on Antimicrobial Resistance was held at the OIE Headquarters in Paris from 26 to 28 September 2006. The meeting was chaired by Dr Herbert Schneider; Mr Christopher Teale from the OIE Reference Laboratory for Antimicrobial Resistance acted as rapporteur. The Agenda and List of Participants are given at Appendices I and II, respectively.

1. Welcome and introductory remarks

Dr Gideon Brückner, Head, OIE Scientific and Technical Department, welcomed participants to the meeting on behalf of Dr Bernard Vallat, Director General of the OIE, and thanked the *ad hoc* Group for its work on antimicrobial resistance. The draft list of veterinary critically important antimicrobials had been presented to the OIE International Committee during the General Session in May 2006 and had stimulated debate and discussion. The Group was asked to consider the comments of OIE Member Countries taking into account the resolution that was adopted. Other areas for the Group to consider were the use of antimicrobials in aquatic animals following the recent expert consultation on antimicrobial use in aquaculture and antimicrobial resistance, and the Codex Task Force on Antimicrobial Resistance. Dr Brückner wished the Group success in addressing these issues and completing these tasks.

2. OIE Resolution No. XXXIII: List of Antimicrobials of Veterinary Importance

The *ad hoc* Group had previously compiled a list of proposed veterinary critically important antimicrobials (VCIA), together with an executive summary, based on the information supplied by OIE Member Countries. The report was submitted for adoption by the OIE International Committee during the General Session in May 2006. OIE Resolution No. XXXIII: List of Antimicrobials of Veterinary Importance relating to this report is attached at Appendix III. Comments were received from several OIE Member Countries relating to the development of a list of antimicrobials of veterinary importance. These comments were noted and the Group agreed to take them into account.

3. Report-back from the Seoul meeting (Joint FAO[1]/WHO[2]/OIE Expert Consultation on Antimicrobial Use in Aquaculture and Antimicrobial Resistance) and expected outcomes

Members of the OIE *ad hoc* Group on Antimicrobial Resistance who had attended the Joint FAO/WHO/OIE Expert Consultation on Antimicrobial Use in Aquaculture and Antimicrobial Resistance gave a brief update to the Group. The meeting complemented the expert consultation meetings held in Geneva, Switzerland and Oslo, Norway relating to terrestrial animals.

1 FAO: Food and Agriculture Organization of the United Nations
2 WHO: World Health Organization

4. Codex Task Force on Antimicrobial Resistance

Dr Francesco Berlingieri, Deputy Head, OIE International Trade Department, presented the discussions held in July 2006 during the meeting of the Codex Alimentarius Commission (CAC) on the Codex Task Force on Antimicrobial Resistance. During this meeting, an in-session Working Group was initially convened to discuss the title, timeframe, objectives and scope; the CAC then agreed on the setting-up of the Task Force and on the terms of reference (which mention that full account should be taken of the work and standards of international organisations, such as FAO, WHO and OIE). The OIE stressed the need for the Codex work to be complementary to existing OIE standards. A circular letter has been issued by the CAC regarding the terms of reference for the Task Force; the Task Force will start its work in 2007 by addressing the points raised in this circular letter.

5. Update on the list of critically important antimicrobials for use in humans

Dr Awa Aidara-Kane, Microbiologist, Food Safety Department, WHO, presented to the *ad hoc* Group the WHO approach, developed during a WHO expert consultation meeting in Canberra, Australia in February 2005, to establishing a list of critically important antimicrobials in humans.

6. Next FAO/OIE/WHO Consultation on Antimicrobial Resistance: Agenda items and possible dates

A further joint FAO/OIE/WHO expert consultation meeting is proposed to discuss the lists of important antimicrobials in human and veterinary medicine developed by WHO and OIE. It is recommended by the *ad hoc* Group that this joint expert consultation, if considered necessary, is to take place once the *ad hoc* Group's recommendations have been accepted by the OIE International Committee at the General Session in May 2007 and after the first session of the Codex Task Force on Antimicrobial Resistance.

7. Discussion on issues raised at the OIE General Session in May 2006 and Resolution No. XXXIII

The *ad hoc* Group took note of all recommendations contained in Resolution No. XXXIII adopted at the OIE General Session in May 2006 and concluded the following:

- Use in food-producing animals. It was agreed that the refined list should refer only to antimicrobial use in food-producing animals and that use in non-food-producing animals be excluded. However, it was noted that antimicrobial use in non-food-producing animals should be subject to the prudent use provisions of the OIE *Terrestrial Animal Health Code* (*Terrestrial Code*).
- Each and every compound included in the list is used for treatment, prevention or control of disease in animals in at least one country but the majority of compounds are so used in many countries world-wide.
- It was agreed that chloramphenicol, use of which is banned in many countries in food-producing animals because of its toxicity, should not be included in the list.
- Antimicrobials used solely as growth promoters are not included in the list.
- The Group discussed and reaffirmed that the answers collected give a world-wide picture of the situation and indicate a solid basis for the interpretation of the data. Those OIE Member Countries of the European Union that had not replied individually to the questionnaire were attributed, by default, the answer of the European Community.

The *ad hoc* Group is aware that available information, as contained in the replies to the questionnaire, may not be complete as far as antimicrobial usage in aquaculture is concerned and it is thus recommended that the refined list be referred to the Aquatic Animal Health Standards Commission for further consideration.

The OIE *ad hoc* Group agreed that the list has no mandatory aspect.

The initial aims regarding the development of a list of antimicrobials of veterinary importance have been previously defined and were agreed at the OIE General Session in May 2005. The OIE *ad hoc* Group discussed the comments regarding the aim of the list, but agreed not to change it.

8. Further analysis of the questionnaire results on critically important antimicrobials for veterinary medicine

The *ad hoc* Group reviewed the comments made at the 74th General Session of the OIE International Committee (May 2006), and Resolution No. XXXIII of the 74th General Session. Dr Gérard Moulin of the OIE Collaborating Centre for Veterinary Medicinal Products presented a further analysis of the results. This working document listed antimicrobial usage by species and summarised all responses to the questionnaire that had been received. The *ad hoc* Group considered a range of options to refine and further develop the list, in accordance with these comments and based on further analysis of the data previously supplied by OIE Member Countries. The final recommendations of the *ad hoc* Group are included in Appendix IV.

Criteria used for categorisation of Veterinary Important Antimicrobials

In developing the list, the Group agreed that any antimicrobial authorised for use in veterinary medicine according to the criteria of quality, safety and efficacy as defined in the *Terrestrial Code* (Appendix 3.9.3. Guidelines for the responsible and prudent use of antimicrobial agents in Veterinary Medicines) is important. Therefore, the Group decided to address all antimicrobials used in food-producing animals to provide a comprehensive list, divided into critically important, highly important and important antimicrobials.

In selecting the criteria to define veterinary important antimicrobials, one significant difference between the use of antimicrobials in humans and animals has to be accounted for: the many different species that have to be treated in veterinary medicine.

After in-depth discussion, the following criteria were selected to determine the degree of importance for classes of veterinary antimicrobials.

Criterion 1. Response rate to the questionnaire regarding Veterinary Critically Important Antimicrobials

This criterion was met when a majority of the respondents (more than 50%) identified the importance of a given antimicrobial class in their response to the questionnaire.

Criterion 2. Treatment of serious animal disease and availability of alternative antimicrobials

This criterion was met when compounds within the class were identified as essential against specific infections and there was a lack of sufficient therapeutic alternatives.

On the basis of these criteria, the following categories were established:
- Veterinary **Critically Important Antimicrobials**: are those that meet **BOTH** criteria 1 **AND** 2
- Veterinary **Highly Important Antimicrobials**: are those that meet criteria 1 **OR** 2
- Veterinary **Important Antimicrobials**: are those that meet **NEITHER** criteria 1 **OR** 2

The Group noted that, within the Highly Important category, some classes are critically important for particular animal species. References to this point are made in the comments in the table (Appendix IV)

The refined list by the OIE *ad hoc* Group shows examples of compounds of each antimicrobial class, and is not meant to be inclusive of all compounds or to comprehensively reflect the usage in all species; in particular specific comments relating to important uses in minor species may not be included. In developing the list, the OIE *ad hoc* Group considered that no antimicrobial or class of antimicrobial used in veterinary medicine could be considered unimportant.

It is recommended that the OIE should preside over any consideration or decision to change the status of an antimicrobial. However, considerations such as costs and availability of antimicrobials in various geographical areas as well as local resistance rates could cause the category of *Critically important* agents to be expanded for regional use (e.g. an antimicrobial agent categorised *Highly important* may become *Critically important* in a particular region).

9. Date of the next meeting

To be decided.

…/Appendices

Appendix I

MEETING OF THE OIE *AD HOC* GROUP ON ANTIMICROBIAL RESISTANCE

Paris, 26–28 September 2006

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Agenda

1. Welcome and introductory remarks
2. OIE Resolution No. XXXIII: List of Antimicrobials of Veterinary Importance
3. Report-back from the Seoul meeting (Joint FAO/WHO/OIE Expert Consultation on Antimicrobial Use in Aquaculture and Antimicrobial Resistance) and expected outcomes
4. Codex Task Force on Antimicrobial Resistance
5. Update on the list of critically important antimicrobials for use in humans
6. Next FAO/OIE/WHO Consultation on Antimicrobial Resistance: Agenda items and possible dates
7. Discussion on issues raised at the OIE General Session in May 2006 and Resolution No. XXXIII
8. Further analysis of the questionnaire results on critically important antimicrobials for veterinary medicine
9. Date of next meeting

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Appendix II

MEETING OF THE OIE *AD HOC* GROUP ON ANTIMICROBIAL RESISTANCE
Paris, 26–28 September 2006

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Appendix III

RESOLUTION No. XXXIII

List of Antimicrobials of Veterinary Importance

CONSIDERING THAT

Antimicrobial agents are essential drugs for human and animal health and welfare. Antimicrobial resistance is a global public and animal health concern that is influenced by both human and non-human antimicrobial usage. The human, animal and plant sectors have a shared responsibility to prevent or minimise antimicrobial resistance selection pressures on both human and non-human pathogens.

The second joint FAO/OIE/WHO workshop on management options for non-human antimicrobial usage in March 2004 in Oslo and recommended that the concept of "critically important" classes of antimicrobials for human usage should be developed by WHO. A similar list for animal usage should be pursued by the OIE.

The list of Critically Important Antibacterial Agents (CIA) for Human Medicine was established in February 2005, in a working group meeting in Canberra.

The OIE *ad hoc* Group on Antimicrobial Resistance prepared a questionnaire to collect proposals on antimicrobials of veterinary importance. The questionnaire was sent to the 167 OIE Member Countries and to International Organisations having signed a co-operation agreement with OIE in order to establish such a list. A list of antimicrobials of veterinary importance was compiled from the information received from Member Countries and was circulated to OIE Member Countries.

When finalised, the list could complement the OIE Guidelines for the responsible and prudent use of antimicrobial agents in veterinary medicine (OIE *Terrestrial Animal Health Code* Appendix 3.9.3).

The list could be useful for the risk assessment of antimicrobial resistance in accordance with OIE *Terrestrial Code* Appendix 3.9.4. In this context, lists of CIA for humans and for animals are elements that could be taken into account in a risk assessment process.

THE COMMITTEE

RESOLVES

To publish a preliminary list of antimicrobials of veterinary importance based on the list compiled by the OIE *ad hoc* Group from the answers received to the questionnaire sent to OIE Member Countries.

To ask the Director General to reconvene the *ad hoc* Group to further refine the list and consider breaking it down into subcategories according to type of usage.

To use the list further refined within the framework of the work in progress with the WHO, FAO and the Codex Alimentarius Commission on antimicrobial resistance.

To regularly update the list in accordance with new scientific information.

(Adopted by the International Committee of the OIE on 25 May 2006)

Appendix IV

Final recommendations of the *ad hoc* Group on refining and further developing the list of antimicrobials of veterinary importance

The designation of an antimicrobial as being a veterinary important antimicrobial (VIA) has been done on the basis of replies received in response to a questionnaire circulated to OIE Member Countries. These replies have been collated by the OIE Collaborating Centre for Veterinary Medicinal products and reviewed by the OIE *ad hoc* Group on Antimicrobial Resistance. Account has also been taken of comments made by OIE Member Countries following the OIE General Session held in May 2006.

Criteria used for categorisation of Veterinary Important Antimicrobials

In developing the list, the *ad hoc* Group agreed that any antimicrobial authorised for use in veterinary medicine according to the criteria of quality, safety and efficacy as defined in the *Terrestrial Animal Health Code* (Appendix 3.9.3. Guidelines for the responsible and prudent use of antimicrobial agents in Veterinary Medicine) is important. Therefore, the Group decided to address all antimicrobials used in food-producing animals to provide a comprehensive list, divided into critically important, highly important and important antimicrobials.

In selecting the criteria to define veterinary important antimicrobials, one significant difference between the use of antimicrobials in humans and animals has to be accounted for: the many different species that have to be treated in veterinary medicine.

The following criteria were selected to determine the degree of importance for classes of veterinary antimicrobials.

Criterion 1. Response rate to the questionnaire regarding Veterinary Critically Important Antimicrobials

This criterion was met when a majority of the respondents (more than 50%) identified the importance of the antimicrobial class in their response to the questionnaire.

Criterion 2. Treatment of serious animal disease and availability of alternative antimicrobials

This criterion was met when compounds within the class were identified as essential against specific infections and there was a lack of sufficient therapeutic alternatives.

On the basis of these criteria, the following categories were established:
- Veterinary **Critically Important Antimicrobials**: are those that meet **BOTH** criteria 1 **AND** 2
- Veterinary **Highly Important Antimicrobials**: are those that meet criteria 1 **OR** 2
- Veterinary **Important Antimicrobials**: are those that meet **NEITHER** criteria 1 **OR** 2

Within the Highly Important category, some classes are critically important for particular animal species. References to this point are made in the comments in the table.

- Use in food-producing animals: the refined list refers only to antimicrobial use in food-producing animals; use in non-food-producing animals is excluded. Antimicrobial use in non-food-producing animals should be subject to the prudent use provisions of the OIE *Terrestrial Animal Health Code*.
- All compounds included in the list are used for treatment, prevention or control of disease in animals in at least one country but for the majority in many countries world-wide.
- Chloramphenicol and some other substances, use of which are banned in many countries in food-producing animals, are not included in the list.
- Antimicrobials used solely as growth promoters are not included in the list.

Abbreviations:

Animal species in which these antimicrobials are used are abbreviated as follows:

AVI:	avian	EQU:	equine
API:	bee	LEP:	rabbit
BOV:	bovine	OVI:	ovine
CAP:	caprine	PIS:	fish
CAM:	camel	SUI:	swine

VCIA: Veterinary Critically Important Antimicrobials
VHIA: Veterinary Highly Important Antimicrobials
VIA: Veterinary Important Antimicrobials

CATEGORISATION OF VETERINARY IMPORTANT ANTIMICROBIALS FOR FOOD-PRODUCING ANIMALS

ANTIMICROBIAL FAMILY	SPECIES	% quotations	Specific comments	C1: Quotation > 50%	C2: Essential or Few alternatives	VCIA	VHIA	VIA
AMINOGLYCOSIDES								
AMINOCYCLITOL								
Spectinomycin	AVI, BOV, CAP, EQU, LEP, OVI, PIS, SUI							
AMINOGLYCOSIDES								
Streptomycin	API, AVI, BOV, CAP, EQU, LEP, OVI, PIS, SUI		The wide range of applications and the nature of the diseases treated make aminoglycosides extremely important for veterinary medicine. Aminoglycosides are of importance in septicaemias; digestive, respiratory and urinary diseases. Gentamicin is indicated for *Pseudomonas aeruginosa* infections with few alternatives. Spectinomycin is used only in animals. Few economic alternatives are available.					
Dihydrostreptomycin	AVI, BOV, CAP, EQU, LEP, OVI, SUI							
Framycetin	BOV, CAP, OVI	77,1%		Y	Y	Y		
Kanamycin	AVI, BOV, EQU, PIS, SUI							
Neomycin	API, AVI, BOV, CAP, EQU, LEP, OVI, SUI							
Paromomycin	CAP, OVI, LEP							
Apramycin	AVI, BOV, LEP, OVI, SUI							
Gentamicin	AVI, BOV, CAM, CAP, EQU, LEP,OVI, SUI							
Tobramycin	EQU							
Amikacin	EQU							
ANSAMYCIN – RIFAMYCINS								
Rifampicin	EQU		This antimicrobial class is authorised only in a few countries and with a very limited number of indications (mastitis) and few alternatives, e.g. treatment of *Rhodococcus equi* infections in foals. **Rifampicin is critically important in the equine.**					
Rifaximin	BOV, CAP, EQU, LEP, OVI, SUI	30%		N	Y		Y	
BICYCLOMYCIN								
Bicozamycin	BOV, PIS	1,4%	Biclomycin is listed for digestive and respiratory diseases in cattle and septicaemias in fishes.	N	N			Y

ANTIMICROBIAL FAMILY	SPECIES	% quotations	Specific comments	C1: Quotation > 50%	C2: Essential or Few alternatives	VCIA	VHIA	VIA
CEPHALOSPORINS								
CEPHALOSPORIN 1G								
Cefacetrile	BOV							
Cefalexin	BOV, CAP, EQU, OVI, SUI							
Cefalotin	EQU							
Cefapyrin	BOV		Cephalosporins are used in the treatment of septicemias, respiratory infections, and mastitis. Alternatives are limited in efficacy through either inadequate spectrum or presence of antimicrobial resistance.					
Cefazolin	BOV, CAP, OVI							
Cefalonium	BOV, CAP, OVI							
CEPHALOSPORIN 2G								
Cefuroxime	BOV	58,6%		Y	Y	Y		
CEPHALOSPORIN 3G								
Cefoperazone	BOV, CAP, OVI							
Ceftiofur	AVI, BOV, CAP, EQU, LEP, OVI, SUI							
Ceftriaxone	AVI, BOV, OVI, SUI							
CEPHALOSPORIN 4G								
Cefquinome	BOV, CAP, EQU, LEP, OVI, SUI							
FOSFOMYCIN								
Fosfomycin	AVI, BOV, PIS, SUI	7,1%	This antimicrobial is authorised only in a few countries. Fosfomycin has a limited number of alternatives in some fish infections. **Critically important for fish[3].**	N	Y		Y	
FUSIDIC ACID								
Fusidic acid	BOV, EQU	1,4%	Fusidic acid is used in the treatment of ophtalmic diseases in cattle and horses.	N	N			Y
IONOPHORES								
Lasalocid	AVI, BOV, LEP, OVI							
Maduramycin	AVI							
Monensin	API, AVI, BOV, CAP							
Narasin	AVI							
Salinomycin	AVI, LEP	42,9%	Ionophores are essential for animal health because they are used to control intestinal parasitic coccidiosis. (*Eimeria* spp.) where there are few or no alternatives available. **Ionophores are critically important in poultry.** **Ionophores are used only in animals**	N	Y		Y	
Semduramicin	AVI							

3 Under study

ANTIMICROBIAL FAMILY	SPECIES	% quotations	Specific comments	C1: Quotation > 50%	C2: Essential or Few alternatives	VCIA	VHIA	VIA
LINCOSAMIDES								
Pirlimycin	BOV							
Lincomycin	API, AVI, BOV, CAP, OVI, PIS, SUI	51,4%	Lincosamides are essential in the treatment of Mycoplasmal pneumonia, infectious arthritis and hemorrhagic enteritis of pigs.	Y	N		Y	
MACROLIDES								
AZALIDE								
Tulathromycin	BOV, CAP, LEP, OVI, SUI							
MACROLIDES C14								
Erythromycin	API, AVI, BOV,CAP, EQU, LEP, OVI, PIS, SUI		Macrolides are used to treat Mycoplasma infections in pig and poultry, hemorraghic digestive disease in pigs and liver abscesses (*Fusobacterium necrophorum*) in cattle, where they have very few alternatives. Macrolides are also used for respiratory infections in cattle					
MACROLIDES C16								
Josamycin	AVI, PIS							
Kitasamycin	AVI, SUI	77,1%		Y	Y	Y		
Spiramycin	AVI, BOV, CAP, EQU, LEP, OVI, PIS, SUI							
Tilmicosin	AVI, BOV, CAP, LEP, OVI, SUI							
Tylosin	API, AVI, BOV, CAP, LEP, OVI, SUI							
Mirosamycin	API, AVI, SUI							
Terdecamycin	AVI							
NOVOBIOCIN								
Novobiocin	BOV, CAP, OVI, PIS	31,4%	Novobiocin is used in the treatment of mastitis in the form of intramammary creams and in sepsis of fishes. **Novobiocin is only used in animals**	N	N			Y
ORTHOSOMYCINS								
Avilamycin	AVI, LEP	4,3%	Avilamycin is used for digestive diseases of poultry and rabbits: avilamycin is used to treat necrotic enteritis in chickens where available. **The antimicrobial class is used only in animals.**	N	N			Y

ANTIMICROBIAL FAMILY	SPECIES	% quotations	Specific comments	C1: Quotation > 50%	C2: Essential or Few alternatives	VCIA	VHIA	VIA
PENICILLINS								
NATURAL PENICILLINS								
Benzylpenicillin	AVI, BOV, CAM, CAP, EQU, LEP, OVI, SUI							
Penethamate hydroxide	BOV, SUI							
Penicillin procaine	BOV, CAM, CAP, EQU, OVI, SUI							
AMDINOPENICILLINS								
Mecillinam	BOV, SUI							
AMINOPENICILLINS								
Amoxicillin	AVI, BOV, CAP, EQU, OVI, PIS, SUI							
Ampicillin	AVI, BOV, CAP, EQU, OVI, PIS, SUI		Penicillins are used in the treatment of septicaemias, respiratory and urinary tract infections.					
Hetacillin	BOV							
AMINOPENICILLIN PLUS BETALACTAMASE INHIBITOR		87,1%	They are very important in the treatment of many diseases in a broad range of animal species.	Y	Y	Y		
Amoxicillin_Clavulanic Acid	AVI, BOV, CAP, EQU, OVI, SUI							
CARBOXYPENICILLINS			Few economical alternatives are available.					
Ticarcillin	EQU							
Tobicillin	PIS							
UREIDO PENICILLIN								
Aspoxicillin	BOV, SUI							
PHENOXYPENICILLINS								
Phenoxymethylpenicillin	AVI, SUI							
Phenethicillin	EQU							
ANTISTAPHYLOCOCCAL PENICILLINS								
Cloxacillin	BOV, CAP, EQU, OVI, SUI							
Dicloxacillin	BOV, CAP, OVI							
Nafcillin	BOV, CAP, OVI							
Oxacillin	BOV, CAP, EQU, OVI							

ANTIMICROBIAL FAMILY	SPECIES	% quotations	Specific comments	C1: Quotation > 50%	C2: Essential or Few alternatives	VCIA	VHIA	VIA
PHENICOLS Florphenicol Thiamphenicol	AVI, BOV, CAP, EQU, LEP, OVI, PIS, SUI AVI, BOV, CAP, OVI, PIS, SUI	51,4%	Phenicols are of particular importance in treating some fish diseases, in which there are no or very few treatment alternatives. Phenicols also represent a useful alternative in respiratory infections of cattle, swine and poultry. Phenicols, and in particular florfenicol, are used to treat pasteurellosis in cattle and pigs.	Y	Y	Y		
PLEUROMUTILINS Tiamulin Valnemulin	AVI, CAP, LEP, OVI, SUI AVI, SUI	48,6%	The class of pleuromutilins is essential against respiratory infections in pigs and poultry. **This family is critically important against swine dysentery** (*Brachyspira hyodysenteriae*) because there are no alternatives in many regions. **Pleuromutilins are used exclusively in animals.**	N	Y		Y	
POLYPEPTIDES Enramycin Gramicidin Bacitracin **POLYPEPTIDES CYCLIC** Colistin Polymixin	AVI, SUI EQU AVI, BOV, LEP, SUI AVI, BOV, CAP, EQU, LEP, OVI, SUI BOV, CAP, EQU, LEP, OVI, AVI	64,3%	Bacitracin is used against necrotic enteritis in poultry where available. Polypeptides are indicated in septicaemias, colibacillosis, salmonellosis, and urinary infections. Cyclic polypeptides are widely used against Gram negative digestive infections.	Y	N		Y	

ANTIMICROBIAL FAMILY	SPECIES	% quotations	Specific comments	C1: Quotation > 50%	C2: Essential or Few alternatives	VCIA	VHIA	VIA
QUINOLONES								
QUINOLONES 1G								
Flumequin	AVI, BOV, CAP, EQU, LEP, OVI, PIS, SUI							
Miloxacin	PIS							
Nalidixic acid	BOV		Quinolones of the 1st and of 2nd generations are used in septicemias and in infections such as colibacillosis, which cause serious losses in poultry, cattle, swine, fish and other species. Fluoroquinolones have no equally efficacious alternative in the treatment of chronic respiratory disease in poultry (*E. coli*)					
Oxolinic acid	AVI, BOV, LEP, PIS, SUI							
QUINOLONES 2G (FLUOROQUINOLONES)								
Ciprofloxacin	AVI, BOV, SUI							
Danofloxacin	AVI, BOV, CAP, LEP, OVI, SUI	68,6%		Y	Y	Y		
Difloxacin	AVI, BOV, LEP, SUI							
Enrofloxacin	AVI, BOV, CAP, EQU, LEP, OVI, PIS, SUI							
Marbofloxacin	AVI, BOV, EQU, LEP, SUI							
Norfloxacin	AVI, BOV, CAP, LEP, OVI, SUI							
Ofloxacin	AVI, SUI							
Orbifloxacin	BOV, SUI							
QUINOXALINES		4,3%	Quinoxalines (carbadox) is used for digestive disease of pigs (e.g. swine dysentery).	N	N			Y
Carbadox	SUI							

ANTIMICROBIAL FAMILY	SPECIES	% quotations	Specific comments	C1: Quotation > 50%	C2: Essential or Few alternatives	VCIA	VHIA	VIA
SULFONAMIDES								
Sulfachlorpyridazine	AVI, SUI							
Sulfadiazine	BOV, CAP, OVI, SUI							
Sulfadimerazin	AVI, BOV, LEP							
Sulfadimethoxine	AVI, BOV, CAP, EQU, LEP, OVI, PIS, SUI							
Sulfadimidine	AVI, BOV, CAP, EQU, LEP, OVI, SUI							
Sulfadoxine	EQU, SUI							
Sulfafurazole	PIS							
Sulfaguanidine	CAP, OVI							
Sulfamethazine	SUI							
Sulfadimethoxazole	AVI, BOV, SUI							
Sulfamethoxine	AVI, PIS, SUI	70%	Several sulfonamides alone or in combination with diaminopyramidines are very essential because of diseases covered (bacterial, coccidial and protozoal infections), and use in multiple animal species. This is essential for treatment of cattle, pigs, sheep, poultry, fish or other species. Few economical alternatives are available.	Y	Y	Y		
Sulfamonomethoxine	AVI, PIS, SUI							
Sulfanilamide	BOV, CAP, OVI							
Sulfaquinoxaline	AVI, BOV, CAP, LEP, OVI							
SULFONAMIDES+DIAMINOPYRIMIDINES								
Sulfamethoxypyridazine	AVI, BOV, EQU							
Trimethoprim+Sulfonamide	AVI, BOV, CAP, EQU, LEP, OVI, PIS, SUI							
DIAMINOPYRIMIDINES								
Baquiloprim	SUI							
Trimethoprim	AVI, BOV, CAP, EQU, LEP, OVI, SUI							
STREPTOGRAMINS								
Virginiamycin	AVI, BOV, OVI, SUI	5.7%	Virginiamycin is an important antimicrobial in the prevention of necrotic enteritis (*Clostridium perfringens*)	N	N			Y

ANTIMICROBIAL FAMILY	SPECIES	% quotations	Specific comments	C1: Quotation > 50%	C2: Essential or Few alternatives	VCIA	VHIA	VIA
TETRACYCLINES								
Chlortetracycline	AVI, BOV, CAP, EQU, LEP, OVI, SUI		Tetracyclines are very important in the treatment of many bacterial and chlamydial diseases in a broad range of animal species. There are no alternatives to tetracyclines in the treatment of animals against heartwater (*Ehrlichia ruminantium*) and anaplasmosis (*Anaplasma marginale*). Few economical alternatives are available					
Doxycycline	AVI, BOV, CAM, CAP, EQU, LEP, OVI, PIS, SUI							
Oxytetracycline	API, AVI, BOV, CAM, CAP, EQU, LEP, OVI, PIS, SUI	87,1%		Y	Y	Y		
Tetracycline	API, AVI, BOV, CAM, CAP, EQU, LEP, OVI, PIS, SUI							

Appendix VI

Original: English
October 2006

REPORT OF THE MEETING OF THE OIE *AD HOC* GROUP ON BIOTECHNOLOGY

Paris, 30–31 October 2006

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A meeting of the OIE *ad hoc* Group on Biotechnology was held at the OIE Headquarters in Paris from 30 to 31 October 2006. The meeting was chaired by Prof. Paul-Pierre Pastoret. Dr Cyril G. Gay acted as rapporteur. The Agenda and List of Participants are given at Appendices I and II, respectively. It was agreed that the next meeting of the *ad hoc* Group on Biotechnology would be 6 months from the date of this meeting.

1. Introduction

The *ad hoc* Group was welcomed by Dr Gideon Brückner, Head of the OIE Scientific and Technical Department, of behalf of Dr Bernard Vallat, Director General of the OIE.

2. Revised objectives for the *ad hoc* Group received from the Biological Standards Commission

Since the last meeting, the OIE Biological Standards Commission, to which the *ad hoc* Group reports, had revised the Group's objectives. The suggested revisions to the objectives, currently termed 'mission' are:

Objectives

1. To develop guidelines for new vaccine technologies (e.g. DNA vaccines, plant-expressed antigens).
2. To develop guidelines on the animal health risks arising from somatic cell nuclear transfer cloning of production animals, including criteria for assessing the health of embryos and animals derived from such cloning.
3. To monitor developments in the applications of nanotechnology related to animal health and to inform OIE.
4. To advise OIE on suitable procedures for the identification and tracing of animals and animal products that have resulted from biotechnological interventions.

The OIE *ad hoc* Group on Biotechnology agreed to adopt the revised objectives as written.

3. Designation of the Chairman

As Prof. Paul-Pierre Pastoret will become a full-time member of the OIE staff from January 2007, he cannot continue after this meeting to be the Chairman of the *ad hoc* Group. The OIE will propose a new chairperson at a later date.

4. Review of draft Guidelines on Reproductive Animal Biotechnology

To achieve its objectives, the *ad hoc* Group had created three subgroups at its meeting in April. These were Subgroup 1: Reproductive Animal Biotechnologies; Subgroup 2: Vaccines; and Subgroup 3: Nanotechnology and Animal Health.

Dr Harpreet Kochhar provided an update on the work of Subgroup 1 Reproductive Animal Biotechnology. An initial draft of the first guideline prepared by the Subgroup had been provided prior to the meeting. Since the last meeting in April 2006, the parent committee, the Biological Standards Commission, provided guidance to the OIE *ad hoc* Group on Biotechnology, by providing the following revised objective:

To develop guidelines on the animal health risks arising from somatic cell nuclear transfer cloning of production animals, including criteria for assessing the health of embryos and animals derived from such cloning.

The OIE *ad hoc* Group on Biotechnology agreed to revise the first draft based on the new objective and scope. The OIE will lead in the development of guidelines as they relate to animal health for transgenic and cloned animals. This first phase of guideline development will focus on mammals with consideration being given to transgenic fish and insects at a later date.

Issues discussed and taken into account included:

- The OIE has already developed definitions of animal welfare and animal health and risk analysis.
- Following in-depth discussions on definitions with respect to the development of new techniques, with specific reference to donor material, recipient material, transfer/fusion methodology, and progeny of clones, it was decided to restrict these guidelines to SCNT[1].
- The Group raised the concern associated with the potential reduction of genetic diversity associated with animal cloning.
- The present draft does not address the following issues:
    - aquatic animals and wildlife,
    - trade implications and horizontal issues,
    - public perception, ethical issues,
    - cost–benefit analysis as a part of risk analysis exercise,
    - feed safety and food safety component as it relates to OIE mandate,
    - Environmental aspects not addressed.

Finally, the OIE *ad hoc* Group agreed to limit the guideline to production livestock animals and horses. The title of the guideline was changed to: Guidelines for Somatic Cell Nuclear Transfer in Production Livestock and Horses. The revised draft guideline is provided at Appendix III.

5. Codex Alimentarius Commission – Codex Ad Hoc Intergovernmental Task Force on Food Derived from Biotechnology, Chiba, Japan, 27 November to 1 December 2006

Michel Thibier will provide an update at a later date.

6. New vaccine technologies (e.g. DNA vaccines, plant-expressed antigens)

6.1. Update on revised chapter from the OIE *Terrestrial Manual* on Principles of veterinary vaccine production

An updated version of the *Terrestrial Manual* chapter on Principles of veterinary vaccine production had been adopted by the International Committee in May and is available as the 2006 version on the chapter on the OIE website. As the *ad hoc* Group's review (see Section 5 of the previous report) was submitted too late for the authors to fully consider before the General Session, they had been asked after the General Session to address the comments. Once the chapter is received, it will be sent to Member Countries for comment before it is presented to the International Committee for adoption in May 2007.

1 SCNT: somatic cell nuclear transfer

6.2. Framework for guidelines on biotechnology-derived vaccines

The *ad hoc* Group on Biotechnology nominated Dr Cyril G. Gay to take the lead in reviewing the section of the chapter on recombinant-derived vaccines to ensure the information is adequate. Dr Gay will also lead the Subgroup 2: Vaccines, which will draft guidelines for DNA vaccines and plant-expressed antigens.

7. Update on nanotechnology

Dr Anne MacKenzie provided an update on the work of the OIE *ad hoc* Group's Subgroup 3 on Nanotechnology and Animal Health. Potential applications include:

1. Smart drug delivery
2. Disease diagnosis and treatment
3. Identify preservation
4. Animal breeding

The *ad hoc* Group agreed to the following recommendations:

a) The OIE is encouraged to consider the implications of nanotechnology relative to its mandate in light of the speed at which advances are being made in this area.

b) The following questions were identified by the *ad hoc* Group and need particular attention:

   1. What objectives of the OIE would be fulfilled by the consideration of further work relating to the animal health applications of nanotechnology?
   2. If an animal has been the subject of a nanotechnology application, will this have any influence on the safety of the products or animals for human or animal consumption?
   3. If nanotechnology has been used in the production of plants or microorganisms destined to be used as feed, will there be any impact on animal health?
   4. Will nanotechnology applications have any environmental or wildlife impacts that may be used in animal health, including wildlife?
   5. Will there be occupational hygiene issues for animal health workers?
   6. What are the toxicology aspects of nanoparticles in drug delivery as applied to animal health therapeutics?
   7. As there is opinion that the Agreement on Sanitary and Phytosanitary Measures (SPS) would apply to human, animal or plant health related measures regulating trade in nanotechnology, what would be the relevance of any such measures for the OIE?
   8. What specific work should the OIE undertake on the animal health applications of nanotechnology in regards to the development of OIE Standards?

c) The OIE could consider organising a common meeting with WHO[2] and FAO[3] to discuss the implications of nanotechnology in relation to the three international standard-setting bodies recognised under the SPS Agreement (OIE, Codex Alimentarius, IPPC[4]).

8. Update on the organisation of the International Symposium 'Animal Genomics for Animal Health'

Dr Cyril G. Gay, Chair of the Organising Committee, provided an update on the organisation of the International Symposium 'Animal Genomics for Animal Health.' The Symposium will take place at OIE Headquarters, from 23 to 25 October, 2007. The Symposium will focus on science and, due to space restrictions, will be limited to 240 participants. The idea to host the Symposium came from human health

2 WHO: World Health Organisation
3 FAO: Food and Agriculture Organization of the United Nations
4 IPPC: International Plant Protection Convention

initiatives and animal genome studies; these developments are providing revolutionary opportunities for animal health, disease control and biomedical research. Although other developments exist, such as marker-assisted selection, of the purpose of this Symposium is primarily to bring together the two communities of (i) disease experts with (ii) genome experts to look at the availability of new genomics tools to study animal diseases. Opportunities to fund research in this new area will be identified and strengthened and collaboration will be fostered at the international level. The Scientific Committee members have been selected to ensure a global representation. The symposium agenda was reviewed and endorsed by the OIE *ad hoc* Group on Biotechnology in its entirety.

The first announcement leaflet, which is available as a PDF file and can be used to publicise the symposium electronically (by e-mail) or printed, was presented. The call for papers (second announcement) is currently scheduled for November 2006 and will include keynote speakers and a link to the symposium web site, which is currently under development by USDA-ARS[5]. The deadline for submitting abstract will be 1 March 2007. The Scientific Committee will select papers by 15 April 2007. Deadline for early registration will be 1 May 2007.

The *ad hoc* Group expressed concern regarding the space limitation and recommended that the Scientific Committee be diligent in selecting participants who can make scientific contributions, enable the Symposium's objectives to be achieved, and include participation from the animal health research community and representation from South America and Asia.

At the next meeting of the *ad hoc* Group, the list of speakers selected by the Scientific Committee will be provided along with an update on registrations.

The *ad hoc* Group expressed its support for the Symposium and members agreed to help disseminate the announcement and call for papers to facilitate participation of the global animal health research community.

9. Suitable procedures for the identification and tracing of animals and animal products that have resulted from biotechnological interventions

Subgroup 1: Reproductive Animal Biotechnology will develop a discussion white paper on identification and tracing of animals and animal products that have resulted from biotechnological interventions. The scope of the white paper is as follows:

- Identify available technologies,
- Identify best technologies to apply for categories of biotechnology-derived animals,
- As a way forward, identify a step-by-step process starting with transgenic animals and taking into account existing work by relevant organisations.

10. Follow-up from previous meeting – the scope and definitions of biotechnology in relation to the OIE Mandate

OIE, Codex, IPPC, FAO, and other organisations do not necessarily agree on the definition of biotechnology. The *ad hoc* Group recommends an examination of existing definitions in other international organisations. This will facilitate the work of the *ad hoc* Group on identifying the most appropriate definitions for its own needs in the framework of the OIE mandate.

5 USDA-ARS: United States Department of Agriculture-Agricultural Research Service

11. Programme for the OIE Seminar on Biotechnology to be held in conjunction with the WAVLD[6] Symposium

The OIE *ad hoc* Group on Biotechnology discussed the preliminary programme and suggested speakers for OIE Seminar on Biotechnology to be held in conjunction to the WAVLD Symposium, Melbourne, Australia, 2007.

12. Finalisation and adoption of the report

The next meeting of the *ad hoc* Group is scheduled for 12–14 June, 2007.

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…/Appendices

6 WAVLD: World Association of Veterinary Laboratory Diagnosticians

Appendix I

MEETING OF THE OIE *AD HOC* GROUP ON BIOTECHNOLOGY

Paris, 30–31 October 2006

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Agenda

1. Introduction
2. Revised objectives for the *ad hoc* Group received from the Biological Standards Commission
3. Designation of the Chairman
4. Review of draft Guidelines on Reproductive Animal Biotechnology (prepared by Dr Kochhar)
5. Codex Alimentarius Commission – Codex Ad Hoc Intergovernmental Task Force on Food Derived from Biotechnology, Chiba, Japan, 27 November to 1 December 2006
6. New vaccine technologies (e.g. DNA vaccines, plant-expressed antigens)
   - 6.1. Update on revised chapter from the OIE *Terrestrial Manual* on Principles of veterinary vaccine production
   - 6.2. Framework for guidelines on biotechnology-derived vaccines
7. Update on nanotechnology
8. Update on the organisation of the International Symposium 'Animal Genomics for Animal Health'
9. Suitable procedures for the identification and tracing of animals and animal products that have resulted from biotechnological interventions
10. Follow-up from previous meeting – the scope and definitions of biotechnology in relation to the OIE Mandate
11. Programme for the OIE Seminar on Biotechnology to be held in conjunction with the WAVLD Symposium
12. Finalisation and adoption of the report

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Appendix II

MEETING OF THE OIE *AD HOC* GROUP ON BIOTECHNOLOGY

Paris, 30–31 October 2006

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Appendix III

Draft Guidelines for Somatic Cell Nuclear Transfer in Production Livestock and Horses

Preface

Following the first meeting of the OIE ad hoc *Group on Biotechnology held from 3 to 5 April 2006, the Biological Standards Commission suggested restricting the mandate "to develop guidelines on the animal health risks arising from SCNT[7] cloning of production animals, including criteria for assessing the health of embryos and animals derived from such cloning." The following document is a starting point for identifying, characterising and providing a basis for discussion on the animal health risks associated with SCNT cloning technology.*

Overview

At the first meeting of the *ad hoc* Group on Biotechnology, it was recommended that the Subgroup on Reproductive Animal Biotechnologies should draft guidelines on risk analysis, based on the life-cycle approach, for biotechnology-derived animals. The definition of 'Reproductive Animal Biotechnology' was proposed as "the generation of animals through the use of ART[8], which range from artificial insemination through to technologies involving a significant *in-vitro* component, such as *in-vitro* fertilisation, embryo transfer, embryo splitting and including asexual reproduction such as nuclear transfer". The following draft is restricted to SCNT and is based on a risk analysis approach to biotechnology-derived animals categorised according to the life-cycle approach consisting of: i) embryos, ii) recipients, iii) offspring, and iv) progeny of animal clones.

Scope

These guidelines address animal health and welfare-related aspects of production animals derived from some reproductive biotechnologies.

Recognising the mandate of the OIE and the suggestion of the Biological Standards Commission, it is the recommendation of the *ad hoc* Group on Biotechnology to identify risk analysis parameters for animal health and their implication for environmental safety and food and feed safety. These guidelines will focus initially on the scientific basis for the risk assessment aspects, prevention measures and guidance for production livestock and horses derived from ART. This is without prejudice to the addition of any relevant issue at a later stage. At present, these guidelines include the following:

- Identification of animal health risks and recommendations for management of those risks in embryos, recipients, animal clones and progeny of clones;
- Risk and prevention measures related with SCNT cloning technology;
- Welfare issues.

Recognising further that the following issues have been discussed or may be addressed by other bodies or instruments, or that they may be addressed at a later stage by the OIE, the document does not address:

- Safety and nutritional aspects of food derived from ART, for example transgenics (addressed by Codex);
- Risks related to the environmental release of animal clones;
- Risks related to transgenic animals that have not involved SCNT or other cloning technology;

7 SCNT: somatic cell nuclear transfer
8 ART: assisted reproductive technologies

- Non-reproductive animal biotechnologies;
- Risks related to animals produced for xenotransplantation or organ donors;
- Technologies related to stem cells;
- Risk related to aquatic animal health, including fish clones;
- Risks related to other terrestrial animals, such as wild mammals and non-mammals, including insects.

Background

Risk analysis– general principles

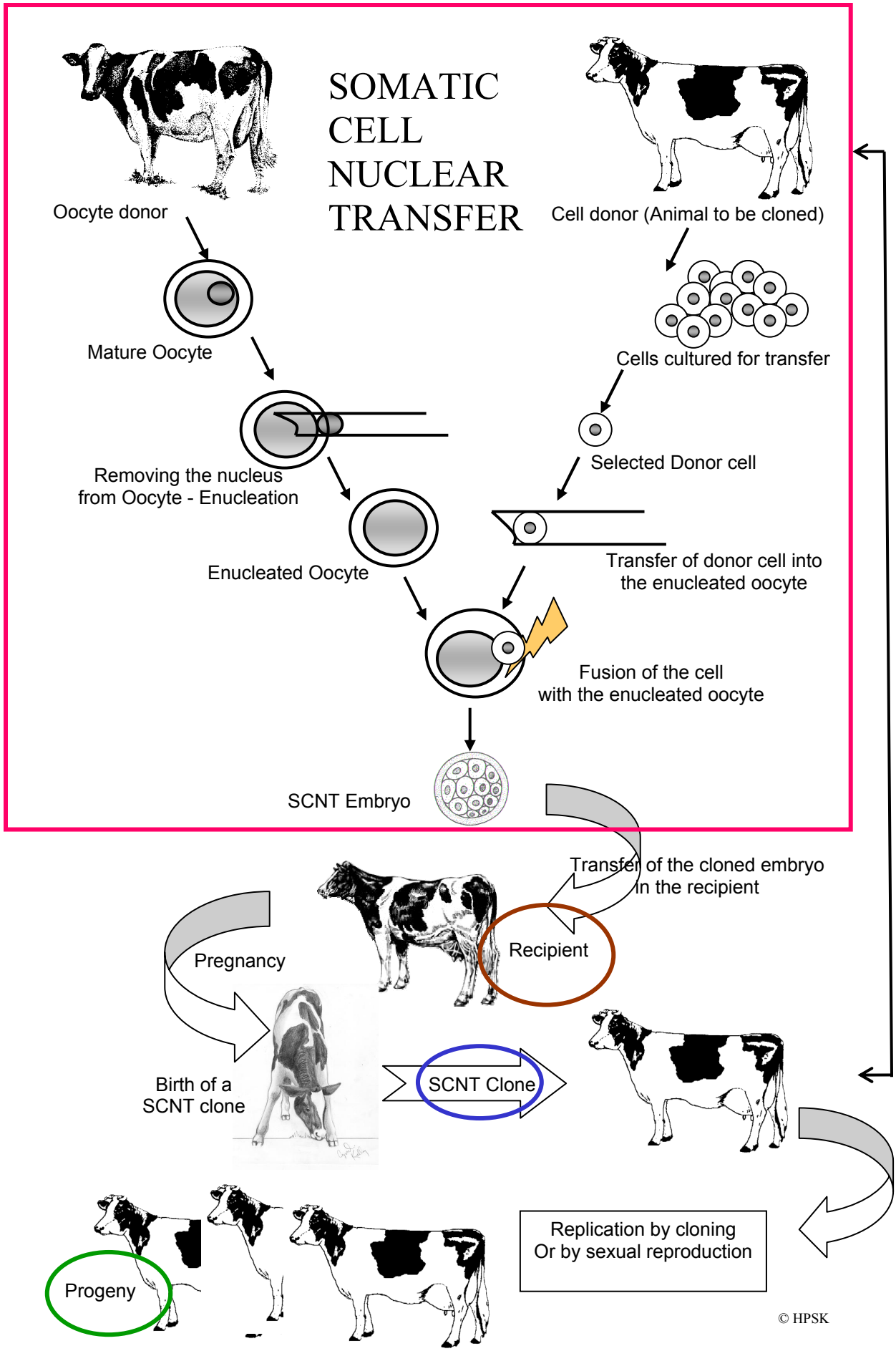
Risk analysis in general includes hazard identification, risk assessment, risk management and risk communication. The risk assessment is the component of the analysis that estimates the risks associated with a hazard (OIE *Terrestrial Animal Health Code*, 2006, Chapter 1.3.1). These principles are routinely used by regulators in making decisions about experimental or commercial releases. These analyses can then be used to determine whether the outcomes require management or regulation. Risk management is the process by which risk managers evaluate alternative actions or policies in response to the result(s) of the risk assessment taking into consideration the various social, economic, and legal considerations that form the environment in which such activities occur.

For animal diseases, particularly those listed in the OIE *Terrestrial Code*, there is broad agreement concerning the likely risks and these risks can be qualitative or quantitative (OIE *Terrestrial Code*, Chapter 1.3.1). In disease scenarios it is more likely that a qualitative risk assessment is all that is required. Qualitative assessments do not require mathematical modelling to carry out routine decision-making. Quantitative or semi-quantitative risk assessments assign magnitudes to the risks in numerical (e.g. 1/1,000,000) or verbal (high/medium/low) terms.

In the context of animal cloning, two broad categories of risk assessments are considered: absolute risk assessment and comparative risk assessments. Absolute risk assessments characterise risk independent of a comparator (e.g. the likelihood of an animal transmitting a specific livestock disease). A comparative risk assessment (or relative risk assessment) puts the risk in the context of a comparator. For example the degree to which an animal produced by one reproductive technology can transmit a particular disease to another animal of the same species compared with the degree to which a similar animal produced by another reproductive technology transmits the same disease to another animal of same species.

Regardless of the methodology used, hazard identification is an early step in all science-based risk assessments. In the context of assessing the risks associated with animal cloning (SCNT) and starting with the embryo and moving on through animal clone development and subsequent progeny, it is important to be clear at this juncture that only a comparative semi-quantitative risk assessment can be completed. A systematic, absolute, quantitative risk assessment of potential risks is difficult, due to the relative newness of the technology, and the variability in outcomes among laboratories and species cloned. Furthermore, with the technology of SCNT there is no introduced hazard (which may potentially happen in transgenesis). Thus, to analyse what factors contribute to animal health risks, the existing baseline must be analysed.

In short, the specific points where the risk assessment needs to be focused need to be identified. As illustrated in the accompanying diagram – the focus is to look at the basics of creating an embryo – using current terminology, starting from the selection of donor of oocyte and the cells to the creation of an embryo by the cloning methodology. The second phase will focus on the recipient of the embryo clone and the animal health and care considerations for the animals. The actual embryo clone that is born as an offspring is the third part of the paradigm that needs clear guidelines for assessment, and the next generation, either the progeny of the animal clone (which is a result of normal sexual reproduction) or animals produced by recloning (clones of clones) is the fourth and final stage.


SOMATIC CELL NUCLEAR TRANSFER
Oocyte donor
Cell donor (Animal to be cloned)
Mature Oocyte
Cells cultured for transfer
Removing the nucleus from Oocyte - Enucleation
Selected Donor cell
Enucleated Oocyte
Transfer of donor cell into the enucleated oocyte
Fusion of the cell with the enucleated oocyte
SCNT Embryo
Transfer of the cloned embryo in the recipient
Recipient
Pregnancy
Birth of a SCNT clone
SCNT Clone
Progeny
Replication by cloning Or by sexual reproduction
© HPSK

Managing Animal Health Risks associated with embryos

Embryo production by *in-vitro* techniques has been applied for many years. Although the additional steps involved in cloning add a new dimension to this procedure, many of the risks associated with SCNT have previously been identified for established ART (OIE *Terrestrial Code*, Appendix 3.3.2). An analysis of SCNT methodology allows the procedural details to be categorised into:

i) Oocytes (obtained from the abattoir, recovered from trans-vaginal ultrasound-guided procedures or by laparotomy procedures).
The primary risks are associated with the health status of the animal from which the ovaries are harvested and the quality of the oocytes.

ii) Donor cells (cells obtained from animals chosen to be cloned – by biopsy, harvesting at slaughter or after death).
Currently there are no specific new risks identified with SCNT cloning. There is a proposed risk related to activation of endogenous retroviruses during cell transfer procedures, however, this may be more theoretical than practical. In some current experimental procedures, the donor cell may be treated with chemicals to modify its composition, for example cell cycle inhibitors or chromatin modifiers.

iii) *In-vitro* culture of reconstructed embryos (procedure used to fuse the donor and recipient material and to culture the reconstructed embryo).

Risks associated with the method of fusing donor cells with enucleated recipient oocytes and with culture conditions.

In addition, the practitioner should ensure that the clone pregnancy is compatible to the surrogate dam's breed, anatomy and physiology.

Oocytes

- The laboratory or the producer should establish a detailed record of ovaries – their origin, health of the animal from which the ovaries are obtained, details of any systemic lesion on the animal and proper herd data. This is particularly useful where the pooling of ovaries may provide cross-contamination of ovarian tissue.
- Follicular fluids may carry various infectious agents like bovine viral diarrhoea virus (BVDV) and can contaminate pooled follicular fluid from healthy animals. Furthermore, the technique for collecting oocytes, such as aspiration or slicing of the ovarian follicles, determines the extent of blood contamination or extraneous material. A representative sample to demonstrate the absence of infectious biological material should be done with each pooled batch.
- Oocytes are matured as cumulus oocyte complexes (COCs) and then matured in most instances in the culture/maturation media. Care and efforts should be taken to carefully select and mature the oocytes from the pools that are morphologically good; also the media used should have been quality tested. Use of serum or protein components from an undefined or untested source should be avoided. Addition of proper and safe antibiotics in the culture media to control opportunistic bacteria should be encouraged.
- Use of proper sanitary and disinfection procedures is of utmost importance and should be emphasised in any *in-vitro* fertilisation (IVF) laboratory. Proper handling and following sanitary protocols during the maturation and further culture of embryos should be encouraged.

Donor cells

In order to minimise risks

- Donor cells should be properly harvested from the animal and cultured under proper sanitary conditions using good laboratory practices.
- When applicable, the passaging of the cells used for the cloning procedure should be documented and at different stage sampling may be warranted to look at the chromosomal component of the cell lines. If possible, procedures should be in place for regular sampling of the cells for morphological and other characteristics.

- Master cell lines (to be used for cloning at a later stage) should be stored under conditions found to be optimal for maintaining viability. Freedom from extraneous agents should be established by testing for bacteria, fungi, mycoplasmas or viruses, using appropriate tests (IETS[9] Manual, 1998).

Cloning procedures/reconstruction

- The cloning procedure that employs the use of chemicals or other reagents should be carefully evaluated, in terms of the quality of embryos and overall efficiency.
- During the fusion of recipient and donor material by chemical or physical means care and control should be employed. The optimisation of the procedure based on the laboratory protocols or published reports should be determined to avoid early embryonic mortalities.
- If co-culture of the cell is used for the culture procedure after reconstruction of embryos, proper screening of the co-culture cells should be done. A sample of each batch may be tested for the bacterial, fungal, mycoplasmal or viral component.
- Embryos should be cultured and harvested for an appropriate time and stage to transfer them or to cryo-preserve them for later use. Proper procedures based on the international standards (IETS Codes of Practice) for washing and preservation of the embryos should be followed.
- Care should be taken with regard to grading the embryos before transfer (OIE *Terrestrial Code*, Appendices 3.3.1 and 3.3.2).

Managing animal health risks related to the recipients (surrogate dams)

1. Animal health risks to the surrogate dams

Currently, when compared with *in-vitro* produced embryos, SCNT has a higher rate of pregnancy failure and, in some species, placental abnormalities. Loss due to defects in the embryo or failure to implant in the uterus of the surrogate dam does not pose a hazard to the dam. Rather, the surrogate dam simply resorbs any embryonic tissue and returns to cycling. Mid- and late-term spontaneous abortions may be hazardous to surrogates if they are unable to expel the fetus and its associated membranes. Most abortions in natural service and artificial insemination (AI) pregnancies in cattle remain undiagnosed due to the expense of laboratory work and the low profit margin in both the beef and dairy industry. Producers and veterinarians become concerned when the rate of abortion exceeds 3–5% in a herd. The same potential impact of external influences should be considered with pregnancy evaluation with SCNT and other reproductive technologies. Disease, under-nutrition, and severe environmental conditions are stressors known to interfere with animal fertility and embryo survival. Under these circumstances, the risk to the pregnancy is directly related to stress factors and not to the technology used.

To date, a species-specific effect has been seen. Abnormalities in clones may result from incomplete reprogramming of the donor nucleus. Epigenetic reprogramming occurs at different times in embryos in different species. Many of the abnormalities reported in cattle and sheep pregnancies have not been noted in goats or swine carrying SCNT clones. The amount of *in-vitro* manipulation of an embryo inversely correlates to the chances for successful pregnancy outcomes. This has been observed in both SCNT embryos and *in-vitro* produced fertilised embryos. Unlike other forms of other reproductive technologies SCNT pregnancy losses occur at all stages of gestation in cattle. Clone pregnancies have been lost during the second and third trimesters and have been accompanied by reports of hydrops, enlarged umbilicus, and abnormal placentation.

2. Animal health risks posed by the surrogate dam to the clone embryos

No new animal health risks have been identified for the developing clone fetus from the surrogate dam compared with conventional pregnancies. The latter include vertically transmitted diseases and abnormalities due to metabolic or physiological stress.

9 IETS: International Embryo Transfer Society

With respect to the animal health risks associated with the surrogate dam, it is difficult to document the relative frequency of early stage losses of SCNT embryos compared with early stage losses of other pregnancies as these abortions are not typically diagnosed with other reproductive technologies. Additionally, external stressors will similarly impact SCNT pregnancies.

Veterinarians should monitor the progress of pregnancy as the common gestational anomalies seen in other assisted reproductive technologies may be exhibited and diagnosed during the physical examination. A database of commonly encountered problems in clone pregnancies would be useful if available to animal health experts.

- Care should be taken to assess the general health of the recipient dam before selection to carry the embryo clones. The general health status of the recipient should be determined in terms of freedom from infection and disease, proper vaccination and follow up, and, if applicable, proof of earlier uneventful pregnancies, absence of birthing problems, and proper post-pregnancy recovery.
- Pregnancy loss is greatest with SCNT embryos prior to 60 days' gestation in cattle. This is similar to the pattern seen with other reproductive technologies. However, in clones, high pregnancy losses during this time of placental formation (between 45–60 days) suggest that embryonic death may be a consequence of faulty placentation. Abnormal placentation may lead to a build up of wastes in the fetus and associated membranes, or inadequate transfer of nutrients and oxygen from the dam to the fetus. Care should be taken to monitor the recipient dam during pregnancy. Once the pregnancy is established and confirmed, regular veterinary assessments and monitoring of animal health status is desirable up to the birth of the offspring.
- To ensure that the recipient is pregnant and to monitor its health during the first trimester, it is useful to perform ultrasonographic assessments, determine hormonal profiles and assess the general physiological parameters. Based on these profiles, proper attention should be paid to aid in the proper establishment of pregnancy by providing proper husbandry conditions and nutrition.
- The animals should be observed carefully for the signs of labour nearing the time of birth. In some species, one of the more common problems is uterine inertia and the absence of contractions. The absence of contractions may result in prolonged pregnancies with associated sequellae that may require assistance with deliveries.
- A surgical intervention should be decided and should be available for the near term animal if the situation so warrants. Proper procedures should be employed to ascertain the proper handling of the offspring and the surrogate dam.
- Health concerns may arise as a result of surgical procedures, excessive traction, or other complications such as retained fetal membranes. In these cases *post-partum* care may be necessary.

Managing animal health risks of animal clones

The health problems of individual clones can be observed *in utero* and *post-partum*. These appear to be the same as observed in other ART, but they may be more common in clones. It is important to determine whether the abnormalities are of genetic or epigenetic origin. LOS[10] and placental abnormalities are particularly observed in sheep and cattle.

- Appropriate husbandry practices are important to the health of animal clones. Care should be taken to provide colostrums and a clean and hygienic environment, supervision for the first few weeks after birth should be practiced.
- The animal clones must be checked routinely for the most common phenotypic anomalies, such as atresia anii, umblical hernia, flexor muscle contractions, respiratory or cardiac insufficiency, and failure to suckle. This will allow proper treatment and care of the newborn and increase the survival of the young one.
- To consolidate current understanding of the health status of animal clones, a comprehensive veterinary examination should be performed to monitor the progress of the clone, as unexplained fatalities or fatalities arising from systemic complications have been reported. It is encouraged to follow the health profile of the animals to at least the reproductive maturity stage, and to record the ability to reproduce (fertility index).

10 LOS: large offspring syndrome

- Animal welfare concerns ranging from LOS to serious abnormalities are notable in the debates pertaining to cloning technology. Proper research and peer-reviewed data should be generated. The animal clones should undergo species-specific basic welfare assessments. If welfare concerns are detected at initial screening, a more extensive characterisation of that phenotype should be performed to document the animal welfare concerns.
- Proper monitoring of the animal population during different stages of life from birth to puberty should be documented to address and validate the genomic potential of the animal clones.

Managing animal health risks related to sexually reproduced progeny of clones

Presently there is no evidence of an increased health risk if sexual reproduction is used for obtaining progeny. Some data indicate that the reprogramming errors during the cloning process may actually be corrected during the natural mating and reproduction process.

- Characterisation of the health profile, including health status and data on animal welfare, would consolidate the knowledge of sexually reproduced progeny.
- Monitoring the reproductive performance of sexually reproduced progeny of clones would be useful to assess their reproductive capacity in comparison with their conventional counterparts.

Managing animal health risks associated with re-cloning/clones of clones

There is a lack of information on recloning. It is therefore necessary to follow the approach below:

- The health profile (health status and data on animal welfare) should be characterised to consolidate the knowledge.
- The reproductive performance of clones of clones should be monitored to assess the capacity of the animals to perform in comparison with their conventional counterparts.

Breeding regimes should consider genetic diversity effects in relation to the desired use of SCNT technology.

Review of guidelines

The goal of these guidelines is to provide a scientific basis and recommendations on animal health and welfare risks to animals involved in SCNT cloning compared with other ART. These guidelines will focus initially on the scientific basis for the risk assessment aspects, prevention measures and guidance for production livestock and horses derived from ART and should be reviewed in light of new scientific information.

Glossary:

Hazard: (as defined in OIE)
Hazard means a biological, chemical or physical agent, or a condition of, an animal or animal product with the potential to cause an adverse health effect.

A hazard is an element or event that poses potential harm; an adverse event or adverse outcome. A hazard is identified by describing what might go wrong and how that might happen (2). Covello and Merkhofer (11) defined a hazard as a (potential) source of risk that does not necessarily produce risk. A hazard produces risk only if an exposure pathway exists and if exposures create that possibility of adverse consequences. Hazard identification is the process of identifying new agents in sources of risk. Risk sources may release risk agents into the environment.

Risk:
Risk means the likelihood of the occurrence and likely the magnitude of consequences of an adverse event to animal or human health during a specified time period, as a result of hazard.

The likelihood of the occurrence and the magnitude of the consequences of an adverse event; a measure of the probability of harm and the severity of impact of a hazard. Objective measurement and scientific repeatability are hallmarks of risk. In risk studies it is common, especially in oral communication, to use "risk" synonymously with the likelihood (probability or frequency) of occurrence of a hazardous event. In such instances, the magnitude of the event is assumed to be significant (2, 4).

Risk analysis:
Risk analysis means the process composed of hazard identification, risk assessment, risk management and risk communication.

The process of risk analysis includes risk assessment, risk management and risk communication (11, 4).

Risk Assessment:
Risk assessment means the evaluation of the likelihood and biological and economic consequences of entry, establishment, or spread of a pathogenic agent.

The process of identifying a hazard and evaluating the risk of a specific hazard, either in absolute or relative terms. The risk assessment process involves four interrelated assessment steps: release assessment, exposure assessment, consequence assessment and risk estimation. It includes estimates of uncertainty in process, and is an objective, repeatable, scientific process. Quantitative risk assessment characterises the risk in numerical representations (2, 4). Qualitative risk assessment characterises the outputs on the likelihood of the outcome or the magnitude of the consequences in qualitative terms such as “high”, “medium”, “low” or “negligible” (17).

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EXPERT SURVEILLANCE PANEL ON EQUINE INFLUENZA VACCINES

Mill Hill London (United Kingdom), 16 January 2007

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Conclusions and recommendations

These recommendations relating to the composition of equine influenza vaccines for 2007 were made following review of the data arising from equine influenza surveillance by the panel of international collaborators for the period January 2006 – January 2007. **The recommendations for vaccine strains remain as for 2005**.

Influenza activity January 2006–January 2007

Minor outbreaks of equine influenza were reported during 2006 in Canada, France, Germany, Ireland, Sweden, the United Kingdom and the United States of America. Some outbreaks occurred in vaccinated animals but disease was generally mild.

All influenza activity was due to H3N8 viruses. There were no reports of serological or virological evidence of H7N7 (equine-1) subtype viruses circulating in the equine population. Nevertheless, diagnostic laboratories should continue serological and virological monitoring for these viruses and when using polymerase chain reaction (PCR) for rapid diagnosis, should ensure that primers specific for H7N7 viruses as well as H3N8 viruses are used.

Characteristics of recent isolates

All viruses characterised antigenically and/or genetically from Europe and North America during 2006 belonged to the 'American' lineage. Most of the viruses tested by haemagglutination inhibition (HI), using post-infection ferret antisera, were closely related to the recommended vaccine strain A/eq/South Africa/4/2003 and the reference strain A/eq/Newmarket/5/2003. With one exception, the HA1 sequences of the viruses fell within a single phylogenetic sub-group comprising the sequences of American lineage viruses isolated since 2003 in America, Europe and South Africa. The viruses isolated in North America since 2003 (represented by A/eq/South Africa/4/2003 and A/eq/Ohio/2003) are characterised by two further amino acid changes in antigenic sites of HA compared with the viruses isolated in Europe; these additional changes appear to contribute to greater antigenic drift from A/eq/Newmarket/1/93-like viruses currently included in vaccines. One European isolate was more closely related antigenically and genetically to A/eq/Newmarket/1/93.

Recommendations for the composition of equine influenza vaccines

During the period January 2006 to January 2007, H3N8 viruses of the 'American' lineage continued to circulate in Europe and North America with some vaccinated horses affected. Most of these viruses, like those responsible for the 2003/4 outbreaks in South Africa and those circulating recently in North America and Europe were antigenically closely related to the currently recommended A/eq/South Africa/4/2003-like vaccine strains.

No 'European' lineage viruses were detected. Nonetheless, the recommendation remains that a European lineage virus be included in vaccines, and surveillance of these viruses will continue in order to monitor their circulation.

It is recommended, therefore, that vaccines contain the following:

- *an A/eq/South Africa/4/2003* (*H3N8*)-*like virus* (*American lineage*)[1]

  [1]A/eq/Ohio/2003 is acceptable as A/eq/South Africa/4/2003-like virus

- *an A/eq/Newmarket/2/93* (*H3N8*)-*like virus* (*European lineage*)[2]

  [2]A/eq/Suffolk/89 and A/eq/Borlänge/91, currently used vaccine strains, continue to be acceptable.

Reference reagents

Reference reagents specific for the recommended European lineage vaccine strains are available for standardisation of vaccine content by single radial diffusion (SRD) assay and can be obtained from the National Institute for Biological Standards and Control (NIBSC). Preparation of reagents for the South Africa/4.2003-like viruses is planned.

Four equine influenza horse antisera (anti-A/eq/Newmarket/77 [H7N7], anti-A/eq/Newmarket/1/93 [H3N8], anti-A/eq/Newmarket/2/93 [H3N8]) and anti-A/eq/South Africa/4/2003 [H3N8] are available as European Pharmacopoeia Biological Reference Preparations (EP BRPs) for serological testing of equine influenza vaccines by the single radial haemolysis assay. These antisera are also available from the Office International des Epizooties International (OIE) Reference Laboratory in Newmarket (UK) for use as primary standards in diagnostic serological testing.

SRD reference reagents	***EP BRPs for serological testing of equine influenza vaccines***	***OIE primary standards for diagnostic serological testing***
NIBSC, Blanche Lane, South Mimms, Potters Bar, Herts, EN6 3QG, UK Fax: (+44-1707) 64.67.30 enquiries@nibsc.ac.uk	European Directorate for the Quality of Medicines, BP 907, F-67029 Strasbourg Cedex, France http://www.pheur.org	Animal Health Trust, Lanwades Park, Kentford, Newmarket, Suffolk, CB8 7UU, UK Fax: (+44 -8700) 50.24.61 info@aht.org.uk

OIE Guide 1:

OIE Guidelines for the Validation of Diagnostic Assays for Infectious Diseases

1. Introduction

1.1. Purpose

This document provides guidelines for the validation of test methods (assays) for infectious diseases and is an adjunct to Chapter I.1.3, Principles of Validation of Diagnostic Assays for Infectious Diseases, *OIE Manual of Standards for Diagnostic Tests and Vaccines*, 2004 and Jacobson R.H. (1998). Validation of serological assays for diagnosis of infectious diseases. *Rev. sci. tech. Off. Int. Epiz.,* **17** (2), 469–486.

1.2. Scope

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

2. Definition of a Validated Assay

A validated assay consistently provides test results that identify animals as positive or negative for a particular analyte or process (e.g. antibody, or antigen or induration at skin test site), or reaction (e.g. induration at a skin test site) and, by inference, accurately predicts the infection status of the animal with a predetermined degree of statistical certainty. From an assay developer's perspective, it includes the development and verification of test method performance characteristics at a defined level of statistical confidence.

3. Essential Prerequisites before Validation of an Assay

3.1. Selecting an assay fit for its intended purpose

Diagnostic assays should be developed only after feasibility studies have taken place. First and foremost, new assays must be capable of fulfilling their intended purpose. These purposes are broadly defined:

i) Demonstrate freedom from infection in a defined population (country/zone/compartment/herd) (prevalence apparently zero)

   a) 'Free' with and/or without vaccination,

   b) Historical freedom,

   c) Re-establishment of freedom after outbreaks;

ii) Certify freedom from infection or agent in individual animals or products for trade/movement purposes;

iii) Eradication of infection from defined populations;

iv) Confirmatory diagnosis of suspect or clinical cases (includes confirmation of positive screening test);

v) Estimate prevalence of infection or exposure to facilitate risk analysis (surveys, herd health status, disease control measures);

vi) Determine immune status of individual animals or populations (post-vaccination).

3.2. Considerations for achieving assay performance characteristics suitable for the intended purpose

An assay type should be selected for evaluation only if it is anticipated that it will have sufficient analytical sensitivity (defined below) with respect to the type and concentration of analyte or level of reaction to be detected, and analytical specificity with respect to the organism in question should be appropriate for the intended application of the assay.

Minimum acceptable requirements for diagnostic sensitivity and specificity need to be pre-established for the intended application of the assay.

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

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

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

3.3. Feasibility of selected assay type

Upon selection of an assay type, reagents, and a preliminary protocol, feasibility studies are required to determine preliminary physical parameters and workable chemical and biological reagent concentrations. Such preliminary studies often indicate whether an assay is worthy of further optimisation to achieve performance characteristics consistent with its intended use.

4. Assay Validation – Part 1

4.1. Standardisation of protocol parameters and optimal reagent concentrations

Further experiments are then done to establish optimal concentration of chemicals and biologicals (e.g. antigens, antibodies, controls, enzyme/substrate systems, etc.). Determination of a linear operating range for the assay is essential. Required physical parameters (e.g., reaction times and incubation temperatures) as well as storage conditions and preparation of all reagents are determined and described or referenced in the protocol. Procedures for titration of reagents and for their calibration against international reference standards (if applicable) should be included in a typical standard operating procedures manual, within a quality assurance programme.

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

4.2. Repeatability estimates

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

4.3. Analytical sensitivity and specificity

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

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

5. Assay Validation – Part 2

5.1. Determination of assay performance characteristics

5.1.1. Estimates of diagnostic sensitivity and specificity

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

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

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

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

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

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

5.1.2. Selection of positive/negative cut-off

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

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

5.1.3. Calculation of diagnostic sensitivity and specificity

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

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

Diagnostic sensitivity = TP / (TP + FN)
Diagnostic specificity = TN / (TN + FP)

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

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

5.1.4. Other standards of comparison

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

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

Relative sensitivity = TP / (TP + FN)
Relative specificity = TN / (TN + FP)

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

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

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

5.1.5. DSe and DSp estimates based on animals with infection status not defined

DSe and DSp can be estimated when infection or analyte status of the animals are not defined; however, these latent class statistical models are complex. Expert advice should be sought not only in the design of the evaluation study but the interpretation of the estimates of DSe and DSp as well. It has been recommended to the OIE that an expert group be formed to address the application of latent class models and to draft guidelines for models as they apply to the validation and certification assays by the OIE.

6. Assay Validation – Part 3

6.1. Reproducibility and enhanced repeatability estimates

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

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

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

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

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

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

7. Assay Validation – Part 4

7.1. Programme Implementation

Ultimate proof of the usefulness of an assay is its successful application(s). These would include international, regional or national programs. As new and improved assays are developed and come on-line, they will ultimately replace existing assays if they prove a better fitness for purpose. However, this will only happen if they are actually put into routine use and their usefulness

documented over time. In the natural progression of diagnostic and/or technological improvement, some new assays will become the new standard of comparison. As such, they may progressively achieve national, regional and international recognition. As a recognised standard, these assays will also be used to develop reference reagents for quality control, proficiency and harmonisation purposes. These reference reagents may also become international standards, as well. The last level of validation in the OIE Registry involves documentation related to actual application and levels of recognition for the assay in question. This is intended to provide potential users with an informed and unbiased source of information.

7.2. Validity of assay results: predictive value

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

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

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

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

7.3. Maintenance and enhancement of validation criteria

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

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

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

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

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

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

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NEW GUIDELINE

OIE Guide 2:

Validation and Quality Control of Polymerase Chain Reaction Methods used for the Diagnosis of Infectious Diseases

1. Background

1.1. Purpose

This document provides guidelines for the validation of polymerase chain reaction (PCR) methods (assays) used in the diagnosis of infectious diseases, and is an adjunct to Chapter 1.1.4 Validation and quality control of polymerase chain reaction methods used for the diagnosis of infectious diseases, OIE *Manual of Standards for Diagnostic Tests and Vaccines*, 2008.

1.2. Scope

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

1.3. Validated assay

When performing diagnostic analyses of clinical material, it is important to produce data of good quality. For this, some key criteria have to be fulfilled. The establishment of quality assurance (QA) and quality control (QC) systems is required, i.e. a set of quality protocols, including the use of control samples that ensure that the system is working properly and confirms data reproducibility and quality. QA and QC systems, together with trained and competent personnel, have already been established in many laboratories worldwide. Assay validation is another essential factor for assuring that test results reflect the true status of the samples. To predict the diagnostic performance of a diagnostic assay, it is necessary to use a validation methodology to document the expected analytical performance of the assay in question. Validation is the evaluation of a diagnostic assay for the purpose of determining how fit the assay is for a particular use.

1.4. Intended fitness for purpose

The fitness of PCR assays for various purposes is broad. Wherever there is a need for direct detection of an infectious agent, it is generally possible to use PCR. During the first years of PCR diagnostic development, many laboratories had problems with contamination and performance and thus PCR had a poor reputation as a technique suitable for diagnostic use. Achievements in later years have totally reversed that view. New technology (i.e. real-time PCR) has made the technique safer and easier to use. Automated extraction and pipetting procedures using robots have substantially lowered the costs and reduced the required work-load. During the 'early years' many in-house assays were developed, and harmonisation and validation were poor or non-existent. Today, the OIE and

Community Reference Laboratories (CRLs) have an important role in driving the validation and harmonisation work forward. It is fair to say that PCR, as it is performed today, is safe, frequently validated and very fit for its intended diagnostic purposes.

2. Assay Validation – Introduction

2.1. Initial assay development considerations

2.1.1. Precautions and controls

Considering the uncertainty about the safety and reliability of the PCR in routine diagnosis, special precautions should be applied in any laboratory using PCR for detecting infectious agents in order to avoid false-positive or false-negative results. These, together with internal controls (e.g. mimics) assure the safe evaluation of the results.

2.1.2. Precautions taken to avoid false-positive results

False-positive results (negative samples showing a positive reaction) may arise from either laboratory-related issues, such as cross-contamination, or assay-related factors, such as inefficient optimisation or assay performance. Product carry-over from positive samples or, more commonly, from cross-contamination by PCR products from earlier experiments is a possible source of error. Various practices and tools have been applied to prevent false-positive PCR results. Localities for performing the different steps in the analysis chain should be carefully planned and separated (Figure 1). Both positive and negative control samples should be interspersed routinely with diagnostic samples to assess PCR assay performance.

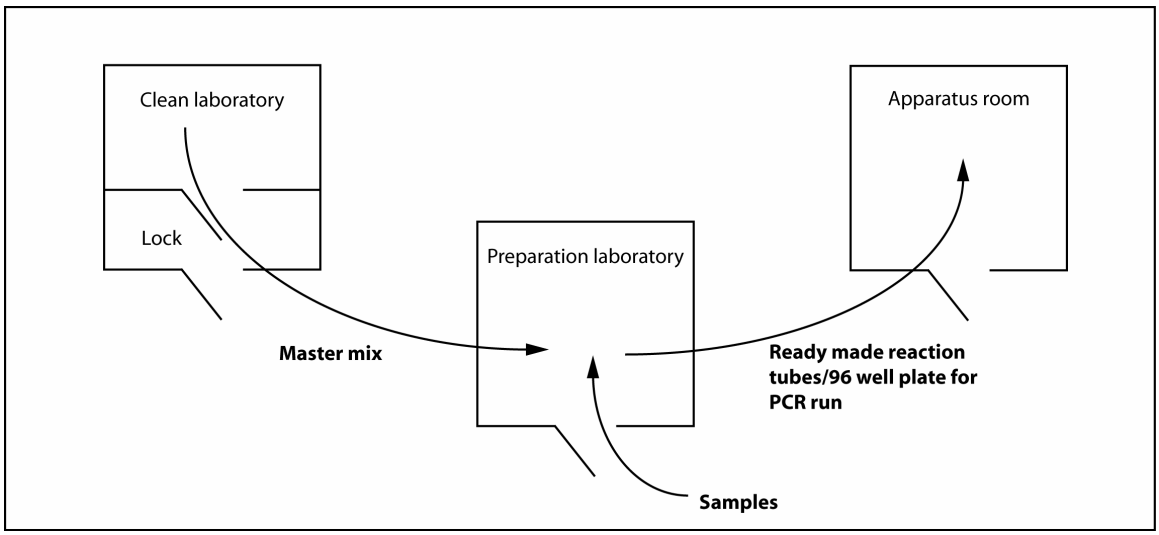

Figure 1. *Typical laboratory set-up for diagnostic real-time PCR.*

3. Assay Validation – Part 1

3.1. Optimisation and standardisation of reagents and determination of critical control parameters

Sample collection, preparation and transport (see OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*, chapter 1.1.1) and nucleic acid extraction methods (see *Terrestrial Manual* chapter 1.1.8) are all critical parameters in test performance and should be optimised for disease diagnosis. Suitable methods vary depending on sample and organism type. In general, blood serum, body tissues and swab samples are suitable samples for easy extraction of target nucleic acids, while faeces and semen samples are more difficult to handle. Extraction of RNA targets differs from

extraction of DNA targets, and RNA is more prone to degradation. Both commercial (robotic, spin columns, magnet-based extractions, etc.) and standard chemistry-based methods are used for DNA or RNA extraction. It is crucial to determine the most reproducible and efficient extraction method before further validation of the assay is performed. If the method of extraction is changed, equivalency data should be generated or the entire validation procedure should be repeated.

All equipment used during the process must be properly maintained. Apparatus (heating blocks, refrigerators, freezers, thermocyclers, pipettes, etc.) that require calibration must be calibrated according to the laboratory's quality assurance protocols. It is also important to properly validate equipment and protocols used. One good example is the rather recent implementation of robotic extraction methods in routine diagnostic laboratories. It is not sufficient to compare the characteristics with that of the priorly used extraction method. It is also necessary to validate the robot and the protocol for cross contamination, e.g. by running a set of mixed positive and negative samples.

When developing 'classical' or real-time PCR assays, all parameters, protocols and reagents need to be optimised. A standardised assay is a method that consistently gives the same result for a given sample when repeated several times and when performed by different analysts in different laboratories.

During the optimisation of the PCR assay, it is also possible to estimate the capacity of the method to remain unaffected by small changes in the main parameters. Documentation of intentional variations during performance of the assay is necessary to characterise critical parameters in the assay. Examples of such parameters include: incubation times and temperatures, concentrations of buffers, primers, $MgCl_2$, etc., pH, amounts of other components added (e.g. dNTP, bovine serum albumin, etc.). The characterisation of critical control parameters is crucial for identifying critical points that must be properly controlled in the assay.

3.2. Repeatability

Agreement between replicates within and between runs of the assay should be accessed at this stage. This gives important information about the assay before further validation is carried out. If excessive variability is encountered, it should be corrected before continuing the validation process.

3.3. Determination of analytical specificity and sensitivity

Analytical specificity is defined as the ability of an assay to distinguish the target agent from other infectious agents. This ability is determined by analysing genetically related pathogens and clinical material obtained from animals with diseases that may mimic that for which the assay is being designed. Acceptable cross-reactivity is largely dependent on the intended purpose of the test and must be discussed from case to case.

Analytical sensitivity (or limit of detection) is defined as the smallest amount of an agent detected by the assay, and may be represented as the number of genome copies, infectious dose, colony-forming units, plaque-forming units, etc. of the agent that can be detected and distinguished from a zero result. To determine analytical sensitivity, an end-point dilution is used until the assay can no longer detect the target in question in more than 5% of the replicates (2 standard deviations). Estimates of analytical sensitivity can vary substantially for the same assay in different sample materials.

4. Assay Validation – Part 2

4.1. Determining assay performance characteristics

Reference animal populations

i) *Negative reference animals*

True negative samples, i.e. samples that are surely 100% negative, can sometimes be difficult to obtain. Often it is possible to collect samples from countries that have eradicated the disease in question.

ii) *Positive reference animals*

It is generally problematic to find positive reference animals in sufficient numbers. Naturally infected or experimentally infected animals are needed and their positive status is best demonstrated by virus isolation.

iii) *Reference animal status determined by other assays*

The term 'gold standard' is commonly used to describe any standard of comparison and should be limited to methods that unequivocally classify animals as infected or uninfected. New PCR assays are generally expected to outperform any already existing 'gold standard' method and thus the 'gold standard' could be difficult to use as a comparison, at least to demonstrate that a negative reference animal is truly negative.

4.2. Threshold determination

Diagnostic sensitivity (D-SN; proportion of known infected reference animals that are tested positive in the assay) and specificity (D-SP; proportion of known uninfected reference animals that are tested negative in the assay) are the most important parameters obtained during the validation of an assay. They form the basis for calculating other parameters and hence they are critical to the whole validation process. The number of reference samples required to determine estimates and allowable error of both D-SN and D-SP can be calculated. To do this, a reasonable prediction of both D-SN and D-SP must be used. Generally, confidence in the estimate is set at 95%. However, no formula can account for the numerous host/organism factors that can affect the outcome of the test. The use of spiked samples in PCR is not appropriate as these might not be representative of naturally infected samples and thus the whole validation process could potentially be jeopardised.

5. Assay Validation – Part 3

5.1. Establishing reproducibility of the assay

Reproducibility is an important parameter in assay precision. Reproducibility is determined in several laboratories using the identical assay (protocol, reagents and controls).

6. Assay Validation – Part 4

6.1. Programme implementation

Reference laboratories are of major importance in implementing new or existing molecular assays. Both OIE Reference Laboratories and CRLs are urged to drive the implementation of promising, new assays for their disease of interest, as has been done for example, during the implementation of avian influenza molecular diagnostics in Europe.

6.2. Monitoring validity of assay performance

6.2.1. Interpretation of test results – factors affecting assay validity

The estimation of the prevalence of an infectious agent in the population is necessary for calculating the predictive value of positive (PV+) or negative (PV–) test results. This applies equally to molecular test methods as it does to other methods, such as the enzyme-linked immunosorbent assay.

Reference Laboratories are encouraged to determine values for D-SN and D-SP as accurately as possible, as these are extremely important for judging the real performance of an assay when used in the field. It is also important to estimate the predictive values (PV+ or PV–) in the local situation.

6.2.2. Maintenance of validation criteria

When the assay is used as a routine test, it is important to maintain the internal QC. The assay needs to be consistently monitored for repeatability and accuracy. Reproducibility between laboratories (proficiency testing) is recommended by the OIE to be estimated at least twice a year and is usually administered by a reference laboratory that distributes panels of samples, receives the results from the laboratories, analyses the data, and reports the results back to the laboratories. If the assay is to be applied in another geographical region and/or population, it might be necessary to revalidate or document equivalency under the new conditions. Revalidation may also be necessary if the test is applied to a different sample matrix, e.g. validated on blood and used on another tissue, or validated for cattle tissue and used on another species. This is especially true for PCR assays as it is very common for point mutations to occur in the genomes of many infectious agents (i.e. RNA viruses). Mutations, which may occur within the primer or probe sites, can affect the efficiency of the assay and, by doing so, the established performance criteria are no longer valid. It is also advisable to regularly confirm the target sequence at the selected genomic regions for national or regional isolates of the infectious agents. This is especially true for the primer sites, to ensure that they remain stable so that validation of the assay cannot be questioned.

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OIE Guide 4:

Laboratory Proficiency Testing

1. Introduction

1.1. Purpose

This document provides guidelines for evaluation of veterinary laboratory capability to conduct diagnostic tests for infectious diseases.

1.2. Scope

These guidelines are intended for use by OIE Member Countries as part of the evaluation of laboratories that are carrying out tests to qualify animals and animal products for international movement. These guidelines should be used in conjunction with the OIE Guidelines for Laboratory Quality Evaluation for overall assessment of laboratory quality and capability.

This guide is based on the relevant requirements of the ISO[1] 9000 series of standards, ISO/IEC[2] 17025 and ISO Guide 43.

1.3. Interlaboratory test comparisons

Interlaboratory test comparisons may be undertaken for a variety of reasons which may include:

i) Determining a laboratory's capability to conduct specific diagnostic tests,

ii) Checking or certifying the performance of individual operators,

iii) Checking or certifying the calibration of instrumentation,

iv) Harmonising existing test methods,

v) Evaluating new test methods,

vi) Assigning values and ranges to standard materials,

vii) Resolving interlaboratory differences.

1.4. Proficiency testing

When an interlaboratory test comparison is conducted for the express purpose of determining a laboratory's capability to conduct specific diagnostic tests, i.e. 1.3.i above, it is referred to as proficiency testing. Proficiency testing is an integral part of laboratory accreditation programmes.

Proficiency testing schemes are based on defined sets of highly characterised test materials which are sometimes referred to as check sample panels. These panels are simultaneously sent to participating laboratories for testing. The results are collected and analysed against the intended result in order to determine the capability of a participating laboratory to conduct a diagnostic test and produce correct results.

1 ISO: International Organisation for Standardisation
2 IEC: International Electrotechnical Commission

1.5. Accreditation

An accreditation programme is a formal process for recognition of laboratory quality and capability by an independent authority. It requires that laboratories successfully participate in an accreditation programme on an ongoing basis in order to maintain their recognition status. The independent authority awards or denies recognition based on stipulated requirements for quality and capability.

In the initial stage of accreditation, laboratories are required to demonstrate a specified and sustainable level of quality. Ideally this would involve compliance with ISO 9000 and ISO/IEC 17025 General Requirements for the Competence of Calibration and Testing Laboratories (1990) in order to qualify for entry into the programme. However, it is recognised that in many circumstances such a high level may be difficult to achieve for a variety of reasons. The OIE Guidelines for Laboratory Quality Evaluation were prepared in order to establish a minimum acceptable level of quality.

The second stage of accreditation entails regularly scheduled proficiency testing for the evaluation of a laboratory's capability to conduct specific diagnostic tests. As proficiency testing schemes are a form of interlaboratory comparison, they must involve two or more laboratories. There is no agreed standard for proficiency testing in veterinary diagnostics, although several schemes are in operation at international and national levels. The present guidelines have been prepared to be used in conjunction with the OIE Guidelines for Laboratory Quality Evaluation. Together, these guidelines form an acceptable basis for a quality assurance programme.

2. Authority and recognition

Accreditation programmes and proficiency testing schemes should be operated by an independent authority in order to prevent any bias in the award or denial of recognition.

Participation in an international accreditation programme and proficiency testing scheme should be voluntary. Lack of participation or failure to achieve recognition should not prevent a laboratory from conducting diagnostic tests or a country from entering into trade agreements.

Participation and recognition status should be made available by the independent authority to trading partners only at the request of or with the consent of the participating laboratory or country authority.

Such a programme and scheme may involve a cost to the participating laboratories for this service.

3. Organisation and management

Details of the proficiency testing scheme and its purpose, eligibility of participating laboratories and disposition of the results should be documented by the coordinating organisation to ensure the protection of proprietary rights and confidential information.

A programme manager should have overall responsibility for the operation, quality and security of the proficiency testing scheme.

It is also the responsibility of the programme manager to ensure that laboratories involved in the production of test materials are compliant with the relevant requirements of the ISO 9000 series of standards and ISO/IEC 17025.

Employees should be free from pressure or inducements that might unduly influence the analysis of proficiency testing results or the recognition status of the participating laboratory.

Adequate supervision and security should be provided by staff involved in either the production and distribution of test materials to be used in the proficiency testing scheme or the receipt and analysis of test results submitted by participating laboratories.

4. Standard methods

For the characterisation of test materials to be used in check sample panels, the standard method should meet or exceed the minimum diagnostic performance characteristics required for eligibility as a prescribed test in the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*.

The standard test should be calibrated against international standard materials, if these are available. Participating laboratories should also be encouraged to calibrate their own assays against the same international standards.

5. Selection and composition of check sample panel

5.1. General principles

For the purpose of selection of test materials for inclusion in the check sample panel, the initial assessment of the status and/or reactivity of the sample will be determined by the producing laboratory, using the standard method.

Acceptance of test materials into the proficiency panel should be based on repeated testing by more than one analyst conducting multiple runs of the test on different days. Sufficient values should be generated to assure the unequivocal status of the test material, including homogeneity.

5.2. Composition of the proficiency panel

The number of test samples that constitute a check sample panel is not well defined. This will be dictated by the type of analysis to be performed on the results and the numbers required to ensure statistical validity.

Irrespective of the type of test, a minimum of three samples should be included:

i) An unequivocal strong positive,

ii) An unequivocal weak positive,

iii) An unequivocal negative.

However, using only three samples of this nature would render the results very predictable after a few rounds of proficiency testing. It would be advisable, therefore, to add at least two more samples to the check sample panel which could be varied from one proficiency test round to the next. This would prevent participating laboratories from anticipating the expected outcome. The additional samples could be different from the above or replicates of the above or a combination.

In planning the overall process for preparation, testing and distribution of test materials and test items, the provider shall provide for, where appropriate, procedures and resources for:

i) material selection;

ii) maintaining suitable environments for preparation and testing of test material;

iii) material preparation;

iv) measuring and testing;

v) calibration/validation of equipment and measurement methods;

vi) assessing test material homogeneity;

vii) assessing test material stability;

viii) organising interlaboratory test comparisons with collaborators, where necessary; (see Note 1 below)

ix) ensuring adequate storage facilities and conditions;

x) ensuring adequate packaging and labelling;

xi) ensuring appropriate transport and distribution arrangements;

xii) statistical analysis of test results and assigning values of measurands and associated uncertainties;

xiii) ensuring adequate reporting service to participants.

6. Statistical analysis

6.1. Types of data

The choice of statistical analysis will in part be determined by the type of data generated by the test method in question. Qualitative data such as 'positive', 'negative' and/or 'suspicious' are somewhat limited in the statistical procedures which may be applied to them. Quantitative data such as end-point titres, and semi-quantitative data such as percentage inhibition values are more flexible with respect to the types of statistical analysis possible.

Irrespective of the type of data to be analysed, it is important that the data from all of the participating laboratories be compatible. In some cases, this may require that participating laboratories be instructed to use a specific dilution series or to express their data against a common standard.

6.2. Assigned values

Either of two approaches may be used:

a) Assigning of target value before issue

In the initial selection of test materials for the check sample panel, the producing laboratory will have assigned a preliminary value, range or status to the sample. For qualitative data, the assigned value may be the only acceptable value. If this is to be the case, then the producing laboratory should verify the status on a battery of tests to increase the confidence that the assigned value is in fact correct. However, as a goal, at least 80% of the participating laboratories should obtain the same result in proficiency tests. For quantitative and semi-quantitative data, the assigned value should be recalculated after proficiency testing results are submitted, and it should be taken as the mean value after removal of outliers.

b) Assigning target value on the consensus value of the returned participants results

6.3. Statistical methods

Many statistical procedures have been applied to interlaboratory comparisons, some being far more sophisticated than others. As a general rule, the statistics being applied should be valid, straightforward and meaningful to the participating laboratories.

Frequency analysis is a simple and meaningful method for participating laboratories to see where their performance lies with respect to the other laboratories in the proficiency testing scheme.

Measures of intra- and interlaboratory variance through repeatability and reproducibility indices will often provide valuable information on the precision and robustness of the test methods.

Youden analysis is a useful indicator of systematic or random error sources that may be causing problems in individual laboratories.

7. Pass/fail criteria

Decision criteria with regards to passing or failing a laboratory on a proficiency test should be clearly documented. These criteria must take into consideration factors which may vary from one disease to another and between types of tests. Once established, the criteria must be applied uniformly.

The types of statistical analyses chosen should assist in making pass/fail decisions. Laboratories submitting results that fall outside ranges established by statistical means should be identified. Results of tests that would potentially lead to a false-negative classification of an infected animal would have to be weighed against results that would potentially lead to a false-positive classification of a healthy animal. In most instances, the former type of error should not be tolerated as it indicates that there is a problem with diagnostic sensitivity. However, there may be some latitude in awarding a provisional status to laboratories experiencing problems with diagnostic specificity.

8. Frequency of proficiency testing

It is recommended that proficiency testing be done on a twice yearly basis, where possible. Depending on the country and disease, some consideration should be given to peak testing periods. Whenever possible, at least one of the proficiency tests should be scheduled to coincide with active testing periods.

Twice yearly, provides sufficient time between proficiency tests to undertake any corrective actions which might prevent a participating laboratory from losing its recognition status.

9. Laboratory recognition

The criteria for awarding, denying or withdrawing recognition should be clearly documented.

10. Logistics

10.1. Eligibility and acceptance

Eligible laboratories should be sent a comprehensive outline of the quality assurance programme and the proficiency testing scheme. This outline should include details pertaining to frequency of testing, commitments and deadlines, methods of data analysis, reporting structure, criteria for recognition, disposition of results and confidentiality. In addition, a form to be signed and returned to the coordinating organisation should be included which indicates that the eligible laboratory accepts the terms and conditions of the programme.

10.2. Notification and shipment of panels

Participating laboratories should be notified at least 1 month in advance of a pending proficiency test. Notification should also include the projected date and method of shipment of the check sample panel. Longer notification may be required by those laboratories in countries requiring import permits for the check sample panels.

Test materials in the check samples should be coded so as not to indicate their expected result. The coding may be alphabetic or numeric. A unique set of codes helps to prevent collusion between laboratories.

All shipments should be by the most expedient and direct method. All shipments should comply with IATA[3] regulations concerning the shipment of biological materials.

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3 IATA: International Air Transport Association

Upon shipment, the recipient laboratories should be informed of pertinent details (i.e. method of shipment, carrier, air-way bill, etc.) in order to facilitate rapid retrieval and clearance of the shipment upon arrival.

Check sample panels arriving in a damaged or questionable condition should be replaced immediately.

10.3. Testing and return of results

Participating laboratories should be given an adequate volume of test material and adequate time to complete the testing of the check sample panel to their satisfaction. The panel may be tested more than once and by more than one person in the participating laboratory. However, only one set of results should be returned to the coordinating organisation for analysis. Normally, the person responsible for running the test routinely should be selected to run the check sample panel.

The check sample panel should be accompanied by a complete set of instructions with respect to reconstitution, storage and handling, special testing requirements, data expression and deadline for the submission of results.

Results must be returned in the proper format and on time. Failure to do so could lead to omission from the round of proficiency testing and loss or downgrading of recognition status.

The coordinating organisation should acknowledge receipt of the results and their acceptance into the analysis.

10.4. Analysis and reporting

Analysis and reporting should be completed in a timely fashion after the deadline for the receipt of results.

A general report summarising the results of all of the analyses should be prepared for distribution to all participating laboratories. Participating laboratories should be randomly assigned a code to ensure anonymity in the general report. Individual laboratories should be informed of their unique code for this run of proficiency tests.

Individual laboratories should also receive a summary of their own performance and their recognition status. This summary should indicate clearly all factors contributing to any change in their status. Where the status has been downgraded, it is especially important to indicate real or potential causes which may have contributed to downgrading. In some instances, it may be pertinent to re-issue a second, identical panel after corrective actions have been taken.

A statement of status may also take the form of an official certificate.

All data, results of analyses and the recognition status of participating laboratories should be kept in confidence at all times.

11. Disclosure

The primary purpose of these guidelines is to remove trade barriers and not to create them. It would be expected that participating laboratories having achieved full recognition status may request that official verification of their status be made available to trading partners from the independent authority or coordinating organisation. This should only be done at the request of or with the consent of the participating laboratory or country authority.

12. References

ISO/IEC INTERNATIONAL STANDARD 17025 (2005). General requirements for the competence of testing and calibration laboratories. International Organisation for Standardisation (ISO)/International Electrotechnical Commission (ISO/IEC), ISO Central Secretariat, 1 rue de Varembé, Case Postale 56, CH - 1211, Geneva 20, Switzerland.

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ISO INTERNATIONAL STANDARDS 9000:2005, 9001:2000; 9004:2000 (2000–2005). Quality management and quality assurance. International Organization for Standardization (ISO), ISO Central Secretariat, 1 rue de Varembé, Case Postale 56, CH - 1211, Geneva 20, Switzerland