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REPORT OF THE MEETING OF THE OIE BIOLOGICAL STANDARDS COMMISSION

Paris, 13–15 September 2006

The OIE Biological Standards Commission met at the OIE headquarters from 13 to 15 September 2006. Dr Bernard Vallat, Director General of the OIE, welcomed the members of the Commission, Professor Steven Edwards, President, Dr Beverly Schmitt, Vice-President, Dr Mehdi El Harrak, Secretary General, and Dr S.K. Bandhopadhyay, 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 from Fisheries and Oceans Canada.

Dr Vallat stressed the importance of the work of the Biological Standards Commission in the field of diagnosis both for the eradication of diseases and for improving the quality of vaccines. The Commission also plays a key role in the organisation of the network of 180 OIE Reference Laboratories and Collaborating Centres, as well as in the selection of new laboratories for this network, and biosecurity measures for laboratories that handle pathogenic organisms. Given the importance of these tasks, it was proposed to increase the number of members of the Commission at the last election from three to five members and to make more use of specialised *ad hoc* Groups. Dr Vallat reminded the meeting that the OIE had more than 120 members from developing countries and that the laboratories in these countries should be more involved in the network, but this required a long-term effort that could start with a global programme for twinning the laboratories in developed countries with those in developing countries.

Professor Edwards mentioned the forthcoming International Conference of OIE Reference Laboratories and Collaborating Centres to take place in Florianopolis, Santa Catarina, Brazil, in December 2006, which provides an excellent opportunity to promote these twinning programmes. Dr Vallat also stressed the importance of the meeting of the Presidents of the Specialist Commissions in October to forge links between them and to work in close collaboration.

Professor Edwards then introduced the new Members of the Commission, welcoming them and giving a brief explanation of the working procedure within the Commission.

The agenda and the list of participants are found in Appendices I and II, respectively.

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

1. OIE Reference Laboratories and Collaborating Centres

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

The Commission recommended acceptance of the following candidates as OIE Collaborating Centres and Reference Laboratories:

OIE Collaborating Centre for Research on Emerging Avian Diseases
Southeast Poultry and Research Laboratory (SEPRL), United States Department of Agriculture (USDA), Agricultural Research Service, 934 College Station Road, Athens, Georgia 30605, UNITED STATES OF AMERICA.
Tel.: (+1-706) 546.3433; E-mail: dswayne@seprl.usda.gov

OIE Reference Laboratory for New World Screwworm
COPEG (Panama–US Commission for the Eradication and Prevention of NWS), Apartado Postal 0816-07636, PANAMA.
Tel.: (507) 232.60.44; Fax: (507) 232.61.92; E-mail: veter56@yahoo.com; tinso24@hotmail.com
Designated Reference Expert: Official letter was sent to the Delegate of Panama requesting this information.

OIE Reference Laboratory for Glanders
Friedrich-Loeffler-Institute, Institute of Bacterial Infections and Zoonoses, Naumburger Str. 96a, 07743 Jena, GERMANY
Tel.: (+49-3641) 80.42.00; Fax: (+49-3641) 80.42.28; E-mail: heinrich.neubauer@fli.bund.de
Designated Reference Expert: Dr Heinrich Neubauer

OIE Reference Laboratory for Chlamydiosis (*ovine and avian*)
Friedrich-Loeffler-Institute, Institute of Bacterial Infections and Zoonoses, Naumburger Str. 96a, 07743 Jena, GERMANY
Tel.: (+49-3641) 80.43.34; Fax: (+49-3641) 80.42.28; E-mail: konrad.sachse@fli.bund.de
Designated Reference Expert: Dr Konrad Sachse

1.2. Updating the list of Reference Laboratories

The OIE was informed of changes in the experts involved in work at the OIE Reference Laboratories. The Commission recommends acceptance of these new experts:

Contagious bovine pleuropneumonia
Dr Ana Rosa Pombo Botelho to replace Dr José Regalla at the Laboratório Nacional de Investigaçâo Veterinária, Lisbon, PORTUGAL.

Control of Veterinary Medicinal Products in Sub-Saharan Africa
Dr Assiongbon Teko-Agbo to replace Dr François Abiola at the EISMV, Dakar, SENEGAL.

Brucellosis
Dr Falk Melzer to replace Dr Konrad Sachse at the Friedrich-Loeffler-Institute, Institute of Bacterial Infections and Zoonoses, Jena, GERMANY.

Avian influenza
Dr Timm C. Harder to replace Dr Ortrud Werner at the Friedrich-Loeffler-Institute, Federal Research Centre for Virus Diseases of Animals (BFAV), Insel Riems, GERMANY.

Newcastle disease
Dr Christian Grund to replace Dr Ortrud Werner at the Friedrich-Loeffler-Institute, Federal Research Centre for Virus Diseases of Animals (BFAV), Insel Riems, GERMANY.

1.3. Activities of Reference Laboratories concerning zoonotic agents

The Commission agreed to a proposal put forward by Professor Ilaria Capua that all Reference Laboratories dealing with zoonotic agents should be encouraged to share sequence data and other relevant information with medical laboratories, as has been initiated in the case of OIE Reference Laboratories for avian influenza (AI) through the activities of the OFFLU network.

1.4. Promoting the concept of twinning

The Commission continues to encourage the concept of twinning between laboratories in developed countries with laboratories in developing countries and pointed out that the forthcoming Conference of OIE Reference Laboratories and Collaborating Centres to be held in Brazil would provide an opportunity to promote such twinning and to advance the programme.

1.5. First International Conference of OIE Reference Laboratories and Collaborating Centres, Brazil, December 2006

The Commission reviewed and suggested minor amendments to the agenda of the First International Conference of OIE Reference Laboratories and Collaborating Centres, to be held at Florianopolis, Santa Catarina, Brazil, from 3 to 5 December 2006. It then examined the draft questionnaires that would be sent to OIE Collaborating Centres and Reference Laboratories. The purpose of the questionnaire is to gather information on the network of laboratories and centres and on their working methods. The responses will be analysed and the findings presented by Dr Gideon Brückner at the Conference in Brazil for the benefit of the participating experts and OIE Delegates.

2. International Standardisation of diagnostic tests and vaccines

2.1. OIE standardisation programmes for diagnostic tests

Highly pathogenic avian influenza (*HPAI*) *– Coordinator: Dr P. Selleck, Australian Animal Health Laboratory* (*AAHL*)*, Geelong, Victoria, Australia*

Dr Selleck informed the Commission work was still in progress on the preparation of OIE international reference serum for the AI AGID[3] test. The project has suffered delays due to the heavy demands on the Reference Laboratories, because of the current avian influenza situation. The Commission noted this and encouraged Dr Selleck to continue with his efforts.

Enzootic bovine leukosis (*EBL*) *PCR – Coordinator: Dr L. Renström, National Veterinary Institute, Uppsala, Sweden*

Dr Renström reported no progress on the standard protocol for EBL PCR[4]. It was hoped to discuss this further among the experts during the Conference in Brazil.

EBL standard reference serum – Coordinator: Dr Dagmar Beier, Friedrich Loeffler Institute, Wusterhausen, Germany

The Commission reviewed an extensive dossier submitted by the OIE Reference Laboratory for EBL in Germany on the validation of an international standard serum to replace the current OIE standard serum (E4) supplies of which are now very limited. After examining the data, the Commission recommended the adoption of the new standard serum (E5).

Ovine and caprine brucellosis – Coordinator: Mrs J. Stack, VLA Weybridge, UK

Mrs J. Stack communicated the first results obtained in a ring trial of the candidate sera. Further results from the Reference Laboratories are awaited.

Caprine arthritis/encephalitis and maedi-visna – Coordinator: Dr Gérard Perrin, AFSSA Niort, France

Dr Perrin had submitted results provided by Institut Pourquier, which is carrying out the work on behalf of the OIE Reference Laboratory. The Commission requested OIE to seek clarification of some technical aspects of the report.

Dourine – Coordinator: Prof. V.T. Zablotsky, All-Russian Research Institute for Experimental Veterinary Medicine (*VIEV*), *Moscow, Russia*

3 AGID: agar gel immunodiffusion
4 PCR: polymerase chain reaction

Prof. Zablotsky had informed the Commission that additional evaluations were being carried out on the candidate preparation as the reference serum. The Commission was eager to obtain more information and would ask Dr Drygin to pursue this.

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

Dr Nielsen informed the Commission that kits had been distributed to the Reference Laboratories. Some technical difficulties had been encountered so further distributions had been made. He had not yet received results from this ring trial. The Commission wished to encourage the participating laboratories to send their results to Dr Nielsen.

Equine influenza – Coordinator: Dr J. Daly, Animal Health Trust, Newmarket, UK

The European Directorate for the Quality of Medicine (EDQM) had provided a full validation dossier on an equine influenza antiserum for subtype 2 American-like strain A/Eq/South Africa/4/03. The Commission agreed to adopt the antiserum as an OIE international standard serum, in addition to the existing OIE standard sera.

2.2. Standardisation of vaccines

Dr J. Mumford, OIE expert on equine influenza, had submitted various correspondence concerning the implementation by vaccine companies of the recommendations of the expert surveillance panel for this disease. The issues will be pursued by Prof Edwards in discussion with Dr Mumford.

3. List of prescribed tests and substitution tests

3.1. Real-time PCR for detection of infectious bovine rhinotracheitis (IBR) virus in extended bovine semen

Following the report of experts on this test validation dossier (see report of the Commission for January 2006), further data had been submitted and again reviewed by OIE experts. The Commission agreed that there was now sufficient evidence to recommend use of the real-time PCR test for detection of IBR virus in extended bovine semen as a prescribed test for trade. The protocol can be found at Appendix III and will be added to the web-version of the *Terrestrial Manual* if adopted by the Committee.

4. *Ad hoc* Groups

4.1. *Ad hoc* Group on Biotechnology

For this session the Commission was joined by Prof. Paul-Pierre Pastoret, Chairman of the *ad hoc* Group on Biotechnology. Some revisions to the Terms of Reference of the Group were proposed and will be put to the Group at its next meeting. The Commission agreed the Group's proposal to continue its work in three select subgroups. The priorities of the *ad hoc* Group should be vaccines, and health aspects of animal cloning technology. An information brief should be maintained on nanotechnology. The report of the *ad hoc* Group was accepted and can be found at Appendix IV.

4.2. *Ad hoc* Group on Antimicrobial Resistance

Prof Edwards informed the Commission of the discussion at the General Session in May 2006 concerning the list of antimicrobials of veterinary importance. In accordance with Resolution No. XXXIII from the General Session, the *ad hoc* Group would meet at the end of September 2006 to further refine the list. A dialogue was being maintained with the Codex Alimentarius Commission on this topic.

4.3. *Ad hoc* Group on Biosecurity

Dr Schmitt reported to the Commission on the state of progress of the *ad hoc* Group's activities. Work on writing the 'Veterinary Biosafety Facility Construction Handbook' had been largely completed and the Booklet would be presented during a meeting in Singapore before publication by the OIE. The Commission also took note of the latest version of a WHO paper entitled "Biorisk management: Laboratory Biosecurity Guidance". The OIE had the opportunity to provide input during the development of this document. The Commission considered the document was now well balanced, but understandably focused on the risks to human rather than animal populations. It would be complementary to the OIE standards published in the *Terrestrial Manual*.

4.4. *Ad hoc* Group on BSE[5] diagnostic tests

The Commission noted a paper produced by the OIE reference laboratory for BSE, Weybridge, UK, entitled 'The evaluation of commercial confirmatory tests for BSE'.

4.5. *Ad hoc* Group on OIE Guidelines for International Reference Sera for Antibody Assays

The *Ad hoc* Group had, as agreed, worked by electronic communication. Its report was presented by Dr Adama Diallo, and is included at Appendix V. The principal recommendations, which were accepted by the Commission, were that the OIE Guidelines should be revised, in particular to recognise either gamma irradiation or bromoethyleneimine (BEI) chemical treatment as acceptable alternatives to inactivate adventitious agents in international reference sera.

5. Review of OIE guidelines

Acknowledging the need to revise the OIE Guidelines on preparation of international reference sera (see 4.5 above) the Commission decided to undertake a full revision of the booklet "OIE Quality Standard and Guidelines for Veterinary Laboratories". This will include the updating of the OIE Quality Standard to bring it in line with ISO 17025-2000, which had already been completed by Mr François Diaz and Dr Peter Wright. A review will also be commissioned of the OIE Guideline on Proficiency Testing to keep it in line with ongoing developments in ISO[6] and ILAC[7].

6. 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 Country comments had been received on the three batches of draft chapters that had been sent to Member Countries so far (68 chapters in total). The comments received were reviewed and the chapters were modified where necessary.

In view of the existence of the register of OIE validated and certified diagnostic tests, it was agreed that references to individual commercial kits should, as far as possible, be removed from the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (*Terrestrial Manual*). A disclaimer would be added at the beginning of the *Terrestrial Manual* stating that "reference to commercial kits does not mean their endorsement by the OIE. All commercial kits should be validated; tests on the OIE register have already met this condition."

The Commission discussed the issue of the necessity of carrying out chicken pathogenicity testing on all sequence-determined low pathogenicity AI (LPAI) isolates of H5 and H7 from wild birds where sequence information on numerous isolates is similar. The Commission supports the concept that when designing surveillance programmes, individual countries can determine for themselves the necessity to perform chicken pathogenicity tests on sequence-determined LPAI isolates for wild birds.

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5 BSE: Bovine spongiform encephalopathy
6 ISO: International Organization for Standardization
7 ILAC: International Laboratory Accreditation Cooperation

The Commission believed that the state of progress of the sixth edition of the *Terrestrial Manual* was satisfactory and that it would be published in the first quarter of 2008 as planned.

7. Register of OIE validated and certified diagnostic tests

Mr François Diaz reported on the state of progress of the four dossiers so far submitted to the OIE. Prof Edwards reminded the Commission that it would be necessary to clear some submissions by correspondence/email once the reports of the expert panels were finalised.

Mr Diaz presented some of the problems encountered, in particular the lack of experts for rare or exotic diseases and the absence of guidance on the 'level of perfection' of the dossier to request of an applicant. Differences were also highlighted in the evaluations depending on the make up of the expert panels.

The Commission approved the modifications made by Mr Diaz to the standard operating procedure (SOP).

8. Review of the OIE web pages concerning the Commission

The Commission asked its members to make enquiries amongst their colleagues concerning the difficulties and problems encountered when consulting the OIE web pages in order to suggest improvements.

9. Liaison with the other Commissions

For this session the Commission was joined by Dr A. Thiermann, President of the OIE Terrestrial Animal Health Standards Commission **(**Code Commission), and Dr Sarah Kahn, Head, OIE International Trade Department.

- **TERRESTRIAL ANIMAL HEALTH STANDARDS COMMISSION**

9.1. Diagnostic methods for paratuberculosis

The Code Commission wished to know if there were any recent improvements in diagnostic tests for this disease. The BSC responded that this remained problematical, but an Australian expert in paratuberculosis will be contacted with a view to providing additional information on diagnostic methods. The recently developed PCR method seemed promising but a comparative assessment with traditional techniques was necessary. The issue was not just the laboratory test itself, but the sampling strategy and the interpretation of the results.

9.2. Transport of pathogens

A Member Country had expressed concern that if the proposal to move the table on Guidance on the laboratory requirements for the different containment groups from the *Terrestrial Animal Health Code* (*Terrestrial Code*) to the *Terrestrial Manual* is not carefully synchronised, this valuable information may be temporarily unavailable. Both Commissions agreed that no change would be made to the *Terrestrial Code* until the *Terrestrial Manual* is adopted and available on-line, thus avoiding the temporary loss of information.

The Commission discussed the continuing dialogue and input to the UN Committee of Experts on the Transport of Dangerous Goods. The OIE continued to press for a proportionate approach on transport of avian influenza diagnostic materials, and also on transport of carcases.

9.3. Border disease

The Code Commission reported that Appendix 3.2.1 on small ruminant semen had been amended this year in response to Member Country comments, and no longer required testing of semen donors for border disease. The *Terrestrial Manual* continues to recommend such testing for rams used for breeding. The Commission was surprised at this change in the *Terrestrial Code*, but would seek advice from experts concerning the likely risk from border disease virus in semen.

9.4. Rabies recombinant vaccines for international trade

Following a recommendation from the OIE Conference on Rabies in Europe, held in June 2005 in Kiev, Ukraine, the Commission reiterated its advice (see report for Sept 2005) that parenteral vaccination of domestic animals using recombinant vaccines expressing the rabies virus glycoprotein in a live virus vector such as canary pox, should not be considered as live rabies virus vaccines. The *Terrestrial Manual* chapter had been modified to reflect this view, and a suitable text had been provided to the Code Commission for the *Terrestrial Code* chapter.

- **SCIENTIFIC COMMISSION FOR ANIMAL DISEASES**

9.5. Bluetongue

In Article 2.2.13.2, paragraph 3a of the *Terrestrial Code* chapter on bluetongue, a waiting period of 60 days is recommended before vaccinated ruminants and other susceptible herbivores can be shipped to a bluetongue virus free country or zone. Following consultation with an expert, the Commission recommends modifying the text such that it is clear that the 60-day waiting period applies only to live vaccines; in the case of vaccination using inactivated vaccine, there is no need for a waiting period of 60 days.

9.6. Vaccines for avian influenza (AI)

Following discussion at the General Session (para 338) the Commission noted that the AI chapter in the *Terrestrial Manual* (online version as updated in 2005) included standards and general advice on the production and use of vaccines. Further advice on the application of different types of vaccine in different epidemiological circumstances should be sought from the Scientific Commission. A draft OIE information document on AI vaccination provided the essential background, but the Commission recommended this should be revised and updated.

10. Miscellaneous questions

10.1. Update on OFFLU[8]

A meeting of the Steering Committee had been held at VLA Weybridge, UK, on 19th July 2006, together with Drs Vallat and Domenech. It was agreed that OFFLU itself would not be tasked with the organisation of Missions to affected countries, but would supply lists of experts to the FAO/OIE Crisis Management Centre based in Rome. Good progress was being made in persuading laboratories world-wide to share virus strains and/or sequence data with the global scientific community, and OFFLU had been a co-signatory to the Global Initiative on Sharing Avian Influenza Data (GISAID). Issues continue over the commitment of members of the OFFLU network, and progress on acquiring resources and appointing staff to advance the aims of the network was disappointingly slow.

10.2. Programme for the OIE Seminar on Biotechnology to be held in conjunction with the WAVLD[9] Symposium

Dr Edwards asked the members of the Commission to propose subjects and speakers for inclusion in the OIE Seminar on Biotechnology to be held in conjunction with the WAVLD Symposium in Melbourne, Australia in 2007. These will be further discussed with the *ad hoc* Group on Biotechnology, and Dr Edwards will liaise with the local organisers in Melbourne.

10.3. Request from WHO[10] to provide guidance to countries on how to transfer strain prototypes in the event of an avian influenza pandemic

A letter had been received from the WHO regarding OIE and WHO recommendations on the transfer of strain prototypes in the event of an avian influenza pandemic. The Commission recognises in principle the WHO's concern. WHO was seeking advice whether *in-vitro* tests could be performed to determine the inoffensive nature of prototype vaccine strains before their transfer, enabling evaluation

8 OFFLU: OIE/FAO Network on Avian Influenza
9 WAVLD: World Association of Veterinary Laboratory Diagnosticians
10 WHO: World Health Organization

work to proceed without a requirement for high biosecurity containment. The Commission agreed that in an emergency situation, these *in-vitro* tests are sufficient without resorting to *in-vivo* inoculation tests. The WHO could produce an instructive text for its members.

10.4. Update on VICH[11]: Examination of live veterinary vaccines in target animals for absence of reversion to virulence

A draft guideline on the above had been received from VICH. The OIE representative at VICH would be asked to compare the VICH guidelines with OIE Standards to make sure that they are in harmony and that there are no contradictions. The VICH glossary would also be compared with the glossary in the OIE *Terrestrial Manual*.

10.5. Follow-up of questions raised during the General Session: equine influenza in dogs in the United States of America

In response to a question raised at the General Session in May 2006 (paragraph 337 of the Final Report), the United States of America had provided information to the Commission regarding canine influenza. Cases of this have become widespread in the USA both in racing greyhounds and in pet dogs. The strain has been characterised as H3N8, closely related to equine influenza of the same subtype (*Science* [2005], **310**, p. 482). The Cornell University website in September 2006 recorded 715 positive samples from 4306 tested (http://diaglab.vet.cornell.edu/issues/civ.asp). Information is also available at www.avma.org/public_health/influenza/default.asp#canine. The Commission understands that transmission is occurring from dog to dog, but there is no evidence as yet of transmission from dogs to horses or other species.

10.6. Consultants Meeting on "Standards, references and validation" at the IAEA headquarters in Vienna, Austria from 21 to 24 November 2006

Dr Adama Diallo, representing the OIE Collaborating Centre for ELISA and molecular techniques in animal disease diagnosis, presented the topics to be discussed at the above-named Consultants Meeting. The Commission strongly recommends that the OIE send a member of staff to participate at this meeting.

10.7. Dates of the next meeting of the Biological Standards Commission

The next meeting of the Commission is scheduled for 23 to 25 January 2007. The following meeting is scheduled for 25–27 September 2007.

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.../Appendices

11 VICH: International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products

Appendix I

MEETING OF THE OIE BIOLOGICAL STANDARDS COMMISSION

Paris, 13–15 September 2006

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Agenda

1. OIE Reference Laboratories and Collaborating Centres
2. International Standardisation of Diagnostic Tests and Vaccines
3. List of Prescribed and Alternative Tests
4. Expert, *ad hoc* and Working Groups
5. Review of the OIE guidelines
6. *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*
7. OIE Register of diagnostic tests
8. Review of the OIE web pages concerning the Commission
9. Liaison with other Commissions
10. Any other business

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MEETING OF THE OIE BIOLOGICAL STANDARDS COMMISSION
Paris, 13–15 September 2006

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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
Infectious bovine rhinotracheitis/ infectious pustular vulvovaginitis	VN, ELISA, Agent id. (semen only), PCR (semen only)	–

ELISA = Enzyme-linked immunosorbent assay
PCR = Polymerase chain reaction
VN = Virus neutralisation

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

Real-time polymerase chain reaction (a prescribed test for international trade)

The following real-time PCR test method has been developed to detect BoHV-1 in extended bovine semen intended for trade. The method has been validated according to chapters 1.1.3 and 1.1.4, and includes a comprehensive international inter-laboratory comparison involving six collaborating laboratories with specialist status in IBR testing.

A number of studies has shown that PCR assays are more sensitive than virus isolation (9, 11, 12, 15). Real-time PCR has been used for detection of BoHV-1 and BoHV-5 in experimentally infected cattle and mice (1, 4) and a number of conventional PCR assays have been used for the detection of BoHV-1 DNA in artificially or naturally infected bovine semen samples (2, 3, 5, 10, 15, 16). Conventional detection of amplified PCR products relies on gel electrophoresis analysis. Sequence-specific primers have been selected to amplify different parts of conserved glycoprotein gene of BHV-1 genome, including glycoprotein B (gB) gene (3, 8), gC gene (9, 11), gD gene (9, 15), gE gene (3), and the thymidine kinase (tk) gene (6, 17).

Real-time PCR differs from standard PCR in that the amplified PCR products are detected directly during the amplification cycle using a hybridisation probe, which enhances assay specificity. Real-time PCR assays have several advantages over the conventional PCR methods. Real-time PCR assays using only one pair of primers are able to provide sensitivity close or equal to nested PCR methods with a much lower risk of contamination. The amplification and detection of target is conducted simultaneously. There is no post-amplification PCR product handling, which significantly reduces the risk of contamination, and it is possible to perform quantitative analysis with real-time PCR systems.

The real-time PCR described here uses a pair of sequence-specific primers for amplification of target DNA and a 5'-nuclease oligoprobe (TaqMan) for detection of amplified products. The oligoprobe is a single, sequence-specific oglionucleotide labelled with two different fluorophores, the reporter/donor, 5-carboxyfluorescein (FAM) at the 5' end, and the acceptor/quencher 6-carboxytetramethylrhodamine (TAMRA) at the 3' end. This real-time PCR assay is designed to detect viral DNA of all BHV-1 strains, including subtype 1 and 2, from extended bovine semen. The assay selectively amplifies a 97 basepair sequence of the glycoprotein B (gB) gene. Details of the primers and probes are given in the protocol outlined below.

- **Sample preparation, equipment and reagents**

i) The samples used for the test are, typically, extended bovine semen stored in liquid nitrogen. The semen samples can be transported to the laboratory in liquid nitrogen, or shipped at 4°C, and stored in liquid nitrogen or at –70°C (for long-term storage) or 4°C (for short-term storage). Storing semen at 4°C for a short period (up to 7 days) does not appear to affect PCR test result.

ii) Three straws from each batch of semen to be tested should be processed. Duplicate PCR amplifications should be carried out for each DNA preparation (six amplifications in total) to ensure the detection of DNA in samples containing low levels of virus.

iii) A real-time PCR detection system, and the associated data analysis software, is required to perform the assay. A number of real-time PCR detection systems are available from various sources. In the procedure described below, a RotorGene 3000, Corbett Research Ltd, Australia, was used. Other real-time PCR detection systems can also be used. Other equipment required for the test includes a micro-centrifuge, a heating block, a boiling water bath, a micro-vortex, magnetic stirrer and micropipettes. Real-time PCR assays are able to detect very small amounts of target nucleic acid molecules therefore appropriate measures are required to avoid contamination[1].

iv) The real-time PCR assay described here involves two separate procedures. Firstly, BoHV-1 DNA is extracted from semen using Chelex-100 chelating resin, along with proteinase K and DL-Dithiothreitol (DTT). The second procedure is the amplification and detection of the extracted DNA template by a real-time PCR detection system using a PCR reaction mixture: Platinum Quantitative PCR SuperMix-UDG, Invitrogen Technologies (note that there are a number of other commercial real-time PCR amplification kits available from various sources and the kit selected needs to be compatible with the real-time PCR

1 Sources of contamination may include product carry-over from positive samples or, more commonly, from cross-contamination by PCR products from earlier experiments. Samples and reagents should be handled in separate areas, with separate equipment for reagent and sample preparation and amplification/detection.

platform selected). The required primers and probes can be synthesised by various commercial companies. In this protocol, all the primers and probes used were synthesised by Sigma-Genosys.

- **Extraction of DNA**

i) In a screw top 1.5 ml tube, add:

Chelex 100 sodium (Sigma) (10% w/v in distilled deionised water)	100 µl.
Proteinase K (10 mg/ml, Sigma)	11.5 µl
DL-Dithiothreitol (1 M, Sigma)	7.5 µl
Nuclease-free water	90 µl
Semen sample	10 µl

Mix gently by pipetting[2].

ii) The sample tubes are incubated at 56°C for 30 minutes and then vortexed at high speed for 10 seconds.

iii) The tubes are then incubated in a boiling water bath for 8 minutes and then vortexed at high speed for 10 seconds.

iv) The tubes are centrifuged at 10,000 ***g*** for 3 minutes.

v) The supernatant[3] is transferred into a new microtube and can be used directly for PCR, or stored at –20°C for testing at a later date.

- **Preparation of reagents**

The real-time PCR reaction mixture (Platinum Quantitative PCR SuperMix-UDG, or other reaction mixture) is normally provided as a 2 × concentration ready for use. The manufacturer's instructions should be followed for application and storage.

Working stock solutions for primers are made with nuclease-free water at the concentration of 4.5 µM and 3 µM, respectively. The stock solution of primers and probe are stored at –20°C and the probe solution should be kept in the dark. Single-use aliquots can be prepared to limit freeze-thawing of primers and probes and extend their shelf life.

- **Real-time PCR test procedure**

i) Primers and probe sequences

Selection of the primers and probe are outlined in Abril *et al*. (2004) and described below.

Primer gB-F: 5'-TGT-GGA-CCT-AAA-CCT-CAC-GGT-3' (position 57499–57519 GenBank®, accession AJ004801)

Primer gB-R: 5'-GTA-GTC-GAG-CAG-ACC-CGT-GTC-3' (position 57595–57575 GenBank®, accession AJ004801)

TaqMan Probe: 5'-FAM-AGG-ACC-GCG-AGT-TCT-TGC-CGC-TAMRA-3' (position 57525–57545 GenBank®, accession AJ004801)

ii) Preparation of reaction mixtures

The PCR reaction mixtures are prepared in a clean laboratory room. All the reagents except the test samples are mixed before distribution to each individual reaction tube. For each PCR test, appropriate controls should be included. As a minimum, a no template control (NTC, reagent only), appropriate negative controls, i.e. 1 per 10 test samples, and two positive controls (strong and weak) should be included. Each test sample and control is tested in duplicate. The PCR amplifications are carried out in a volume of 25 µl.

a) PCR reagent mixtures are added in a clean room (no viral cultures, DNA extracts or post-amplification products should be handled here)

2 × Platinum Quantitative PCR SuperMix-UDG	12.5 µl
ROX reference dye (optional)	0.5 µl

2 It is important that Chelex 100 sodium be distributed evenly in the solution while pipetting, as Chelex 100 sodium is not soluble. This can be done by putting the vessel containing Chelex-100 solution on a magnetic stirrer while pipetting.

3 Some DNA samples can become cloudy and a thin white membrane may form occasionally after freezing and thawing. This appears to have no influence on the PCR performance. No heating or re-centrifuging of the samples is necessary.

Forward primer (gB-F, 4.5 µM)	1 µl
Reverse primer (gB-R, 4.5 µM)	1 µl
Probe (3 µM)	1 µl
Nuclease free water	4 µl

b) 5 µl of the DNA template are added to the PCR reagent mixture to a final volume of 25 µl. DNA samples are prepared and added in a separate room.

iii) Real-time (TaqMan) polymerse chain reaction

The PCR tubes are placed in the real-time PCR detection system in a separate, designated PCR room.

The PCR detection system is programmed for the test as follows:

PCR Reaction Parameters[4]

One cycle:	Hold 50°C	2 minutes
One cycle:	Hold 95°C	2 minutes[5]
45 cycles:	Hold 95°C	15 seconds
	Hold 60°C	45 seconds

iv) Analysis of real-time PCR data

The threshold level is usually set according to the manufacturers instructions for the selected analysis software used. Alternatively, virus isolation negative semen samples, from sero-negative animals, can be run exhaustively (e.g. up to 60 amplification cycles) to determine the background reaction associated with the detection system used.

- **Interpretation of results**

  - **Test controls**

Positive and negative controls, as well as reagent controls, should be included in each PCR test. Negative semen, from virus isolation negative sero-negative bulls, can be used as a negative control. Positive semen from naturally infected bulls is preferable as a positive control. However, this might be hard to obtain. Alternatively, positive controls can be derived from negative semen spiked with known quantities of BoHV-1 virus.

  - **Test results**

Positive result: Any sample that has a cycle threshold (Ct) value equal or less than 45 is regarded as positive. The positive control should have a Ct value within an acceptable range (± 3 Ct values) as previously determined by repeatability testing.

Negative result: Any sample that shows no Ct value is regarded as negative. Negative control and no template control should have no Ct values.

REFERENCES

1. ABRIL C., ENGELS M., LIMAN A., HILBE M., ALBINI S., FRANCHINI M., SUTER M. & ACKERMANN M. (2004). Both viral and host factors contribute to neurovirulence of bovine herpesviruses 1 and 5 in interferon receptor-deficient mice. *J. Virol.,* **78**, 3644–3653.

2. DEKA D., MAITI RAMNEEK N.K. & OBEROI M.S. (2005). Detection of bovine herpesvirus-1 infection in breeding bull semen by virus isolation and polymerase chain reaction. *Rev. sci. tech. Off. int. Epiz.*, **24**, 1085–1094.

3. GROM J., HOSTNIK P., TOPLAK I. & BARLIC-MAGANJA D. (2006). Molecular detection of BHV-1 in artificially inoculated semen and in the semen of a latently infected bull treated with dexamethasone. *Vet. J.,* **171**, 539–544.

4. LOVATO L., INMAN M., HENDERSON G., DOSTER A. & JONES C. (2003). Infection of cattle with a bovine Herpesvirus 1 strain that contains a mutation in the latencyrelated gene leads to increased apoptosis in trigeminal ganglia during the transition from acute infection to latency. *J. Virol.*, **77**, 4848–4857.

4 These PCR parameters are based on those most suitable for the RotorGene 3000, Corbett Research Ltd, Australia, and may vary with different PCR platforms.

5 PCR *Taq* polymerase systems from different commercial sources may require a prolonged initial denaturation (95°C) time up to 10 minutes. Please follow the manufacturer's instructions.

5. MASRI S.A., OLSON W., NGUYEN P.T., PRINS S. & DEREGT D. (1996). Rapid detection of bovine herpesvirus 1 in the semen of infected bulls by a nested polymerase chain reaction assay. *Can. J. Vet. Res.*, **60**, 100–107.

6. MOORE S., GUNN M. & WALLS D. (2000). A rapid and sensitive PCR-based diagnostic assay to detect bovine herpesvirus 1 in routine diagnostic submissions. *Vet. Microbiol*., **75**, 145–153.

7. ROLA J., POLAK M. & ZMUDZINSKI J. (2003). Amplification of DNA of BHV 1 isolated from semen of naturally infected bulls. *Bull. Vet. Inst. Pulaway*, **47**, 71–75.

8. SANTURDE G., SILVA N.D., VILLARES R., TABARES E., SOLANA A., BAUTISTA J.M., CASTRO J.M. & DA SILVA N. (1996). Rapid and high sensitivity test for direct detection of bovine herpesvirus-1 genome in clinical samples. *Vet. Microbiol.,* **49**, 81–92.

9. SMITS C.B., VAN MAANEN C., GLAS R.D, DE GEE A.L., DIJKSTRAB T, VAN OIRSCHOT J.T. & RIJISEWIJK F.A. (2000). Comparison of three polymerase chain reaction methods for routine detection of bovine herpesvirus 1 DNA in fresh bull semen. *J. Virol. Methods,* **85**, 65–73.

10. VAN ENGELENBURG F.A.C., MAES R.K., OIRSCHOT J.T., RIJSEWIJK F.A.M. & VAN ENGELENBURG F.A.C. (1993). Development of a rapid and sensitive polymerase chain reaction assay for detection of bovine herpesvirus type 1 in bovine serum. *J. Clin. Microbiol.,* **31**, 3129–3135.

11. VAN ENGELENBURG F.A.C., VAN SCHIE F.W., RIJSEWIJK F.A.M. & VAN OIRSCHOT J.T. (1995). Excretion of bovine herpesvirus 1 in semen is detected much longer by PCR than by virus isolation. *J. Clin. Microbiol*., **33**, 308–312.

12. VILCEK S., NETTLETON P.F., HERRING J.A. & HERRING A.J. (1994). Rapid detection of bovine herpesvirus 1 (BHV 1) using the polymerase chain reaction. *Vet. Microbiol*., **42**, 53–64.

13. WAGTER L.H.A., GLAS R.D., BLEUMINK PLUYM N., VAN ENGELENBURG F.A.C., RIJSEWIJK F.A.M. & HOUWERS D.J. (1996). A polymerase chain reaction (PCR) assay for the detection of bovine herpesvirus 1 (BHV1) in selectively digested whole bovine semen. *Vet. Res. Comm*., **20**, 401–408.

14. WEIBLEN R., KREUTZ L., CANABOROO T.F., SCHUCH L.C. & REBELATTO M.C. (1992). Isolation of bovine herpesvirus 1 from preputial swabs and semen of bulls with balanoposthitis. *J. Vet. Diag. Invest*., **4**, 341–343.

15. WIEDMANN M., BRANDON R., WAGNER P., DUBOVI E.J. & BATT C.A. (1993). Detection of bovine herpesvirus-1 in bovine semen by a nested PCR assay. *J. Virol. Methods,* **44**, 129–140.

16. XIA J.Q., YASON C.V. & KIBENAGE F.S.B. (1995). Comparison of dot blot hybridization, polymerase chain reaction, and virus isolation for detection of bovine herpesvirus-1 (BHV-1) in artificially infected bovine semen. *Can. J. Vet. Res*., **59**, 102–109.

17. YASON C.V., HARRIS L.M., MCKENNA P.K., WADOWSKA, D. & KIBENAGE F.S.B. (1995). Establishment of conditions for the detection of bovine herpesvirus-1 by polymerase chain reaction using primers in the thymidine kinase region. *Can. J. Vet. Res.,* **59**, 94–101.

Appendix IV

Original: English
April 2006

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

OIE Headquarters, Paris, France, 3–5 April 2006

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A meeting of the OIE *ad hoc* Group on Biotechnology was held at the OIE Headquarters in Paris from 3 to 5 April 2006. The meeting was chaired by Prof. Paul-Pierre Pastoret. Dr Cyril G. Gay and Dr Eric Schoonejans acted as rapporteurs. The Agenda and List of Participants are given at Appendices I and II, respectively.

The *ad hoc* Group was welcomed by Dr Bernard Vallat, Director General of the OIE, who emphasised that the principal mandate of the OIE is to improve animal health worldwide through the implementation development of the international standards to be published by the OIE. He indicated that the OIE recognises the necessity to develop a common understanding and definition of biotechnology for the purpose of animal health[1]. He identified three main priority areas that the *ad hoc* Group should address in the area of biotechnology:

1. *The role of biotechnology:* Dr Vallat emphasised the need for better tools for the improvement of animal health and welfare (vaccines, drugs, etc.); for example, the use of genetic engineering and LMOs[2] for the development of new vaccines and drugs. These were identified as key challenges to meet the OIE mandate;

2. *The safety of cloned and transgenic animals:* During the 73rd General Session of the OIE in 2005, the OIE International Committee adopted a Resolution on biotechnology, following the presentation of the Technical Item by Dr Anne McKenzie, in which she also addressed the safety of GMO[3]/cloned animals. Dr Vallat stressed the need to consider the Resolution in the context of animal and public health, but not in the context of food safety, to the extent that it falls within the terms of reference of Codex Alimentarius and another expert Working Group within the OIE.

3. The need to develop guidelines for research priorities in biotechnology in relation to animal health and welfare that could be conveyed to policy makers and funding agencies.

A number of other considerations were also addressed in the discussion with Dr Vallat:

1. Animal conservation/ecological issues, including consideration of the use of biotechnology in the biological control of invasive species and conservation of animal genetic resources;

2. The safety of feed, including GM[4] feeds, in relation to animal health clearly falls within the OIE mandate.

3. Validation of diagnostic tests, including the OIE commitment to develop standards and certification of diagnostic tests and the quality of vaccines.

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1 The draft comparison chart of "glossary of terms" among international organisations, prepared for the FAO (Food and Agriculture Organization of the United Nations) consultation on Biosafety within a Biosecurity Framework, was provided as a background document to illustrate the need to better harmonise definitions and terminology across international organisations.

2 LMO: Live modified organisms

3 GMO: Genetically modified organisms

4 GM: Genetically modified

4. Insects are to be addressed within OIE mandate, but consideration of the use of biotechnology to control disease vectors (vector-borne diseases) should only be addressed after attention to the main priorities;

5. The issue of pet animals was raised in relation to the current availability of GM pets, including GM fish. The Group was informed that the OIE will address the issue of animal health risks associated with international trade in pets within its working programme.

1. Discussion on the draft Terms of Reference (ToR)

The draft ToR, and the organisation of work were discussed and it was decided to identify subgroups within the *ad hoc* Group on specific topics. It was confirmed that the *ad hoc* Group will assist the OIE to develop general/overarching guidelines that could be made more specific under domestic frameworks.

The initial focus of the Group would be to identify issues that should be taken into consideration in the development of guidelines for all types of vaccines and reproductive animal biotechnologies.

The *ad hoc* Group agreed to avoid duplication of the work already covered by other fora or agencies (e.g. VICH[5], IETS[6], Codex Alimentarius). The relevant work of other international organisations was recognised in relation to the ToR, such as:

- the guidelines of EMEA[7] on DNA vaccines;
- the development of work on diagnostics by the Vienna-based IAEA[8], to evaluate diagnostics at the final product level (this was felt to be key for animal trade and OIE);
- past work of the OECD[9] workshop on recombinant live vaccines;
- Codex Alimentarius discussions on the safety of food derived from biotechnology and DNA vaccines.

Risk analysis issues related to the impacts on the environment and/or biological diversity, and horizontal issues such as animal welfare, public perception, and ethical issues were also identified as important considerations.

The draft ToR were endorsed, with only one correction introduced in the 4th indent, adding the words "**and development**" after the reference to research.

2. Discussion on Resolution No. XXVIII with a view to identifying priority areas and developing a work programme

The Resolution was discussed recognising that it could not be amended.

The Group concluded that "research" as mentioned in priority 1 of Resolution XXVIII and in the ToR, is to be understood as covering research and development.

Priority number 7 (nanosciences/nanotechnology) and the need to commence with work on nanosciences related to animal health, was the subject of more in-depth discussion. The *ad hoc* Group identified the need for a definition of nanoscience/nanotechnology in the context of animal health. Reference was made to nanotechnology within the ISO[10] work, and ongoing activities on research and development in nanotechnologies in different countries, such as Canada and France. Some background documents on Nanotechnologies were provided to the Group[11, 12].

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5 VICH: International Coooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products

6 IETS: International Embryo Transfer Society

7 EMEA: The European Agency for the Evaluation of Medicinal Products

8 IAEA: International Atomic Energy Agency

9 OECD: Organisation for Economic Co-operation and Development

10 ISO: International Organization for Standardization

11 Colvin V.L. (2003). The potential environmental impact of engineered nanomaterials. *Nature Biotechnology*, **21**, 1166–1170.

12 Hoet P.H.M., Brüske-Hohlfeld I. & Salata O. (2004). Nanoparticles – known and unknown health risks. *J. Nanobiotechnology*, **2**, 12.

The *ad hoc* Group recommended that a seminar/workshop on nanotechnology should be convened, as a stock-taking exercise, in cooperation with other interested organisations, such as FAO[13], WHO[14], IAEA and in particular with the three standard-setting bodies: the OIE, Codex Alimentarius and the IPPC[15].

It was noted that the Resolution does not specifically address the safety of feed products derived from biotechnology or "modern biotechnology", nor does it address explicitly non-production animals.

The work of FAO and the IPPC on GM insects as control agents of plant pests, as well as the pest risk analysis of GM insects was noted.

3. Mission report on the FAO Expert Consultation on Biosafety within a Biosecurity Framework: issues arising from this consultation that could be addressed by this *ad hoc* Group

Dr Anne MacKenzie presented her written report on her participation in the FAO consultation on Biosafety within a Biosecurity[16] framework (Rome, Italy, 28 February to 3 March, 2006).

Three areas where the consultation felt that OIE could lead activities as they relate to animal health were emphasised in their relation to the setting of standards, guidelines and recommendations:

1. Transgenic and cloned animals (including fish and insects)

2. LMO vaccines for animals

3. GM feed safety

A number of issues in addition to the written report were conveyed to the Group:

- The need for interagency cooperation and coordination in this context, as mentioned in paragraph 309 of the background document provided to the FAO consultation and to the members of the *ad hoc* Group.

- The call to, and the need for the OIE to address animal health in the context of ecological impact assessment, in particular as it relates to integrated ecosystems, and the assessment of the impact of new biotechnologies on the health of animals in the wild and as part of ecosystems. This area has applications with regard to the development of methods to restrict or manage gene flow.

- The opportunity to establish a link between the implementation of the internationally binding framework of the Cartagena Protocol on Biosafety, and the measures adopted therein, and the technical expertise and work of the OIE as it relates to animal health or live vaccines in the context of biosafety.

- The current ongoing work on the development of national biosafety framework within 120+ countries, mainly under the auspices of environment departments and international funding for the implementation of environmental agreements but with a lack of appropriate expertise in animal health, and the need for OIE guidance to be included in these ongoing national developments.

The Group noted with specific reference to the last two points, that GM animals and live vaccines derived from "modern biotechnology" are to be covered by the national biosafety frameworks currently developed in many countries, fall within the scope of the biosafety protocol, and will soon be addressed in the programme of work thereof.

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13 FAO: Food and Agriculture Organization of the United Nations

14 WHO: World Health Organisation

15 IPPC: International Plant Protection Convention

16 The term "*Biosecurity*" refers here to FAO definition of *Biosecurity* in Food and Agriculture, as defined by the FAO Technical Consultation on Biological Risk Management in Food and Agriculture (Bangkok, January 2003).

The Group endorsed the need to also address environmental assessments/ecological issues as they relate to animal health, e.g. in the case of live vaccines or transgenic animals. Other forthcoming topics, such as transgenic insects or fish were also mentioned, with fish being an important issue to be addressed in future work of the *ad hoc* Group.

The issue of assessing the safety of vaccination and vaccines for wild fauna (conservation medicine) was noted. In this context, the Group concluded that addressing the efficacy, not only the safety, for wildlife should be recommended.

4. Discussion on future work of the *ad hoc* Group on Biotechnology and the allocation of tasks to subgroups

In support of the ToR, the *ad hoc* Group agreed to create three subgroups:

Subgroups	Members
1 : Reproductive Animal Biotechnologies (cloned and transgenic animals, both terrestrial and aquatic)	Lino Baranao Wendelyn Jones Bruce Whitelaw Michel Thibier Harpreet Kochhar
2 : Vaccines	Yiseok Joo Lorne Babiuk Oscar Burrone Hiroshi Yoshikura Sandor Belak Paul-Pierre Pastoret Cyril Gay Anne MacKenzie
3 : Nanotechnology	Anne MacKenzie Michel Thibier Harpreet Kochhar

The *ad hoc* Group agreed to leave the issue of xenotransplantation for the time being because it was not felt to be a priority and there is not a significant need for coverage in the immediate future.

Tasks for Subgroups 1 and 2

The *ad hoc* Group set the followings tasks for Subgroups 1 and 2: to identify the risk analysis parameters (risk assessment, risk management, risk communication), including risk–benefit analysis, for the following areas:

1. Animal health;

2. Environmental safety;

3. Food and feed safety within the context/specificity of the OIE mandate.

Trade implications and horizontal issues (e.g. animal welfare, public perception, ethical issues) were also identified as tasks for these two Subgroups.

Discussions on the need for guidance to address ethical issues within a rationale framework (with scientific methodologies) resulted in the identification of relevant work and publications in this regard[17, 18].

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17 Kaiser M. (2005). Assessing ethics and animal welfare in animal biotechnology for farm production. *Rev. sci. tech. Off. Int. Epiz.*, **24**, 75–85.

18 FAO/WHO (2003). Safety assessment of foods derived from genetically modified animals, including fish. Report of the FAO/WHO Expert Consultation. FAO, Rome, Italy, 17–21 November 2003, p. 22.

The issue of cost/benefit analysis was noted, with the understanding of risk analysis being implemented in a global context, including the risks of not using a technology or a product. It was agreed to include the risk/benefit analysis as part of risk analysis.

The *ad hoc* Group on Biotechnology met in two parallel breakout sessions in the afternoon of the second day, to refine the work and terms of references of the two subgroups on Reproductive Animal Biotechnologies, and on Vaccines.

Subgroup 1: Reproductive Animal Biotechnologies (cloned and transgenic animals, both terrestrial and aquatic)

The *ad hoc* Group discussed the mandate of subgroup 1. The collaborative work of IETS with OIE was emphasised, with reference to the issues of insemination, IVF[19] and embryo transfer, both being well covered, as well as *in vitro* embryo production, for which work is ongoing, and cloned animals and transgenics, for which more work is needed.

As regards food and feed safety, the links with the mandate and the ongoing work of Codex Alimentarius on the safety of foods derived from biotechnology, as well as the OECD work on GM feed safety assessment were referred to.

In relation to the reference to "somatic cell nuclear transfer", it was noted that "stem cell," including cells derived from embryos, may not be covered.

Horizontal issues, such as animal welfare, public perception, should be taken into account.

As regards reproduction technologies (including cloning, transgenesis, and other types of new reproduction technologies) and trade, the work of IETS was discussed. Discussion on the trade dimension identified the need to cover trade in embryos, in animals themselves, as well as offspring (and animal products).

Finally, it was noted that new animal technologies, such as genomics, marker-assisted selection, etc., need also be covered in the mandate of Subgroup 1.

The *ad hoc* Group Recommendations for the Development of Biotechnology Guidelines Focusing on Reproductive Animal Biotechnology were endorsed and can be found at Appendix III.

Subgroup 2: Vaccines

With regard to the future work on live and attenuated vaccines, the *ad hoc* Group agreed on the need to include:

1. Environmental issues; how many/which wild species to be tested as non-target species;

2. Use for conservation medicine (both safety & efficacy);

3. How to apply the three R's rules[20].

As regards safety assessment of vaccines and the need to use appropriate testing, it was recommended that the three R's rules should be taken into account.

The subgroup on vaccines was also tasked with the issues of conservation medicine, including the environmental safety aspect of risk analysis. The working group was also tasked with the identification of guidance for the appropriate implementation of the three R's rules.

The *ad hoc* Group Recommendations for the Development of Biotechnology Guidelines Focusing on Vaccines were endorsed and can be found at Appendix IV.

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19 IVF: *in vitro* fertilisation

20 Three R's rule: replace, reduce, refine. International Association for Biologicals (IABs) (2002). Advancing Science and Elimination of the Use of Laboratory Animals for Development and Control of Vaccines and Hormones, Brown F., Hendricksen C., Sesardic D. & Cussler K., eds. Developments in Biologicals, vol. 111, Karger, Basel, Switzerland.

Subgroup 3: Nanotechnology

With regard to the this subgroup, which was tasked with the preparation of an information-gathering seminar on nanotechnology, it was agreed that the scope would be the potential use of nanotechnologies and nanosciences in relation to animal health and welfare. A seminar could usefully be organised jointly with FAO, IAEA, WHO, Codex Alimentarius and IPPC. It was felt that there is not yet enough information to put forward recommendations, and the purpose of the seminar would thus be a fact-finding activity, with the gathering and assessment of existing data and information that could then lead to proposals or recommendations for future work on nanotechnologies within the following three international standard-setting bodies: OIE, Codex Alimentarius and IPPC. The main task of the seminar would be to identify:

1. Applications of nanotechnologies as they apply to food, animals and plants (i.e. what needs to be addressed);

2. The need for standard development, including risk assessment (i.e. how would it be addressed);

3. An action plan to move forward (i.e. who would address it).

5. Review of draft chapter from the OIE *Terrestrial Manual* on Principles of veterinary vaccine production

The draft chapter was reviewed and amended as appropriate. In addition to specific comments on the text, the following general recommendations were made:

1. Principles of master seed safety and efficacy should be addressed in guideline.
2. OIE should consider the appropriateness and extent of applicability of the definition of "modern biotechnology" as defined in the Cartagena Protocol and adopted by Codex Alimentarius. The *ad hoc* Group on Biotechnology concluded that the definition of "Modern Biotechnology" is not appropriate to fully cover recombinant vaccines. As written, the definition of "modern biotechnology" in the Cartagena Protocol does not fit the definition of a recombinant vaccine: …"in vitro *nucleic acid techniques, including recombinant deoxyribonucleic acid* (*DNA*) *and* direct injection of nucleic acid into cells or organelles*, or…*".
3. Whenever possible, vaccinovigilance information post-marketing should be used to further assess the efficacy profile of the vaccine.
4. Genetic stability of the attenuated vaccine should be taken into account.
5. The section on "Classification of Biotechnology-Derived Vaccines" should be modified by moving DNA-vaccines from Category III to Category I.
6. The Biologics Standards Commission should consider adopting guidelines for risk analysis of live recombinant vaccines[21].
7. Recommend that the three R's rules (replace, reduce, refine) be taken into account.
8. The *ad hoc* Group would need more time to discuss if and how the handling, transport, packaging, and identification (labelling) of LMO veterinary vaccines in the context of transboundary movement as noted within in the Cartegena Protocol (Article 18) may be addressed by OIE guidelines or other existing agreements or bilateral or multilateral agreements could be supported by OIE.

6. Animal Genomics for Animal Health Symposium

The Group discussed the purpose and objectives of the Animal Genomics Symposium planned for October 2007. Knowledge arising from human health initiatives and model animal genome studies are providing new opportunities in the filed of animal health and disease control, as well as in marker-assisted selection. The symposium has as major purpose: to bring together the two communities of (i) disease experts with

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21 Gay C.G. (1994). A Risk Analysis Model for Experimental Veterinary Vaccines. *Biotechnology*, **11**, 826–827; Gay C.G. (1997). Risk Analysis for Veterinary Biologics. *In:* Veterinary Vaccinology, Pastoret P.-P., Blancou J., Vannier P. & Verschueren C., eds. Elseviers Science Publishers B.V. Amsterdam, The Netherlands, 718–725; Roth H.J., & Gay C.G. (1997). Specific Safety Requirements for Products Derived from Biotechnology. *In:* Veterinary Vaccinology, Pastoret P.-P., Blancou J., Vannier P. & Verschueren C., eds. Elseviers Science Publishers B.V. Amsterdam, The Netherlands, 226–239.

(ii) genome experts to look at the availability of new genomics tools to study animal diseases. The symposium will also offer an opportunity to identify and strengthen opportunities to fund research in this new area and foster collaborations at the international level. One major development will be the ability to incorporate pathogen genomics with host responses at the molecular level.

The proposed agenda of the symposium was endorsed by the *ad hoc* Group and can be found at Appendix V.

The *ad hoc* Group made the following additional recommendations:

- The symposium should be science driven.
- Additional members of the steering committee that could make substantial contributions could be identified.
- The scientific committee should be limited to ~15 members.
- FAO, WHO, etc., should be informed through OIE Central Bureau.
- Leaflets for the purpose of advertising the symposium should be prepared.

The *ad hoc* Group will be kept informed of progress in the organisation of the symposium.

7. Report of the OIE Working Group on Animal Production Food Safety: issues for consideration by the *ad hoc* Group

The *ad hoc* Group discussed the relevant recommendations of the Working Group on Animal Production Food Safety:

a) The *ad hoc* Group on Biotechnology had some discussion on the relevance of the definition of the term "biotechnology" and "modern biotechnology" for the purpose of animal health. The members were also informed of the outcome of similar discussions held in the context of the recent FAO consultation on Biosafety within a Biosecurity framework. The matter was referred for further elaboration to the *ad hoc* Group on Biotechnology.

b) The recommendation of the Working Group on Animal Production Food Safety to separate the two issues of "criteria for assessing the health of embryos and production animals" and "develop guidelines for the exclusion of unapproved animals" was also referred for further discussion to subgroup 1 (Reproductive Animal Biotechnologies).

c) The *ad hoc* Group on Biotechnology agrees with the recommendation of the Working Group on Animal Production Food Safety. It noted that it agreed earlier to address horizontal issues such as these as part of the work of the working groups on "Reproductive Animal Biotechnologies" and "vaccines".

d) The *ad hoc* Group on Biotechnology agrees with this recommendation of the Working Group on Animal Production Food Safety, noting in particular the address from the OIE Director General on the OIE mandate as regards food safety, and its limits with regards to the Codex Alimentarius mandate.

e) The *ad hoc* Group on Biotechnology agreed to address the ethical aspects of modern biotechnology. The *ad hoc* Group recommends that the OIE consider ethical aspects of biotechnology as they relate to animal health and welfare. However in order to achieve this, the *ad hoc* Group on Biotechnology would need additional expertise in this area.

8. Recommendations for the future working programme of the *ad hoc* Group

- At the next meeting, the *ad hoc* Group will commence with defining the scope and definitions of biotechnology in relation to the OIE Mandate. Members of the *ad hoc* Group will submit contributions to the OIE before the next meeting.

- It is proposed that a seminar on nanotechnology be integrated into the working programme of the *ad hoc* Group. The rationale for this proposal will be forwarded to the Biological Standards Commission.

- Guidelines on reproductive animal biotechnology will be prepared for discussion at the next meeting. A first draft, elaborated by two members of the Group will be sent to the members of the *ad hoc* Group at the latest 1 month before the date of the next meeting.

- One of the priorities identified by the *ad hoc* Group is to review current OIE texts on vaccines for use in animals in the context of the recommendations made by the *ad hoc* Group. A draft a proposal will be circulated amongst the members of the *ad hoc* Group at the latest 1 month before the next meeting.

- The next meeting of the *ad hoc* Group is scheduled for 30–31 October 2006.

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…/Appendices

Appendix I - Report of the *ad hoc* Group on Biotechnology

MEETING OF THE OIE *AD HOC* GROUP ON BIOTECHNOLOGY

OIE Headquarters, Paris, France, 3–5 April 2006

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Agenda

1. Discussion on the draft Terms of Reference
2. Discussion on Resolution No. XXVIII with a view to identifying priority areas and developing a work programme
3. Mission report from Dr MacKenzie on the FAO Expert Consultation on Biosafety within a Biosecurity Framework: issues arising from this consultation that could be addressed by this *ad hoc* Group
4. Discussion on future work of the *ad hoc* Group on Biotechnology and the allocation of tasks to subgroups
5. Review of draft chapter from the OIE *Terrestrial Manual* on Principles of veterinary vaccine production
6. Animal Genomics for Animal Health Symposium
7. Report of the OIE Working Group on Food Safety: issues for consideration by the *ad hoc* Group
8. Recommendations for the future working programme of the *ad hoc* Group

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MEETING OF THE OIE *AD HOC* GROUP ON BIOTECHNOLOGY

OIE Headquarters, Paris, France, 3–5 April 2006

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Ad hoc Group Recommendations for the Development of Biotechnology Guidelines Focusing on Reproductive Animal Biotechnology

1. To address the limitations in scope afforded by the current definition of "modern biotechnology", i.e. including transgenic but not animal clones, the definition of "Reproductive animal biotechnologies" was proposed as "the generation of animals through the use of ART[38] involving non-natural mating procedures".

2. Risk analysis approach could be categorised according to the life cycle approach consisting of:

   a) embryos

   b) recipient

   c) offspring

   d) progeny of the animal clone

   The four entities shall remain the basis for consideration of clones and transgenics (where appropriate). Furthermore, the issues pertaining to each entity should be assessed under different headings of animal health, environment, feed issues, trade issues and horizontal issues.

3. Animals health risks associated with:

   a) Embryos: the choice of donor cells is crucial in cloning procedures. Therefore, guidelines on the selection of donor cells should be developed so that they induce minimum risk when transferred;

   b) Recipient: present knowledge indicates that active health monitoring should be done on the recipient during gestation through to the post-partum period. A Working Group in the IETS[39] has started work in this area in different mammalian species. This work will aid the subgroup to develop guidelines;

   c) Offspring: active health monitoring should be provided for animal clones up to the puberty stage. Extra veterinary care should be provided when required during the post-natal phase;

   d) Progeny: at present, no apparent health problems have been observed and reported in the progeny of cloned animals.

4. Research on the choice of cells, recipients, offspring, progeny etc., as related to animal health and welfare is needed.

5. Further discussions related to other issues, for example, environment, feed, trade and horizontal issues, will be continued in the subsequent meetings where recommendations will be formulated.

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[38] ART: assisted reproductive technologies

[39] IETS: International Embryo Transfer Society

Appendix IV of the report of the *ad hoc* Group on Biotechnology

Ad hoc Group Recommendations for the Development of Biotechnology Guidelines Focusing on Vaccines

The *ad hoc* Group considered the new tools available for enhancing both the efficacy and safety of vaccines as well as how these vaccines could dramatically enhance both animal welfare and address transmission of animal diseases to humans. These technologies have the potential to dramatically reduce the unnecessary need to kill and dispose of healthy animals. These advances can also be beneficial both in the monitoring of disease progression and eradication in a country, as well as having significant risk benefit. Finally, by employing better technologies, it should be possible to reduce the number of animals, e.g. initial vaccine testing, interference studies, and potency testing (three R's – replace, reduce, refinerule[40]). As many regulations for licensing and monitoring veterinary vaccines are already well established and accepted by the international community, extensive rewriting of the regulations is unnecessary. The Group also recognises that biotechnology-derived vaccines have the potential to be safer than conventional vaccines, therefore the current regulations appear to be adequate for both biotechnology-derived and conventional vaccine development, and post-marketing monitoring.

In producing the Guidelines the following issues need to be considered:

1. Guidelines for assessing live vaccines derived by any means should be consistent.
2. Mechanisms whereby cost/benefit analysis could be performed should be identified. This would encourage countries to use vaccines derived from biotechnology, where appropriate.
3. When developing the guidelines for all vaccines, the application of pathogenomics, epidemiology, vaccinomics and immunology should be used. In the long-term, this will be more economical and would compliment the use of the three R's rule (replace, reduce, refine).
4. The development of companion diagnostics that would allow the differentiation of vaccinated animals from possibly infected animals should be considered thereby reducing slaughter and disposal requirements of healthy animals.
5. Veterinary officials worldwide should be encouraged to pursue measures that could rapidly reduce the occurrence and transmission of diseases of national herds through biotechnology-derived vaccines.
6. The importance of wildlife as sources of infection of livestock and *vice versa* should be recognised, both being sources of human diseases therefore emphasising the importance of vaccine development in this critical area.
7. The use of existing guidelines[41] on the importation of vaccines should be encouraged in order to achieve harmonisation.
8. The use of new technologies to enhance vaccine shelf life and the removal of the need for a cold chain in tropical countries should be considered.
9. The benefits of biotechnology-derived vaccines as opposed to conventional vaccines should be considered as they can be safer, more efficacious, provide a more uniform product and allow stability to be monitored.
10. The use of modern viral detection and identification methods, such as sequencing to ensure viral strain identify in all types of viral vaccines, should be encouraged.
11. Encourage VICH[42] in collaboration with OIE and other relevant international organisations, should be encouraged to expedite international harmonisation in the area of veterinary biologicals, especially in the area of biotechnology-derived vaccines.

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[40] Three R's rule: replace, reduce, refine. International Association for Biologicals (IABs) (2002). Advancing Science and Elimination of the Use of Laboratory Animals for Development and Control of Vaccines and Hormones, Brown F., Hendricksen C., Sesardic D. & Cussler K., eds. Developments in Biologicals, vol. 111, Karger, Basel, Switzerland.

[41] Roth H.J., Gay C.G. & Espeseth D.A. (1994). Models used in the U.S.A in risk assessments for biological s or related products. World Organisation for Animal Health (OIE). First International Symposium on Risk Assessment for Veterinary Biologicals: The Next Step in International Harmonization. Washington D.C

[42] VICH: International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products

Animal Genomics for Animal Health Symposium
October 2007, OIE Headquarters, Paris, France

Proposed Agenda

Steering Committee: OIE, ARS-USDA, INRA, BBSRC, IABs, others to be determined

Collaborating Institutions: OIE, FAO, WHO, BBSRC, IABs, ARS-USDA, EADGENE, (to be contacted: IAEA, CBD, CGIAR-ILRI, etc.)

Financial Contributors: to be determined

Symposium Objective: To identify critical needs and opportunities to advance the use of animal genomics to solve problems in animal health.

Theme for day 1 – How will animal genomics revolutionise animal health research?

Day 1 – Morning

8:00 a.m. **Welcome**
Dr Vallat

1st Half
Objective 1 – What has the human genome accomplished?

The first half of the morning will focus on what has been achieved with the human genome. What are the most important genomics tools that have been developed (*e.g. the Hapmap*)*? What are some of the human health applications? What are some of the expected versus unexpected results?*

Key Note Speaker
The Human Genome – Outcomes and Impact in Biomedical Research

Breakthroughs in understanding human diseases: two presentations

Objective 2 – Short review of where we stand with the animal genomes? What are the tools that are available now (tutorials)? How are these tools integrated to advance research programmes?

Invited speakers and selected abstracts chosen by the scientific committee.

- *Full genome sequence and annotation*
- *Hapmaps and other genetic markers*
- *Transcriptomics; e.g. ESTs for arrays*
- *Proteomics*
- *Animal genomics consortia*
- *Species: Chicken, Cattle, Pigs, Sheep, Aquaculture, dog, horses*

Day 1 – Afternoon

Objective 3 – How can we use genomics tools to advance research programmes in animal health (tutorials)?

Invited speakers and selected abstracts chosen by the scientific committee.

- *Developing phenotypes to identify the genetics affecting health traits*
- *Using genetics and genomics to identify genes affecting health traits*
- *Gene expression data analysis/Functional genomics/Host pathogen interaction*
- *Comparative animal genomics to advance biomedical research in animal and human health*
- *Diagnostic, vaccine, and drug discovery applications*
- *Animal genomics to maintain biodiversity*
- *Animal genomics to understand animal behaviour and improve animal welfare*

Theme for day 2 – Reinforce the utility of the animal genomics approach.

Objective 4 – Examples of research projects that are using animal genomics to understand animal diseases, disease susceptibility, and desirable animal health traits?

Invited speakers and selected abstracts chosen by the scientific committee.

Objective 5 – Examples of research projects that are using animal genomics to discover new tools to prevent and control animal diseases?

Invited speakers and selected abstracts chosen by the scientific committee.

Theme for day 3 – What are the priority research areas and what needs to be done to enable the use of animal genomics to enhance animal health?

Objective 6 - What are the critical needs/ future applications in animal health?

- *Understanding the influence of population genetic on disease epidemiology*
- *Understanding variations of the immune system as it relates to disease susceptibility and resistance*
- *Understanding the genetic and biological determinants that influence disease susceptibility and resistance*
- *Use of genomics in vaccine discovery, selection of responders (Vaccinogenetics)*
- *Use of genomics in diagnostics discovery*
- *Drug discovery/pharmacogenetics*
- *Do we need additional tools: e.g. transcriptomes of additional tissues?*
- *Bioinformatics*
- *Marker-assisted selection/Genome selection/Selection for Robustness*
- *Transgenics to solve agricultural problems*

Objective 7 - Conclusions and Recommendations

Appendix V

Original: English
September 2006

REPORT OF THE OIE *AD HOC* GROUP ON REVISION OF THE OIE GUIDELINES FOR INTERNATIONAL REFERENCE STANDARDS FOR ANTIBODY ASSAYS

September 2006

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1. Background

The World Organisation for Animal Health (OIE) has developed guidelines for the preparation of standard reference sera to be used in animal disease serological diagnostic tests. These guidelines are published in a booklet entitled '*OIE Quality Standard and Guidelines for Veterinary Laboratories: Infectious Diseases*'. As blood and blood products can be vehicles for pathogens, the OIE guidelines recommend treatment of serum samples by gamma irradiation to inactivate potential pathogens. There is however, increasing evidence that gamma irradiation alters the performance of the serum in some serological tests such as the complement fixation test. In view of this, the OIE Biological Standards Commission felt that the guidelines should be revised to take into consideration the drawbacks of using gamma irradiation for pathogen inactivation when preparing OIE standard sera. The Director General of the OIE invited four experts to form an *ad hoc* Group to revise the guidelines; the Group communicated by e-mail (see Appendix I for the list of the *ad hoc* Group members). The Group were sent the OIE Guidelines along with a document on the subject prepared by Dr Adama Diallo, FAO/IAEA in Austria (OIE Collaborating Centre) for the Biological Standards Commission at its meeting in January 2006. The present report is a summary of the *ad hoc* Group's electronic communications.

2. Title of the Guidelines

As the Guidelines deal only with serum and not with antigens, the *ad hoc* Group felt that an appropriate title would be "*OIE Guidelines for International Reference Antibody Standards for Antibody Assays"* instead of "*OIE Guidelines for International Reference Standards for Antibody Assays"*.

3. Treatment of sera to inactivate adventitious agents

Three techniques have been mentioned:

a) *Heat inactivation*

Heat inactivation at 56°C for 30 minutes is recommended where the risk of infection is considered low. In general, heat treatment should be carried out at 65°C for 20 minutes or 56°C for 2 hours.

b) *Gamma irradiation*

For wet samples, the current OIE recommended gamma irradiation dose of 25–30 kilogray (kGy) is appropriate but the irradiation should be carried out at –78°C to preserve the performance of the treated serum. This dose is not enough if the sample has been freeze-dried before irradiation: an irradiation dose of more than 40 kGy is required to eliminate foot and mouth disease virus (FMDV) in freeze-dried samples. It has also been noted in some cases that freeze drying the sera after irradiation affects their biological activity (e.g. anti-Newcastle disease virus and avian influenza virus sera).

c) *Treatment with BEI* (*bromoethyleneimine*)

BEI (bromoethyleneimine) is a chemical reagent that is being used successfully to inactivate FMDV, swine vesicular disease virus (SVDV) and vesicular stomatitis virus (VSV). It is currently the most suitable alternative to the gamma irradiation technique. A protocol in use at the Institute for Animal Health in the UK is given at Appendix II.

4. Innocuity test

Sera samples that have undergone treatment to inactivate potential pathogens should be subjected to appropriate tests for innocuity. An example protocol for checking the presence of live FMDV or VSV is provided at Appendix II.

5. Storage

Paragraph 4.2 of the OIE Guidelines for International Reference Standards for Antibody Assays recommends that the final standard should be freeze dried in sealed glass ampoules rather than rubber caps. An alternative option is to store non-freeze dried reagents in cryotubes at –80°C. This option has the following advantages:

a) It avoids errors in reconstituting the standard, e.g. incorrect volume of diluent used; incorrect diluent used; loss of dried material upon opening; reconstitution is incomplete before the standard is used, etc.

b) Cryotubes have secure closures and the container is not easily broken if roughly handled or dropped.

c) Cryotubes use space efficiently in standard storage systems at –80°C or below.

d) Cryogenic labels are available to ensure appropriate labelling.

The main drawback with this option is that it is not ideal for sending out the samples: there is a risk of samples thawing with no guarantee of their quality being preserved.

6. Conclusions

In conclusion, the *ad hoc* Group suggests the following changes to the OIE Guidelines for International Reference Standards for Antibody Assays:

a) Change the title to '*OIE Guidelines for International Reference Antibody Standards for Antibody Assays'*. The OIE may consider developing other guidelines pertaining to reference antigen preparations.

b) Include both gamma irradiation and BEI treatment as recommended methods for inactivating adventitious agents in reference sera.

c) Indicate clearly that innocuity tests should be carried out on samples of the reference sera.

Based on these suggestions, the Guidelines were reviewed and amended as appropriate (see Appendix III for amended version).

OIE *AD HOC* GROUP ON REVISION OF THE OIE GUIDELINES FOR INTERNATIONAL REFERENCE STANDARDS FOR ANTIBODY ASSAYS

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PROTOCOL FOR BEI INACTIVATION OF SERUM

1. Procedure

i) Prepare 0.1 M BEI (bromoethyleneimine) by first weighing 0.205 g of 2-Bromoethylamine hydrobromide (BEA) and transferring the amount to a glass universal in a fume hood that meets ISO standards of safety.

ii) Add 10 ml of 0.2 M sodium hydroxide to dissolve the BEA.

iii) Place the universal in a water-bath or incubator at 35–39°C for 1 hour to produce BEI.

iv) In a safety cabinet, carefully transfer the serum to be inactivated to a screw capped bottle/vessel (e.g. a sterile 1 litre plastic pot with cap) containing a sterile magnetic bar and avoid contaminating the lip of the bottle where the cap, when fitted, may prevent BEI contact with the serum.

v) Add 10 ml of BEI to every 1000 ml of serum (i.e. a final BEI concentration of 0.001 M). Discard tips and pipettes that have come into contact with the BEI by soaking in a 1% solution of sulphuric acid to inactivate the BEI contamination.

vi) Ensure that the cap of the container is tightened and then invert the container several times to mix the serum and BEI solution thoroughly.

vii) Incubate the mixture at 35–39°C for 24 hours in an incubator or hot room (or alternatively a water-bath). Ensure that all the serum is in contact with BEI by spinning the magnetic bar rod in the solution using a magnetic stirrer. Periodically invert the container to mix the serum plus BEI solution during the course of the day. Measure the temperature at an appropriate frequency and enter in a log book.

viii) After 24 hours, neutralise any remaining BEI in the serum solution by adding a 10% concentration of a 20% solution of sodium thiosulphate solution (i.e. to a final concentration of 2%).

NOTE:

The addition of sodium thiosulphate at this stage is optional. The operation is not essential but the step may be required if the inactivation process is part of a contractual agreement for preparing inactivated material on behalf of an outside third party and consequently written into an agreed protocol.

The step may otherwise be omitted as it is considered impossible for any BEI chemical to remain intact after 24 hours at temperatures between 35 and 39°C.

2. Innocuity test procedure

i) Collect the required number of tubes (or flasks, e.g. 25 cm^2) with confluent monolayers of primary calf thyroid cells (in the case of foot and mouth disease virus), IB-RS-2 cells (in the case of swine vesicular disease) or BHK-21 cells (in the case of vesicular stomatitis virus), to allow for testing of a proportion (e.g. 5%) of the 'inactivated' serum for the presence of any remaining live (infectious) virus.

ii) Wash the cell monolayers by tipping out the spent medium into a beaker (using aseptic technique) and adding an appropriate volume of phosphate buffered saline (PBS) (2 ml/tube, 5–10 ml per flask). Discard the PBS into the beaker.

iii) Add 0.2 ml (200 µl) of inactivated serum to be tested to each tube (or 1 ml of inactivated serum per 25 cm^2 flask).

iv) Incubate the cell cultures in a stationary position at 35–39°C (cell monolayer downwards) for 1 hour in a hot room or incubator.

v) After 1 hour's incubation, return the cultures to the safety cabinet and pour off the test serum into disinfectant solution in the fish kettle and wash the tubes with 2 ml of PBS (or 10 ml per 25 cm^2 flask) and discard media. Repeat this PBS wash procedure a further two times before then adding 2 or 15 ml of complete cell culture medium per tube or flask.

vi) Incubate the tubes (rolling) or the flasks (stationary) at 35–39°C for 3 days, observing the cell monolayer with a microscope each day for a cytopathic effect (CPE). Record the results each day on a data sheet.

vii) The presence of CPE may indicate that virus replication may have taken place but this will need to be confirmed by performing a suitable antigen detection test (e.g. antigen ELISA) on an aliquot of clarified cell culture supernatant fluid collected when the CPE is suitably advanced.

viii) If after 3 days there is no indication of CPE, then freeze the tubes in a freezer at either –30 to –5°C or –90 to –50°C.

ix) Thaw the tubes or flasks and transfer the contents to a sterile container and clarify by centrifugation at approximately 2000 ***g*** (e.g. 4,500 rpm on a bench centrifuge) for 10 minutes. Transfer the supernatant to a new container and store at 1–8°C until required.

x) Collect new cultures and repeat **stages 1 to 6** but on this occasion inoculate a larger volume of the stored supernatant from the first passage, i.e. 1 ml per tube or 5 ml per flask, than used for the first passage described in **stage 3.**

xi) Check the cell cultures as before over the following three days and record the results on a data sheet. If no CPE is observed after three days, the tubes should be frozen and the supernatant harvested after thawing in a similar manner to that described in **stages 8 and 9**. The supernatant should then be tested using a suitable detection test. If the results are negative then the serum is considered inactivated.

xii) The inactivated serum can then be stored in a freezer at –30 to –5°C in labelled containers or stored in bulk.

REFERENCE

BAHNEMANN H.G. (1975). Bromine ethyleneimine as an inactivant for foot and mouth disease virus and its application for vaccine production. *Arch. Virol.,* **47**, 47–56.

OIE GUIDELINES FOR INTERNATIONAL REFERENCE ANTIBODY STANDARDS FOR ANTIBODY ASSAYS

1. Introduction

1.1. Purpose

This document provides guidelines for the preparation, validation and distribution of antibodies as International Reference Standards for antibody assays for infectious diseases of animals. In these guidelines, the term "Standards" refers to antibodies unless indicated otherwise. Such standard preparations are designated by the OIE as primary reference standards for use in conjunction with tests described in the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.*

1.2. Definitions

1.2.1. Standard Test Protocol

Standard Test Protocol refers to a validated, internationally accepted test procedure, often an 'OIE Prescribed Test for International Trade', which is described in the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.*

1.2.2. International Reference Standard

The term International Reference Standard is synonymous with primary reference standard. It represents the standard by which all others are compared and calibrated.

1.2.3. Secondary and Working Standards

Secondary standards are prepared by direct comparison with the International Reference Standard, and should so far as is possible mimic the characteristics of the primary standard when used in the Standard Test Protocol. A Secondary Standard would typically be prepared by a National Reference Laboratory and be designated as the national or local standard.

Working standards may be synonymous with secondary standards, or they may be tertiary standards calibrated against the secondary standard. Working standards should be available in sufficient quantities for use by diagnostic laboratories to standardise routine daily testing.

1.3. Scope

International Reference Standards are necessary to ensure that a given antibody assay is capable of measuring antibody activity to a specified level of diagnostic sensitivity. Diagnostic sensitivity relates to the risk of a false negative reaction occurring in an antibody assay when in fact an animal is, or has been, infected. International Reference Standards are normally for use by international, national and other reference laboratories in calibrating standard assays and as templates for the production of secondary standards. The secondary or other working standard, and not the international standard, are to be used on a daily basis to standardise testing.

For a limited number of diseases, there has been international agreement on a system of 'International Units' of antibody activity. In such cases the International Reference Standards define the scale of such units. In the vast majority of animal diseases no such system exists, and assay systems, working standards, and test samples are defined relative to the International Reference Standards.

1.4. Approach

For most assays, three primary reference standards should be established: a strong positive. a weak positive and negative standard. These standards should be selected and characterised by a designated Reference Laboratory using an internationally accepted Standard Test Protocol and internationally accepted reagents.

The weak positive standard is critical for providing assurance of the diagnostic sensitivity of the test. For non-quantitative assays (e.g. immunodiffusion tests) the weak positive reference standard may be the only positive standard required.

For quantitative, non-titration assays such as indirect ELISA, the strong positive standard should define an arbitrary level of 100% positivity. The weak positive and negative standards should then be assigned a proportional percentage positivity corresponding to their reactivity when tested in the standard test protocol.

2. Selection of Materials for use as Standards

2.1. Types of material

The majority of International Reference Standards will be prepared from blood serum. This should be free from haemolysis and from excessive lipaemia. Antisera should where possible be produced in specific pathogen free or gnotobiotic animals of a species appropriate to the assay being standardised. Other materials, for example defatted milk, or monoclonal antibodies, may be used where appropriate to the assay being standardised.

2.2. Safety

The reference standards should be prepared so that they are free of infectious material. To facilitate shipment between countries it is recommended that the standards in the wet state be either treated with BEI (bromoethyleneimine) or irradiated at 25–30 kilogray (2.5–3.0 Mrad) while keeping the samples at -78°C. Irradiating freeze-dried samples is not recommended as the recommended dose may not be enough for complete pathogen inactivation. After treatment, samples should be submitted to appropriate innocuity tests to ensure that they are free from detectable live agents. Bovine sera should be from a BSE-free source.

2.3. Positive reference standards

Positive reference standards should be selected from animals which exhibit a typical humoral (i.e. antibody) immune response to the organism in question. Hyperimmune animals are not considered typical, and should be avoided if possible. The immune response may be elicited by experimental infection or by immunisation with vaccines. The timing after immunisation for collection of the material should be determined by the response of the animal as measured in the standard test protocol. This may vary according to the nature of the disease and the assay. Full details of the immunisation schedule and the nature of the immunogen must be provided so that secondary standards can be prepared by equivalent methods. The standards should be free from antibodies to organisms that might cross-react in the standard assay or information on this cross-reaction should be provided. The standard may be derived from a single animal or a pool of samples from a number of animals. Exceptionally, naturally infected animals may be used as the source of the standard where controlled immunisation or infection is not feasible.

2.4. Negative reference standards

Negative reference standards should be selected from animals that have never been exposed to, or vaccinated against, the organism in question. They should be free from antibodies to organisms which might cross-react in the standard assay. The negative standard may be derived from a single serum or a pool of sera.

3. Characteristics of International Reference Standards

3.1. Strong positive reference standard

For tests such as complement fixation, virus neutralisation or indirect ELISA, that demonstrate typical sigmoidal dose/response curves, the strong positive reference standard should exhibit an antibody activity which lies on the linear portion of the curve just below the plateau phase. In other tests, the strong positive reference standard should contain sufficient antibody to produce consistently the maximum reaction within the selected limits of the test, e.g. a clear cut line of identity in an immunodiffusion test or 100% inhibition in a competitive/inhibition ELISA.

3.2. Weak positive reference standard

The weak positive reference standard should exhibit an antibody activity which again lies on the linear portion of the curve just above the positive/negative threshold. The reaction produced should never be equivocal. In other tests, the weak positive reference standard should contain sufficient antibody to produce consistently the minimum detectable reaction, e.g. a weak but unequivocal line of identity in an immunodiffusion test. For competitive/inhibition assays which frequently show a sharp transition from positive to negative the selection of the weak positive standard can be particularly difficult. The same priniciples apply, in that the standard should give a consistent positive response, just above the positive/negative threshold, in the Standard Test Protocol.

3.3. Negative reference standard

This standard should always give a reaction below the positive–negative threshold in the Standard Test Protocol. The reaction produced should never be equivocal.

4. Preparation of Reference Standards

4.1. Constitution of the standards

Where possible, the positive reference standards should be prepared from materials showing the desired level of reactivity without further dilution. However in many cases it may be necessary for the Reference Laboratory to make a one time dilution of a positive serum in negative serum in order to achieve the desired level of reactivity as specified in (3) above. In such cases the weak positive reference standard may be derived from the same positive serum stock as the strong positive reference standard.

An International Reference Standard should not require any special manipulation (e.g. predilution) by the recipient laboratory prior to its use in the assay in question. The standard should be tested as would any field sample under routine diagnostic conditions (including any dilution steps which are a normal part of the assay procedure). This prevents the introduction of error or bias related to special handling or preparation. Therefore the amount of antibody activity in a positive reference standard should be within the accurate detection limits of the diagnostic test.

4.2. Stability and storage

All materials should be stored frozen or refrigerated pending evaluation. Repeated freeze–thaw cycles should be avoided. To ensure stability it is recommended that the final standard, after sample treatment to inactivate adventitious agents, be freeze-dried, and it would be advantageous to provide the sterile diluent for reconstitution of the material, along with the freeze-dried standard. Sealed glass ampoules, rather than rubber caps, are preferred for long-term storage. Freeze-dried stocks should be stored at 4°C, although short periods at ambient temperature (e.g. during shipment) should not be deleterious. The freeze-drying process may alter the biological quality of sera; storing the standards in cryotubes at –78°C is the recommended alternative solution.

After freeze-drying, several bottles of the standard should be reconstituted and re-evaluated. There should be no evidence of cross-reacting antibodies or other non-specific factors which interfere with the interpretation of assay results. If there is a possibility of cross-reaction with closely related agents, this information should be indicated.

4.3. Batch control

The original reference material must begin as one single stock with enough to last at least 5 years. This can be kept frozen (preferably at –70°C or below) and a batch can be freeze-dried for a minimum 2-year supply (about 500 tests). For each batch, whether frozen or freeze-dried, batch references must be allocated and full quality control data maintained for each batch.

Each freeze-dried batch must be recalibrated. Each bottle or ampoule should contain 0.5–1 ml.

4.4. Labelling

The label should contain the following minimum information: OIE logo; OIE international reference standard for (disease) (test); specify if strong positive, weak positive or negative; the name of the Reference Laboratory; reconstitution method; and storage conditions. The space available on the label may prevent the inclusion of all these items; abbreviations may be used and some of the items may need to be put on the data sheet instead of on the label.

4.5. Data sheets

OIE Reference Laboratories issuing international reference standard sera should ensure that all aliquots are accompanied by an appropriate Data Sheet. It should be made clear to requesting laboratories that international reference standards are intended for use in the calibration of their own assay and for promotion of international harmonisation.

In order for a diagnostic laboratory to prepare a secondary reference standard for its own use, it will be necessary for the OIE Reference Laboratory to supply specific data on the selection and/or preparation of the primary reference standards. This is especially true when primary reference standards have been prepared by dilution of strong or hyperimmune positives in negative sera.

4.5.1. Data required

The datasheet should repeat all the information specified for the label (see 4.4). The following information must also be supplied in order to facilitate the selection and/or preparation of secondary reference standards which, as closely as possible, duplicate the primary reference standard.

i) Description of donor animal for positive and negative serum, including species, age, reproductive status and origin (i.e. natural production, specific pathogen free, gnotobiotic, etc.).

ii) Nature of antibody response, i.e. to natural infection, experimental infection, immunisation, etc.

iii) Details of organism used to elicit the immune response, i.e. source, strain, serotype, etc.

iv) Details of experimental infection or immunisation protocols, i.e. route, dose, immunisation schedules, method and time of sample collection etc.

v) Reference tests used to select positive and negative reference sera candidates and to characterise the antibody response, e.g. ELISA, agar gel immunodiffusion, virus neutralisation, etc.

vi) Sample of titration profiles of hyperimmune sera and criteria for selection of appropriate dilutions of defined activity.

vii) Presence of heterologous antibodies, if known, and tests used in detection.

viii) Details of any safety testing carried out on the materials

ix) A statement that the standard is for *in vitro* use only.

x) Description of sterilisation methods, including type of irradiation and dose and condition of sample at time of sterilisation (i.e. liquid, frozen, freeze-dried, etc.).

xi) Batch number and date of production.

xii) Recommended reconstitution (type of reconstituting fluid, and volume), handling and storage conditions.

xiii) Full contact address, fax, email of the Reference Laboratory as a source of further information.

5. Approval of Reference Standards by OIE

An International Reference Standard may not be issued under the name of OIE unless it has been endorsed by the OIE Standards Commission acting under authority of the OIE International Committee.

The full technical and statistical data on the evaluation of the candidate reference standards, together with the full data sheet information as specified above, should be submitted to OIE. The OIE Standards Commission will review the information. If the Standards Commission approves, the reference standard will be added to the list of International Reference Standards available. This list will be supplied to all OIE Members Countries on request, and may also be accessed on the OIE Web site (http:\\www.oie.int).

6. References

BAHNEMANN H.G. (1975). Bromine ethyleneimine as an inactivant for foot and mouth disease virus and its application for vaccine production. *Arch. Virol.*, **47**, 47–56.

WORLD HEALTH ORGANIZATION (2002). Guidelines on viral inactivation and removal procedures intended to assure the viral safety of human blood plasma products. WHO Expert Committee on Biological Standardization. WHO, Geneva, Switzerland.

WRIGHT P.F. (1998). International standards for test methods and reference sera for diagnostic tests or antibody detection. *In:* Veterinary Laboratories for Infectious Diseases, Pearson J.E., ed. *Rev. sci. tech. Off. int. Epiz.,* **17** (2), 527–533.