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REPORT OF THE MEETING OF THE OIE BIOLOGICAL STANDARDS COMMISSION

Paris, 8–10 February 2011

The OIE Biological Standards Commission met at the OIE Headquarters from 8 to 10 February 2011. Dr Kazuaki Miyagishima, Head of the OIE Scientific and Technical Department, welcomed the Members of the Commission, Prof. Vincenzo Caporale, President, Dr Beverly Schmitt, Vice-President, Dr Mehdi El Harrak, Secretary General, and Dr Alejandro Schudel, Dr Paul Townsend, members of the Commission, and as well as the expert participant: Dr Peter Wright, Canada.

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

1. OIE Reference Laboratories and Collaborating Centres

1.1. Applications for the status of OIE Reference Laboratory or Collaborating Centre

The Commission recommended acceptance of the following two applications for OIE Collaborating Centre status:

OIE Collaborating Centre for Veterinary Drug Regulatory Programmes

Center for Veterinary Medicine (CVM), Food and Drug Administration (FDA), Department of Health and Human Services, 7519 Standish Place, HFV-1, Room 177, Rockville, Maryland 20855, UNITED STATES OF AMERICA

Tel: (+1-240) 276.90.25; E-mail: merton.smith@fda.hhs.gov;

<http://www.fda.gov/AnimalVeterinary/default.htm>

Contact point: Dr Merton V. Smith

OIE Collaborating Centre for Epidemiology and Diagnosis of Emerging, Re-Emerging and Transboundary Diseases of Animals in the Caribbean and Central America

Centro Nacional de Sanidad Agropecuaria (CENSA), Apdo. 10, San José de las Lajas, CP 32700, Provincia Mayabeque, CUBA

Tel: (+53) 47.86.32.06; E-mail: lydia@censa.edu.cu;

<http://www.censa.edu.cu>

Contact point: Dr Lydia M. Tablada Romero

Both of these Centres were endorsed by the Commission using the current Mandate and Internal Rules. In view of the proposed changes to these documents, the Commission recommended that assurances be sought on the sustainability of the proposed Centres. For the Cuban application, the OIE Regional Commission for the Americas would be asked if it would be more efficient to integrate this Centre into the already existing OIE Collaborating Centre for Animal Disease Surveillance Systems, Risk Analysis and Epidemiological Modelling, in Colorado, USA.

An application had been received for a Collaborating Centre for Research and Diagnosis of Emerging and Existing Pathogens of Wildlife in the USA. In view of the proposal to designate one centre per speciality per region (see item 2.1), this application was put on hold for the present. At a future date, the Centre could be integrated into the already existing OIE Collaborating Centre for Wildlife Disease Surveillance and Monitoring, Epidemiology and Management, in Saskatoon, Canada.

All of these suggestions would be submitted to the Council.

The Commission recommended acceptance of the following two applications for OIE Reference Laboratory status:

OIE Reference Laboratories for Equine influenza and Equine Rhinopneumonitis

Institute of Virology, Veterinary Medicine, Free University of Berlin, Philippstrasse 13, 10115 Berlin, GERMANY

Tel: (+49-30) 20.93.65.63; E-mail: borchers@zedat.fu-berlin.de

Designated Reference Expert: Prof. Kerstin Borchers.

OIE Reference Laboratories for Anaplasmosis and Babesiosis

Centro Nacional de Servicios de Constatación en Salud Animal (CENAPA), Carretera Cuernavaca Cuautla #8534, Colonia Progreso, CB 62550, Jiutepec, Morelos, MEXICO

Tel: (+52-777) 3.19.02.02 / 3.20.43.62; E-mail: para.cen@senasica.sagarpa.gob.mx

Designated Reference Expert: Dr Fernando Parrodi López.

A laboratory in Europe had sent an application for an OIE Reference Laboratory for Swine influenza. Although the application revealed a high level of expertise, it did not include any information on the laboratory's international activities. As the principal role of an OIE Reference Laboratory was to provide its services globally, the institute would be asked to provide information on its international activities and how it could fulfil the mandate of an OIE Reference Laboratory.

An application had been received for an OIE Reference Laboratory for Equine babesiosis in the Americas. Although the applicant was considered technically competent, the application lacked evidence of diagnosis and testing activities. The applicant would be asked to provide this information along with specific examples of international training apart from postgraduate PhD studies.

A laboratory in Europe that had completed a twinning project on avian influenza and Newcastle disease, had applied for OIE Reference Laboratory status. The Commission decided to ask for more information. The application, and another one from the Americas for the designation of an OIE Reference Laboratory for Avian mycoplasmosis, generated a discussion on how best to aid laboratories that successfully complete a twinning project, to reach OIE Reference Laboratory status. Such laboratories would usually lack international activities, and this was identified as their main obstacle to accession. A proposal was made to create an intermediary status. Applicant laboratories would be assessed on a case-by-case basis; a laboratory that had completed a twinning programme and that demonstrated that it had all the technical competence to function as an OIE Reference Laboratory, except international activities would be granted "intermediate status" (a list of such laboratories would be included in the reports of the meetings of the Biological Standards Commission). During this probationary period, the laboratory would be encouraged to engage itself in a post-twinning project specifically focused on increasing its international activities. The Commission supported this proposal, which would be submitted to the Council.

An application from Asia, the Far East and Oceania Region for an OIE Reference Laboratory of Chronic wasting disease and one from Europe for Q fever were both put on hold pending receipt of more information on international activities. Again, the applications revealed the laboratories to be competent national laboratories rather than OIE Reference Laboratories. These applications also prompted a discussion on the benefit of designating laboratories for diseases that are not on the OIE list or for which there was no chapter in the *Terrestrial Animal Health Code*. The Commission would seek inputs from the Council and Delegates on this matter to agree on an organisation-wide policy.

A year ago, an application had been received jointly from two laboratories in Europe for a cross-country OIE Reference Laboratory for dourine. The Commission had then recommended that the laboratories reach an agreement between them on how to organise their activities and re-submit the application as a single laboratory, which should have the most experience in the disease. This re-submitted application was assessed; the reference laboratory would be based in one country but would call upon the expertise of the laboratory in the other country. The Commission felt that the laboratory relied too much on the contribution from this latter laboratory as most of the diagnostic expertise was to be found there. On this basis, the application was rejected.

The Commission did not discuss the standing applications from one laboratory in Europe for OIE Reference Laboratories for Bovine anaplasmosis and piroplasmosis (Bovine babesiosis), which were kept pending until the next meeting of the Commission.

1.2 Changes of experts in the List of Reference Laboratories

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

Bovine viral diarrhoea

Dr Oliver Lung to replace Dr Dirk Dereg at the Canadian Food Inspection Agency, Animal Diseases Research Institute, Lethbridge, Alberta, CANADA

Equine influenza and Equine Rhinopneumonitis

Dr Debra Elton to replace Dr Jenny Mumford at the Animal Health Trust, Centre for Preventive Medicine, Suffolk, UNITED KINGDOM.

Leptospirosis

Dr Jessica Petrakovsky to replace Dr Gleyre Dorta De Mazzonelli at DILAB – SENASA, Martínez, Buenos Aires-ARGENTINA.

Avian influenza and Newcastle disease

Ms Janice Pedersen to replace Dr Brundaban Panigraphy at the National Veterinary Services Laboratories, Ames, Iowa, UNITED STATES OF AMERICA.

Rinderpest and Peste des petits ruminants,

Dr Michael Baron to replace Prof. Tom Barrett at the Institute for Animal Health, Pirbright Laboratory, Surrey, UNITED KINGDOM.

Aujeszky's disease

Dr André Jestin to replace Dr Philippe Vannier at the Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail, Laboratoire de Ploufragan, FRANCE.

Rabies

Dr Chrishine Fehlner-Gardiner to replace Dr Alex Wandeler at the Animal Diseases Research Institute, Nepean, Ontario CANADA.

Enzootic bovine leukosis

Dr Bhudipa Choudhury to replace Dr Chris Venables at the VLA Weybridge, New Haw, Addlestone, Surrey, UNITED KINGDOM.

1.3. Annual reports of Reference Laboratory/Collaborating Centre activities for 2010

Reports had been received from 149 out of 158 Reference Laboratories and from 32 out of 35 Collaborating Centres for terrestrial animal diseases or topics. The Commission expressed its on-going appreciation to the enthusiastic support and expert advice given to OIE by the Reference Laboratories and Collaborating Centres. The full set of reports for 2010 would be supplied to Members and to all the Reference Laboratories and Collaborating Centres on a CD-ROM. The international activities relevant to the work of the OIE are summarised in the following tables:

Reference Laboratories

Activities		Percentage of Laboratories carrying out these activities
General activities		
1	Test(s) in use/or available for the specified disease	99%
2	Production and distribution of diagnostic reagents	89%
Specific OIE activities		
3	International harmonisation/standardisation of methods	78%
4	Preparation and supply of international reference standards	66%
5	Research and development of new procedures	89%
6	Collection, analysis and dissemination of epizootiological data	71%
7	Provision of consultant expertise	84%
8	Provision of scientific and technical training	75%
9	Provision of diagnostic testing facilities	57%
10	Organisation of international scientific meetings	28%
11	Participation in international scientific collaborative studies	65%
12	Presentations and publications	88%
13	Inscription of diagnostic kits on the OIE Register	0.01%

Collaborating Centres

Activities		Percentage of Collaborating Centres carrying out these activities
General activities		
1	Activities as a centre of research, expertise, standardisation and dissemination of techniques	96%
2	Proposal or development of any procedure that will facilitate harmonisation of international regulations applicable to the surveillance and control of animal diseases, food safety or animal welfare	73%
3	Placement of expert consultants at the disposal of the OIE	88%
Specific OIE activities		
4	Provision of scientific and technical training within to personnel from OIE Member Countries and Territories	88%
5	Organisation of scientific meetings on behalf of the OIE	24%
6	Coordination of scientific and technical studies in collaboration with other laboratories or organisations	85%
7	Publication and dissemination of any information that may be useful to OIE Member Countries and Territories	85%

The Commission suggested having occasional visits to certain OIE Reference Laboratories to see if they are accurately reporting their activities and services. Such visits might also stimulate activities of laboratories. This proposal would be considered by the Director General. On the issue of how to critically assess annual reports in a systematic manner, a member of the Commission expressed his willingness to spend the day before the next spring meeting of the Commission to read through the annual reports and compile a report of his findings for review by the Commission.

1.4. Review of new and pending applications for laboratory twinning

Dr Keith Hamilton updated the Commission on the status of the twinning programme. The Commission expressed its positive opinion for a twinning project between France and Burkina Faso for African trypanosomosis; it however sought clarification on several logistical details including the state of the laboratory in Burkina Faso, the degree of involvement of the expert from France, and on quality assurance capacity building. The Commission also expressed its positive opinion for twinning between

Germany and India for glanders; it however requested clarification on some technical aspects of the proposal. The Commission requested the secretariat to convey the Commission's comments to the twinning applicant laboratories and ask them to address these points in revised proposals.

All Commission members were invited to attend the twinning feedback workshop from 30 to 31 March 2011.

2. *Ad hoc* Groups

■ Past *ad hoc* Group meetings

2.1. Report of the second Meeting of the *ad hoc* Group on Scientific Partnerships

Prof. Caporale, who had chaired the *ad hoc* Group, presented the report of this meeting. The meeting reviewed the Mandate and Internal Rules for OIE Collaborating Centres and developed proposals for amendment; it also reviewed the proposed amendments to the Mandate and Internal Rules for OIE Reference Laboratories arising from its previous meeting and adjusted them for consistency. Prof. Caporale reiterated his belief that the provision of scientific and technical advice on disease control measures should be kept in the mandate of OIE Reference Laboratories. The Commission agreed that disease control was one of the main responsibilities of Reference Laboratories, and proposed to the Council that the heading "If requested, OIE Reference Laboratories shall:" be deleted from the mandate.

The *ad hoc* Group had proposed that there should be only one Collaborating Centre per region for the same topic. Should other applications be received within the same region, the centres would be asked to work together in a network to ensure that the centres have the same level of competence. The Commission endorsed this approach and the concept of categorising the specialties covered by Collaborating Centres (see [Appendix IV](#) of the report of the meeting of the *ad hoc* Group). Unlike Reference Laboratories, Collaborating Centres must be approved by the corresponding Regional Commission, which should be done if possible in parallel with the Specialist Commission, and then by the Council.

With regard to the suggestion to designate a substitute expert, the Commission proposed adding text to the Article 6bis of the Internal Rules stating that the designated expert can delegate specific responsibilities to other experts, should the need arise.

The Commission adopted the Report of this *ad hoc* Group, which would be reviewed by the OIE Council. The report can be found at [Appendix III](#) of this report. As these texts were an integral part to the Basic Texts of the OIE, their amendments would be formally proposed by the OIE Council for adoption by the World Assembly of Delegates in May 2011.

■ Planned *ad hoc* Groups

2.2. *Ad hoc* Group on the Quality of Foot and Mouth Disease Vaccines

For this agenda item, the Commission was joined by Dr Yong Joo Kim, Scientific and Technical Department. The Commission reviewed the Terms of Reference (ToRs) for this *ad hoc* Group. Prof. Caporale stated that the main purpose of the Group should be to provide guidance for use by Members when procuring Foot and mouth disease (FMD) vaccines. The output would be a standard describing production, control and marketing of FMD vaccines: how to choose a strain, what is meant by safety, efficacy and potency, and what tests to use to check for these characteristics.

On the question of membership of the Group, Prof. Caporale reiterated his personal disagreement with the new OIE policy of not allowing Members of Specialist Commission to preside over *ad hoc* Groups. Regarding participants from the vaccine manufacturing industry, the Commission was reminded that the OIE had an official agreement with IFAH¹, which had been consulted when the list of participants was drawn up.

The *ad hoc* Group would meet at the OIE Headquarters from 29 to 31 March 2011.

¹ IFAH: International Federation for Animal Health

2.3. *Ad hoc* Group on Validation of Tests in Wildlife

The Commission reviewed the proposed ToRs for this Group. Prof. Caporale confirmed his participation and suggested including the validation of diagnostic tests for diseases of camelids either in the ToRs of this *ad hoc* Group or the *ad hoc* Group for Diseases of Camelids.

The *ad hoc* Group would meet at the OIE Headquarters from 27 to 29 April 2011.

■ Proposed *ad hoc* Groups

2.4. *Ad hoc* Group on Modernising the *Terrestrial Manual*

Recognising the need to improve the quality of the information given in the *Terrestrial Manual* and to harmonise the contents across chapters, the Commission explored possible mechanisms to achieve this goal. One suggestion was that the Commission could extend the length of its meetings by a day or two dedicated to reviewing, with extra invited experts, certain of the proposed revised chapters that would be identified beforehand. It was agreed that it was necessary to find a way to increase the number of people who contribute to the *Terrestrial Manual* chapters and to improve the transparency of the updating process. For certain chapters or groups of chapters for related diseases, a ‘virtual’ group could be convened to exchange ideas electronically. At present, chapters are updated by one or a small number of experts (chosen from among OIE Experts for the diseases for which there are experts) and the other OIE Reference Laboratory experts are then requested to review the draft. From now on, an *ad hoc* Group could be responsible for analysing and editing the *Terrestrial Manual*, proposing changes for review and approval by the Commission. The OIE Reference Laboratories, instead of individual experts, should be the main drivers of the updating process and thus they should be asked, at regular intervals, if the chapter on their disease needed an update, and if so, to identify which sections in particular were out of date. All the OIE Experts for a given disease would consult with each other and provide a consensus document to be sent to the *ad hoc* Group for further processing. The Consultant Editor’s input was deemed important and his contribution should continue to be sought.

All options will shortly be presented to the Director General for final decision.

2.5. *Ad hoc* Group on Biosafety/Biocontainment Standards for Veterinary Laboratories

The Commission reiterated the benefit of developing pragmatic OIE guidance on biosafety and on biosecurity of veterinary laboratories, in collaboration with the WHO². Dr Beverly Schmitt could contribute, if the Group meets back-to-back with the next meeting of the Commission. Prof. Caporale stated that membership of the Group should include participants from all regions and not only experts from countries with national biosafety guidelines, with the aim of representing the widest possible range of needs to assure the applicability of any guidance eventually developed.

2.6. *Ad hoc* Group on Diagnostic Tests Related to New and Emerging Technologies

Following the advice received from an OIE expert, the Commission identified an author to update Chapter 1.1.7 “Biotechnology in the Diagnosis of Infectious Diseases and Vaccine Development” of the *Terrestrial Manual*.

² WHO: World Health Organization

3 International Standardisation/Harmonisation

■ Diagnostic tests

3.1. Progress on the on-going standardisation programmes for reagents (for the harmonisation of diagnostic testing)

The Commission noted the technical report, from the OIE Reference Laboratory for Rabies in Nancy, France (OIE Expert: Dr F. Cliquet), on an inter-laboratory trial to determine the titre of the new batch of the anti-rabies positive reference serum of dog origin. It was also informed that a new second set of OIE Standard Sera for rabies would be produced this year replacing the first set. The protocol proposed for producing this second set was reviewed by the Commission and it had no objections to the principles described. However it was suggested that Dr Cliquet consult the other OIE Reference Laboratories for Rabies on this matter.

3.2. OIE Register of diagnostic tests: review of applications

Dr François Diaz updated the Commission on the applications submitted to the OIE Procedure for Validation and Certification of Diagnostic Assays. After thorough review, the Commission agreed that one diagnostic kit did not meet the standards and conditions prescribed and thus would not be proposed for adoption by the World Assembly. An official letter will be sent to inform the applicant of this decision and mentioning the possibility to appeal it.

Another kit, on salmonella typing, was reviewed in the framework of the appeal procedure. Following submission of additional data and a more limited scope for the proposed purposes of the kit, the Commission decided, based on this new information, which had been evaluated by experts, to propose the kit for adoption by the World Assembly in May 2011.

3.3. Letter from the OIE Delegate of Cuba

The Delegate of Cuba had sent a letter about the in-house validation by veterinary laboratories of diagnostic test methods described in the *Terrestrial Manual*. An updated draft of chapter on validation, including all the 'best practices' appendices, had just been received from the *ad hoc* Group on Validation of Diagnostic Tests. A decision on how best to address the concerns of the Delegate was postponed until the September meeting when the Commission would have reviewed the documents.

■ Reference material

3.4. General discussion on how to improve the availability of reference reagents

The Commission noted that the updated Mandate for OIE Reference Laboratory was being proposed for adoption in May 2011 (see Item 2.1) and that this included the obligation that Reference Laboratories should develop reference material in accordance with OIE requirements. The Commission felt that this was a vital part of a Reference Laboratory's responsibility and discussed ways to emphasise that the new mandate was reinforcing this requirement. Once the new Mandate has been adopted by the World Assembly, the OIE Reference Laboratories would be advised of the amendments. At this time, a short questionnaire could be sent to them to gather information on the current state of affairs and identify possible future steps: which laboratories are preparing reference reagents, what they are preparing and in what quantities, whether they prepare the reagents in collaboration with other Reference Laboratories, etc.

4. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals

For this agenda item, the Commission was joined by the Consultant Editor of the *Terrestrial Manual*, Prof. Steven Edwards.

4.1. Review of the Consultant Editor's report on the *Terrestrial Manual*

As decided at the last meeting of the Commission, the Consultant Editor had reviewed the overall structure and contents of chapters in the *Terrestrial Manual* and had submitted a report of his findings. His main recommendations were:

- A more uniform style and content should be adopted through a combination of instructions to authors and standardised subheadings.
- Summaries should only consist of a brief résumé of the key points in the main chapter (all sections). No information should be included in the summary that is not in the main chapter.
- The Introduction should be approximately between 300–600 words. It should describe the causal pathogen, host range, clinical signs, gross pathology and outline epidemiology (including regional distribution) and the nature of any zoonotic risks, all without going into inappropriate depth of detail. Information should be included on the biosafety level at which the pathogen should be held.
- Greater efforts should be made to get authors to use a consistent framework for the Diagnostic techniques section, following defined subheadings.
- At the start of the section, a brief introduction to the tests available should be followed by a table listing which tests are considered fit for each of the purposes defined in the OIE Validation Template. The purposes could be cited in abbreviated form, such as “animal movement”, “diagnosis”, “surveillance”, etc.
- The concept of Prescribed Tests should be sustained. Constant dialogue should be maintained with the Terrestrial Animal Health Standards Commission to ensure that when it introduces new testing requirements, a suitable test is included in the *Terrestrial Manual*.
- Well established tests using older technologies should remain in the *Terrestrial Manual* for use by laboratories lacking infrastructure to mount high-tech methods, but the limitations of such tests should be clearly stated.
- Tests should be described in sufficient detail to enable a laboratory to set them up and validate them for local use.
- Information should be given for each test regarding its performance characteristics and fitness for different purposes.
- Section C “Requirements for Vaccines” should continue to be restructured in line with the recommendations of the OIE *ad hoc* Group on Vaccines in Relation to New and Emerging Technologies.
- Many chapters have far too many references. Authors should limit these to essential further reading and not to treat the chapters as comprehensive literature reviews.

The Commission approved the recommendations of the Consultant Editor.

Prof. Caporale suggested that common text could be used between the *Terrestrial Manual* chapters on one hand, and the OIE Technical disease cards and the newly published Atlas of Transboundary Animal Diseases, on the other. He went on to propose that no new tests be added to the *Terrestrial Manual* unless they had been validated according to the OIE Standards for the declared purpose. He requested that this obligation be incorporated into the guidelines for authors.

4.2. Review of chapters proposed for adoption in May 2011 before they are sent to Members

The chapter on rabies had been updated by consensus of all the OIE Experts in this disease. It had been circulated last year and received a large number of Member comments, in particular on the vaccine section. The Experts had tried to address all the comments, but unfortunately, the document they returned arrived late with no time for the Commission to review it properly. It was agreed therefore, to circulate the chapter with the updated diagnostic tests section, but with the old vaccine section marked “under study”. The Consultant Editor would continue to work with the authors on the vaccine section.

The chapter can be found at Appendix IV of this report for Member comments. It would be proposed for adoption by the World Assembly in May 2011.

The African horse sickness chapter had been heavily amended by the *ad hoc* Group on African Horse Sickness. It was felt that the proposed new tests had not yet been validated to OIE standards. The Commission decided to postpone proposition of this chapter for adoption until more validation data became available.

4.3. Review of author/reviewer list

See item 2.5 above on modernising the *Terrestrial Manual*.

4.4. Review of chapters ready for circulation and selection of more chapters for proposal in May 2012

Updates to the following chapters were reviewed by the Commission. Circulation of the draft updated chapters would begin shortly. In accordance with the new production procedure, chapters would be circulated a second time to give Members the opportunity to see that comments had been taken into account, and the amended final versions would be proposed for adoption (in May 2012):

- 1.1.3. Quality management in veterinary testing laboratories
- 1.1.6. Laboratory methodologies for bacterial antimicrobial susceptibility testing
- 2.1.2. Aujeszky's disease
- 2.1.16. Trichinellosis
- 2.1.17. *Trypanosoma evansi* infection (surra)
- 2.3.7. Duck virus enteritis
- 2.3.14. Newcastle disease
- 2.4.1. Bovine anaplasmosis
- 2.4.11. Enzootic bovine leukosis
- 2.4.12. Haemorrhagic septicaemia
- 2.4.17. Trichomonosis
- 2.5.7. Equine influenza
- 2.8.1. African swine fever

The following chapters were identified for revision with a view to possible adoption in May 2012. Updates would be commissioned in accordance with the updated Guidelines for authors:

- New chapter on Epizootic haemorrhagic disease
- 2.1.1. Anthrax
- 2.1.8. Leishmaniasis
- 2.1.11. Paratuberculosis (Johne's disease)
- 2.1.14. Rift Valley fever
- 2.1.15. Rinderpest
- 2.3.11. Fowl typhoid and Pullorum disease
- 2.4.3. Brucellosis
- 2.4.9. Contagious bovine pleuropneumonia
- 2.5.2. Contagious equine metritis
- 2.7.11. Peste des petits ruminants
- 2.8.3. Classical swine fever

4.5. Recommendations from the GF-TADs³ workshop on "Rift Valley Fever Vaccine - Development, Progress and Constraints", 19–21 January 2011

The GF-TADs workshop had reported that the chapter on Rift Valley fever needed to be updated, in particular the vaccine section. Noting that the author of this chapter had retired, the Commission proposed that the Director General convene an *ad hoc* Group to address this issue.

³ GF-TADs: Global Framework for the Progressive Control of Transboundary Animal Diseases

4.6. Rinderpest – change in *Terrestrial Manual* chapter brought about by global eradication

In the light of an imminent declaration of global rinderpest freedom, a number of changes would need to be made to the *Terrestrial Manual* chapter on this disease. The Commission noted that the authors of the chapter, who had been involved in the work of the Joint FAO/OIE Committee on Global Rinderpest Eradication, were aware of what needed to be amended in the chapter.

4.7. What to do about chapters in the *Terrestrial Manual* and Reference Laboratories for delisted diseases

A divergence of opinion emerged in the discussions of what should be done with *Terrestrial Manual* chapters when the disease concerned is delisted from the OIE list of notifiable diseases. Some members felt that an interest in the disease and its diagnosis and control would remain and thus the chapters should be kept in the *Terrestrial Manual*; others felt that keeping such chapters in the *Terrestrial Manual* gave the diseases more importance than they should have and proposed that those chapters could be published in a separate publication that would not be considered as OIE Standards. The President of the Commission decided to seek the views of the Council and OIE Members on this matter, with a view to developing an organisation-wide policy applicable to both *Terrestrial* and *Aquatic Manuals*.

In a related discussion, some members of the Commission expressed their concern at certain of the diseases that were being proposed for delisting. It was felt that the Commission should be allowed to comment on the report of the *ad hoc* Group on Notification of Animal Diseases and Pathogenic Agents, which evaluated diseases against the listing criteria.

5. Resolutions

5.1. Review of Resolutions to be presented in May 2011

The Commission noted that the following resolutions would be proposed for adoption at the General Session in May 2011:

- A resolution proposing the adoption of one draft chapter for the *Terrestrial Manual* (rabies but not the vaccine section);
- A resolution proposing the addition of one kit to the OIE Register.

6. Conferences, Workshops, Meetings

6.1. OIE/FAO/IAEA Consultants meeting to develop a roadmap for the implementation of OIE principles and methods of diagnostic test validation

The Commission agreed to the principle of developing a training programme on validation based on the OIE draft *Terrestrial Manual* chapter and ‘best practices’ appendices. The draft text of this chapter and its appendices had just been received. The Commission agreed to discuss how best to take this project forward at its September meeting when it would have reviewed the documents; one possibility would be to convene specialist *ad hoc* Groups.

6.2. Update on OFFLU

The OFFLU network had developed considerably since its launch in 2005, with more than sixty world leading animal influenza experts now participating in OFFLU projects. Achievements include a much stronger functional collaboration with the public health sector; improved laboratory capacity in countries on all continents; widely disseminated guidance on surveillance, control, and biosafety; setting an animal influenza research agenda; and improved sharing of information and biological material. The OFFLU technical meeting was held in Rome in November 2010 and 6 monthly management meetings were held throughout the year. OIE and FAO had signed an OFFLU-WHO cooperation agreement which formalised OFFLU contribution to the WHO Vaccine Strain Selection process. OFFLU presented antigenic and genetic data to the OFFLU WHO Vaccine Composition meeting in February 2011 and was acknowledged in the subsequent recommendations for candidate vaccines to protect against H5N1 and H9N2 viruses. The annual report for 2010 can be found at: http://www.offlu.net/OFFLU%20Site/OFFLU_Annual_Report_2010.pdf

6.3. WAVLD⁴ Conference, 6–8 June 2013, Berlin, Germany

The Commission was informed of the dates and location of the next WAVLD meeting. As is customary, one day would be set aside on the programme for an OIE symposium, organised by the Commission. One possible topic for the symposium would be test validation.

7. Liaison with other Commissions

7.1. Scientific Commission for Animal Diseases

Requests for consideration/noting by the Biological Standards Commission:

Issues leftover from last Biological Standards Commission meeting:

Update on Brucellosis and camelids

Dr Medhi El Harrak updated the Commission on the current situation regarding brucellosis in camelids. A study carried out in the United Arab Emirates had found that *Brucella melitensis* was the cause of disease in dromedary camels (and not in New World camelids), though the signs were different from the signs in cattle. No vaccination protocol yet existed. However, as knowledge was limited, the *ad hoc* Group had not yet recommended having a chapter on brucellosis in camels.

Crimean–Congo haemorrhagic fever: need for diagnostic methods

The Commission agreed that there was a need to provide Members with guidance on diagnostic methods and surveillance for Crimean–Congo haemorrhagic fever. The Reference Laboratory in France, and experts in South Africa and the Centers for Disease Control and Prevention in the USA would be asked if they could collaborate to develop a suitable text.

Rabies: threshold unit

Comments from the *ad hoc* Group and the Scientific Commission on the *Terrestrial Manual* rabies chapter would be forwarded to the authors of the *Terrestrial Manual* chapter.

Last minute requests:

Classical swine fever: problem with vaccines

The Commission agreed to the proposal from the *ad hoc* Group on Classical swine fever to delete all mention of DIVA strategies from the *Terrestrial Manual* chapter. The Commission noted that more information was also needed on vaccination strategies.

Future ad hoc Groups of potential interest to the Biological Standards Commission:

The Commission took note of the following upcoming meetings.

- *Ad hoc* Group on Epizootic Haemorrhagic Disease (March 2011)
- *Ad hoc* Group on Peste des petits ruminants (28–30 June 2011)
- *Ad hoc* Group on Brucellosis (July 2011)

7.2. Terrestrial Animal Health Standards Commission

During the General Session in May 2010, a Member had proposed that the Gamma interferon test be designated a prescribed test for bovine tuberculosis. This issue had been examined by the Biological Standards Commission in 2007–2008. At that time, the Commission made the following decision:

“The Commission had requested advice from the OIE Reference Laboratory experts on the suitability of proposing the gamma interferon as a prescribed test for bovine tuberculosis. Based on the answers received, the Commission recommends that the test be designated at this stage as an alternative test for trade. As the test is only available as a commercial kit, the manufacturer would be encouraged to apply to have the kit included on the OIE register, which would give the opportunity for a fuller evaluation of its validation data. Considering the wider use of the gamma interferon test for diagnosis and surveillance, the Commission noted that the test has higher

⁴ WAVLD: World Association of Veterinary Laboratory Diagnosticians

sensitivity but lower specificity than the tuberculin test and as such might be better used as a screening test with the tuberculin test being used for confirmation. At present, the tests are often used in the reverse manner, which seems inappropriate. It is nevertheless important to take account of practical considerations such as general familiarity with the tuberculin test, and the difficulty in some countries of getting blood samples to the laboratory in time to preserve their lymphocyte viability, which is an essential component of the test.”

The current Commission did not believe that there was any new information that would lead it to change its view.

8. Matters of Interest for Information

8.1. Expert Surveillance Panel on Equine Influenza Vaccine Composition – Conclusions and Recommendations

The Commission noted the report of the Expert Panel, which had met at the OIE Headquarters, 24 January 2011. The recommendations concerning which virus strains to include in the vaccine would be published in the OIE *Bulletin*.

8.2. Other activities on biosecurity/biosafety

The Commission took note of the Working Group for Enhanced Biosafety and Biosecurity Training Coordination initiative. The documents would be given to the *ad hoc* Group on Biosafety/Biocontainment Standards for Veterinary Laboratories (item 2.5)

9. Any Other Business

9.1. Workplan and activities (as of 11 February 2011)

See [Appendix V](#).

9.2. Dates of the next Biological Standards Commission meeting

The Commission noted the dates for its next meeting: 12–16 September 2011, pending confirmation.

9.3. Possibility of giving presentations at the Conferences of the Regional Commissions

The Director General of the OIE was in favour of inviting members of the Commission to participate in Conferences of the OIE Regional Commissions if resources are available and if the Presidents of Regional Commissions agree.

9.4. Criteria for including a network website on OIE website

The Commission reviewed and endorsed the proposed criteria for inclusion, on the Website of the OIE, of a link to websites maintained by a network of OIE Reference Laboratories or Collaborating Centres. A letter would now be sent from the OIE Headquarters to the Bluetongue network, which had requested web links, to ask whether the network would undertake to comply with the criteria. If the response was positive, a link to that network would be added to the OIE website.

The Commission agreed to work on future guidance on how a network of OIE Reference Centres should operate, using the criteria presented above as a starting point.

9.5. Other matters

The Commission members agreed that they did not need to have printed working documents but could work on their laptops from electronic documents. From the next meeting, printed working documents would no longer be provided.

.../Appendices

MEETING OF THE OIE BIOLOGICAL STANDARDS COMMISSION

Paris, 8–10 February 2011

Agenda

1. OIE Reference Laboratories and Collaborating Centres

- 1.1. Applications for the status of OIE Reference Laboratory or Collaborating Centre
- 1.2. Changes of experts in the List of Reference Laboratories
- 1.3. Annual reports of Reference Laboratory/Collaborating Centre reports for 2010
- 1.4. Review of new and pending applications for laboratory twinning

2. *Ad hoc* Groups

Past *ad hoc* Group meetings

- 2.1 Report of the Meeting of the *ad hoc* Group on Scientific Partnerships

Planned *ad hoc* Groups

- 2.3. *Ad hoc* Group on the Quality of Foot and Mouth Disease Vaccines
- 2.4. *Ad hoc* Group on Validation of Tests in Wildlife

Proposed *ad hoc* Groups

- 2.5. *Ad hoc* Group on Modernising the *Terrestrial Manual*
- 2.6. *Ad hoc* Group on Biosafety/Biocontainment Standards for Veterinary Laboratories
- 2.7. *Ad hoc* Group on Diagnostic Tests Related to New and Emerging Technologies

3 International Standardisation/Harmonisation:

a) Diagnostic tests

- 3.1. Progress on the on-going standardisation programmes for reagents (for the harmonisation of diagnostic testing)
- 3.2. OIE Register of diagnostic tests: review of applications
- 3.3. Letter from the OIE Delegate of Cuba

b) Reference material

- 3.4. General discussion on how to improve the availability of reference reagents

4. *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*

- 4.1. Review of the Consultant Editor's report on the *Terrestrial Manual*
- 4.2. Review of chapters proposed for adoption in May 2011 before they are sent to Members
- 4.3. Review of author/reviewer list
- 4.4. Review of chapters ready for circulation and selection of more chapters for proposal in May 2012
- 4.5. Recommendations from the GF-TADs workshop on "Rift Valley Fever Vaccine Development, Progress and Constraints", 19–21 January 2011
- 4.6. Rinderpest – change in *Terrestrial Manual* chapter brought about by global eradication
- 4.7. What to do about chapters in the *Terrestrial Manual* and Reference Laboratories for delisted diseases?

5. Resolutions

- 5.1. Resolutions that will be presented in May 2011

6. Conferences, Workshops, Meetings

- 6.1. OIE/FAO/IAEA Consultants meeting to develop a roadmap for the implementation of OIE principles and methods of diagnostic test validation
6.2. Update on OFFLU
6.3. WAVLD Conference, 6–8 June 2013, Berlin, Germany

7. Liaison with other Commissions

- 7.1. Scientific Commission for Animal Diseases

Requests for consideration/noting by the Biological Standards Commission:

Issues leftover from last Biological Standards Commission meeting:

Update on Brucellosis and camelids
Crimean–Congo haemorrhagic fever: need for diagnostic methods
Rabies: threshold unit

Last minute requests:

Classical swine fever: problem with vaccines

Future ad hoc Groups of potential interest to the Biological Standards Commission:

Ad hoc Group on Epizootic Haemorrhagic Disease (March 2011)

Ad hoc Group on Peste des Petits Ruminants (28–30 June 2011)

Ad hoc Group on Brucellosis (July 2011)

- 7.2. Terrestrial Animal Health Standards Commission

Gamma interferon test for bovine tuberculosis

8. Matters of Interest for Information

- 8.1. Expert Surveillance Panel on Equine Influenza Vaccine Composition – Conclusions and Recommendations
8.2. Other activities on biosecurity/biosafety

9. Any Other Business

- 9.1. Workplan and activities (as of 11 February 2011)
9.2. Dates of the next Biological Standards Commission meeting
9.3. Possibility of giving presentations at their Conferences of the Regional Commissions
9.4. Criteria for including a network website on OIE website
9.5. Other matters
-

MEETING OF THE OIE BIOLOGICAL STANDARDS COMMISSION
Paris, 8–10 February 2011

List of participants

MEMBERS

Prof. Vincenzo Caporale
(President)
 Director, Istituto Zooprofilattico
 Sperimentale dell'Abruzzo
 e del Molise 'G. Caporale'
 Via Campo Boario, 64100 Teramo
 ITALY
 Tel.: (39.0861) 33 22 33
 Fax: (39.0861) 33 22 51
 direttore@izs.it

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

Dr Mehdi El Harrak
(Secretary General)
 Chef Département Virologie, BP 4569,
 Avenue Hassan II, km2, Rabat-Akkari
 MOROCCO
 Tel.: (212-37) 69.04.54
 Fax: (212-37) 69.36.32
 elharrak_m@hotmail.com

Dr Paul Townsend
(Member)
 Veterinary Laboratories Agency
 New Haw
 Addlestone
 Surrey KT15 3NB
 UNITED KINGDOM
 Tel.: (44 1932) 341 111
 Fax: (44 1932) 357 838
 p.townsend@vla.defra.gsi.gov.uk

Dr Alejandro Schudel
(Member)
 Urraca 1366
 Carilo, (7167)
 Partido de Pinamar
 Provincia de Buenos Aires
 ARGENTINA
 Tel: (54) 2254 571563
 Fax: (54) 2254 571563
 alejandro.schudel@gmail.com

Dr Hualan Chen
(Member) (Invited but could not come)
 National Avian Influenza Reference
 Laboratory, Animal Influenza
 Laboratory of the Ministry of
 Agriculture, Harbin Veterinary
 Research Institute, CAAS
 427 Maduan Street, Harbin 150001
 CHINA (PEOPLE'S REP. OF)
 Tel.: (+86-451) 8593.5079
 Fax: (+86-451) 8273.3132
 hlchen1@yahoo.com

EXPERT PARTICIPANT

Dr Peter Wright
 Fisheries and Oceans Canada,
 343 University Avenue, Moncton,
 New Brunswick, NB E1C 9B6
 CANADA
 Tel.: (1-506) 851.29.48
 Fax: (1-506) 851.20.79
 WrightPf@DFO-MPO.GC.CA

**CONSULTANT EDITOR OF THE
 TERRESTRIAL MANUAL**

Prof. Steven Edwards
 c/o OIE 12 rue de Prony
 75017 Paris, FRANCE
 Tel.: (33-1) 44.15.18.88
 Fax: (33-1) 42.67.09.87
 steve-oie@cabanas.waitrose.com

OIE HEADQUARTERS

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

Dr Kazuaki Miyagishima
 Deputy Director General
 Head,
 Scientific & Technical Dept
 k.miyagishima@oie.int

Dr Elisabeth Erlacher-Vindel
 Deputy Head,
 Scientific & Technical Dept
 e.erlacher-vindel@oie.int

Ms Sara Linnane
 Scientific Editor,
 Scientific & Technical Dept
 s.linnane@oie.int

Dr François Diaz
 Secretariat for Validation, Certification
 and Registry of Diagnostic Assays,
 Scientific & Technical Dept
 f.diaz@oie.int

Dr Keith Hamilton
 OFFLU Coordinator
 Scientific & Technical Dept
 k.hamilton@oie.int

**REPORT OF THE SECOND MEETING OF THE OIE *AD HOC* GROUP ON
SCIENTIFIC PARTNERSHIPS AMONG
OIE REFERENCE LABORATORIES AND COLLABORATING CENTRES**

Paris, 18–20 January 2011

1. Opening, designation of the Chair and Rapporteur, and adoption of the Agenda

The second meeting of the OIE *ad hoc* Group on Scientific Partnerships among OIE Reference Laboratories and Collaborating Centres was held from 18 to 20 January 2011, at the OIE Headquarters in Paris. The participants were welcomed by Dr Kazuaki Miyagishima, Deputy Director General of the OIE and Head of the OIE Scientific and Technical Department.

Dr Miyagishima recalled the work done so far by the *ad hoc* Group and the interaction that had taken place between the Biological Standards Commission and the OIE Council, including the points of divergence between these bodies. He clarified that the final proposals for amendments to the Mandates and Internal Rules of OIE Reference Laboratories and Collaborating Centres would be made by the Council for adoption by the World Assembly of Delegates, as these texts form an integral part of the Basic Texts of the OIE.

Prof. Vincenzo Caporale, President of the Biological Standards Commission, pointed out that there were currently (to May 2010) 35 Collaborating Centres covering various topics, but that there was insufficient networking among those Centres.

The meeting was chaired by Prof. Caporale; Dr Alf-Eckbert Füssel was appointed Rapporteur.

The agenda for the meeting was adopted with the understanding that the present meeting should concentrate its discussion on Mandates and Rules for OIE Collaborating Centres. The agenda for the meeting, adopted without amendment, and list of members of the *ad hoc* Group and other participants are given in Appendices I and II.

2. Terms of reference for the *ad hoc* Group meeting

The Terms of Reference had already been adopted at the first meeting of the *ad hoc* Group; they are given at Appendix III.

3. Mandates and Rules for OIE Reference Laboratories and Collaborating Centres

Dr Vallat, who briefly joined the meeting on Wednesday 19 January, reminded the Group that the main purpose of this *ad hoc* Group was to act as a ‘think-tank’ to reflect on how to maintain and improve the quality and networking of OIE Collaborating Centres. Given the large number of existing centres, and the growing number of new applications, the OIE needed a new approach and a quality control policy. The Group was asked to review the mandate and internal rules for OIE Collaborating Centres to clarify procedures, roles and commitments, bearing in mind the need to maintain some flexibility.

Dr Vallat confirmed the recommendation of the Council to have no more than one Collaborating Centre approved for the same speciality in any given region. However, this policy should not affect in the immediate the status of currently designated Collaborating Centres. He underlined the need to extend the network of Collaborating Centres and its geographical coverage to developing countries. In the future, the rules should discourage applicants from establishing intercontinental constructs to form one Collaborating Centre because that complicates the follow-up procedures for the OIE Headquarters.

Dr Vallat updated the Group on collaboration of OIE Reference Laboratories and Collaborating Centres with other organisations: at the forthcoming annual FAO/OIE/WHO tripartite meeting, the OIE would request the FAO and WHO to consider choosing their reference centres taking into account OIE Reference Laboratories and Collaborating Centres.

The Group agreed that the mandate of OIE Reference Laboratories could include references to both diagnosis of disease and provision of technical advice on disease control measures.

The Group felt that during the Collaborating Centre approval process, it was necessary to involve the OIE Regions and, where applicable, Sub-Regions. In addition, despite its approval on a regional basis, an OIE Collaborating Centre has a global mandate. The Group discussed and finally agreed that the designation of Collaborating Centres should be predominantly demand-driven, in contrast to offer-driven.

The Group agreed that a Collaborating Centre could comprise a network of institutions and expertise, provided there is a very clear agreement on responsibilities among the institutions and that the latter belong to one Region.

A review of the current list of Collaborating Centres revealed that some Centres had very broad mandates, which was not always helpful for those seeking specific assistance and support. As the Group saw no reason to limit the number of designations a given institution could have to one, very broad topics could and should be split thus allowing more than one Collaborating Centre to be set up at a single institution. This would also facilitate the annual reporting and management of Collaborating Centres.

The Group drew up an inventory of areas of expertise and competence currently covered by the designated OIE Collaborating Centres, and found that there were 13 categories of specialties (see [Appendix IV](#)). This draft inventory was not exhaustive and specialties might change or would be adapted depending on the demands of the OIE and the applications submitted by institutions. While facilitating a gap analysis, this inventory needed refinement in consultation with the Centres concerned.

The Group agreed that each future application should indicate into which of these topical categories, if any, it fits, thus facilitating the harmonisation of titles and the establishment of networks of Collaborating Centres.

To observe the general rule of having just one Centre for a given specialty per region, certain regions may need to be broken down to sub-regions allowing Collaborating Centres to be designated in each sub-region, taking into consideration the volume of worldwide demands for specific areas of expertise.

The Group recommended that if at present there was more than one Collaborating Centre for the same specialty in any one region, existing designations should not be revoked, but the situation should be carefully reviewed should new applications be submitted. The OIE should however be very stringent in regularly reviewing the work of the Collaborating Centres.

Conclusions:

The Group agreed that:

- there were no strong arguments against the designation of more than one Collaborating Centre at a given single institution;
- Multi-national Collaborating Centres should be possible within a Region;
- Multi-site Collaborating Centres should be possible;

- Multipurpose Collaborating Centres should be split into specific narrow-purpose Collaborating Centres (the designation of Collaborating Centres for specific, well defined purposes should be favoured);
- The designation of Collaborating Centres should be demand-driven and OIE should be proactive in identifying categories of specialities in need and in designing the network of Collaborating Centres by categories;
- The OIE should strive to achieve a regional balance in the distribution of Collaborating Centres.

The Group reviewed the current Mandate of the Collaborating Centres, aligned the wording as far as possible to the wording agreed for Reference Laboratories and introduced the notion of:

- Speciality;
- Networking; and
- Regional distribution.

The text of the revised Mandate for Collaborating Centres is found at [Appendix V](#).

The Group reviewed the Internal Rules for Collaborating Centres, aligned the wording as far as possible to the wording agreed for Reference Laboratories and introduced the notion of:

- Sustainability;
- Responsibility of the Delegate;
- Approval of the Head of the Collaborating Centre;
- Multi-site centres;
- The possibility of having more than one centre at the same institution;
- Categorisation of topics;
- The concept of one centre per region/sub-region; and
- Transparent and objective procedures for the approval and revocation of a designation.

The text of the revised Internal Rules for Collaborating Centres is found at [Appendix V](#).

Based on the modifications made to the Mandate and the Internal Rules, and taking into account a discussion paper drafted by the OIE Working Group on Animal Welfare entitled “Animal Welfare Collaborating Centres – Vision, Strategy, and Qualifying Criteria”, the Group adapted the Guidelines for Applicants for OIE Collaborating Centre Status.

The text of the revised “Guidelines for Applicants for OIE Collaborating Centre Status” is found at [Appendix V](#).

All the amendments made to the documents relating to Collaborating Centres necessitated adjusting the text of the Mandate, the Internal Rules and the Guidelines for Reference Laboratories accordingly. In particular the following changes were proposed to the Mandate of Reference Laboratories:

1. to reflect that the OIE may equally request to carry out and not only to coordinate a study:
 “- ~~carry out and/or~~ coordinate ~~coordination of~~ scientific and technical studies in collaboration with other laboratories, centres or organisations;”
2. to incorporate a recommendation made by the Aquatic Animal Health Standard Commission on validation of tests as follows:
 “- to develop, standardise and validate according to OIE Standards new procedures for diagnosis and control of the designated pathogens and ~~or diseases or topics~~;”

The Group wished to express concerns about the current OIE Standards on validation as they were not in all cases fit for purpose and required a review, taking more account of practicality, cost effectiveness, labour intensity, etc. of tests rather than just being concerned with test performance.

The text of the revised Mandate and Internal Rules for Reference Laboratories are found at Appendix VI.

The *ad hoc* Group's proposals for modifications to the Mandate, Internal Rules and Guidelines for applicants for Reference Laboratory or Collaborating Centre status are presented as appendices to this report. Amendments made to the Mandate, Internal Rules and Guidelines for applicants for Reference Laboratory status at the May 2010 meeting of the *ad hoc* Group, which were circulated to Members in the report of the September 2010 meeting of the Biological Standards Commission, are shown as double underlined text and deleted text in strike through, as are amendments made at the January 2011 meeting of the *ad hoc* Group to the Mandate, Internal Rules and Guidelines for applicants Collaborating Centre status. Amendments made at this meeting to the revisited Mandate, Internal Rules and Guidelines for applicants for Reference Laboratory status, are shown in double underline/strikethrough but are also highlighted with a coloured background to distinguish them.

The Group agreed that the designated "OIE Expert" did not always represent all available disciplines and expertise at an OIE Reference Laboratory. However the principle of designating a "substitute OIE Expert" was related to overarching principles and policies of the OIE and should be continued in the Biological Standard Commission.

4. Other Agenda Items

The Group did not address other Items as had been agreed when adopting the Agenda.

5. Adoption of report

Due to time limitation, the Group could not adopt its report during the session. It was agreed to finalise the report by correspondence.

.../Appendices

Appendix I

**SECOND MEETING OF THE OIE AD HOC GROUP ON
SCIENTIFIC PARTNERSHIPS AMONG
OIE REFERENCE LABORATORIES AND COLLABORATING CENTRES**

Paris, 18–20 January 2011

Agenda

1. Opening, Designation of Chair and Rapporteur, Adoption of Agenda
 2. Terms of Reference of the ad hoc Group Meeting
 3. Mandates and Rules for OIE Reference Laboratories and Collaborating Centres
 - Review and propose update, if necessary
 4. Any other business
 5. Adoption of report
-

Appendix II

**SECOND MEETING OF THE OIE AD HOC GROUP ON
SCIENTIFIC PARTNERSHIPS AMONG
OIE REFERENCE LABORATORIES AND COLLABORATING CENTRES**

Paris, 18–20 January 2011

List of participants

MEMBERS

Prof. Vincenzo Caporale
(President of the OIE Biological Standards Commission)
Director, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale'
Via Campo Boario, 64100 Teramo
ITALY
Tel: (39.0861) 33 22 33
Fax: (39.0861) 33 22 51
direttore@izs.it

Dr Katsuaki Sugiura
Vice-President
Food and Agricultural Materials Inspection Center
Shin-toshin 2-1, Chuo-ku, Saitama-shi
Saitama-prefecture, 330-9731
JAPAN
Tel: (81) 50 3797 1860
Fax: (81) 48 600 2372
katsuaki_sugiura@nm.famic.go.jp

Dr Francisco Javier Reviriego Gordejo
Head of Sector
European Commission
Health & Consumers Directorate-General
DG SANCO/D1
Rue Froissart 101-3/72
1040 Brussels
BELGIUM
Tel: 32 (0) 2 298 47 99
Fax: 32 (0) 2 295 31 44
Francisco.reviriego-gordejo@ec.europa.eu

Dr Kekgonne Edinton Baipoledi
Deputy Director
Veterinary Services
Ministry of Agriculture
Private Bag 0032
Gaborone
BOTSWANA
Tel: (267) 397 4235
Fax: (267) 390 3744
GSM: (267) 71 410 286
kbaipoledi@gov.bw

Dr Alf-Eckbert Füßel
Deputy Head of Unit
European Commission
Health & Consumers Directorate-General
DG SANCO/D1
Rue Froissart 101-3/67
1040 Brussels
BELGIUM
Tel: 32 (0) 2 295 08 70
Fax: 32 (0) 2 295 31 44
alf-eckbert.fuessel@ec.europa.eu

Dr Craig Carter
2011 AAVLD President
Director & Professor, Epidemiology
University of Kentucky
Veterinary Diagnostic Laboratory
P.O. Box 14125
Lexington, KY 40512-4125
UNITED STATES OF AMERICA
Tel: (1.859) 257 8283
Fax: (1.859) 255 1624
craig.carter@uky.edu

OIE HEADQUARTERS

Dr Bernard Vallat
Director General
12 rue de Prony
75017 Paris
FRANCE
Tel: 33 - (0)1 44 15 18 88
Fax: 33 - (0)1 42 67 09 87
oie@oie.int

Dr Elisabeth Erlacher-Vindel
Deputy Head, Scientific and Technical Department
e.erlacher-vindel@oie.int

Ms Sara Linnane
Scientific Editor
Scientific & Technical Dept
s.linnane@oie.int

Dr Kazuaki Miyagishima
Deputy Director General
Head, Scientific and Technical Department
k.miyagishima@oie.int

Appendix III

TERMS OF REFERENCE OF THE AD HOC GROUP

- To consider and review, if necessary, the mandates, rules and procedures for OIE Reference Laboratories and Collaborating Centres;
 - To assess the need for and approaches to scientific partnerships of laboratories (objectives, expected outcomes, incentives);
 - To provide guidance for the management of such scientific partnerships (leadership, reporting rules and procedures, membership, good practices).
-

Appendix IV

**Draft Inventory of the 13 categories of specialties and Mapping
of the currently designated OIE Collaborating Centres**

(The inventory is not exhaustive; new categories may be added or obsolete ones deleted)

REGION	INSTITUTION	DISEASE CONTROL	EPIDEMIOLOGY, SURVEILLANCE, RISK ASSESSMENT, MODELING	TRAINING, CAPACITY BUILDING	ZOOZOSIS	WILDLIFE	AVIAN DISEASES	AQUATIC	ANIMAL WELFARE	DIAGNOSIS, BIOTECHNOLOGY & LABORATORY	VETERINARY MEDICINE	VACCINES	FOOD SAFETY	FEED SAFETY
Africa	Ondestepoort (S. Africa)	X												
Africa	University of Pretoria			X		X								
Africa	University of Dakar (Senegal)			X	X					X				
Americas	CEBASEV (Argentina)			X										
Americas	CFIA (Canada)				X									
Americas	CCWHC (Canada)					X								
Americas	University Austral-Chile								X					
Americas	Cuba									X	X	X		
Americas	Fort Collins (USA)		X											
Americas	NVSL-CVB-IICAB (Ames)									X		X		
Americas	CDC (USA)				X									
Americas	University of Georgia (USA)						X							
Americas	University Minnesota (USA)			X										
Asia/Oceania	Massey University (NZ+A)								X					
Asia/Oceania	CSIRO (Jeggo) (Australia)	X												
Asia/Oceania	CSIRO (Daniels) (Australia)			X										
Asia/Oceania	University Obihiro (Japan)	X												
Asia/Oceania	FAMIC (Japan)													X
Asia/Oceania	University of Tokyo (Japan)												X	
Asia/Oceania	NIAH+NVAL (Japan)	X									X	X		
Europe	CERVA (Belgium)									X		X		
Europe	University of Copenhagen		X											
Europe	CIRAD/EMVT (France)	X												
Europe	Fougères (France)										X			
Europe	ENSV (France)			X										
Europe	Insel Riems (Germany)				X									
Europe	Teramo (Italy)		X	X					X				X	
Europe	IZS Padova (Italy) (Marangon)						X							
Europe	IZS Padova (Italy) Capua				X									
Europe	Moscow (Russia)	X											X	
Europe	Vladimir (Russia)	X												
Europe	Uppsala (Sweden)									X				
Europe	Weymouth (UK)							X						
International	FAO/IAEA									X				
Americas/Europe	University of PEI + Norway							X						

Appendix V

COLLABORATING CENTRES

MANDATE

The principal mandate of Collaborating Centres of the World Organisation for Animal Health (OIE) ~~shall have as their mandate is:~~

- to ~~function operate~~ as a ~~world~~ centres of research, expertise, standardisation of techniques and dissemination of knowledge on their specialty (which is a clearly defined, focused topic/discipline/knowledge domain) ~~techniques within their sphere of competence;~~
- to propose or develop methods and any procedures that ~~will~~ facilitate harmonisation of international standards and guidelines ~~regulations~~ applicable to the designated specialty ~~surveillance and control of animal diseases;~~
- to establish and maintain a network with other OIE Collaborating Centres designated for the same specialty, and should the need arise, with Collaborating Centres in other disciplines;
- to identify and maintain existing expertise, in particular within its region;
- to place expert consultants at the disposal of the OIE.

OIE Collaborating Centres shall ~~In addition they may:~~

- provide services to the OIE, in particular within the region, in their specialty, in support of the implementation of OIE policies and, where required, seek for collaboration with OIE Reference Laboratories;
- within their specialty ~~sphere of competence~~, provide scientific and technical training to personnel from Members of the OIE.
- organise and participate in scientific meetings and other activities on behalf of the OIE;
- carry out and/or coordinate scientific and technical studies in collaboration with other centres, laboratories or organisations;
- gather, process, analyse, publish and disseminate data and information relevant to their specialty ~~in their sphere of competence which may be useful to Members of the OIE.~~

Appendix V (contd)

COLLABORATING CENTRES

INTERNAL RULES

Article 1

Applications for the title of Collaborating Centre of the OIE shall be submitted to the Director General by the Delegate of the Member to which the establishment belongs or by the corresponding Regional Commission.

Such applications should only be filed after the applicant institution has explored all possibilities of collaborating with an existing OIE Collaborating Centre in the same region, or sub-region, if any, having the same specialty as defined by the OIE.

Article 2

Applications received shall be presented by the Director General, after consultation with the Biological Standards Commission or the Aquatic Animal Health Standards Commission, as appropriate, appropriate Specialised Commission and the corresponding Regional Commission, to the Administrative Council for selection at its annual meetings. Applications shall be selected according to the principle of designating no more than one Collaborating Centre for the same category of specialty, as defined by the OIE, in the same region or sub-region and solely on the basis of scientific and technical competence of the candidate Centre and on the commitment by the applicant to sustain its operation as an 'OIE Collaborating Centre'.

Article 3

Applications endorsed by the Council shall be presented to the World Assembly of Delegates to the OIE for approval.

Article 4

The Director General shall notify the OIE Delegate(s) of the Member(s) in which the approved Centre is situated and the Director(s) of the Institution(s) hosting the approved Centre of its designation as an 'OIE Collaborating Centre'.

~~After approval by the Assembly, a letter of notification shall be sent by the Director General to the Director of the Collaborating Centre.~~

Article 5

This notification shall confer on ~~entitle~~ the Centre ~~the right~~ to use the ~~title name~~ 'OIE Collaborating Centre' and to use the OIE emblem on all documents issued by the Centre in its official capacity.

Article 6

The Head of the Collaborating Centre proposed by the Director(s) of the institution(s) hosting this Centre shall, after the approval by the Biological Standards Commission or the Aquatic Animal Health Standards Commission, as appropriate, be responsible for the implementation of all aspects of the mandate and act as the sole interface with the OIE for its activities as an OIE Collaborating Centre.

~~The rights conferred by Article 5 shall require full compliance by the Collaborating Centre with its mandate, within the limits of facilities available, and provision of a brief annual report of its activities at the end of each calendar year of its mandate. This report will be distributed to all Members.~~

Article ~~7~~ 6

The Centre shall provide a brief annual report of its activities as an OIE Collaborating Centre, according to the template established by the OIE Headquarters, at the end of each calendar year. A copy of this report will be made available to all Members.

Article 8 7

The Centre may revoke the designation at any time.

The designation of the Collaborating Centre shall be valid for four years, at the end of which the Director General may propose to the Assembly that it be renewed. Either party may revoke this designation at any time revoked if the Centre fails to comply with the provisions of the Mandate and the present Rules. In such cases, the Director General of the OIE, after consulting the Biological Standards Commission or the Aquatic Animal Health Standards Commission, as appropriate, proposes the revocation to the World Assembly of Delegates.

The same procedure shall be followed should the designation of the Collaborating Centre be considered obsolete.

Article 9 8

Any major change within a Collaborating Centre or in relation to budgetary and legal provisions applicable to it that may impair its competence (particularly changes in personnel and in material or financial resources) shall be reported immediately to the Director General of the OIE.

GUIDELINES FOR APPLICANTS FOR COLLABORATING CENTRE STATUS

Applications shall be submitted in accordance with Article 1 of the Internal Rules and should include the following information:

- Name of applicant institution(s).
- Postal address.
- Name of Director(s) of the institution(s).
- Name of proposed Head ~~contact point for~~ of the Collaborating Centre.
- Email and telephone contact details.
- Website (if any).
- The speciality for which the applicant wishes to be considered ~~Proposed name of the Collaborating Centre and its sphere of competence.~~
- A description or an organisational chart of the proposed Collaborating Centre and the institution(s) hosting it.
- Relevant legal and budgetary provisions in place that provide assurance on the sustainability and functioning of the laboratory.
- Summary of recent activities within the speciality ~~sphere of competence~~ as an international centre of research, scientific expertise, standardisation of techniques and dissemination of knowledge ~~techniques~~.
- Summary of recent activities on the development of methods and procedures that will facilitate harmonisation of international standards and guidelines applicable to the designated speciality ~~within the sphere of competence on harmonisation of international surveillance and control of animal diseases.~~
- Recent provision of expert consultancy, or scientific and technical training for the OIE or OIE Members other than the one in which the proposed Centre is located.
- Recent international scientific meetings organised by the proposed Collaborating Centre.
- List of ~~currently active scientific and technical studies~~ current activities relevant to the mandate and speciality of the Centre carried out in collaboration with other centres, laboratories or organisations.
- List of recent publications of international significance within the proposed speciality ~~sphere of competence~~.
- Information on professional experience ~~summaries~~ and relevant expertise of the proposed Head of the Collaborating Centre and the of scientists who will work within the proposed Collaborating Centre demonstrating their competence in the speciality.
- Where the proposed Centre involves more than one institution or research group, robust governance arrangements should be documented, to ensure clear lines of communication and accountability. Direct OIE Member involvement in governance arrangements, with appropriate financial support, is recommended.

The application will be processed by OIE in accordance with Articles 2, 3 and 4 of the Internal Rules.

Appendix VI

REFERENCE LABORATORIES

MANDATE

The principal mandate of Reference Laboratories of the World Organisation for Animal Health (OIE) is shall have as their principal mandate:

- to function as world reference a centres of expertise on and standardisation for a designated pathogens and ,or diseases or topics;
- to use, promote and disseminate diagnostic methods validated according to OIE Standards;
- to develop reference material in accordance with OIE requirements, and implement and promote the application of OIE Standards;
- to store and distribute to national laboratories biological reference products and any other reagents used in the diagnosis and control of the designated pathogens and ,or diseases or topics;
- to develop, standardise and validate according to OIE Standards new procedures for diagnosis and control of the designated pathogens and ,or diseases or topics;
- to gather, process, analyse and disseminate epizootiological data relevant to their speciality;
- to place expert consultants at the disposal of the OIE.

Reference Laboratories of the OIE shall:

- respect the intellectual property rights on samples received and not use those results, without consent, for more than determining the principal characteristics of the pathogen necessary for the country of origin to carry out an epidemiological inquiry and to decide about its control strategy;
- establish and maintain a network with other OIE Reference Laboratories designated for the same pathogen and disease and organise regular inter-laboratory proficiency testing to ensure comparability of results;
- in the case of results that are confirmed positive for any OIE listed disease, immediately inform the OIE Delegate of the Member from which the samples originated as well as the OIE Headquarters;

If requested, OIE Reference Laboratories should They may also contribute to:

- provide ~~provision of~~ scientific and technical training for personnel from OIE Members;
- recommend the prescribed and alternative tests or vaccines;
- provide ~~provision of~~ diagnostic testing facilities, and scientific and technical advice on disease control measures to Members:

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

- organise ~~organisation of~~ and participate in scientific meetings on behalf of the OIE;

- ~~carry out and/or coordinate~~ carry out and/or coordinate ~~coordination of~~ scientific and technical studies in collaboration with other laboratories, Centres or organisations;
 - organise inter-laboratory proficiency testing with laboratories other than OIE Reference Laboratories for the same pathogens and diseases to ensure equivalence of results;
 - publish publication and disseminate dissemination ~~of~~ any information in their sphere of competence that may be useful to OIE Members.
-

REFERENCE LABORATORIES

INTERNAL RULES

Article 1

Applications for the title of Reference Laboratory of the OIE shall be submitted to the Director General by the Delegate of the Member to which the laboratory belongs ~~or by the corresponding Regional Commission.~~

Article 2

Applications received shall be presented by the Director General, after consultation with the Biological Standards Commission or the Aquatic Animal Health Standards Commission, as appropriate, to the Council for selection at its annual meetings. Applications shall be selected solely on the basis of scientific and technical competence of the candidate establishment, in particular its commitment to its sustainable operation as an 'OIE Reference Laboratory' and the excellence of the proposed specialist.

Article 3

Applications endorsed by the Council shall be presented to the World Assembly of Delegates of the OIE for approval.

Article 4

The Director General shall notify the OIE Delegate of the Member in which the approved laboratory is situated and the Head of the approved laboratory upon the designation as an 'OIE Reference Laboratory'.

Article 5

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

Article 5

The Head of the laboratory shall be responsible for the overall implementation of the Mandate.

Article 6

The specialist proposed by the laboratory may, after the approval by the Biological Standards Commission or the Aquatic Animal Health Standards Commission, as appropriate, use the title of "OIE Expert".

Article 6b

The OIE Expert is responsible for the implementation of the technical aspects of the mandate and is subject to the OIE Experts exercise their function within the "rules applicable to OIE Experts". OIE Experts can delegate specific responsibilities to other disease experts in the Reference Laboratory.

Article 7

The rights conferred by Article 5 ~~upon a laboratory~~ and by Article 6 ~~an expert~~ require full compliance with the Mandate of an OIE Reference Laboratory. ~~within the limits of facilities available, and~~ The Laboratory shall provide provision of a brief report of activities as an OIE Reference Laboratory, according to the template established by the OIE Headquarters, at the end of each calendar year of their mandate. A copy of this report will be made available distributed to all OIE Members.

Article 7b

The OIE Reference Laboratory shall maintain a system of quality assurance, biosafety and biosecurity relevant for the pathogen and the disease concerned.

Article 8

The Laboratory may revoke the designation at any time.

The designation shall be withdrawn if the Laboratory fails to comply with the provisions of the Mandate and the present rules. In such case valid for four years, at the end of which the Director General of the OIE, after consulting the Biological Standards Commission or the Aquatic Animal Health Standards Commission, as appropriate, may proposes the withdrawal to the World Assembly of Delegates ~~that it be renewed by either party.~~

The same procedure shall be followed should the designation of the Reference Laboratory be considered obsolete.

~~The Laboratory may revoke the designation at any time.~~

Article 9

Any major change within the Laboratory or in relation to budgetary and legal provisions applicable to it that may impair its competence (particularly the retirement of a designated expert) shall be reported immediately to the Director General of the OIE.

**GUIDELINES FOR APPLICANTS FOR DESIGNATION
AS OIE REFERENCE LABORATORY STATUS**

1. Name of expert (an ~~a~~ brief informal curriculum vitae and documented proof of international recognition for his/her expertise, e.g. publications in peer-reviewed journals, awards, membership in high-profile academic boards, should be included).
2. Name and address of laboratory (telephone and fax numbers, e-mail address, Web site).
3. Name of the Head of laboratory (Responsible Official) Director.
- 3a. Relevant legal and budgetary provisions in place that provide assurance on the sustainability and functioning of the laboratory.
4. Experience in diagnostic testing for the disease according to the OIE Standards (approximate number of tests performed annually for each technique).
5. Additional expertise in diagnostic techniques ~~the disease~~ (agent characterisation techniques, molecular techniques, monoclonal antibody techniques, etc.), epidemiology and control of the disease.
6. Experience in standardisation and validation of diagnostic tests.
7. Reagent production capability (provide details of current stock of reagents for the disease).
8. Capability for timely international shipment and receipt of samples in accordance with the requirements for postage and packaging of biological materials described in ~~Chapter 1.1.1. of the OIE Manual of Diagnostic Tests and vaccines for Terrestrial Animals, Sixth Edition, 2008~~ and the OIE Terrestrial Animal Health Code or the OIE Aquatic Animal Health Code.
- 8a. Guarantees to ensure that staff respect the confidential nature of certain subjects, results or communications
9. Current and completed research and methods development projects on the disease, including a list of relevant publications.
10. Training and consultation experience for the disease in the last two years (courses provided, number of people trained, examples of international consultation).
11. Contribution to the preparation or reviewing of reference documents (chapters for the *OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*, *OIE Manual of Diagnostic Tests for Aquatic Animals*, disease cards, etc.).

The application will be processed by OIE in accordance with Articles 2, 3 and 4 of the Internal Rules.

CHAPTER 2.1.13.

RABIES

SUMMARY

Rabies is a major zoonosis for which diagnostic techniques have been standardised inter-nationally. As there ~~is no~~ are neither gross pathognomonic lesions nor specific and constant clinical signs for rabies, accurate diagnosis can only be made in the laboratory. Laboratory techniques are preferably undertaken ~~conducted~~ on central nervous system (CNS) tissue removed from the cranium (specifically, brain stem, Ammon's horn, thalamus, cerebral cortex and medulla oblongata ~~and the cerebellum~~). A composite of CNS samples should be tested and the brain stem is the most important component of the sample.

Identification of the agent: Agent identification is preferably undertaken ~~done~~ using the fluorescent antibody test (FAT). A drop of purified immunoglobulin previously conjugated with fluorescein isothiocyanate (FITC) is added onto an acetone-fixed brain tissue smear, preferably made from several parts of the brain stem central nervous system. FAT provides a reliable diagnosis in 98–100% of cases for all rabies virus strains serotypes if a potent conjugate is used. For a large number of samples, as in an epidemiological survey, the polymerase chain reaction (PCR) ~~or immunoenzyme techniques~~ can provide rapid results in specially equipped laboratories.

Infected neuronal cells have been demonstrated by histological tests and these procedures will reveal aggregates of viral material ('Negri bodies') in the cytoplasm of neurones. However, ~~the sensitivity of~~ histological techniques is are much less sensitive than ~~that of~~ immunological methods, especially if there has been some autolysis of the specimen in the case of autolysed specimens. Consequently, histological techniques can no longer be recommended for primary diagnosis ~~and should only be used for confirmatory purposes~~.

~~As a single negative test on fresh material does not rule out the possibility~~ In cases of infection, inconclusive results from FAT, or in all cases of human exposure, further tests (cell culture or mouse inoculation tests) on the same sample or repeat FAT on other samples are recommended should be carried out simultaneously. A monolayer culture of susceptible cells is inoculated with a pool of several CNS tissues, including the brain stem. FAT undertaken ~~carried out~~ after appropriate incubation will demonstrate the presence or absence of viral antigen. Alternatively, newborn or 3–4-week-old mice may be inoculated intracerebrally with a similar pool of tissues and then kept under observation for 28-days. For any mouse that dies between 5 and 28-days post-inoculation, the cause of death should be confirmed by FAT. Wherever possible, virus isolation in cell culture should replace mouse inoculation tests.

The identification of the agent can be supplemented in specialised laboratories by identifying any variant virus strains through the use of monoclonal antibodies, specific nucleic acid probes, or the polymerase chain reaction followed by DNA sequencing of genomic areas. Such techniques can distinguish between field and vaccine strains, and identify the geographical origin of the field strains. These very sensitive tests should be used by well trained personnel in specialised laboratories.

Serological tests: Virus neutralisation (VN) assays in cell cultures are ~~the~~ prescribed tests for checking vaccination responses prior to international animal movement or trade. Results are expressed in International Units relative to an international standard antiserum. Alternatively, use may be made of a validated tests that is are known to correlate with these, notably an enzyme-linked immunosorbent assays using antibody to the G protein or the whole virus neutralisation test in mice. Results are expressed in International Units or equivalent units relative to an international standard antiserum.

Requirements for vaccines: Rabies vaccines for use in animals contain either live virus attenuated for the target species (such as Flury low egg passage, Flury high egg passage, Street-Alabama-Dufferin or Kelev), or virus inactivated by chemical or physical means, or recombinant vaccines. The virus is cultivated in embryonated egg, or in cell cultures.

Rabies vaccines are usually lyophilised, but inactivated virus vaccines, preferably with an adjuvant, may be stored in liquid form.

Before newly developed vaccines can be licensed, the duration of immunity resulting from their use should be determined in vaccinated animals of the target species. Vaccines should confer protective immunity for at least 1 year.

For live virus vaccines, the minimum virus content that will elicit an adequate a protective immune response must be established.

The potency of inactivated virus vaccines is established and controlled by mouse vaccination followed by intracerebral challenge using tests formulated by the United States Department of Agriculture in the United States of America or the European Pharmacopoeia elsewhere. The final products of both types of vaccine are subjected to tests for innocuity and absence of toxicity.

For live vaccines that are prepared for oral vaccination of wild (or domestic) animals, safety and efficacy in target animals and safety in non-target species must be demonstrated.

A. INTRODUCTION

Rabies is caused by a neurotropic virus of the genus *Lyssavirus* in the family *Rhabdoviridae*, and is transmissible to all mammals. As the viruses it is are transmissible to humans by inoculation or inhalation of infectious virus, all suspected infected material must be handled under the appropriate safety conditions specified by the World Health Organization (WHO, 1996).

Seven Eleven distinct genetic lineages species can be distinguished within the genus, *Lyssavirus* by cross-protection tests and molecular biological analysis (Bourhy *et al.*, 1993) namely classical rabies virus itself (RABV, genotype 1, serotype 1), Lagos bat virus (LBV, genotype 2, serotype 2), Mokola virus (MOKV, genotype 3, serotype 3), Duvenhage virus (DUVV, genotype 4, serotype 4), European bat lyssaviruses type-1 subdivided into two biotypes (EBLV1, genotype 5) and type-2 (EBLV2, genotype 6), and Australian bat lyssavirus (ABLV, genotype 7) and four lyssaviruses (Aravan virus [ARAV], Khujand virus [KHUV], Irkut virus [IRKV], and West Caucasian bat virus [WCBV]), which have been isolated from Eurasian bats, and have recently been ratified as new lyssavirus species (ICTV). In addition, a newly identified lyssavirus (Shimoni bat virus) has been isolated from a bat in Kenya (Kuzmin *et al.* 2010) and is awaiting official classification. Viruses of serotypes 2–4, EBLV and ABLV are known as rabies-related viruses.

The use of monoclonal antibodies (MAbs) directed against viral nucleocapsid or glycoprotein antigens, and the sequencing of defined genomic areas has made possible the definition of numerous subtypes within each serotype. Lyssaviruses RABV is found worldwide, and is responsible for the overwhelming majority of reported animal and human rabies cases. Other lyssaviruses appear to have more restricted geographical and host range, with the majority having been isolated from bats. However, all lyssaviruses tested to date cause clinical disease indistinguishable from classical rabies. Conserved antigenic sites on the nucleocapsid proteins permit recognition of all lyssaviruses with modern commercial preparations of anti-rabies antibody conjugates used for diagnostic tests on brain tissue.

There exist two The Lyssaviruses have been divided into two phylogroups with distinct pathogenicity and immunogenicity (Badrane *et al.*, 2001). For RABV, DUVV, EBLV and ABLV, conserved antigenic sites on the surface glycoproteins allow cross-neutralisation and cross-protective immunity to be elicited by rabies vaccination. A reduced protection with pre-exposure vaccination and with conventional rabies post-exposure prophylaxis was observed against IRKV, ARAV, and KHUV (Hanlon *et al.*, 2005) and all of the above-mentioned lyssavirus species were assigned to phylogroup 1. Little or no cross-protection against infection with the members of phylogroup 2 (MOKV or and LBV) is elicited by rabies vaccination and most anti-rabies virus antisera do not neutralise these lyssaviruses (Badrane *et al.*, 2001). WCBV does not cross-react serologically with any of the two phylogroups. Four new lyssaviruses rabies-related viruses (Aravan, Khujand, Irkut, and West Caucasian bat viruses) have been isolated recently from Eurasian bats, and have been ratified as are described new putative lyssavirus species. There is a reduced protection with pre-exposure vaccination and with conventional rabies post-exposure prophylaxis against all four new bat variants of rabies virus (Hanlon *et al.*, 2005). In addition, newly identified bat lyssaviruses have been isolated from Africa and are awaiting official classification (Centers for Disease Control and Prevention, [CDC], USA, unpublished data).

98 ~~Humans working with suspect material must be vaccinated against lyssaviruses or other pathogens that may be present~~
 99 ~~in diagnostic samples. The laboratory~~ Laboratories working with lyssaviruses or suspect material must comply with
 100 national biocontainment and biosafety regulations ~~to protect staff from contact with pathogens and~~ they should also
 101 comply with the guidelines for Risk Group 3 pathogens in Chapter 1.1.2 Biosafety and biosecurity in the veterinary
 102 microbiological laboratory and animal facilities.

103 The WHO recommends the preventive immunisation of all exposed staff handling infected or suspect material. The
 104 immunisation protocol includes three injections, e.g. at days 0, 7, and 28. The serological evaluation of immunisation is
 105 made 1–3 weeks after the last injection, and checked every 6 months in the case of laboratory workers or every 2 years
 106 for other diagnosticians. Booster vaccination must be given when the titre falls below 0.5 International Units (IU) per ml.
 107 In the absence of serological monitoring, the vaccination regimen should consist of a booster vaccination at 1 year and
 108 thereafter every 1–3 years.

109 As no clinical sign or gross post-mortem lesion can be considered pathognomonic in domestic or wild animals, the
 110 diagnosis of rabies has to rely on laboratory testing. Serological ~~testing evidence of infection~~ is rarely useful for ante-
 111 mortem diagnosis because of late seroconversion and the high mortality rate of host species, but is very useful for
 112 assessing sero-conversion following vaccination and for epidemiological studies ~~although such data may be used in~~
 113 ~~some epidemiological surveys.~~

114 B. DIAGNOSTIC TECHNIQUES

115 1. Identification of the agent

116 Clinical observation may only lead to a suspicion of rabies because signs of the disease are not characteristic and may
 117 vary greatly from one animal to another (43). The only way to undertake ~~perform~~ a reliable diagnosis of rabies is to
 118 identify the virus or some of its specific components using laboratory tests.

119 As rabies virus is rapidly inactivated, refrigerated diagnostic specimens should be sent to the laboratory by the fastest
 120 means available. Shipment conditions must be considered to be part of the 'rabies diagnostic chain' and should follow
 121 international guidelines.

122 Several laboratory techniques may be used, and have been detailed and standardised in the fourth edition of the WHO's
 123 *Laboratory Techniques in Rabies* (WHO, 1996). The methods vary in their efficiency, specificity and reliability. They are
 124 classically applied to brain tissue, but they can also be applied with variable sensitivity and specificity ~~though less~~
 125 ~~effectively~~ to other organs (e.g. salivary glands). In the brain, rabies virus antigen is particularly abundant in the
 126 thalamus, pons and medulla. ~~The hippocampus (Ammon's horn), cerebellum and different parts of the cerebrum have~~
 127 ~~been reported to be negative in 3.9–11.1% of the positive brains. The structure of choice is the thalamus as it was~~
 128 ~~positive in all cases. It is recommended that a pool of brain tissues that includes the brain stem should be collected and~~
 129 ~~tested (Bingham & Van der Merwe, 2002).~~ The most widely used test for rabies diagnosis is the fluorescent antibody
 130 test (FAT), which is recommended by both WHO and OIE, and is sensitive, specific and cheap. The fluorescent
 131 antibody test (FAT) is sensitive, specific and cheap. In cases of inconclusive results from FAT, or in all cases of human
 132 exposure, further tests on the same sample or repeat FAT on other samples are recommended. This is particular
 133 important where sample autolysis is confirmed or suspected. To reach these parts of the brain, it is necessary to remove
 134 the entire organ after having opened the skull in a necropsy room. Under some conditions (e.g. in the field or when
 135 sampling for large epidemiological studies), a simplified method of sampling through the occipital foramen (11), or
 136 through the orbital cavity (32), can be used.

137 Precautions should be taken when handling central nervous system tissues from suspected rabies cases. Gloves
 138 should always be worn and precautions must be taken to prevent aerosols. ~~The use of~~ Cutting tools, scissors and
 139 scalpels, should be used with care to prevent injury and contamination.

140 a) Collection of brain samples

141 Usually the brain is collected following the opening of the skull in a necropsy room, and the appropriate samples
 142 are collected preferably Ammon's horn, thalamus, cerebral cortex and medulla oblongata. Under some conditions
 143 (e.g. in the field or when sampling for large epidemiological studies, this step may be impractical under certain
 144 hazardous if laboratory technicians are not fully trained, or under field conditions. In such cases, there are two
 145 possible methods of collecting some brain samples without opening the skull:

- 146 • Occipital foramen route for brain sampling

147 A 5 mm drinking straw (Barrat & Blancou, 1988) or a 2 ml disposable plastic pipette (17) is introduced into the
 148 occipital foramen in the direction of an eye. Samples can be collected from the rachidian bulb, the base of the
 149 cerebellum, hippocampus, cortex, and medulla oblongata. When using a straw it should be pinched between the
 150 fingers to prevent material escaping when withdrawing. Bovine spongiform encephalopathy

(BSE) should be considered in the differential diagnosis of most cattle that are considered to be 'rabies suspect'. Sampling of Brain specimens for both diseases from cattle can also be done sampled using the 'brain scoop or tool' developed for bovine spongiform encephalopathy (BSE) tissue sampling rather than, yielding a sample suitable for diagnosis of both BSE and rabies straw or pipette. The resulting samples are relatively easily recognised as to the area of brain sampled.

- **Retro-orbital route for brain sampling**

In this technique (Montano Hirose *et al.*, 1991), a trocar is used to make a hole in the posterior wall of the eye socket, and a plastic pipette or straw is then introduced through this hole. The sampled parts of the brain are the same as in the former technique, but they are taken in the opposite direction.

ba) Shipment of samples

During the shipment of Suspect material should be shipped according to for diagnosis (animal heads, brain or other tissue samples), no risk of human contamination should arise: brains must be placed in a leak-proof rigid container (animal heads will be wrapped in absorbent material) as prescribed in the International Air Transport Association (IATA) Dangerous Goods Regulations. These regulations are summarised in Chapter 1.1.1. Collection and shipment of diagnostic specimens.

When it is not possible to send refrigerated samples, other preservation techniques may be used. The choice of the preservative is dependent on closely linked to the tests to be used for diagnosis:

- Formalin inactivates the virus, thus virus isolation tests cannot be used and diagnosis depends on using a modified direct fluorescent antibody test (FAT), polymerase chain reaction (PCR), (less sensitive than FAT these tests on fresh tissue), immunohistochemistry or histology (Warner *et al.*, 1997);
- Infectivity at room temperature may be extended for several days if brain material is kept in a mixture of 50% glycerol in phosphate buffered saline (PBS). Glycerol/PBS slows bacterial action and therefore protects against the chemical and biological effects of putrefaction. It does not protect against titre decline due to thermal conditions and therefore, because rabies is thermo-labile, the virus titre will decline during glycerol/PBS storage. Under normal transport conditions in the tropics, this protection may only be effective for a matter of several days. Therefore, whenever possible samples in glycerol/PBS saline should be kept refrigerated. As the virus is not inactivated by glycerol/PBS, all laboratory tests can be used on these samples.
- An alternative for the transport of samples for molecular techniques is the use of FTA Gene Guard system (Picard-Meyer *et al.*, 2007). The FTA paper preserves rabies virus RNA within the fibre matrix allowing the transport of samples at ambient temperature without specific biohazard precautions for further characterisation of rabies strains.

c) Routine Laboratory tests

i) Immunochemical identification of rabies virus antigen

- *Fluorescent antibody test*

The most widely used test for rabies diagnosis is the FAT, which is recommended by both WHO and OIE. This 'gold-standard' test may be used directly on a smear, and can also be used to confirm the presence of rabies antigen in cell culture or in brain tissue of mice that have been inoculated for diagnosis. The FAT gives reliable results on fresh specimens within a few hours in more than 95–99% of cases. The FAT is sensitive, specific and cheap. The sensitivity of the FAT depends on the specimen (the degree of autolysis and how comprehensively the brain is sampled, see Section B.1) (Barrat & Aubert, 1995), on the type of lyssavirus and on the proficiency of the diagnostic staff. Sensitivity may be lower in samples from vaccinated animals due to localisation of antigen, which is confined to the brainstem. For direct rabies diagnosis, smears prepared from a composite sample of brain tissue, that includes the brain stem, are fixed in 100% high-grade cold acetone for at least 20 minutes, air dried and then stained with a drop of specific conjugate for 30 minutes at 37°C. Anti-rabies fluorescent conjugates may be prepared in the laboratory. Those available commercially are either polyclonal or monoclonal antibodies (MAbs), specific to the entire virus or to the rabies nucleocapsid protein, conjugated to a fluorophore such as fluorescein isothiocyanate (FITC) conjugates specific to the entire virus or specific to the rabies nucleocapsid protein, or they may be prepared from a mix of different MAbs. FAT slides should then be examined for specific fluorescence using a fluorescent microscope and filter appropriate for the wavelength of the fluorescent conjugate used, for instance FITC, the most commonly used, is excited at 490 nm and re-emits at 510 nm. Aggregates of nucleocapsid protein are identified by specific fluorescence of bound conjugate. It is recommended that two independent trained operators read each FAT slide. Fluorescent antibody conjugates may be made locally, but should be fully validated for specificity and sensitivity before use, including its ability to detect lyssaviruses other than rabies. This validation would need to include species of lyssavirus other than rabies before ruling out a lyssavirus infection on the basis of a negative FAT result. In the FAT, the specific aggregates of nucleocapsid protein are identified by their fluorescence. The specificity and sensitivity of these anti-rabies fluorescent conjugates for locally predominant virus variants should be checked before use.

The FAT may be applied to glycerol-preserved specimens after a washing step. If the specimen has been preserved in a formalin solution, the FAT may be used only after the specimen has been treated with a proteolytic enzyme (Warner *et al.*, 1997). However, the FAT on formalin-fixed and digested samples is always less reliable and more cumbersome than when performed on fresh tissue (Barrat, 1992).

In cases of inconclusive results from FAT, or in all cases of human exposure, further tests on the same sample or repeat FAT on other samples are recommended. This is particularly important where sample autolysis is confirmed or suspected.

- *Immunochemical tests*

~~The antibody may be conjugated to an enzyme such as peroxidase instead of fluorescein isothiocyanate (FITC). This conjugate may be used for direct diagnosis. Immunoperoxidase methods can be used as an alternative to FAT with the same sensitivity as FAT (Genovese & Andral, 1978 Lembo *et al.*, 2006), but attention should be paid to the risk of nonspecific false-positive results. This risk is considerably reduced by the thorough training of the technicians. It must also be emphasised that this technique needs one incubation step more than the FAT.~~

Peroxidase conjugate may also be used on fresh brain tissue or sections of formalin-fixed tissue for immunohistochemical tests.

- Enzyme-linked immunosorbent assay (ELISA)

An ELISA that detects rabies antigen is a variation of the immunochemical test. It is useful for large epidemiological surveys (Xu *et al.*, 2007). The specificity and sensitivity of such tests for locally predominant virus variants should be checked before use. In case of human contact these tests should be used in combination with confirmatory tests such as FAT or virus isolation

- Rapid immunodiagnostic test (RIDT)

A rapid immunodiagnostic test (RIDT) was developed recently (Kang *et al.*, 2007). This simple test can be used under field conditions and in developing countries with limited diagnostic resources.

Generally, tests other than the gold standard FAT should only be used after validation in multiple laboratories.

ii) Detection of the replication of rabies virus after inoculation

These tests detect the infectivity of a tissue suspension in cell cultures or in laboratory animals. They should be used if the FAT gives an uncertain result or when the FAT is negative in the case of known human exposure. Wherever possible, virus isolation on cell culture should be considered in preference to the mouse inoculation test (MIT). They should be used if the FAT gives an uncertain result or when the FAT is negative in the case of known human exposure. Cell culture tests are as sensitive as MIT (Rudd & Trimarchi, 1989) but are less expensive, give more rapid results and avoid the use of animals.

- *Cell culture test*

~~Neuroblastoma cells lines, e.g. N2a, CCL-131 in the American Type Culture Collection (ATCC)¹ are highly susceptible to infection with lyssaviruses is used for routine diagnosis of rabies. The cells are grown in Dulbecco's modified Eagle's medium (DMEM) with 5% fetal calf serum (FCS), incubated at 36°C with 5% CO₂. Its sensitivity has been compared with that of Baby hamster kidney (BHK-21) cells (34) are also This cell line is sensitive to most street isolates without any adaptation step, but should be checked for susceptibility to locally predominant virus variants before use (Rudd & Trimarchi, 1987). Presence of rabies virus in the cells is revealed by the FAT. The result of the test is obtained after at least 18 hours (one replication cycle of virus in the cells); generally incubation continues for 48 hours (10) or in some laboratories up to 4 days. Cell culture tests may be undertaken in multi-well plastic plates, multi-chambered glass slides or on glass cover-slips. The use of one 4-day passage in four wells of a 96-well microtitre plate has been shown to have comparable sensitivity to MIT for rabies strains (Rudd & Trimarchi, 1989). However additional passages could be considered to increase sensitivity. Cytotoxicity is a commonly reported factor limiting test robustness. Techniques proposed to reduce cytotoxicity include adding antibiotics, reducing the time before changing media (to as short as 35 minutes) and dilution of samples. Cell culture tests and their variations should be fully validated before use.~~

1 American Type Culture Collection (ATCC), P.O. Box 1549, Manassas, Virginia 20108, United States of America (USA)

Suggested protocol in multiple wells of for a 96-well plate: 100 µl of clarified brain homogenate (20% in phosphate buffered saline, 0.1 M, pH 7.4) is added to 200 µl of a 2×10^5 cells/ml suspension, freshly prepared from a sub-confluent flask in four wells of a 96-well plate. After 24 hours incubation at 5% CO₂ and 35–37°C, the supernatant from each well is removed and 200 µl of fresh medium is added to each well. After a further 72 hours incubation the supernatant is removed by pipette and kept. The cells are fixed with 80% acetone and stained with fluorescent antibody according to manufacturers recommendations. Variations include staining after 24 or 48 hours, reduced incubation time before changing media to reduce cell toxicity, the use of cell permeability agents (e.g. DEAE-dextran), and further passages. Up to three passages may be considered to increase sensitivity. It is recommended that 24- and 72-hour supernatants are kept for further passage.

This test is as sensitive as the mouse inoculation test. Once a cell culture unit exists in the laboratory, this test should replace the mouse inoculation test as it avoids the use of live animals, is less expensive and gives more rapid results.

It is often advisable to carry out more than one type of test on each sample, particularly when there has been human exposure.

Suggested protocol for use in 8-chamber Lab-Tek® slides: 50 µl of clarified brain homogenate (20% in a grinding substrate made of PBS, 0.1 M, pH 7.4 with heat-inactivated new-born calf serum) is added to 400 µl of a 10^5 cells/ml suspension, freshly prepared from a subconfluent flask. After 24 hours incubation at 5% CO₂ and 35.5°C, the supernatant from each chamber is removed and 400 µl of fresh medium is added to each chamber. After a further 24 hours incubation (or more) the supernatant is removed, chamber structure removed, cells layer dried and fixed with pure high grade cold acetone. The fixed cell layer is then stained with fluorescent antibody according to laboratory procedures. Variations include incubation time, use of cell permeability agents and further passages. Removed supernatants should be kept for possible further passage.

• Mouse inoculation test

Three Five to ten mice, 3–4 weeks old (12–14 g), or a litter of 2-day-old newborn mice, are inoculated intracerebrally. The inoculum is the clarified supernatant of a 10–20% (w/v) homogenate of brain material including brainstem (e.g. cortex, Ammon's horn, cerebellum thalamus, medulla oblongata) in an isotonic buffered solution containing antibiotics. To reduce animal pain, Mice should be anaesthetised when inoculated. The young adult mice are observed daily for 28 days, and every dead mouse is examined for rabies using the FAT. For faster results in newborn mice, it is possible to check one baby mouse by FAT on days 5, 7, 9 and 11 post-inoculation. Any deaths occurring during the first 4 days are regarded as nonspecific (due to stress/bacterial infection etc.).

Once a validated and reliable cell culture unit exists in the laboratory, consideration should be given to replacing the mouse inoculation test with cell culture whenever possible as it avoids the use of live animals, is less expensive and gives more rapid results. However, advantages of MIT are that This *in vivo* test should be avoided when possible on animal welfare grounds. It is also expensive, particularly if SPF mice are used, and does not give rapid results (compared with *in vitro* inoculation tests), but when the test is positive, a large amount of virus can be isolated from a single mouse brain for strain identification purposes and Another advantage of this low tech test is that it can be easily and practicably applied in situations where skills and facilities for other tests (e.g. cell culture) are not available. MIT may also detect viruses other than rabies virus.

iii) Molecular techniques

Various molecular diagnostic tests, e.g. detection of viral RNA by reverse transcription PCR (RT-PCR), PCR, ELISA, hybridisation *in situ* and real-time PCR are used as rapid and sensitive additional techniques for rabies diagnosis (Fooks *et al.*, 2009). The principle of lyssavirus-specific PCRs is a reverse transcription of the target RNA (usually parts of the N gene) into complementary DNA followed by the amplification of the cDNA by PCR. Although those molecular tests have the highest level of sensitivity, their use is currently not recommended for routine post-mortem diagnosis of rabies (WHO Expert Committee on Rabies, 2005) due to high levels of false positive or false negative results without standardisation and very stringent quality control. Nevertheless, they are useful for confirmatory diagnosis, as a first step in virus typing (see below).

iii) Histological identification of characteristic cell lesions

Negri bodies correspond to the aggregation of viral proteins, but the classical staining techniques detect only an affinity of these structures for acidophilic stains. These histological methods, especially the Techniques that stain sections of paraffin embedded brain tissues (e.g. Mann's technique) are time consuming, less sensitive and more expensive than FAT. Seller's method on unfixed tissues, can no longer be recommended because they have tissue smears has a very low sensitivity and should be discarded is only suitable for perfectly fresh specimens. These methods are no longer recommended for routine diagnosis. Immunohistochemical tests are the only histological methods test specific to rabies.

An unfixed tissue smear may be stained by the Seller's method, diagnosis is then obtained in under 1 hour. Generally, histological tests, such as Mann's test, are performed on fixed material after a paraffin-embedding step, and the result of the test is obtained within 3 days. These techniques have the advantage that the laboratory equipment needed to perform them is inexpensive and any need to keep specimens cold after fixation is avoided. Whichever staining method is used, the evidence of infection is provided by intracytoplasmic acidophilic bodies. These histological methods, especially the Seller's method, can no longer be recommended because they have very low sensitivity and should be discarded/abandoned.

d) Other identification tests

An enzyme-linked immunosorbent assay (ELISA) that detects rabies antigen is a variation of the immunochemical test. It is useful for large epidemiological surveys (Xu *et al.*, 2007). It should only be used after validation against numerous samples in different laboratories. The specificity and sensitivity of these anti-rabies enzyme conjugates for locally predominant virus variants should be checked before use. This test should be used in combination with confirmatory tests by FAT or virus isolation.

The tests above describe methods to accurately diagnose rabies and to isolate and identify the virus. Typing of the virus can provide useful epidemiological information and should be carried out/undertaken in specialised laboratories (such as OIE or WHO Reference Laboratories). These techniques would include the use of MAbs, nucleic acid probes, or the PCR, followed by DNA sequencing of genomic areas for typing the virus (Bourhy *et al.*, 1993). These characterisations enable, for instance, a distinction to be made between vaccine virus and a field strain of virus, and possibly identify the geographical origin of the latter.

Participation in inter-laboratory proficiency testing is highly encouraged as part of quality assurance schemes; such tests should be organised for Regional Laboratories by the National Reference Laboratories, while the latter in turn should participate in international proficiency tests organised by OIE reference laboratories.

2. Serological tests

Serological tests are rarely used in epidemiological surveys, due to late seroconversion and the low percentage of animals surviving the disease and therefore having post-infection antibodies. The main application of serology for classical rabies is to determine responses to vaccination, either in domestic animals prior to international travel, or in wildlife populations following oral immunisation of rabies reservoirs. In addition, serological surveys have been used to provide information on dynamics of lyssaviruses in bats (Turmel *et al.*, 2009). In accordance with the WHO recommendations (WHO Expert Committee on Biological Standards, 1985), 0.5 IU per ml of rabies antibodies is the minimum measurable antibody titre considered to represent a level of immunity in humans that correlates with the ability to protect against rabies infection. The same measure is used in dogs and cats to confirm a satisfactory response to vaccination. As neutralising antibodies are considered a key component of the adaptive immune response against rabies virus (Hooper *et al.*, 1998) the gold standard tests are virus neutralisation (VN) tests. However, indirect ELISAs have been developed that do not require high-containment facilities and produce rapid results. Care should be taken when correlating results between virus neutralisation tests and ELISAs owing to the inherent differences between them. Multiple publications demonstrate a variable sensitivity and specificity for ELISAs in both humans and animals. Although VN tests are recommended where specific assessment of protection is required, both tests are useful for detecting responses to vaccination if appropriate cut-offs are used. Poor quality sera can cause cytotoxicity in VN tests, which could lead to false-positive results. For such samples, the use of an indirect ELISA has been shown to be as sensitive and specific as the VN test (Servat *et al.*, 2007). For follow-up investigations in oral vaccination campaigns, virus neutralisation (VN) tests in cell culture are preferred. However, if poor quality sera are submitted, the VN tests in cell culture are sensitive to cytotoxicity, which could lead to false-positive results. For such samples, the use of an indirect ELISA with rabies glycoprotein-coated plates has been shown to be as sensitive and specific as the VN test on cells (22).

Serological surveys have also been used to provide information on dynamics of lyssaviruses in bats although standardisation of serological tests for bats is still needed.

a) Virus neutralisation test in cell culture: fluorescent antibody virus neutralisation test (a prescribed test for international trade)

The principle of the fluorescent antibody virus neutralisation (FAVN) test (Cliquet *et al.*, 1998) is the *in vitro* neutralisation of a constant amount of rabies virus ('challenge virus standard' [CVS-11] strain adapted to cell culture) before inoculating cells susceptible to rabies virus: BHK-21 C13 cells (ATCC number: CCL-10).

The serum titre is the dilution at which 100% of the virus is neutralised in 50% of the wells. This titre is expressed in IU/ml by comparing it with the neutralising dilution of the OIE serum of dog origin under the same experimental conditions. The WHO standard for rabies immunoglobulin [human]² No. 2, or an internal control calibrated against the international control may be used. The WHO standard or internal control should only be used as a control in the test and should not be used to calculate the IU/ml titre of the sera.

This microplate method uses 96-well plates, and is an adaptation of the technique of Smith *et al.* (1973), modified by Zalan *et al.* (47) and by Perrin *et al.* (33). Several publications (18, 21) have shown that the FAVN test and the rapid fluorescent focus inhibition test (RFFIT) give equivalent results (Cliquet *et al.*, 1998).

• Essential equipment

Humidified incubator at 35°C/37°C with 5% CO₂; dry incubator at 37°C; biocontainment cabinet; fluorescence microscope suitable for FITC fluorescence equipped with ×10 eye-piece and ×10 objective. The global magnification of the microscope ranges between ×100 and ×125 due to the extra magnification of some epi-fluorescence systems.

• Reagents and biologicals

PBS buffer, pH 7.2, without Ca²⁺ and Mg²⁺, stored at 4°C;

Trypsin ethylene diamine tetra-acetic acid (EDTA);

High-grade acetone 80% (diluted with deionised water), stored at 4°C;

Dulbecco's modified Eagle's medium (DMEM) + 10% heat-inactivated FCS;

FITC anti-rabies conjugate;

Cells: BHK-21 C13 (ATCC CCL-10) maintained in GMEM with 10% FCS and antibiotics;

Virus: CVS-11 (previously ATCC reference VR 959) strain, which is available from the ATCC or the OIE Reference Laboratory for Rabies, Nancy, France (see Table given in Part 3 of this *Terrestrial Manual*). Vials are stored at –80°C;

OIE Standard Serum of dog origin (OIE Reference Laboratory for Rabies, Nancy, France [see Table given in Part 3 of this *Terrestrial Manual*] stored at +4°C and diluted to 0.5 IU/ml with sterile deionised or distilled water according to the titre of the batch). This control serum may be used to calibrate an internal control that is used for regular FAVN testing;

Naive serum: The pool of negative dog sera is stored at –20°C.

• CVS production

i) *Cell growth*: the BHK-21 C13 cells (ATCC CCL-10) used to produce the CVS virus (ATCC VR 959 CVS-11) are trypsinised during the rapid growth phase, i.e. cells are in the exponential phase of their kinetic growth. If the confluence of the layer is complete, a new passage should be made. The cells in the cell suspension should not be aggregated; 2×10^7 cells are used needed for a 75 cm² cell culture flask. Cells are collected within a volume of 20–30 ml in cell culture medium with 10% heat-inactivated FCS.

ii) *Infection of cells*: the multiplicity of infection (number of infective particles per cell) is adjusted to between 0.1 and 0.5. The glass bottle containing the virus/cell suspension is incubated for 60 minutes at 35.5–37°C. The contents of the bottle are gently stirred every 10–15 minutes.

iii) *Virus growth*: the virus/cell suspension is then centrifuged at 800–1000 *g* for 15 minutes and the cell pellet is resuspended in cell culture medium mixed with 10% heat-inactivated FCS. Virus is harvested 2 days later.

iv) *Harvest and storage*: the supernatant is centrifuged at 800–1000 *g* for 15 minutes at 4°C. If several flasks have been used, the different centrifuged supernatants are mixed and then aliquoted and frozen at –80°C. The infective titre of the harvest is established at least 3 days after freezing.

• Titration of virus in TCID₅₀ (50% tissue culture infective dose)

This titration method uses BHK-21 C13 cells (ATCC CCL-10) in microtitre plates.

² National Institute for Biological Standards and Control (NIBSC), Blanche Lane, South Mimms, Potters Bar, Hertfordshire EN6 3QG, United Kingdom (UK).

Different steps in this procedure may be adapted according to the safety requirements and to the working practices of the laboratory, but the following should not be changed:

- inoculation of a 24-hour cell layer,
- tenfold dilutions prepared using 0.9 ml of diluent and 0.1 ml of virus suspension,
- ~~four to~~ six 50 µl replicates per dilution,
- incubation for 72 hours,
- qualitative reading (i.e. the well is positive or negative),
- in every titration session, a vial of a control batch of virus is titrated and this titre is integrated in a control card to validate the titration process,
- calculation according to neoprobbit graphic or Spearman–Kärber methods.

i) *Cell suspension:* the day before titration, a cell suspension containing 10^5 cells/ml is prepared in cell culture medium containing 10% heat-inactivated FCS, and is distributed, 200 µl per well, into 96-well microtitre plates. The plates are then incubated for 24 hours at 35.5°C–37°C with 5% CO₂.

ii) *Dilution of the virus:* the serial dilutions are performed in 5 ml tubes using a cell culture medium without FCS as diluent. Ten-fold dilutions from 10^{-1} to 10^{-12} are prepared (0.9 ml of diluent with 0.1 ml of the previous dilution).

iii) *Infection of the cells:* the medium in the microtitre plates is discarded using an aspiration system. Fifty µl of each virus dilution is distributed per well. Six replicates are used per dilution. The microtitre plate is then incubated for 1 hour at 35.5–37°C with 5% CO₂. Then 200 µl of cell culture medium, containing 5% FCS, is added.

iv) *Incubation:* incubate for 3 days at 35.5–37°C in 5% CO₂.

v) *Staining and calculation of titre:* The cells are stained using the FAT, as detailed below. Reading is qualitative, every well that shows specific fluorescence is considered to be positive. The titre calculation is made using either the neoprobbit graphic method (2) or the Spearman–Kärber formula (WHO, 1996).

vi) The CVS titration must be performed by FAVN test to establish the infective dose in TCID₅₀.

• Test procedure

i) The microplates are used according to the pattern shown in Figure 1. Plate No. 1 is used for the titration of CVS (rows 1 to 4), and for the controls, standard sera and naive dog serum are used. All other plates are used for the sera to be tested.

ii) Medium is added to the wells as follows: plate 1, rows 1 to 4 and cells A9 to A12: add 150 µl per well; in the other plates, rows 6 and 12: add 200 µl per well; all other wells: add 100 µl.

iii) Sera to be tested are heat inactivated for 30 minutes at 56°C. As indicated in Figure 1, 50 µl of each undiluted serum to be tested is added to four adjacent wells.

iv) Dilutions of sera are conducted in the microplates as follows:

OIE serum, the WHO serum, the internal control and the naive dog serum: with a 50–200 µl multichannel pipette, mix the first dilution wells by sucking in and out at least eight times, transfer 50 µl from one row to the next one, until the last one is reached. Discard 50 µl from the last row.

If there is a serum to be tested on the control plate, see below for the dilution step.

A minimum of four three-fold dilutions is required.

Sera being tested (all plates): as above, transfer successively 50 µl from one row to the following one until rows 5 and 11 (dil. $10^{-2.39}$). With a 5–50 µl multichannel pipette, transfer 10 µl from rows 5 and 11 to rows 6 and 12, respectively (from dil. $10^{-2.39}$ to dil. $10^{-4.23}$). Using a multichannel pipette adjusted to 90 µl, mix rows 6 and 12 and discard 180 µl. Then add 70 µl of medium to these rows. This final step does not lend itself to high throughput testing. To attain or exceed the recommended final dilution alternative procedures may be used. These may require modifications to the plate layout.

Fig. 1. Proposed use of microplates for the fluorescent antibody virus neutralisation test. Wells to which undiluted sera must be added are filled with the indicated '50 µl'. Wells to which 50 µl of diluted challenge virus standard must be added are shaded. Dilutions are given in log₁₀.

Plate 1: Controls									
	H	G	F	E	D	C	B	A	
Challenge virus standard titration									1
									2
									3
									4
OIE standard serum (0.5 IU/ml)	50 µl					50 µl			5
	50 µl					50 µl			6
	50 µl					50 µl			7
	50 µl					50 µl			8
Serum or internal positive control or WHO Standard serum	50 µl								9
	50 µl								10
	50 µl								11
	50 µl								12
log (dilution)	0.48	0.95	1.43	1.91	2.39	2.87	CVS virus control	Cells control	

Naive dog serum (negative)

Plate 2: Sera to be tested

		log dilution											
		0.48	0.95	1.43	1.91	2.39	4.23	0.48	0.95	1.43	1.91	2.39	4.23
Serum 1	A	50 µl						50 µl					
	B	50 µl						50 µl					
	C	50 µl						50 µl					
	D	50 µl						50 µl					
Serum 2	E	50 µl						50 µl					
	F	50 µl						50 µl					
	G	50 µl						50 µl					
	H	50 µl						50 µl					

Serum 3

Serum 4

• Addition of challenge virus standard

- Stock CVS is stored in 1 ml microtubes at -80°C . One tube is thawed rapidly under cold running water, and placed in melting ice.
- One dilution from this tube is prepared in order to obtain 100 TCID₅₀ in 50 µl. Of this dilution, 50 µl is added to each serum-filled well (see Figure 1). For virus titration, 50 µl is added to wells H1 to H4 (plate 1). Next, transfer 50 µl from row to row (plate 1, lines 1–4). Discard 50 µl from the last row (plate 1, wells A1 to A4). No virus is added to wells A9 to A12 of plate 1 (controls). The range allowed for the virus dose titre must be between 30 and 300 TCID₅₀/50 µl.
- Incubate the microplates at 35–37°C in a humid incubator with 5% CO₂ for 1 hour.
- Addition of cells:** trypsinise a subconfluent culture of 3-day-old BHK-21 cells. Resuspend the cells to obtain a 4×10^5 cells/ml suspension in DMEM supplemented with 10% heat-inactivated FCS. Add 50 µl of the cell suspension to each well.
- Incubate the microplates for 48 hours at 35–37°C in a humid incubator with 5% CO₂.

• **Fixation and staining**

- i) After the 48-hour incubation period, the medium is discarded, and the microplates are rinsed once in PBS, pH 7.2, and once in 80% acetone. The microplates are then fixed in 80% acetone at room temperature for 30 minutes, and are dried at room temperature for at least 30 minutes.
- ii) Add 50 µl of the FITC anti-rabies conjugate, at the working dilution, to each well, gently rock the microplates and incubate at 35–37°C for 30 minutes. Discard the fluorescent conjugate and rinse the microplates twice with PBS. Excess PBS is removed by briefly inverting the microplates on absorbent paper.

• **Reading and interpreting the results**

- i) The total surface of each well is observed. The reading evaluation is qualitative (plus or minus): no fluorescent cell – a minus score is recorded for the well; fluorescent cells (one cell or more) – a plus score is recorded for the well.
- ii) Cell and virus controls are read first. For titration of CVS, naïve serum, and OIE standard serum, titres are calculated according to the Spearman–Kärber method or the neoprobit graphic method (WHO, 1996).
- iii) Results of titration of CVS (TCID₅₀), naïve serum (D₅₀ [median dose]) and positive standard (D₅₀) are reported on a control card for each of these three controls. The control results of the current test are compared with the accumulated control test results from previous tests using the same batch of control. The test is validated if the values obtained for the three controls in the current test are not statistically different from the mean (± 2 SD) of all the values obtained in the tests conducted previously according to this technique.
- iv) The result of the test corresponds to the non-neutralised virus after incubation with the reference serum or with the serum to be tested. These titres are calculated with the neo-probit graphic method ~~(2)~~ or with the Spearman–Kärber formula (WHO, 1996). The comparison of the measured titre of the tested sera with that of the OIE positive standard serum of a known neutralising titre allows determination of the neutralising titre of the tested sera in IU/ml. The conversion to IU/ml can be made by using either the log D₅₀ value of the day or the mean value of the OIE standard serum.

- Formula to convert the log D₅₀ value in IU/ml titre:

$$\text{Serum titre (IU/ml)} = \frac{[(10^{\text{(serum log D}_{50} \text{ value)})} \times \text{theoretical titre of OIE serum 0.5 IU/ml}]}{(10^{\text{(log D}_{50} \text{ of OIE serum 0.5 IU/ml)})}$$

Example of conversion:

- log D₅₀ of the serum = 2.27
 - theoretical titre of OIE serum 0.5 IU/ml = 0.5 IU/ml
 - log D₅₀ of OIE serum = 1.43
- (for the log D₅₀ of OIE, the value of the day or the mean value can be considered)

$$\text{Serum titre (IU/ml)} = [(10^{2.27} \times 0.5 = 3.46 \text{ IU/ml})]$$

$$(10^{1.43})$$

The following parameters have to be strictly respected:

- Rabies virus: only the CVS-11 strain (~~ATCC number = VR 959~~) should be used.
- Cells culture: only BHK-21 cells (ATCC number – CCL 10) should be used.
- The FAVN test must be performed only in 96 wells microplate.
- Control charts should be used for rabies virus, naïve serum and OIE positive standard serum of dog origin.
- The back titration of the CVS virus, as well as naïve serum and OIE positive standard serum of dog origin, must be present on control plate.
- A minimum of four three-fold dilutions of sera are required. The reading method is 'all or nothing' only.

- 522 • Four replicates of each serum should be diluted.
- 523 • For the conversion of log D₅₀ in IU/ml, the laboratories should use only the log D₅₀ value of the **OIE**
- 524 **positive** standard serum of dog origin.
- 525 **b) The rapid fluorescent focus inhibition test (RFFIT) for determining rabies virus-neutralising antibody (a**
- 526 **prescribed test for international trade)**
- 527 • **Standard procedure (from WHO Laboratory Techniques in Rabies, 1996)**
- 528 • **Preparation of seed virus suspension**
- 529 i) Trypsinise one 3-day-old 150 ml flask culture of mouse neuroblastoma (MNA) cells. ~~These cells prefer an~~
- 530 ~~acidic medium, supplemented with vitamins (31).~~ A similar cell line (CCL-131) may be obtained on request
- 531 from the ATCC (see footnote 1).
- 532 ii) Resuspend 3×10^7 cells in a 50 ml conical centrifuge tube in 2.7 ml of Eagle's minimal essential medium
- 533 supplemented with 10% fetal bovine serum (EMEM-10).
- 534 iii) Using standard rabies safety procedures, add 1×10^7 infectious units of CVS-11 rabies virus (**previously**
- 535 ATCC **reference** VR959) and vortex/mix once. Incubate the cells and virus for 15 minutes at 37°C; vortex/mix
- 536 the cells once during this time.
- 537 iv) Add 10 ml EMEM-10, vortex/mix, and centrifuge the cells at 500 **g** for 10 minutes.
- 538 v) Discard the supernatant. Resuspend the cells in 30 ml of growth medium and transfer to a 150 ml flask.
- 539 vi) Gently rock the flask to mix the cell suspension, and then prepare three eight-well tissue-culture chamber
- 540 slides by pipetting 0.2 ml of the cell suspension into one well of each slide.
- 541 vii) Incubate the flask and slides at 37°C in a humidified incubator with 0.5% carbon dioxide (CO₂). The flask
- 542 should be incubated as a closed culture (tighten the cap).
- 543 viii) At 20, 40 and 64 hours after infection, acetone fix and stain one slide using an immunofluorescence
- 544 technique (Cliquet *et al.*, 1998) to determine the virus infectivity. The supernatant should be harvested
- 545 24 hours after the cells reach 100% infectivity (typically 40 hours after infection).
- 546 ix) Transfer the supernatant to a 50 ml centrifuge tube and centrifuge at 4000 **g** for 10 minutes.
- 547 x) Distribute the supernatant into 0.5 ml aliquots and store at –70°C.
- 548 • **Titration of seed virus suspension**
- 549 i) Thaw one aliquot of the seed virus and prepare serial tenfold dilutions (from 10⁻¹ to 10⁻⁸) in EMEM-10.
- 550 ii) Distribute 0.1 ml of each virus dilution into one well of an eight-well tissue-culture chamber slide. Add 0.2 ml
- 551 of MNA cells suspended in EMEM-10 (concentration 5×10^4 cells per 0.2 ml) to each well.
- 552 iii) Mix the cells and virus by gently rocking the slide, then incubate at 37°C in a humidified incubator with 0.5%
- 553 CO₂ for 40 hours.
- 554 iv) Acetone fix and stain the slide using an immunofluorescence technique. Evidence of virus infection should be
- 555 observed at the 10⁻⁶ dilution of virus, indicating a virus stock suspension containing at least 1×10^6 infectious
- 556 units per 0.1 ml. Prepare sufficient seed virus so that frequent serial passage of the virus is unnecessary.
- 557 • **Preparation of stock virus suspension**
- 558 i) Infect 3×10^7 MNA cells with 1×10^7 infectious units of the seed virus preparation (see above).
- 559 ii) Harvest the supernatant 24 hours after the cells reach 100% infectivity (typically 40 hours after infection).
- 560 iii) Distribute the supernatant into 0.5 ml aliquots and store at –70°C.
- 561 • **Titration of stock virus suspension**
- 562 i) Thaw one aliquot of the stock virus and use this to prepare serial tenfold dilutions (from 10⁻¹ to 10⁻⁶) in
- 563 EMEM-10.
- 564 ii) Distribute 0.1 ml of each virus dilution into one well of an eight-well tissue-culture chamber slide. Add 0.2 ml
- 565 of MNA cells suspended in EMEM-10 (concentration 1×10^5 cells per 0.2 ml) to each well.
- 566 iii) Mix the cells and virus suspension by gently rocking the slide, then incubate at 37°C in a humidified incubator
- 567 with 0.5% CO₂ for 20 hours.
- 568 iv) Acetone fix and stain the slide using an immunofluorescence technique.
- 569

Each well of an eight-well tissue-culture chamber slide contains 25–50 distinct microscopic fields when observed at $\times 160$ –200 magnification. One unit of virus for the RFFIT is determined as the dilution at which 50% of the observed microscopic fields contain one or more foci of infected cells (the focus-forming dose, FFD₅₀). The stock virus suspension should contain at least 1×10^4 FFD₅₀ per 0.1 ml (i.e. the well with cells infected with the 10^{-4} dilution of the virus should contain at least one focus of infected cells in 50% of the observed microscopic fields). A stock virus suspension of this titre can then be diluted to $10^{-2.3}$ to obtain a challenge virus containing 50 FFD₅₀.

• Reference sera

A national or international reference serum standard diluted to a potency of 2.0 IU/ml should be included in each test. The reference serum used at the Centres for Disease Control and Prevention is the first second international standard for rabies immunoglobulin (Montano-Hirose *et al.*, 1991; Lyng, 1994), which may be obtained from the NIBSC (see footnote 2). The reference serum should be maintained as frozen aliquots in amounts sufficient for 1 week of tests. A positive serum control standard diluted to a potency of 0.5 IU/ml and a negative serum control standard with a potency of <0.1 IU/ml should also be prepared by the laboratory and included in each test.

• Test sera

Serum samples should be heated at 56°C for 30 minutes before testing in order to inactivate complement. If sera are frozen, they should be reheated after thawing. Serial dilutions of test sera may be prepared in an eight-well tissue-culture chamber slide. Screening dilutions of 1/5 and 1/50 are sufficient for routine evaluation of vaccination efficacy and may be made as follows:

- i) Prepare a 1/2.5 dilution by adding 0.1 ml of inactivated serum and 0.15 ml of EMEM-10 to one of the slides. Mix by gently rocking the slide.
- ii) Transfer 0.05 ml of the 1/2.5 dilution to a second well containing 0.45 ml of EMEM-10. Discard all but 0.1 ml from the well containing the 1/2.5 dilution.
- iii) Mix the second well and discard all but 0.1 ml.
- iv) Add 0.1 ml of the challenge virus preparation (containing 32–100 FFD₅₀) to all serum dilutions.
- v) Mix and incubate at 35°C in a humidified incubator with 0.5% CO₂ for 90 minutes.

• Addition of cells

- i) During the incubation period, trypsinise a stock culture of 3–5-day-old MNA cells.
- ii) Resuspend the cells in EMEM-10 to give a final concentration of 1×10^5 cells per 0.2 ml.
- iii) Distribute 0.2 ml of the cell suspension into each well of the slide and incubate at 35°C in a humidified incubator with 0.5% CO₂ for a further 20 hours.

• Acetone fixation and staining by immunofluorescence

- i) After 20 hours, remove the slides from the incubator and pour off the medium into a virucidal solution.
- ii) Rinse the slides once in PBS and then fix for 10 minutes at room temperature in cold acetone (–20°C).
- iii) Leave the slides to dry for 10 minutes before adding FITC-conjugated anti-rabies serum. The conjugate may be prepared in EMEM-10 or PBS; there is no need to adsorb the conjugate with tissue or cells. The working dilution of the conjugate should be determined by titration. The slides should be stained for 20–30 minutes at 37°C and then rinsed in PBS and distilled water, respectively.
- iv) Observe the slides under a fluorescence microscope.

• Calculation of virus-neutralising antibody titres

Residual virus is detected using a standard fluorescence microscope. The serum neutralisation end-point titre is defined as the dilution factor of the highest serum dilution at which 50% of the observed microscopic fields contain one or more infected cells (i.e. a 97% reduction in the virus inoculum). This value may be obtained by mathematical interpolation. Alternatively, a 100% neutralisation titre may be determined by recording the highest serum dilution at which 100% of the challenge inoculum is neutralised and there are no infected cells in any of the observed fields. For both titration methods, the titre of antibody in the test serum (in IU/ml) can be obtained by comparison with the titre of the national reference standard included in each test. It should be noted that it is also valid to perform the RFFIT using BHK-21 cells instead of neuroblastoma cells. A modified protocol for this has been published (WHO, 1996).

The following parameters have to be strictly adhered to:

- Rabies virus; only the CVS-11 strain (ATCC number VR-959) should be used.
- Cells cultures: only BHK-21 cells (ATCC number CCL10) or MNA cells (ATCC number CCL131) should be used.
- The test should be performed only on Lab-tek chamber slides.
- Control charts should be used for rabies virus, naïve serum and OIE positive standard dog serum.
- The back titration of the CVS virus, as well as the naïve serum and OIE positive standard dog, must be present on control plate.
- Reading method for the test: each chamber slide should contain 25–50 fields and be observed at ×160–200 magnification.
- A minimum of three-to-five-fold dilutions of sera is required.
- For the conversion of log D₅₀ to IU/ml, only the log D₅₀ value of the OIE positive standard serum of dog origin should be used.

c) Virus neutralisation in mice

This method is no longer recommended by either OIE or WHO and should be discontinued.

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

The ELISA provides a rapid (~ 4 hours) test that avoids the requirement to handle live rabies virus. Commercial indirect ELISA kits are available for indirect ELISA that allow a qualitative detection of rabies antibodies in individual dog and cat serum samples following vaccination. In accordance with the WHO recommendations (WHO Expert Committee on Biological Standards, 1985), 0.5 IU per ml of rabies antibodies is the minimum measurable antibody titre considered to represent a level of immunity that correlates with the ability to protect against rabies infection. The ELISA provides a rapid (~ 4 hours) test that does not require handling of live rabies virus, to determine if vaccinated dogs and cats have sero-converted. The sensitivity and specificity of any kit used should be determined by comparison with virus neutralisation methods. The ELISA is acceptable as a Prescribed Test for international movement of dogs or cats provided that a kit is used that has been validated and adopted on the OIE Register as fit for such purposes (see http://www.oie.int/vcda/eng/en_VCDA_registre.htm?e1d9)). Virus neutralisation methods may be used as confirmatory tests if desired. In 2007, the International Committee adopted such methods as Prescribed Tests for evaluating vaccine responses in dogs and cats prior to international movement, provided a kit is used that has been validated and adopted on the OIE Register as fit for such specific purposes³. The validation for this purpose should include a comparison with, and calibration against prescribed methods for virus neutralisation.

Other ELISA methods are also or kits should not be regarded as prescribed but may be useful for monitoring of vaccination campaigns in wildlife populations, provided the kit used has been validated for the wildlife species under study.

C. REQUIREMENTS FOR VACCINES AND DIAGNOSTIC BIOLOGICALS

NB: SECTION C IS “UNDER STUDY”. THIS IS THE LAST ADOPTED VERSION PUBLISHED IN 2008

Rabies vaccines prepared from Pasteur's original 1885 strain and its derivative strains (Pasteur Virus, Challenge Virus Standard, Pitman-Moore, etc.), and strains isolated more recently (Flury, Street-Alabama-Dufferin [SAD], Vnukovo and Kelev), protect against all strains of genotype 1 isolated so far. Conventional rabies virus vaccines may not provide adequate cross-protection against other lyssaviruses, especially in phylogroup II; there is no protection provided against Mokola virus (37) and the recently identified West Caucasian Bat Virus (Hanlon *et al.*, 2005). Cross neutralisation using conventional rabies virus vaccines has been demonstrated against two phylogroup I viruses: EBLV type-1 and EVLV type-2 (20). The principles governing the preparation of inactivated rabies vaccines are identical whether they are to be used in humans or animals, although an adjuvant may be added to vaccines for animal use.

³ see http://www.oie.int/vcda/eng/en_VCDA_registre.htm?e1d9

Recombinant vaccine (e.g. vaccinia rabies-glycoprotein recombinant) has also proved to be effective (19, 31). The rabies-glycoprotein recombinant vaccines are not live rabies vaccines. They are prepared by inserting non-infectious rabies nucleic acid into a vector such as vaccinia or canary pox. Since these do not contain live rabies virus, animals vaccinated with rabies-glycoprotein recombinant vaccines should not be restricted from entry into countries that have restrictions on entry of animals vaccinated with live rabies vaccines.

For animals, live and recombinant vaccines are effective by the oral route and can be distributed in baits in order to immunise wild (or domestic) animals.

Guidelines for the production of veterinary vaccines are given in Chapter 1.1.8 Principles of veterinary vaccine production. The guidelines given here and in Chapter 1.1.8 are intended to be general in nature and may be supplemented by national and regional requirements.

Different standards apply to vaccines containing live virus modified by passage in eggs or cell cultures to reduce its virulence for the target animal, and to vaccines prepared from inactivated virus. Both types of vaccine have their advantages and disadvantages (6), but they can both be used to immunise animals for periods of between 1 and 3 years. Live attenuated rabies vaccines are not accepted in some countries. They are not to be relied on to protect previously unvaccinated animals that have been exposed to infection (15). Only in humans has the efficiency of post-exposure prophylaxis with vaccine alone been proven and even in these cases there is an additional strong recommendation to administer anti-rabies immunoglobulin.

All handling of the virus during manufacture and testing of vaccines must conform to the strict safety precautions specified by WHO (43, 45), the OIE (Chapter 1.1.2) and to national guidelines and regulations.

1. Seed management

a) Characteristics of the seed

Any strain belonging to serotype 1, which has been proved to protect against field rabies viruses (currently found in the country where the vaccine is to be used), is suitable. The strain of virus used should have well-known biological (e.g. pathogenicity) and antigenic properties (typing by MAbs). If it is to be used as a live vaccine, the master seed virus must be shown not to cause clinical rabies. At least two animals (preferably five to six per group) of each of the species for which the vaccine is intended and, so far as possible, any species that might be in contact with vaccine or vaccinated animals, should be tested. This can be done by inoculating in or adjacent to a major nerve, a dose equivalent to ten times the intended viral titre in one dose of the proposed final product. Animals should be observed for at least 90 days for any adverse effect attributable to the master seed.

b) Method of culture

A master cell stock of the seed virus should be prepared and kept at or below -70°C . Subculture from this stock will be used for vaccine production. Virus multiplication is verified by titration during growth of the seed virus.

c) Validation as a vaccine

Before a vaccine is licensed, evidence of efficacy should be established by the challenge of vaccinated and control animals of each target species. The challenge should be performed at the end of the period after vaccination for which the manufacturer claims maintenance of immunity. Antibody kinetics should also be determined in order to establish the correlation between antibody titre and resistance to challenge.

The efficacy of the produced vaccine is assessed by studies on every target species previously vaccinated as recommended. Protection at the end of the period of immunity is monitored by a measurement of specific neutralising antibodies and by challenge with rabies virus. The experimental conditions of this challenge should mimic the natural conditions of infection. The challenge virus should preferably be prepared from locally isolated strains. In animals vaccinated with inactivated vaccines, the percentage of seroconversion and the mean level of antibody allow a good prognosis for survival to challenge (3).

The correlation between potency in the target species and antigenic value as estimated in mice should be established (see Section C.4.c below).

For the purposes of licensing a vaccine, safety tests should be conducted in the target species. In the case of live virus vaccines used in oral vaccination campaigns (including recombinant vaccines), safety tests should also be carried out on those other species that live in the area of vaccination and could become exposed to the vaccine (6).

Vaccine stability is ascertained by testing batches after prolonged storage, usually 1–2 years. A process of accelerated ageing, by storage at 37°C for 1 week, is sometimes used. The storage life claimed by the manufacturer is checked by the national licensing authority. In general, it is 12–18 months for fluid vaccines, and possibly 24 months for lyophilised vaccines.

2. Method of manufacture

Whatever method is adopted, close attention should be paid to the quality of the substrate. Eggs should be of SPF origin, and the cell cultures, such as BHK cell lines, should conform to international standards of sterility and innocuity.

During manufacture, the multiplication of the virus in one of the substrates mentioned above is monitored, followed by harvesting at the most appropriate time, usually 4–6 days after inoculation of eggs or cell cultures. The virus harvest is suspended in a buffer solution at a dilution that will provide an optimum antigenicity of the end-product. If required, the suspension is either inactivated or lyophilised. An adjuvant is recommended for vaccines prepared from inactivated virus, as well as for other vaccine antigens that may be incorporated in polyvalent vaccines.

a) In cell cultures

Cultures are infected with cell-culture-adapted strains of rabies virus and incubated at 35–36°C. These may then be used as live virus vaccines (as in Flury and SAD vaccines), or as inactivated vaccines after the addition of phenol (Semple vaccine) or some other chemical, such as beta-propiolactone.

Cell culture can also be used to grow the vector viruses (e.g. vaccinia virus) harbouring the gene coding for the expression of rabies virus glycoprotein (31).

b) In eggs

A modified egg-adapted strain of virus is inoculated into SPF-embryonated chicken eggs, which are then incubated at 38°C for 5–6 days. The virus is harvested in the form of infective embryo tissues, and is usually lyophilised and used as a live vaccine. Examples of such vaccines include those that contain the Flury low egg passage (LEP), or the more desirable high egg passage (HEP) variant strain, which is safer for some animal species such as the cat.

c) In animals

Nervous tissue vaccines prepared in animals are no longer considered safe or effective, and their use should be discontinued.

3. In-process control

This consists of monitoring virus growth to provide an optimum titre and ensure the absence of undesirable microbial contamination.

In live virus vaccines, kinetics of virus growth should be established in order to ensure a final titre of virus correlated to the desired protection in target species.

In inactivated virus vaccines, immunogenic properties of the final product may be evaluated by *in-vitro* techniques (e.g. ELISA, agar gel immunodiffusion, antibody-binding tests or infected cell staining). These evaluations will indicate the best time for harvesting the virus in cell cultures.

4. Batch control

a) Sterility

Tests for sterility and freedom from contamination of biological materials may be found in Chapter 1.1.9.

b) Safety

Safety tests for batches of inactivated virus vaccines are carried out by inoculation of cell culture or intracerebrally into mice to detect viable virus. A suitable safety test for live rabies vaccines should be carried out on each lot of vaccine, in the intended host species. At least three, preferably five to six animals of the intended host species should be given a dose equivalent to ten times the recommended field dose, by the recommended route of administration. The animals should be observed for 180 days for adverse reactions attributable to the vaccine (23).

758 **c) Potency/biological activity**

759 The amount of virus present in live attenuated and recombinant vaccines is determined by titration. Once a
 760 correlation has been established between the activity of the vaccine in the target species and virus titres, virus
 761 titrations become reliable indicators of vaccine efficacy. This is carried out using cell cultures or by the
 762 intracerebral inoculation of unweaned mice (in mice it is only possible with a few attenuated viruses). Recombinant
 763 vaccines should be monitored for the expressed rabies protein until assured that expression stability is maintained
 764 in the manufacturing process. Titre of the vector can then be used as a reliable indicator of vaccine efficacy.

765 For inactivated virus vaccines, correlation between potency in the target species and antigenic value as estimated
 766 in mice provides a reliable indicator of vaccine activity. The potency of the vaccine is established in the USA by the
 767 National Institutes of Health (NIH) test. Elsewhere, the European Pharmacopoeia test is widely adopted.

768 Groups of at least ten mice, aged 3–4 weeks, are inoculated with single, decreasing doses of vaccine in
 769 accordance with the European Pharmacopoeia (23), or with two doses, 1-week apart, according to the NIH test
 770 (45). A sufficient number of dilutions of vaccine are compared to estimate the dilution at which 50% of the mice are
 771 protected against intracerebral challenge 14 days later (23, 45).

772 A WHO international standard vaccine is available (see footnote 2) for calibration of national standards, so that the
 773 results of testing for antigenicity can be expressed in IUs. The test is not valid unless:

- 774 i) For both the vaccine to be examined and the standard preparation, the PD_{50} (50% protective dose) lies
 775 between the largest and smallest doses given to the mice.
- 776 ii) The titration of the challenge virus suspension shows that 0.03 ml of the suspension contained 25 mouse
 777 intra-cranial LD_{50} (MIC LD_{50}). The challenge dose should be in the range 12–50 LD_{50} for a valid test.
- 778 iii) The confidence interval ($p = 0.95$) for the test should not be less than 25% and not more than 400% of the
 779 estimated potency: statistical analysis should show a significant slope and no significant deviations from
 780 linearity or parallelism of the dose–response lines.

781 The vaccine passes the test if the estimated potency is not less than 1 IU per dose, in the smallest prescribed
 782 dose.

783 A simplified test can also be used for the purpose of anticipating which vaccines are likely to be of an antigenic
 784 value ≥ 1 IU per dose (4). This test used as a screening test is a good way to reduce the number of mice used in
 785 vaccine potency control tests.

786 **d) Duration of immunity**

787 Duration of immunity must be established for the product licence in the target species with a defined vaccination
 788 protocol.

789 **e) Stability**

790 The proposed shelf life must be verified by appropriate tests. These experiments include biological and physico–
 791 chemical stability tests, and should be performed on a sufficient number of batches of vaccine stored under
 792 recommended conditions.

793 The thermostability of live virus vaccines in liquid form is generally poor. For freeze-dried inactivated virus
 794 vaccines, stability is generally granted for 2 years at 4°C.

795 **f) Preservatives**

796 Inactivated virus vaccines may contain preservatives (formalin, merthiolate). The nature and quantity of these
 797 preservatives should comply with national control regulations.

798 **5. Tests on the final product**799 **a) Safety**

800 See Section C.4.b.

801

802 **b) Potency**

803 See Section C.4.c.

804 **6. Oral vaccination**

805 The concept of oral vaccination is unique: as stray or wild animals are out of physical reach, dropping vaccine baits into
 806 their environment is the only way to immunise them. In the 1980s and 1990s, the Veterinary Public Health Department
 807 of WHO organised several meetings of rabies experts to define the requirements for guaranteeing the safety and
 808 efficacy of vaccines both for the target species (red fox, raccoon dog, skunk, dog, etc.) and nontarget species, namely
 809 wild rodents and any other wild and domestic species that might be in contact with baits or a recently vaccinated animal
 810 (42, 44).

811 Several guidelines have been established for the quality criteria that vaccines have to satisfy before marketing; the most
 812 precise documents are those produced by WHO, the European Pharmacopoeia and the European Commission (24, 25,
 813 44). Available oral vaccines have been extensively tested by different routes (cerebral, muscular and oral) in a variety of
 814 species: puppies and adults of carnivores, avian species, nonhuman primates, rodents and immunocompromised mice.
 815 Nonhuman primates have been added to this list since the discovery in 1992 that the original SAD Bern strain is highly
 816 pathogenic for baboons by the oral route (12).

817 All vaccines currently used for oral vaccination programmes are either modified live-virus vaccines or live recombinant
 818 vaccines. At the present time, two oral vaccines are recommended by WHO (44): a recombinant vaccine – VRG
 819 vaccine, and a highly attenuated vaccine – SAG2.

820 The production controls are closely related to the ones used for parenteral vaccines. The major differences concern
 821 three points:

- 822 i) Safety of the vaccine for man, target and non-target species.
- 823 ii) Efficacy of the protection induced by the vaccine.
- 824 iii) Monitoring of the impact of oral vaccination campaigns in the field.

825 **a) Safety considerations**

826 For oral vaccination, either attenuated rabies strains or live-recombinant vaccines may be used. The vaccine
 827 should not induce any adverse signs in target and nontarget species. For vaccines used for dog immunisation,
 828 saliva should be checked for the absence of vaccinal virus because of possible contact with humans.

829 The attenuated rabies virus-based vaccines must achieve the lowest residual pathogenicity for target and
 830 nontarget species (24); this is of utmost importance in the case of oral vaccination of dogs as dogs are often in
 831 close contact with humans (44).

832 The recombinant vaccines cannot induce any risk of rabies; the safety controls concern only the possible residual
 833 pathogenicity of the recombined parental virus.

834 **b) Protection induced by the vaccine**

835 The protection induced by the vaccine must be tested not only with the virus itself (to determine the minimal
 836 vaccinating dose) but also with manufactured baits ready to be used in the field. For foxes for instance, the vaccine
 837 should have a minimal titre corresponding to at least ten times the 100% protective dose (obtained with the same
 838 vaccine experimentally by direct oral instillation) (14).

839 The protection status cannot be then checked by serology only; a virulent challenge with the homologous street
 840 rabies virus is necessary because of the important implication of cell-mediated immunity in response to oral
 841 vaccination (25).

842 **c) Monitoring the impact of oral vaccination**

843 The stability of the vaccine in the field is important. The European Commission stresses the importance of
 844 checking the 100% protective dose after 7 days of exposure at 25°C (24). Each vaccine bait should be tested for
 845 stability with a melting point above 40°C, and the blister or sachet containing the vaccine should still be covered by
 846 the bait casing after 7 days exposure at 40°C (24).

847

848 Aerial distribution of baits is the only way to perform an homogenous, rapid and sufficient distribution for wildlife
 849 vaccination. Quality control measures should be used to monitor different key points of baiting; control of vaccine
 850 titre, control of area coverage by air and of baiting density should at least be constantly monitored. The cross
 851 border cooperation between neighbouring countries is also needed to avoid any unvaccinated area along the
 852 border.

853 For wildlife in Europe, two campaigns are performed yearly: the spring one aims at vaccinating the young
 854 population of the target species, its period should then be fixed according to the biology of the target species. The
 855 autumn campaign concerns both adult and young animals. It is generally admitted that four campaigns (i.e. 2
 856 years) should be conducted after the last rabies diagnosis.

857 The impact of vaccination on the host/vector population is monitored in two different ways:

- 858 • Directly by measuring the bait uptake by the wild target species. This supposes that a biomarker (generally
 859 tetracycline) is included in the bait casing. The same examination allows the age of animals to be determined.
- 860 • Directly by measuring the serological response of target animals. This serological control is better done using
 861 validated ELISA techniques (22, Servat *et al.*, 2007) as they are more robust than seroneutralisation tests
 862 when testing poor quality field specimens.
- 863 • Indirectly by measuring the incidence of rabies in the vaccinated area. Typing of field isolates should be
 864 performed (44) either by using MAbs or by sequencing positive samples from areas where the target species
 865 have been vaccinated with attenuated vaccines to possibly distinguish vaccine and field virus strains.

866 The first two controls should be performed on specially killed animals to collect good quality samples. Rabies
 867 monitoring is more sensitive when performed in found dead or ill animals.

868 REFERENCES

- 869 3. AUBERT M.F.A. (1992). Practical significance of rabies antibodies in cats and dogs. *Rev. sci. tech. Off. int. Epiz.*,
 870 **11**, 735–760.
- 871 4. AUBERT M.F.A. & BLANCOU J. (1982). Test simplifié de contrôle d'activité des vaccins antirabiques à virus inactivés.
 872 *Rev. sci. tech. Off. int. Epiz.*, **1**, 811–822.
- 873 BADRANE H ., BAHLOUL C ., PERRIN P.&, TORDO N . (2001). Evidence of two Lyssavirus phylogroups with distinct
 874 pathogenicity and immunogenicity. *J. Virol.*, **75**, 3268–3276.
- 875 6. BAER G.M. (1991). The Natural History of Rabies, Second Edition. CRC Press, Boca Raton, Florida, USA, 620 pp.
- 876 BARRAT J. (1992). Experimental diagnosis of rabies. Adaptations to field and tropical conditions. *SEARG proceedings*,
 877 72–83. BARRAT J. & AUBERT M.F.A. (1995). Diagnostic de la rage animale en France de 1991 à 1993, bilan de CNEVA
 878 laboratoire d'études sur la rage et la pathologie des animaux sauvages en France. *Revue Méd. Vét.*, **146**, 561–566.
- 879 BARRAT J. & AUBERT M.F.A. (1995). Diagnostic de la rage animale en France de 1991 à 1993, bilan de CNEVA
 880 laboratoire d'études sur la rage et la pathologie des animaux sauvages en France. *Revue Méd. Vét.*, **146**, 561–566.
- 881 BARRAT J. & BLANCOU J. (1988). Technique simplifiée de prélèvement, de conditionnement et d'expédition de matière
 882 cérébrale pour le diagnostic de rage. *Doc. WHO/Rab. Res./88.27*.
- 883 6. BINGHAM J., FOGGIN C.M., GERBER H., HILL F.W.G., KAPPELER A., KING A., PERRY B.D. & WANDELER A. (1992).
 884 Pathogenicity of SAD vaccine rabies vaccine given orally in Chacma baboons (*Papio ursinus*). *Vet. Rec.*, **131**, 55–
 885 56.
- 886 BINGHAM J. & VAN DER MERWE M. (2002). Distribution of rabies antigen in infected brain material: determining the reliability
 887 of different regions of the brain for the rabies fluorescent antibody test. *J. Virol. Methods*, **101**, 85–94.

888

- 889 14. BLANCOU J., SCHNEIDER L.G., WANDELER A. & PASTORET P.P. (1986). Vaccination du renard roux (*Vulpes vulpes*)
890 contre la rage par voie orale. Bilan d'essais en station expérimentale. *Rev. Ecol. (Terre Vie)*, **40**, 249–255.
- 891 15. BLANCOU J., SORIA BALTAZAR R., MOLLI I. & STOLTZ J.F. (1991). Effective postexposure treatment of rabies-infected
892 sheep with rabies immune globulin and vaccine. *Vaccine*, **9**, 432–437.
- 893 BLANTON J.D., SELF J., NIEZGODA M., FABER M.L., DIETZSCHOLD B. & RUPPRECHT C. (2007). Oral vaccination of raccoons
894 (*Procyon lotor*) with genetically modified rabies virus vaccines. *Vaccine*, **25** (42), 7296–7300.
- 895 BOURHY H., KISSI B. & TORDO N. (1993). Molecular diversity of the lyssavirus genus. *Virology*, **194**, 70–81.
- 896 19. BROCHIER B., KIENY M.P., COSTY F., COPPENS P., BAUDUIN B., LECOCQ J.P., LANGUET B., CHAPPUIS G., DESMETTRE P.,
897 AFIADAMANYO K., LIBOIS R. & PASTORET P.-P. (1991). Large-scale eradication of rabies using recombinant vaccinia-
898 rabies vaccine. *Nature*, **354**, 520–522.
- 899 20. BROOKES S.M., PARSONS G., JOHNSON N., MCELHINNEY L.M. & FOOKS A.R. (2005). Rabies human diploid cell vaccine
900 elicits cross-neutralising and cross-protecting immune responses against European and Australian bat
901 lyssaviruses. *Vaccine*, **23**, 4101–4109.
- 902 CLIQUET F. & AUBERT M. (2004). ELIMINATION OF TERRESTRIAL RABIES IN WESTERN EUROPEAN COUNTRIES. *DEV. BIOL. (BASEL)*,
903 **119**, 185–204.
- 904 CLIQUET F., AUBERT M. & SAGNE L. (1998). Development of a fluorescent antibody virus neutralisation test (FAVN test) for
905 the quantitation of rabies-neutralising antibody. *J. Immunol. Methods*, **212**, 79–87.
- 906 22. CLIQUET F., SAGNE L., SCHEREFFER J.L. & AUBERT M.F.A. (2000). ELISA tests for rabies antibody titration in orally
907 vaccinated foxes sampled in the fields. *Vaccine*, **18**, 3272–3279.
- 908 23. COUNCIL OF EUROPE (2005). *Vaccinum rabiei perorale vivum ad vulpen*. Rabies vaccine (live, oral) for foxes.
909 European Pharmacopeia, Fifth Edition. Monograph 074663. Editions of the Council of Europe, Strasbourg, France,
910 792.
- 911 DATO V., MOOSE C., REA N., FRASER G., SEIDERS J., RITTLE C., URDANETA V., OST S., ENCARNACION C., REYNOLDS M., DAMON
912 I., LI Y., DAVIDSON W., WILKINS K., MC DOWELL E., RUPPRECHT C.E., ORCIARI L., SMITH S. & ROESS A. (2009). Human
913 vaccinia infection after contact with a raccoon rabies vaccine-bait – Pennsylvania, 2009. JAMA, 302, 2426–2428.
- 914 24. EUROPEAN COMMISSION (2002). The oral vaccination of foxes against rabies. Report of the Scientific Committee on
915 Animal Health and Animal Welfare, adopted 23 October 2002. European Commission, Health and Consumer
916 Protection Directorate-General, Directorate C – Scientific Opinions, C2 – Management of Scientific Committees;
917 Scientific Co-Operation and Networks, 55 pages.
- 918 25. EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES (2007). Rabies Vaccine (live, oral) for foxes (2007).
919 European Pharmacopoeia 06, 01: 952–953.
- 920 FOOKS A.R., JOHNSON N., FREULING C.M., WAKELEY P.R., BANYARD A.C., MCELHINNEY L.M., MARSTON D.A., DASTJERDI A.,
921 WRIGHT E., WEISS R.A. & MULLER T. (2009). Emerging technologies for the detection of rabies virus: challenges and
922 hopes in the 21st century. PLoS Negl. Trop. Dis., 3, e530.
- 923 GENOVESE M.A. & ANDRAL L. (1978). Comparaison de deux techniques utilisées pour le diagnostic de la rage:
924 l'immunofluorescence et l'immunoperoxydase. Rec. Med. Vet., 154 (7–8), 667–671.
- 925 HANLON C.A., KUZMIN I.V., BLANTON J.D., WELDON W.C., MANANGAN J.S. & RUPPRECHT C.E. (2005). Efficacy of rabies
926 biologics against new lyssaviruses from Eurasia. *Virus Res.*, **111**, 44–54.
- 927 HOOPER D.C., MORIMOTO K., BETTE M., WEIHE E., KOPROWSKI H. & DIETZSCHOLD B. (1998). Collaboration of antibody and
928 inflammation in clearance of rabies virus from the central nervous system. *J. Virol.*, **72**, 3711–3719.
- 929 KNOWLES M.K., NADIN DAVIS S.A., SHEEN M., ROSATTE R., MUELLER R. & BERESFORD A. (2009). Safety studies on an
930 adenovirus recombinant vaccine for rabies (AdRG1.3-ONRAB) in target and non target species. Vaccine, 27, 6619–
931 6626.
- 932

- 933 [ICTV \(International Committee on Taxonomy of viruses\): http://www.ictvonline.org/virusTaxonomy.asp?bhcp=1](http://www.ictvonline.org/virusTaxonomy.asp?bhcp=1)
- 934 KANG B., OH J., LEE C., PARK B.K., PARK Y., HONG K., LEE K., CHO B. & SONG D. (2007). Evaluation of a rapid
935 immunodiagnostic test kit for rabies virus. *J. Virol. Methods*, **145**, 30–36.
- 936 31. KIENY M.P., LATHE R., DRILLIEN R., SPEHNER D., SKORY S., SCHMITT D., WIKTOR T., KOPROWSKI H. & LECOCQ J.P.
937 (1984). Expression of rabies virus glycoprotein from a recombinant vaccinia virus. *Nature*, **312**, 163–166.
- 938 KUZMIN I.V., MAYER A.E., NIEZGODA M., MARKOTTER W., AGWANDA B., BREIMAN R.F. & RUPPRECHT C.E. (2010). Shimoni bat
939 virus, a new representative of the *Lyssavirus* genus. *Virus Res.*, Feb 6. [Epub ahead of print]
- 940 LEMBO T., NIEZGODA M., VELASCO-VILLA A., CLEAVELAND S., ERNEST E. & RUPPRECHT C.E. (2006). Evaluation of a direct,
941 rapid immunohistochemical test for rabies diagnosis. *Emerg. Infect. Dis.*, **12** (2), 310–313.
- 942 LYNNG J. (1994). Calibration of a replacement preparation for the international standard for rabies immunoglobulin.
943 *Biologicals*, **22** (3), 249–255.
- 944 MONTANO HIROSE J.A., BOURHY H. & SUREAU P. (1991). Retro-orbital route for brain specimen collection for rabies
945 diagnosis. *Vet. Rec.*, **129**, 291–292.
- 946 NADIN-DAVIS S.A. & FEHLNER-GARDINER C. (2008). Lyssaviruses – Current Trends. *Adv. Virus Res.*, **71**, 207–250.
- 947 PICARD-MEYER E., BARRAT J. & CLIQUET F. (2007). Use of filter paper (FTA) technology for sampling, recovery and
948 molecular characterisation of rabies viruses. *J. Virol. Methods*, **140**, 174–182.
- 949 ROOT J.J., MCLEAN R.G., SLATE D., MACCARTHY K.A. & OSORIO J.E. (2008). Potential effect of prior raccoonpox virus
950 infection in raccoons on vaccinia-based rabies immunization. *BMC Immunol.*, **9**, 57–63.
- 951 ROSATTE R.C., POWER M.J., DONOVAN D., DAVIES J.C., ALLAN M., BACHMANN P., STEVENSON B., WANDELER A. & MULDOON F.
952 (2007). Elimination of arctic variant rabies in red foxes, metropolitan Toronto. *Emerg. Inf. Dis.*, **413** (1), 25–27.
- 953 RUDD R.J. & TRIMACHI C.V. (1989). Development and evaluation of an *in vitro* virus isolation procedure as a replacement
954 for the mouse inoculation test in rabies diagnosis. *J. Clin. Microbiol.*, **27**, 2522–2528.
- 955 RUPPRECHT C.E., BLASS L., SMITH K.C., ORCIARI L.A., NIEZGODA M., WHITFIELD S., GIBBONS R.V., GUERRA M. & HANLON C.A.
956 (2001). Human infection due to recombinant vaccinia-rabies glycoprotein vaccinia-rabies glycoprotein virus. *N. Engl. J.
957 Med.*, **345** (8), 582–586. Erratum in: *N. Engl. J. Med.*, (2001) **345** (24), 1784.
- 958 SERVAT A., FEYSSAGUET M., BLANCHARD I., MORIZE J.L., SCHEREFFER J.L., BOUÉ F. & CLIQUET F. (2007). A quantitative
959 indirect ELISA to monitor the effectiveness of rabies vaccination in domestic and wild carnivores. *J. Immunol. Methods*,
960 **318**, 1–10.
- 961 SMITH J.S., YAGER P.A. & BAER G.C. (1973). A rapid reproducible test for determining rabies neutralizing antibody. *Bull.*
962 *WHO*, **48**, 535–541.
- 963 TURMELLE A.S., ALLEN L.C., JACKSON F.R., KUNZ T.H., RUPPRECHT C.E. & MCCracken G.F. (2009). Ecology of rabies virus
964 exposure in colonies of Brazilian free tailed bats (*Tadarida brasiliensis*) at natural and man made roosts in texas. *Vector
965 Borne Zoonotic Dis.*, Jun 3. [Epub ahead of print].
- 966 37. VON TEICHMAN B.F., DE KOKER W.C., BOSCH S.J., BISHOP G.C., MERIDITH C.D. & BINGHAM J. (1998). Mokola virus
967 infection: description of recent south African cases and a review of the virus epidemiology. *J. S. Afr. Vet. Assoc.*,
968 **69**, 169–171.
- 969 WARNER C.K., WHITFIELD S.G., FEKADU M. & HO H. (1997). Procedures for reproducible detection of rabies virus antigen
970 mRNA and genome *in situ* in formalin-fixed tissues. *J. Virol. Methods*, **67**, 5–12.
- 971 WEYER J., RUPPRECHT C.E. & NEL L.H. (2009). Poxvirus-vectored vaccines for rabies – a review. *Vaccine*, **27** (51), 7198–
972 7201.
- 973 WORLD HEALTH ORGANISATION. Recombinant vaccines for oral immunization of wildlife:
974 <http://www.who.int/rabies/vaccines/recombinant/en/index.html>
- 975

- 976 42. WORLD HEALTH ORGANIZATION (1989). Report of WHO Consultation on requirements and criteria for field trials on
977 oral rabies vaccination of dogs and wild carnivores, Geneva, 1–2 March 1989, *Doc. WHO/Rab.Res./89.32*.
- 978 WORLD HEALTH ORGANISATION (1996). Laboratory Techniques in Rabies, Fourth Edition, Meslin F.-X., Kaplan M.M. &
979 Koprowski H., eds. WHO, Geneva, Switzerland.
- 980 WORLD HEALTH ORGANIZATION EXPERT COMMITTEE ON BIOLOGICAL STANDARDS. THIRTY-FIFTH REPORT (1985). World Health
981 Organisation Technical Report Series No. 725. WHO, Geneva, Switzerland.
- 982 43. WORLD HEALTH ORGANIZATION EXPERT COMMITTEE ON RABIES. EIGHTH REPORT (1992). World Health Organisation
983 Technical Report Series No. 824, 84 pp.
- 984 WORLD HEALTH ORGANIZATION EXPERT COMMITTEE ON RABIES, EIGHTH REPORT (2005). World Health Organisation, WHO
985 Technical Report Series, **931**, WHO, Geneva, Switzerland, 1–87.
- 986 XU G., WEBER P., HU Q., XUE H., AUDRY L., LI C., WU J. & BOURHY H. (2007). A simple sandwich ELISA (WELYSSA) for the
987 detection of lyssavirus nucleocapsid in rabies suspected specimens using mouse monoclonal antibodies. *Biologicals*,
988 **35**, 297–302.
- 989 ~~ZHANG S., LIU Y., FOOKS A.R., ZHANG F. & HU R. (2008). Oral vaccination of dogs (*Canis familiaris*) with baits containing~~
990 ~~the recombinant rabies-canine adenovirus type 2 vaccine confers long-lasting immunity against rabies. *Vaccine*, **26** (3),~~
991 ~~345–350.~~
- 992 *
- 993 * *
- 994 **NB:** There are OIE Reference Laboratories for Rabies (see Table in Part 3 of this *Terrestrial Manual* or consult the OIE
995 Web site for the most up-to-date list: www.oie.int).

Work plan and activities (as of 11 February 2011)

Topic	Progress made	Actions
1. Updating the <i>Manual of Diagnostic Tests and Vaccines for Terrestrial Animals</i>	<i>Modus operandum</i> and timetable discussed; Consultant Editor's recommendations for streamlining of <i>Manual</i> Chapters endorsed.	- Extend the next BSC meeting by 1 day - Identify additional experts to assist in the review of proposed revised chapters - Update Guidelines for Authors - Seek OIE-wide policy on non-listed diseases in the <i>Manual(s)</i>
2. <i>Manual</i> Chapters for adoption in May 2011	Chapters for adoption in May 2011 reviewed	Circulation for Member comments
3. <i>Manual</i> Chapters for adoption in May 2012	Chapters for adoption in May 2012 identified	- Circulation of those already ready for Member comments - Ask OIE Experts to confirm the need for revision - Ask OIE Experts to start revision work
4. Mandate and Internal Rules for Reference Laboratories and for Collaborating Centres	Proposed amendments prepared by AHG on Partnerships	- Forward the proposals on Mandate and Internal Rules to the Council - Update guidelines for applicants
5. Designation of Reference Centres	On-going	Seek OIE-wide policy on Reference Laboratories for non-listed diseases
6. Review template for annual reports of Reference Laboratory/Collaborating Centre activities	Consultant Editor's report on 2009 annual reports reviewed	Modify template to give clearer instructions
7. Review of annual 2010 reports of Reference Laboratory/Collaborating Centre activities		- Identify new mechanisms for the review of 2011 reports - Discuss the possibility of BSC members visits to selected laboratories

Ad hoc Groups

Existing Groups		
Title of <i>ad hoc</i> Group	Progress by Feb. 2011 BSC meeting	Action by Sept. 2011 BSC meeting
Scientific Partnerships among Reference Centres	Review of Mandate and Internal Rules Completed.	Develop operational guidance on networks (could be done by BSC itself if not by AHG)
Quality of FMD Vaccines	Draft ToRs approved and possible members identified	Meeting will take place in 29–31 March 2011. Report will be available for BSC
Validation of Tests in Wildlife	Draft ToRs approved and possible members identified	Meeting will take place 27–29 April 2011. Report will be available for BSC
Future Groups		
Biosafety/Biocontainment Standards for Veterinary Laboratories	Draft ToRs approved	Identify possible members and dates
Diagnostic Tests Related to New and Emerging Technologies	Sought advice of an expert who believes it is necessary to convene this Groups	Commission identified author to update <i>Manual</i> Chapter on Biotechnology in the Diagnosis of Infectious Diseases and Vaccine Development

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