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

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REPORT OF THE MEETING OF THE OIE BIOLOGICAL STANDARDS COMMISSION

Paris, 14–16 September 2011

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The OIE Biological Standards Commission met at the OIE Headquarters from 14 to 16 September 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, Vice-President, and Dr Hualan Chen, member of the Commission. The other two members, Dr Paul Townsend and Dr Alejandro Schudel, could not attend.

Dr Miyagishima informed the Commission of the updated version of the OIE Basic Texts that had been adopted by the World Assembly in May 2011. The texts included updated Terms of Reference for OIE Reference Laboratories and Collaborating Centres (now collectively called Reference Centres) and for the OIE Specialist Commissions. New texts regarding confidentiality and conflicts of interest would be implemented soon. All members of OIE bodies, whether on Commissions, Working or *ad hoc* Groups, and directors and OIE Experts in the OIE Reference Centres, would be asked to sign the relevant forms once finalised.

Under the updated Terms of Reference for the four OIE Specialist Commissions, new applications for OIE Collaborating Centre status would be assigned to one of the four Commissions for approval according to the proposed specialty and the competence of respective Commissions. This meant a departure from the former system whereby the Biological Standards Commission oversaw all applications (apart for ones for aquatic animals), even for applications that required advice from other Specialist Commissions and their Working Groups. The Commission strongly opposed the new system and urged the Director General and the Council to reconsider this change. The Commission believed that it was no longer clear who had the overall responsibility for Reference Centres, including for their designation in cases of overlapping competencies, for ensuring their compliance with their mandates, for all follow-up and administrative steps, etc.

Dr Bernard Vallat, Director General of the OIE, who joined the meeting on Wednesday, informed the Commission that the Council would be meeting the following week and would welcome any proposed improvements to the Basic Texts. He reiterated that all applications for Reference Laboratory status (for diseases of terrestrial animals) would remain the responsibility of the Biological Standards Commission. He saw little difference in asking the Biological Standards Commission its opinion on an OIE Collaborating Centre application for a topic not linked to diagnostics and vaccines, where the Commission would seek the advice of another Specialist Commission, and asking that Specialist Commission directly. He agreed to make the Council aware of the Commission's misgivings[1].

Dr Vallat went on to congratulate the Commission for its work, in particular on vaccine quality – which was becoming more and more important, and for its commitment to ensuring that the *Terrestrial Manual* was a manual of Standards, as referred to in the Agreement on the Application of Sanitary and Phytosanitary Measures of the World Trade Organization (SPS Agreement).

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

1 The Council, which met on 20–22 September 2011, considered this matter and reaffirmed that the provisions of the Basic Texts as revised in May 2011 were appropriate as regards the terms of reference of Specialist Commissions.

1. OIE Reference Centres

1.1. Applications for the status of OIE Reference Centre

Following the adoption by the World Assembly in May 2011 of the new Terms of Reference for OIE Collaborating Centres and, in particular, the requirement to limit the mandate of each Centre to a specific defined area of competence, a request had been received from the OIE Collaborating Centre in Teramo, Italy, along with the relevant documentation, to divide the Centre into four Centres, one each for Animal Welfare, Food Safety, Epidemiology and Veterinary Training. The Biological Standards Commission accepted this proposal. In accordance with the new procedure, the request would also be reviewed by the Code Commission (Animal Welfare, Food Safety, and Veterinary Training) and the Scientific Commission for Animal Diseases (Epidemiology)[2].

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

OIE *Reference Laboratory for Epizootic haemorrhagic disease*
French Agency for Food, Environmental and Occupational Health and Safety (Anses), Laboratoire de santé animale de Maisons-Alfort, National Reference Laboratory for BT/EHD and AHS Diseases, UMR 1161 Virology, 23 Avenue de Général De Gaulle, 94703 Maisons-Alfort, FRANCE
Tel: (+33[0]1) 43.96.72.82; Fax: (+33[0]1) 43.96.73.96; E-mail: s.zientara@vet-alfort.fr or stephan.zientara@anses.fr
Designated Reference Expert: Dr Stephan Zientara.

OIE *Reference Laboratory for Porcine reproductive & respiratory syndrome*
Veterinary Diagnostic Laboratory, China Animal Disease Control Center, No. 2 Yuanmingyuan West Road, Haidian District, Beijing, CHINA (PEOPLE'S REP. OF) 100193
Tel: (+86-010) 62.89.12.57 / 58; Tel: (+86-010) 62.89.35.07; E-mail: cadczhen@agri.gov.cn
Designated Reference Expert: Dr Kegong Tian.

OIE *Reference Laboratory for Newcastle disease*
National Diagnostic Center for Exotic Animal Diseases, China Animal health and Epidemiology Center, Ministry of agriculture, No. 369Nanjing Road, Qingdao 266032, CHINA (PEOPLE'S REP. OF)
Tel: (+86-532) 87.83.91.88; Tel: (+86-532) 87.83.99.22; E-mail: zlwang111@yahoo.com.cn
Designated Reference Expert: Dr Zhiliang Wang.

OIE *Reference Laboratory for Foot and mouth disease*
National Veterinary Services Laboratories, USDA-APHIS-VS, Foreign Animal Disease Diagnostic Laboratory, Plum Island Animal Disease Center, P.O. Box 848, Greenport, NY 11944, UNITED STATES OF AMERICA
Tel: (+1-631) 323.32.56; Tel: (+1-631) 323.33.66; E-mail: Consuelo.Carrillo@aphis.usda.gov
Designated Reference Expert: Dr Consuelo Carrillo.

OIE *Reference Laboratory for Swine influenza*
National Reference Laboratory for Animal Influenza, Viral Disease and Epidemiology Research Division, National Institute of Animal Health, National Agriculture and Food Research Organization, Kannondai, Tsukuba, Ibaraki, 305-0856, JAPAN
Tel: (+81-29) 838.79.14; Tel: (+81-29) 838.79.14; E-mail: taksaito@affrc.go.jp
Designated Reference Expert: Dr Takehiko Saito.

2 The Council, which met on 20–22 September 2011, while agreeing that the request to split the Collaborating Centre into four Centres was receivable, expressed the view that the Biological Standards Commission had no power to approve new Centres in the four mentioned areas of specialty, in accordance with the applicable Basic Texts. The Council requested that the Director General refer the applications to the relevant Specialist Commissions and to the Regional Commission for Europe for review. The Council also agreed that careful consideration should be given to the breadth of specialty assigned to a Collaborating Centre; for instance, animal welfare could cover a very side range of subjects (production animal, companion animal, animals in experiments) and it was unlikely that one centre could offer the world's best expertise in all these areas; the same could hold for wildlife.

OIE *Reference Laboratory for Swine influenza*
Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Via Antonio Bianchi No. 9, 25124 Brescia, ITALY
Tel: (+39-[0]521) 29.37.33; Fax: (+39-[0]521) 29.35.38
Email: emanuela.foni@izsler.it
Designated Reference Expert: Dr Emanuela Foni.

OIE *Reference Laboratory for Enzootic bovine leukosis*
Institute of Virology, Centre for Infectious Diseases, Faculty of Veterinary Medicine, Leipzig University, An den Tierkliniken 29, 04103 Leipzig, GERMANY
Tel: (+49-341) 97.38.201; Fax: (+49-341) 97.38.219
Email: Thomas.vahlenkamp@uni-leipzig.de
Designated Reference Expert: Prof. Thomas Vahlenkamp.

OIE *Reference Laboratory for Chronic wasting disease*
Prion Disease Research Laboratory, Division of Foreign Animal Disease, Animal, Plant and Fisheries Quarantine and Inspection Agency (QIA), 335 Anyang-ro, Manan-gu, Anyang, Gyeonggi, 430-757, KOREA (REP. OF)
Tel: (+82-31) 467.18.67; Fax: (+82-31) 467.18.30
Email: shonhj@korea.kr or shonhjoo@hanmail.net
Designated Reference Expert: Dr Hyun-Joo Sohn.

OIE *Reference Laboratory for Avian mycoplasmosis*
MYCOLAB (Laboratorio para diagnóstico de micoplasmas), Centro nacional de sanidad Agropecuaria, CENSA, San José de las Lajas, Provincia Mayabeque, CUBA
Tel: (+53-47) 86.33.14 ext. 153; Fax: (+53-47) 86.38.97
Email: elobo@censa.edu.cu or evelynlobo68@hotmail.com
Designated Reference Expert: Dr Evelin Lobo Riveroi.

A laboratory in Europe had sent an application to transfer its OIE Reference Laboratory for Paratuberculosis to another location within the country. Although the newly proposed laboratory revealed a high level of expertise, the application 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 experience in accordance with the terms of reference of an OIE Reference Laboratory.

At its February 2011 meeting, the Commission had considered an application from a laboratory in Europe for designation as an OIE Reference Laboratory for Q fever. The Commission had then asked to provide more information on its international activities. The Commission reviewed the extra information received, but maintained its position that the laboratory did not demonstrate the level of international collaboration and provision of expertise that was expected of an OIE Reference Laboratory. The application was therefore rejected[3].

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 had decided at its last meeting to collect more information on whether the laboratory was yet in a position to fulfil the mandate of an OIE Reference Laboratory. Following the advice received on the technical aspects of the dossier, the Commission agreed that it was premature to accord the laboratory OIE Reference Laboratory status.

1.2 Changes of experts in the List of Reference Laboratories

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

3 The Council, which met on 20–22 September 2011, expressed the view that applications for OIE Reference Laboratory status for Q fever should be encouraged, as there was none at the moment and OIE Member States needed urgently international expert support in dealing with this disease.

Avian influenza

Dr Chakradhar Tosh to replace Dr Shiv Chandra Dubey at the High Security Animal Disease Laboratory, Indian Veterinary Research Institute, Indian Council of Agricultural Research, Bhopal, INDIA.

1.3. New mandates and rules for OIE Reference Centres

The Commission noted with satisfaction that the newly adopted revised terms of reference and internal rules for OIE Reference Centres included many of the proposals of the *ad hoc* Group on Scientific Partnerships, most importantly the requirement to commit to the Centre's sustainability.

1.4. Updating the annual report template

Following the adoption of new terms of reference and internal rules for OIE Reference Centres, the Commission recommended that a question regarding sustainability be added to the annual report template, and that the question on production and supply of reference reagents be reworded to elicit more exact and useful responses.

1.5. Review of new and pending applications for laboratory twinning

Dr Keith Hamilton updated the Commission on the current status of the twinning programme. The Commission was satisfied about the technical content of the following twinning project proposals and felt that no further clarification was needed: Italy with Turkey for West Nile virus; United Kingdom with India for equine influenza; and France with Morocco for peste des petits ruminants. The Commission also reviewed a proposal for twinning between Ethiopia and United Kingdom for foot and mouth disease and, whilst satisfied with the principle of the proposal, the Commission agreed that the proposal should be resubmitted to the Commission in February 2012 with more detail about activities. The Commission agreed that Candidate Laboratories should focus on one application at a time.

1.6. Twinning Guide update

Dr Keith Hamilton updated the Commission on improvements to the twinning guidance document ("OIE Laboratories Twinning Guide") that reflect recommendations from twinning project audits and a twinning workshop held in Paris in March 2011; the Commission supported the changes to the Twinning Guide.

2. *Ad hoc* Groups

- **Past *ad hoc* Group meetings**

2.1. Reports of the three Meetings of the *ad hoc* Group on Vaccine Quality related to Foot and Mouth Disease (29–31 March 2011; 8–9 June 2011; 5–6 September 2011)

Prof. Caporale presented the reports of the three meetings of the *ad hoc* Group. Over the course of the three meetings the Group had devised a "skeleton" outline of what information needed to be included in the vaccine section of the chapter on FMD, and applied it to the current FMD chapter. It was hoped that this could be used as a template for all chapters on diseases for which vaccines existed and were used. Prof. Caporale requested that the reference to DIVA (detection of infection in vaccinated animals) strategies be removed from the text.

Further consultations revealed that the Group had not had the time to fully develop a comprehensive text regarding DIVA. It may therefore be envisaged to convene an *ad hoc* Group to draft a text either for the FMD chapter or as an independent chapter on this topic.

The Commission adopted the reports, the last one of which can be found at Appendix III of this report. The Commission agreed to circulate the proposed revised chapter for Member Country comment by **8 January 2012**.

2.2. Report of the Meeting of the *ad hoc* Group on Validation of Diagnostic Tests for Wildlife (27–28 April 2011)

Dr François Diaz presented the report of the meeting of the *ad hoc* Group. Of particular interest was the proposal to create a category of tests that were "provisionally validated" once the procedure had reached the beginning of stage 2. The Commission adopted the report, which can be found at Appendix IV of this report.

■ Planned *ad hoc* Groups

2.3. *Ad hoc* Group on the Biosafety and Biosecurity in Veterinary Laboratories

The Commission noted the Terms of Reference for this meeting. The *ad hoc* Group would meet at the OIE Headquarters from 19 to 21 September 2011.

■ Proposed *ad hoc* Groups

2.4. *Ad hoc* Group on Rift Valley Fever (Vaccines)

The Commission had already been aware that the vaccine section of the *Terrestrial Manual* chapter on Rift Valley fever was in need of an update and had listed this issue as top priority. The tentative dates for the meeting of this *ad hoc* Group were 6–8 December 2011.

2.5. *Ad hoc* Group on Vaccine Quality related to Rabies

Following the completion of the work of the *ad hoc* Group on Vaccine Quality related to Foot and Mouth Disease (see item 2.1 above), the next priority disease had been identified as rabies. The tentative dates for the meeting of this *ad hoc* Group were 10–12 January 2012.

2.6. *Ad hoc* Group on Vaccine Quality related to Classical swine fever

Classical swine fever was the third disease on the priority list for review of vaccine quality. The meeting could not be held before the next meeting of the Commission, but it was hoped that an ad hoc Group could be convened sometime after February 2012.

2.7. *Ad hoc* Group on Scientific Partnerships among OIE Reference Centres: networking

This *ad hoc* Group had already provided input to the Commission and through the Commission to the Council, which led to the adoption of revised terms of reference for OIE Reference Centres in May2011. The remaining task of this *ad hoc* Group was: 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). The tentative dates for the meeting of this *ad hoc* Group were 17–19 January 2012.

2.8. *Ad hoc* Group on How to include Genomic Sequencing in the OIE Information System

It was proposed to defer discussions on this item to the next meeting of the Commission. In the meantime, the item would be discussed internally at the OIE, in particular the OIE Animal Health Information Department would be asked to provide an opinion.

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 reiterated its request to the OIE Reference Laboratory for Rabies in Nancy, France (OIE Expert: Dr Florence Cliquet) to produce a new second set of OIE Standard Sera for rabies using the protocol that was reviewed by the Commission at its last meeting. The sera set should include a strong positive, a weak positive and a negative serum. If production of a weak positive proved problematic, other OIE Reference Laboratories would be approached for assistance.

The Commission reviewed and approved the final report and data sheet for a *Brucella suis* Positive International Standard Antiserum.

3.2. New mandates and rules: survey of reagents being produced

See Item 1.4 above.

3.3. Prescribed and Alternative Tests – update on application from Canada

Some time ago, the Canadian Food Inspection Agency, Ottawa Laboratory, had submitted a dossier entitled Application for Certification of a Monoclonal Antibody-Based Antigen Capture ELISA for Detection of *Campylobacter fetus* in Preputial Washings and Other Diagnostic Samples. The dossier had been evaluated by a validation expert and the OIE Designated disease expert for their opinion. As the test had been developed as a new test method and not in kit form, the OIE expert and author of the *Terrestrial Manual* chapter on Bovine genital campylobacteriosis had been asked to consider whether it should be included in the *Terrestrial Manual*. The expert had some further questions that would be submitted to the test developers. The Commission would review the dossier at its next meeting.

3.4. OIE Register of diagnostic tests: review of applications

Dr François Diaz updated the Commission on the current status of the OIE Procedure for Validation and Certification of Diagnostic Assays: dossiers that are in the pipeline, the number of fully approved kits on the register, etc.

He informed the Commission that the kit for confirmation and serotyping of presumptive *Salmonella* spp. had been adopted by the Assembly at the last General Session in May. He proposed for approval a summary of the validation studies carried out for the kit. The Commission gave its approval. The summary once approved by the kit manufacturer will be available on the OIE register webpage.

Dr Diaz also proposed some amendments to the Standard Operating Procedure (SOP) to clarify the pathway of the procedure, to streamline the renewal procedure and to harmonise with the current OIE editing policy. The Commission approved the proposed changes; the revised SOP will be forwarded for approval to the Director General of the OIE.

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. Follow-up from brainstorming meeting for modernising the *Terrestrial Manual*

Dr Beverly Schmitt updated the Commission on the discussions that took place during the brainstorming meeting for modernising the *Terrestrial Manual* (Paris, 12-13 September 2011). One of the main outcomes was to recognise the Manual as a manual of *Standards*; guidelines or other general recommendations should either be removed from the *Terrestrial Manual* or placed at the end in a section that is clearly separate. The Commission endorsed the report of the brainstorming meeting, which included a series of ideas and recommendations on how to improve the *Terrestrial Manual*. The report can be found at Appendix V of this report. It was agreed that this report would need further discussion at the next meeting of the Commission. Rather than implementing the report and its recommendations straightaway, it would be used as a guide and its recommendations would be implemented on a step-by-step basis.

4.2. Review of chapters proposed for adoption in May 2012

Chapters already sent: review of first round of comments

In March 2011, thirteen chapters had been sent for Member Country comment. The Consultant Editor had amended the chapters according to the comments received. Some of the comments concerned matters of policy and principle and for these the Editor requested the advice of the Commission. Once all the comments have been addressed, the chapters would be marked to show the new changes and would be sent again to Member Countries, this time as the final texts for which approval would be sought at the next meeting of the Commission.

The Commission agreed that the glossary in the *Terrestrial Manual* did not require an update as it did not conflict with the glossary in the *Terrestrial Code*.

At the February meeting, the Commission noted that a number of changes would need to be made to the *Terrestrial Manual* chapter on rinderpest in the light the declaration of global rinderpest freedom. 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 and had provided an updated chapter. The President of the Commission would have liked to receive some explanation of certain of the proposed amendments directly from the authors. The chapter has thus been put on hold until the next meeting when the experts would be invited to attend that agenda item.

Chapters ready to be sent for first round of comments

The Commission noted that a small number of chapters had been updated and would shortly be ready for first round circulation to Member Countries. If the schedule allowed the time, these might be ready for second circulation in time for proposal for adoption by the Assembly in May 2012.

4.3. Discussion on the validation chapter and appendices

The draft chapter on the Principles and methods of validation of diagnostic assays for infectious diseases and the seven ‘best practices’ appendices, which had been received at the last meeting were discussed. It was agreed that the best way forward would be to send the chapter and appendices to Member Countries for comment, in particular asking if the documents are clear or if and how they could be improved. All comments received, along with the report of the *ad hoc* Group on Validation of Diagnostic Tests for Wildlife (see item 2.2) and comments provided by the President of the Commission would be sent to the authors. The authors’ response to the comments would be reviewed at the next meeting of the Commission. The Commission would then decide whether or not to submit the Chapter and appendices for adoption by the World Assembly.

The Commission agreed that a paragraph on in-house validation could usefully be added to the chapter. The Commission believed that the concerns expressed by the Delegate of Cuba that the validation procedure was too complex had now been addressed in the chapter, which allowed for provisional validation at the beginning of stage 2.

5. Follow-up from General Session

5.1. Update on the status of two Collaborating Centres adopted at the General Session

The Commission noted the excerpt from the Final Report of the General Session explaining that two Collaborating Centres in the region of the Americas had been adopted by the Assembly even though their mandates were already partly covered by existing Collaborating Centres in the same region and their designation should be re-considered under the principle of designating no more than one Collaborating Centre per region per speciality, and exceptionally per sub region. The Council recommended and the Assembly agreed that both applicants seek conformity with the provisions in the revised Basic Texts. The Centres had been requested to submit revised dossiers by December 2011.

6. Conferences, Workshops, Meetings

6.1. Upcoming technologies for the early and rapid diagnosis of infectious diseases, IAEA, Vienna, Austria, 18–20 May 2011

Prof. Caporale reported to the Commission that he represented the OIE and the Commission at this meeting.

6.2. Perspectives of a potential global, real-time microbiological genomic identification system – implications for national and global detection and control of infectious diseases, Brussels, Belgium, 1–2 September 2011

Prof. Caporale represented the OIE and the Commission at this meeting. He stated that this was an important area and the OIE should keep track of developments in genomic sequencing. Prof. Caporale expressed his concern about the control of the sequence data: he believed that OIE Reference Laboratories should be encouraged to do whole genomic sequencing and place the resulting data in a databank such as the OIE information system databank (see item 2.8).

6.3. OIE/FAO/WHO Global Conference on Rabies Control, Incheon-Seoul, Korea, 7–9 September 2011

The Commission was satisfied that this Global Conference had been very successful. One of the priority issues identified was vaccine, in particular a genetically modified recombinant vaccine that protects against rabies and was a contraceptive device for controlling stray dog populations.

6.4. Workshop on Alternative Methods for Human and Veterinary Rabies Vaccine Testing: State of the Science and Planning the Way Forward, Ames, Iowa, 11–13 October

Prof. Caporale informed the Commission that he would represent the OIE and the Commission at this meeting.[4]

6.5. Veterinary Vaccines, Brussels, Belgium, 30 November–1 December 2011

Prof. Caporale would represent the OIE and the Commission at this meeting

6.6. Mission to South America (Ecuador and Brazil) to investigate vaccine matching

The Commission was reminded that FAO had been overseeing an FMD control and eradication programme in Ecuador. In accordance with OIE recommendations, vaccination campaigns had been organised and carried out, but the results had not been satisfactory and the FMD outbreaks continued. In response to the apparently limited effect of the vaccination campaigns, FAO sent field isolates to four laboratories: three OIE Reference Laboratories and an FAO Reference Centre. Three of the four found that the vaccine did not effectively protect against the strain circulating in the region (immunogenic coverage or vaccine-matching), the fourth found no such problems. That there was a difference of opinion between the world's leading laboratories, especially OIE Reference Laboratories, was considered problematic for the OIE.

4 Since the Commission meeting, Prof. Caporale had to cancel his participation in this Workshop.

It had been decided therefore to provide and publish an *official* OIE view on the problem. To do this, an expert mission to South America (Ecuador) would be organised under the auspices of the OIE Biological Standards Commission. This expert mission was planning to visit laboratories and conduct a 3-day field visit to veterinary services in Ecuador, focusing on the vaccine-matching question, followed by a 2-day debriefing at the Panaftosa headquarters in Brazil. It was envisaged that the mission would take place in November 2011 and would be led by Prof. Caporale[5].

6.7. Update on OFFLU

Prof. Edwards, Chair of the Steering Committee, updated the Commission on OFFLU - the joint OIE-FAO network of expertise on animal influenza, as chairperson of its Executive Committee. Both the OIE and the FAO had reaffirmed their commitment to supporting this influenza network; the WHO also maintained close ties with OFFLU. Following the Technical Meeting held in Rome, Italy last year, OFFLU was hoping to host a satellite symposium during the International Influenza Symposium that will be held in the UK in April 2012.

7. Liaison with other Commissions

7.1. Scientific Commission for Animal Diseases

Update on on-going issues or issues from last Biological Standards Commission meeting:

African horse sickness: the updated chapter for the *Terrestrial Code* was being finalised for proposal for adoption by the Assembly. The OIE Reference Laboratory experts would be asked to review the updated *Terrestrial Manual* chapter.

Surra: the *Terrestrial Manual* chapter was one of the chapters that would be circulated soon for Member Country comment (first round).

Crimean–Congo haemorrhagic fever – need for diagnostic methods: an author had been identified by the Biological Standards Commission and had agreed to provide a *Terrestrial Manual* chapter by the end of the year.

Rabies – vaccine section: this issue would be addressed by the *ad hoc* Group (see item 2.5).

Classical swine fever – problem with vaccines: this issue would be addressed by the *ad hoc* Group (see item 2.6)

Rinderpest – sequestration guidelines: see item 4.2.

Epizootic haemorrhagic disease (EHD): the OIE Reference Laboratory experts on Bluetongue had agreed on the need for a *Terrestrial Manual* chapter on EHD and had proposed to oversee the draft in collaboration with EHD experts.

Requests for consideration/noting by the Biological Standards Commission:

Report of the *ad hoc* Group on Peste des petits ruminants: Dr Joseph Domenech presented the report of this Group and noted that the Biological Standards Commission had not been consulted when the Terms of Reference were drafted. He assured the Commission that it would be involved in any future meetings of the Group as it would related to diagnostic and vaccines. In the meantime, the Commission accepted the revised *Terrestrial Manual* chapter.

Working Group on Wildlife Diseases – need to update *Terrestrial Manual* chapter on Zoonoses transmissible from non-human primates: the Biological Standards Commission agreed that this was a highly specialised area that would need to be addressed by a specialised Group at a future date.

5 Since the Commission meeting, the 3-day field visit Ecuador has been cancelled. The 2-day meeting at the Panaftosa Headquarters in Brazil will go ahead. The ultimate aim of the meeting remains unchanged: to provide the Director General with advice that could be formulated into an official OIE opinion vaccine matching in the region.

7.2. Terrestrial Animal Health Standards Commission

The Biological Standards Commission requested clarification from the Code Commission regarding its question on Aujeszky's disease vaccination.

8. Matters of Interest for Information

8.1. Need for an official OIE statement on the use of Thiomersal in animal vaccines

Those OIE Collaborating Centres dealing with vaccines would be asked if they had and could provide information on whether thiomersal was used in animal vaccines and if there were any toxicity data.

8.2. 19th Conference of the OIE Regional Commission for Africa, 14–18 February 2011: Recommendation Technical Item II: Main pathologies of camels, breeding of camels, constraints, benefits and perspectives

The first meeting of a network of camelid experts would be held in Teramo, Italy in October 2011.

8.3. Need to update OIE Booklet (Standard and Guidelines)

This agenda item would be discussed at the February 2012 meeting.

8.4. Update on VICH and on CAMEVET

This agenda item would be discussed at the February 2012 meeting

9. Any Other Business

9.1. Workplan and activities (as of September 2011)

See Appendix VI.

9.2. Dates of the next Biological Standards Commission meeting

The Commission noted the dates for its next meeting: 7–9 February 2012.

9.3. Other matters

This meeting was successfully held as the first "paper free" meeting of an OIE Specialist Commission, with all documentation provided electronically to the members of the Commission.

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.../Appendices

Appendix I

MEETING OF THE OIE BIOLOGICAL STANDARDS COMMISSION

Paris, 14–16 September 2011

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Agenda

1. OIE Reference Centres

1.1. Applications for the status of OIE Reference Centre
1.2. Changes of experts at OIE Reference Centres
1.3. New mandates and rules for OIE Reference Centres
1.4. Updating the annual report template
1.5. Review of new and pending applications for laboratory twinning
1.6. Twinning guide update

2. *Ad hoc* Groups

Past *ad hoc* Group meetings: reports for adoption:

2.1. Report of the three Meetings of the *ad hoc* Group on Vaccine Quality related to Foot and Mouth Disease
2.2. Report of the Meeting of the *ad hoc* Group on Validation of Diagnostic Tests for Wildlife (linked to item 4.3)

Planned *ad hoc* Groups:

2.3. *Ad hoc* Group on the Biosafety and Biosecurity in Veterinary Laboratories (to be held 19–21 September 2011)

Proposed *ad hoc* Groups: prioritisation of work and draft ToRs

2.4. *Ad hoc* Group on Rift Valley Fever (Vaccines)
2.5. *Ad hoc* Group on Vaccine Quality related to Rabies
2.6. *Ad hoc* Group on Vaccine Quality related Classical swine fever
2.7. *Ad hoc* Group on Scientific Partnerships among OIE Reference Centres: networking

3 International Standardisation/Harmonisation:

a) Diagnostic tests

3.1. Progress on the on-going standardisation programmes for reagents
3.2. New mandates and rules: survey of reagents being produced
3.3. Prescribed and Alternative Tests – update on application from Canada
3.4. OIE Register of diagnostic tests: review of applications

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

4.1. Follow-up from brainstorming meeting for modernising the *Terrestrial Manual*
4.2. Review of chapters proposed for adoption in May 2012:
Chapters already sent: review of first round of comments
Chapters ready to be sent for first round of comments
4.3. Discussion on the validation chapter and appendices (including follow-up from the letter from the OIE Delegate of Cuba [dated 10 January 2011] about the in-house validation of diagnostic test methods described by the *Terrestrial Manual* in veterinary laboratories)

5. Follow-up from General Session

5.1. Update on the status of two Collaborating Centres adopted at the General Session

6. Conferences, Workshops, Meetings

6.1. Upcoming technologies for the early and rapid diagnosis of infectious diseases, IAEA, Vienna, Austria, 18–20 May 2011
6.2. Perspectives of a potential global, real-time microbiological genomic identification system – implications for national and global detection and control of infectious diseases, Brussels, Belgium, 1–2 September 2011
6.3. OIE/FAO/WHO Global Conference on Rabies Control, Incheon-Seoul, Korea, 7–9 September 2011
6.4. Workshop on Alternative Methods for Human and Veterinary Rabies Vaccine Testing: State of the Science and Planning the Way Forward, Ames, Iowa, 11–13 October
6.5. Veterinary Vaccines, Brussels, Belgium, 30 November–1 December 2011
6.6. Mission to South America (Ecuador and Brazil) to investigate vaccine matching
6.7. Update on OFFLU

7. Liaison with other Commissions

7.1. Scientific Commission for Animal Diseases

Update on on-going issues or issues from last Biological Standards Commission meeting:

African horse sickness: validation of newly proposed diagnostic tests
Surra: differential diagnosis
Crimean–Congo haemorrhagic fever: need for diagnostic methods
Rabies: vaccine section
Classical swine fever: problem with vaccines
Rinderpest: sequestration guidelines
Epizootic haemorrhagic disease

Requests for consideration/noting by the Biological Standards Commission:

Report of the *ad hoc* Group on Peste des petits ruminants

Working Group on Wildlife Diseases – need to update *Terrestrial Manual* chapter on Zoonoses transmissible from non-human primates

7.2. Terrestrial Animal Health Standards Commission

Question on Aujeszky's disease

8. Matters of Interest for Consideration

8.1. Need for an official OIE statement on the use of Thiomersal in animal vaccines
8.2. 19th Conference of the OIE Regional Commission for Africa, 14–18 February 2011: Recommendation Technical Item II: Main pathologies of camels, breeding of camels, constraints, benefits and perspectives
8.3. Need to update OIE Booklet (Standard and Guidelines)
8.4. Update on VICH and on CAMEVET

9. Any Other Business

9.1. Workplan
9.2. Dates of the next Biological Standards Commission meeting: 7–9 February 2012
9.3. Other matters

MEETING OF THE OIE BIOLOGICAL STANDARDS COMMISSION

Paris, 14–16 September 2011

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Appendix III

Original English
September 2011

MEETING OF THE OIE *AD HOC* GROUP ON VACCINE QUALITY RELATED TO FOOT AND MOUTH DISEASE (FMD)

Paris, 5–6 September 2011

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The third meeting of the OIE *ad hoc* Group on Vaccine Quality related to Foot and Mouth Disease (FMD) was held at the OIE Headquarters, Paris from 5 to 6 September 2011. This meeting had been scheduled to complete the revision of Sections C and D of Chapter 2.1.5 Foot and Mouth Disease of the *Terrestrial Manual* that had been started at the two previous meetings on 29–31 March 2011 and 8–9 June 2011.

1. Opening and purpose of the meeting

Dr Kazuaki Miyagishima, Deputy Director General, welcomed the participants on behalf of Dr Bernard Vallat, the Director General of the OIE. He underlined the importance of this *ad hoc* Group by making reference to the OIE's initiative to establish an FMD vaccine bank for Asia, the development of an OIE/FAO Global Strategy for FMD Control, and the recent outbreaks of FMD in Africa and Asia.

2. Adoption of agenda and appointment of a chairman and a rapporteur

The meeting was chaired by Dr Michel Lombard and Dr Alf-Eckbert Füssel was designated as rapporteur. Dr Lombard introduced the provisional agenda, which was subsequently adopted by the Group. The adopted agenda and list of participants are attached as Appendices I and II, respectively.

3. Update and revision of Section C (Vaccine Matching Tests) and Section D (Requirements for Vaccines and Diagnostic Biologicals) of Chapter 2.1.5 Foot and Mouth Disease of the *Terrestrial Manual*

At its first meeting, the Group had developed a "skeleton" outline for a revised vaccine section for the FMD chapter (see Appendix III of this report). The Group had applied this template in its review of the FMD vaccine section and hoped, should it prove successful for FMD, to use it as a blueprint for other disease chapter vaccine sections, starting with rabies.

It was noted that the order of Section C and Section D had been reversed following discussion at the first meeting. Dr Lombard proposed to finalise the review of the last sub-sections of the "new" Section C on "Requirements for Vaccines" before continuing to work on the review of the first sub-sections of Section C.

The Group commenced its work with a brief review of comments made by participants on the amendments made to the first two parts of the new Section C during the June 2011 meeting. After discussion the Group agreed that for a 50% protective dose (PD_{50}) potency test, it was not acceptable to use animals that were seropositive for antibodies to any of the seven FMDV types.

There was extensive discussion on paragraph C.5.c. relating to *Purity* and the testing for antibody against non-structural proteins (NSP).

The Group agreed that the tests for purity should be carried out on at least eight cattle that might be the same cattle used for the safety test described in C.5.b. The sero-negative cattle should receive three single doses of vaccine at 3- to 4-week intervals. The vaccine should contain the maximum number of strains and amounts of antigens permitted on the authorisation. Cattle should be sampled for testing for antibody to NSP before each revaccination and between 30 and 60 days after the last administration of the vaccine.

The Group considered the test scheme as sufficiently sensitive to support a claim that the vaccine would not induce antibody to NSP for the number of injections tested.

In this respect, the Group also discussed the sensitivity and specificity of tests prescribed for the detection of antibody to NSP. Dr Onkabetse George Mathlo pointed to the increasing importance of NSP testing in SAT (Southern African Territory) virus settings and requested an OIE expert group to address the performance of available NSP tests under SAT virus settings. Dr Susanne Münstermann made the Group aware of a literature review carried out by Dr P. Roeder in the framework of the EU-funded SADC[1]-FMD project that indicated that the performance of available NSP test kits was less sensitive and specific following infection with SAT viruses as compared with infection with FMD viruses of the A, O and Asia serotypes.

The Group then agreed that the *Duration of immunity* (C.5.d) was a relevant criterion for the selection of a vaccine and therefore adequate information based on appropriate studies was necessary in a vaccine registration dossier to justify the vaccination scheme recommended by the manufacturer. As regards the interference with maternal antibodies, the Group agreed that field data on the dynamics of maternal antibodies should also be provided, as such field data better reflect the epidemiological situation.

The *Stability* requirements in paragraph C.5.e were reviewed to make them consistent.

The Group agreed that point C.5.f on *Preservatives* was better placed in Section C.2. on *Method of manufacture* and moved the text there for subsequent revision.

As regards Section C.6. on *Storage and monitoring of antigen concentrates*, the Group agreed to link this intermediate product ("concentrated antigens") to the text provided in C.2. on *Method of manufacture*. The Group then introduced a reference to Chapter 1.1.10, Guidelines for International Standards for Vaccine Banks, which also provided guidance for the storage of antigens of the FMD virus.

It was furthermore decided to abandon the entire paragraph C.6.a on *Prestorage conditions* by removing certain redundancies in that point and inserting the text on "emergency procedures for provisional acceptance of new MSV, and subsequent release of formulated vaccines" as a new paragraph (d) in Section C.1. on the *Seed virus management*.

The remaining part of Section C.6. was then divided into *Storage conditions* (C.6.a) and a simplified point on *Monitoring* (C.6.b) for which requirements for representative samples of the stored antigens were included.

In addition, a new Section C.7. on *Emergency release of vaccines prepared from concentrated antigens* was added that included provisions based on European Pharmacopoeia requirements for emergency release of a vaccine reconstituted from previously tested concentrated antigens.

In paragraph a) of C.1. *Characteristics of the seed virus*, the text required the use of an official recommended nomenclature that did not exist as such. To solve the problem, the Group agreed on a "unique" nomenclature instead.

As a consequence of the wording finally introduced, the Group agreed to recommend the OIE to address the need for an acceptable nomenclature for all viruses mentioned in the *Terrestrial Manual* and *Terrestrial Code.*

In relation to paragraph c) of C.1. *Validation as a vaccine strain*, the Group recalled that it had concluded at the meeting in June 2011 that:

> "The extent of characterisation of the master seed virus required (antigenic and/or genetic) was also extensively discussed. Observers from International Federation for Animal Health (IFAH) were strongly of the opinion that genetic characterisation added no significant advantages but other participants thought that this information could facilitate selection of vaccine strains and also tracing of vaccine virus strains. No definitive conclusion could be reached and final decision was deferred to a later time."

1 SADC: Southern African Development Community

Following up on this recommendation the Group discussed the need for genetic characterisation as a prerequisite for the selection of a master seed and concluded that the isolates must be well characterised, without pointing particularly to antigenic and genetic characterisation. The isolates for the selection of master seed strains should preferably come from and be characterised by an OIE Reference Laboratory.

Paragraph c) of C.3. *Non-capsid proteins control* stated that there was currently no OIE-validated test for testing for non-capsid proteins in industrial inactivated antigens, although several commercially available tests were used in practice. As this statement did not contain any obligation or requirement, the Group agreed to delete paragraph C.3.c.

In paragraph C.4. *Final product batch test*, the Group agreed to delete the introductory sentence: "Both, inactivated antigen as well as formulated vaccine, can be considered as final product.", as it did not provide any additional information. Instead, a reference to *concentrated antigens* was introduced to maintain consistency in terminology.

The Group took note of a general problem with any reference to "Good Manufacturing Practice" or "GMP" as this was not necessarily a globally accepted standard. The Group agreed to use in appropriate text passages a reference to standard requirements mentioned in Chapter 1.1.8 of the *Terrestrial Manual*, for example in paragraph C.4.c *Viral Non Structural Protein testing:*

> Non-structural proteins refer to proteins not present in the FMD viral capsid. Only products claiming to be purified from NSPs have to demonstrate their level of purification. Unless consistency of purification is demonstrated and approved in the registration dossier, and the production process is approved for consistency in accordance with the standard requirements referred to in Chapter 1.1.8, NSP lack of reactivity has to be demonstrated in the final product (see Section C.5. *Requirements for registration of vaccine*).

Small changes were made to safety testing, in particular the administration of a double dose was replaced by the administration of the *recommended* dose.

Discussing paragraph C.4.e on *Potency*, the Group concluded that the Expected Percentage of Protection (EPP) procedure should not appear in paragraph C.5.iv on *Efficacy* as it was nowhere included in the registration file, but should be added to section C.4. on *Final product batch test* because it was used as such in South America. The Group therefore agreed on a revised paragraph C.4.e on *Potency* that contained a shortened description of the EPP procedure.

The Group reviewed Section C.5 on *Requirements for registration of vaccine* and made minor editorial modifications. The Group agreed on a modified definition of DIVA as Detection of Infection in Vaccinated Animals.

The Group finalised the draft text for Section C *Requirements for Vaccines* and the new Section D *Vaccine matching tests*, as attached at Appendix IV of the present report for Member Country comment by **8 January 2012**.

Prof. Vincenzo Caporale pointed out that Chapter 1.1.2 of the *Terrestrial Manual* – Biosafety and Biosecurity in the Veterinary Microbiology Laboratory and Animal Facilities – required revision.

4. Other matters

The Group reviewed the OIE template for FMD-vaccine tenders and finalised an amended ~~version~~proposal.

5. Finalisation and adoption of the draft report

The Group reviewed the preliminary outline of the draft report provided by the rapporteur. The Group agreed that the draft report and revised parts of the chapter on FMD would be subject to a short period of circulation within the Group for comments and final adoption by participants.

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…/Appendices

Appendix I

MEETING OF THE OIE *AD HOC* GROUP ON VACCINE QUALITY RELATED TO FOOT AND MOUTH DISEASE (FMD)

Paris, 5–6 September 2011

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Agenda

1. Opening and purpose of the meeting
2. Adoption of the agenda and appointment of a chairman and a rapporteur
3. Update and revision of Section C (vaccine matching tests) and Section D (requirements for vaccines and diagnostic biologicals) of Chapter 2.1.5 Foot and Mouth Disease of the *Terrestrial Manual*
4. Other matters
5. Finalisation and adoption of the draft report

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Appendix II

MEETING OF THE OIE *AD HOC* GROUP ON VACCINE QUALITY RELATED TO FOOT AND MOUTH DISEASE (FMD)

Paris, 5–6 September 2011

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Appendix III

"Skeleton" outline for a revised vaccine section for the FMD chapter[2]

C. REQUIREMENTS FOR VACCINES

1. Introduction

OIE general policy

Short introduction of new OIE endorsement programme

Appropriate strain selection

Compliance with OIE *Terrestrial Manual* (Chapter1.1.8) + biosecurity

Vaccines for regular vaccination

Vaccines for emergency vaccination

Vaccines for new strains ≠ current vaccine strains

Antigen for banks (test as a vaccine), consideration of the different combinations

Multi-strain aspect

(Purity), non-structural protein (NSP) interference

2. Outline of production and minimum requirements for FMD vaccines and antigen

Generalities about biosecurity of the buildings (FAO) + former version + OIE chapter

Basic steps in outline of production with specific focus on interest points and quality assurance/quality control (QA/QC) aspects - approved cell line + explants problems

Preference for ingredients – sera – products of biological origin (POBs)

a) Characteristics of the seed

Attention to the OIE listed transmissible spongiform encephalopathies (TSEs) + former text

i) *Biological characteristics*

Reference to vaccine/field strain matching

Former text

ii) *Quality criteria* (*sterility, purity, freedom from extraneous agents*)

Keep former text

b) Method of manufacture

Former text without primary culture

One sentence at the end for explanation of method

i) *Procedure*

Former text

ii) *Requirements for substrates and media*

Salts – pharmacological grade quality

POBs – see chapter 1.1.9 (should be revised and updated)

Antibiotics – see chapter 1.1.8 (should be revised and updated)

2 This "skeleton" outline for a revised vaccine section for the FMD chapter had been developed by the Group and applied to the FMD vaccine section. Should it prove successful for FMD, it would be used as a blueprint for other disease chapter vaccine sections, starting with the rabies chapter.

Preservatives – see chapter 1.1.8 (should be revised and updated)

Cells – see chapter 1.1.8 master cell stocks (to be revised)

- Sentence on the Frenkel method

iii) *In-process controls*

Former text

iv) *Final product batch tests*

Consider antigen + vaccine

- Sterility/purity

  Sterility: put in general chapter, but does not currently exist in chapter 1.1.8, nor in 1.1.9 – new paragraph on sterility needed in 1.1.8, protocols in 1.1.9

  Purity: Tests on the final product, C) purity,

  Compromise

  QA/QC scheme + registration

  Batch control NSP – risk low

  In absence of NSP detection in inactivated bulk antigen

  Non-QA/QC scheme

  Batch control NSP – risk high

  90 days

- Safety (p.211 of the *Terrestrial Manual*)
- Batch potency

  In-vivo tests: target animals

  In-vitro tests: serological method enzyme-linked immunosorbent assay (ELISA), virus neutralisation test (VNT)

c) Requirements for authorisation/licensing (former text: tests on the final product)

Benefits of QA/QC & good manufacturing practice (GMP) scheme

Three consecutive batches, more than 1/3 industrial batch volume

i) *Safety requirements*

Innocuity

Safety – target animals, one dose eight animals (normal, pregnant, minimum age)

Max 146s + max valencies

Repeated doses PV + booster 1 + booster 2

Environmental consideration – No

Residues/food animal problem

ii) *Efficacy requirements*

- Efficacy at T_0 – Potency test, vaccination-challenge test, per strain? type?; target animals
- Duration of efficacy- indirect

iii) *Stability = shelf life = batch release test*

Three different batches

d) FMD combined vaccine: FMD components shall be checked following the above requirements point by point

D. VACCINE MATCHING TESTS

1. Introduction

Mention OIE FMD Reference laboratories

Update literatures

Definition Potency/Efficacy

Antigen drift FMDV

Presentation general for methods (immunological, serological)

Former text

2. Selection of field viruses for vaccine matching

According to:

Chapter 2.1.5. Section B, Diagnostic Techniques

Chapter 1.1.1. Collecting and shipment of diagnostic specimens

At natural level

At laboratory level (OIE level 4)

3. Selection of vaccine strains

Former text

4. Vaccine matching tests

Introduction, golden standard, laboratory

a) Field strain characterisation

r_1 value

Keep the order of former text

ELISA (ref. literature)

VNT (ref. literature)

b) Fitness for purpose

Challenge

EPP (ref. literature)

Appendix IV

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Draft Text Chapter 2.1.5 (Sections A and B of the Chapter remain unchanged)

CHAPTER 2.1.5.

FOOT AND MOUTH DISEASE

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C. ~~D~~ REQUIREMENTS FOR VACCINES ~~AND DIAGNOSTIC BIOLOGICALS~~

The control of FMD is usually a national responsibility and, in many countries, the vaccine may be used only under the control of the Competent Authority.

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. Varying requirements relating to quality, safety and efficacy apply in particular countries or regions in order for manufacturers to obtain an authorisation or licence for a veterinary vaccine. Where possible, manufacturers should seek to obtain such a license or authorisation for their FMD vaccines as independent verification of the quality of their product.

Virulent FMDV must be used to produce FMD vaccine; consequently, the FMD vaccine production facility should operate under the appropriate biosecurity procedures and practices. The facility should meet the requirements for Containment ~~Group 4 pathogens as~~ outlined in Chapter 1.1.2 of this *Terrestrial Manual.*

Routine vaccination against FMD is used in many countries or zones recognised as free from foot and mouth disease with vaccination and in countries where the disease is endemic. In contrast, a number of disease-free countries have never vaccinated their livestock but have preferred the use of strict movement controls and culling of infected and contact animals when outbreaks have occurred. Nevertheless, many disease-free countries maintain the option to vaccinate and have their own strategic reserves of highly concentrated inactivated virus preparations. Such antigen reserves offer the potential of supplying formulated vaccine in an 'emergency' at short notice (Doel *et al.*, 1994). Chapter 1.1.10 of this *Terrestrial Manual* provides Guidelines for international standards for vaccine banks.

FMD vaccines may be defined as a fixed formulation containing defined amounts (limits) of one or more chemically inactivated cell-culture-derived preparations of a seed virus strain blended with a suitable adjuvant/s and excipients.

Antigen banks may be defined as stockpiles of antigen components, registered or licensed according to the finished vaccine, and which can be stored for a very long time for subsequent formulation into vaccine as and when required.

The vaccines are formulated for their specific purpose and in the case of vaccines destined for use in cattle, both aluminium hydroxide saponin adjuvanted and oil adjuvanted vaccines may be used. For use in swine, double oil emulsions are preferred due to their efficacy. ~~Oil adjuvanted vaccines in ruminants can also be used in ruminants and may have advantages in terms of less interference from maternal antibody and a longer duration of immunity.~~

FMD vaccines may be classified as either 'standard' or 'higher' potency vaccines. Standard potency vaccines are formulated to contain sufficient antigen to ensure that they meet the minimum potency level required (recommended at Section D.4.b as 3 PD_{50} [50% protective dose]) for the duration of the shelf life claimed by the manufacturer. This kind of vaccine is usually suitable for use in routine vaccination campaigns. For vaccination in naïve populations to control FMD outbreaks, higher potency vaccines (e.g.> 6 PD_{50} for the duration of the shelf life claimed by the manufacturer) are recommended for their wider spectrum of immunity as well as their rapid onset of protection.

~~Higher potency vaccines are formulated with an increased amount of antigen such that the potency is in excess of the minimum requirement to provide particular features such as a more rapid onset of immunity, and a wider spectrum of immunity against relevant field viruses. Higher potency vaccines are thus well suited for emergency use.~~

~~Live FMD vaccines are not acceptable due to the danger of reversion to virulence and as their use would prevent the differentiation of infected from vaccinated animals.~~

Conventional live FMD vaccines are not acceptable due to the danger of reversion to virulence and as their use would prevent the detection of infection in vaccinated animals.

Because of the presence of multiple serotypes of the virus ~~many FMD vaccines are multivalent and~~ it is common practice to prepare vaccines from two or more different virus serotypes ~~strains~~. In certain areas, it may be advisable to include more than one virus strain per serotype to ensure broad antigenic coverage against prevailing viruses.

1. Seed virus management

a) Characteristics of the seed virus

Selection of master seed viruses (MSVs) should ideally be based on their ease of growth in cell culture, virus yield, stability and broad antigenic spectrum (Samuel *et al.*, 1990). Isolates to prepare MSVs should be characterised and distributed, preferably by the ~~official control~~ OIE FMD Reference laboratories ~~in regions where such laboratories exist~~; they should be selected in accordance with the epidemiological importance of each variant.

The exact source of the isolate should be recorded and should include details such as the location, species and the type of material from which the virus was derived. Unique nomenclature should be used to identify the FMDV strain. The *in-vitro* passage history of the virus and details of main components should be recorded in accordance with Chapter 1.1.8. of this *Terrestrial Manual.*

b) Method of culture

Methods of culture shall comply with the Chapter 1.1.8 of this *Terrestrial Manual*. Where no suitable established vaccine strain exists, new vaccine strains are derived through the establishment of MSVs from local field isolates by adapting them to growth in suspension or monolayer cells by serial passage. In order to remove the risk of contaminating lipid-enveloped viruses, it is recommended that putative MSVs undergo a validated organic solvent treatment prior to, or during, adaptation. ~~It is preferable to keep the number of passages in cell culture to a minimum as there is evidence of antigenic 'drift' of FMDV during this procedure.~~

c) Validation as a vaccine strain

MSVs must be ~~antigenically and, if possible genetically~~, well characterised and proven to be pure and free from all extraneous agents in accordance with Chapter 1.1.9 and those listed by the appropriate licensing authorities. Homology should be established with the original candidate isolates and effectiveness against the circulating strains from which they were developed should be proven. This often encompasses a number of methods, the most reliable being *in-vivo* ~~cross~~ protection assays. Alternatively, *in-vitro* tests (preferably virus neutralisation) can also be used, which require the availability of post-vaccination sera against these master seeds (see Section D of this chapter).

Seed viruses may be stored at ~~–20°C if glycerinated or at a lower~~ low temperature (e.g. –70°C) ~~if not glycerinated~~ or freeze-dried. Working seed viruses may be expanded in one or a few more passages from the master seed stock and used to infect the final cell culture ~~at an approximate rate of 1 PFU (plaque-forming unit) per 100 cells. Whenever possible, the exact source of the isolate should be recorded and should include details such as the location, species and the type of material from which the virus was derived. The *in-vitro* passage history of the virus should be recorded.~~

Consideration should also be given to minimising the risk of transmission of transmissible spongiform encephalopathy agents (TSEs) by ensuring that TSE risk materials are not used as the source of the virus or in any of the media used in virus propagation

d) Emergency procedures for provisional acceptance of new MSV, and subsequent release of formulated vaccines

In the case of incursion in a region of a new strain that is antigenically distinct from existing vaccine strains, it may be necessary to develop a new vaccine strain from a representative field isolate. Before the new MSV can be accepted, full compliance should be demonstrated with the relevant guidelines to demonstrate freedom from all extraneous agents listed by the appropriate licensing authorities using both general and specific tests, and to establish homology to the original candidate isolates. The time taken to raise the specific antisera necessary to neutralise the new strain for use in the general tests for detection of extraneous agents and to conduct other specific tests that require specialised techniques may be lengthy. Therefore, in emergency situations where there is insufficient time to complete full testing of the MSV, provisional acceptance of the new strain should be based on a risk analysis of the possibility of contamination of the antigen produced from the new MSV with

extraneous agents. This risk assessment should take into account that a validated procedure to inactivate enveloped viruses must be used when establishing the MSV and that the virus is inactivated using a chemical inactivant with first order kinetics. Further assurance is provided by the requirement for the kinetics of inactivation to be monitored and recorded for each production batch.

In order to accelerate the release of batches of vaccine formulated to contain new vaccine strains, it may be acceptable for batch potency testing to be carried out using a vaccine formulated using an intermediate antigen lot pending production of all of the batches of antigen that are intended to constitute the final antigen lot. This will allow the potency of antigen derived from a new MSV to be determined whilst the manufacturer continues to build up stocks of this new antigen.

2. Method of manufacture

The recommended method of virus propagation for antigen production is the growth of FMDV in large-scale suspension cultures or monolayers using cell lines under sterile conditions.

Cattle tongue epithelium in surviving conditions in medium with salts but without products of biological origin, may be acceptable for vaccine production but only if the method of production is entirely compliant with the standard requirements referred to in Chapter 1.1.8 of the *Terrestrial Manual*. In addition, in order to remove the risk of contaminating lipid-enveloped viruses, the harvested virus suspension must undergo a validated organic solvent treatment prior to BEI/EI inactivation. A validated procedure is applied to ensure inactivation of all possible extraneous agents and each batch is independently tested in an official laboratory for absence of extraneous agents. Adequate in-process and final products tests are in place to ensure consistency and safety of the final product. Consideration should also be given to minimising the risk of transmission of transmissible spongiform encephalopathy agents (i.e. BSE) by ensuring safe sourcing of the epithelium.

~~Primary cell culture may be acceptable for vaccine production in some countries but only if the method of production is entirely compliant with Good Manufacturing Practice, a validated procedure is applied to ensure inactivation of all possible extraneous agents and adequate in-process and final products tests are in place to ensure consistency and safety of the final product. It is essential that all pipework and vessels be thoroughly sterilised ensuring that no areas in the system harbour microorganisms. In addition to general considerations of sterility, it is important to note that the virus is vulnerable to attack by proteolytic enzymes, such as those produced by microorganisms (Doel & Collen, 1982). Control of pH and temperature are also critical because of the acid and temperature lability of the virus (Doel & Baccarini, 1981). Optimum temperature for cell, virus growth and inactivation, normally around 37°C and 26°C, respectively, should be precisely controlled. During other stages of manufacture, the temperature should be reduced to 4–6°C. Virus should be maintained at approximately pH 7.6 and should never be below pH 7.0.~~

A suitable strain of the virus is used to infect a suspension or monolayers of an established cell line, such as BHK. Such cell cultures should be proven to be free from contaminating microorganisms.

It is common practice to keep stocks of BHK cells over liquid nitrogen and revive as necessary. On revival, they are expanded in nutrient medium to a volume and cell density appropriate to seeding the main culture. ~~As an approximation, the main culture is seeded to give an initial density of 0.2–0.5 × 10^6 cells/ml, which is allowed to multiply to 2–5 × 10^6 cells/ml before being infected with virus.~~

When the virus ~~has~~ is expected to have reached its maximum ~~titre, which is variously determined by infectivity, CF or other tests~~ yield, the culture is clarified, often by chloroform treatment followed by centrifugation and/or filtration. The virus is subsequently inactivated by addition of an inactivant of first order, usually ethyleneimine (EI) in the form of binary ethyleneimine (BEI) ~~This is usually prepared by dissolving, to a concentration of 0.1 M, 2-bromoethylamine hydrobromide in 0.2 N sodium hydroxide solution, and incubating at 37°C for 1 hour~~ (Bahnemann, 1975; 1990). It is important that the necessary safety precautions for working with BEI/EI are fully observed.

The BEI ~~formed~~ is ~~then~~ added to a virus suspension ~~held at 26°C~~, to give a predetermined final concentration ~~of 3 mM~~. Inactivation ~~is usually continued for 24 hours, followed by a second dose of BEI for a further 24 hours~~ must be duly validated and documented to show the inactivation kinetic and the results of the inactivation controls. The time period for BEI treatment and temperature used for inactivation must be validated for the actual conditions and equipment used. ~~After inactivation any residual BEI in the harvest can be neutralised by adding sodium thiosulphate solution to a final concentration of 2%.~~

To decrease the likelihood of live virus failing to contact the ~~EI at the second application~~ inactivant, e.g. EI/BEI, it is essential to transfer the vessel contents immediately to a second sterile vessel where inactivation is allowed to go to completion ~~at 48 hours~~ according to the validated inactivation kinetic and taking into account possible regulatory requirements for additional waiting times.

During inactivation, the virus titre is monitored by a sensitive and reproducible technique. The inactivation procedure is not satisfactory unless the decrease in virus titre, plotted logarithmically, is linear and extrapolation indicates that there is less than 1 infectious virus unit per 10^4 litres of liquid preparation at the end of inactivation.

After inactivation any residual EI/BEI in the harvest can be removed, or neutralised, for example by adding excess sodium thiosulphate solution to a final concentration of 2%.

The inactivated virus may be concentrated/purified by procedures such as ultrafiltration, polyethylene glycol ~~precipitin~~ precipitation or polyethylene oxide adsorption (Adamowicz *et al.,* 1974; Wagner *et al.,* 1970). Concentrated inactivated virus may be purified further by procedures such as chromatography. These concentrated and purified antigens can be ~~kept at –70°C or lower~~ formulated into vaccines or stored at low temperatures for many years ~~if necessary~~, and made into vaccine when required by dilution in a suitable buffer and addition of adjuvants (Doel & Pullen, 1990).

Conventional FMD vaccines are usually formulated as oil adjuvanted or aqueous. ~~The aqueous vaccine, which is most commonly used for cattle is prepared by adsorbing the virus on to aluminium hydroxide gel, one of the adjuvant constituents of the final vaccine blend. Other components of the final blend include antifoam, phenol red dye (if permitted by the country requiring vaccine), lactalbumin hydrolysate, tryptose phosphate broth, amino acids, vitamins and buffer salts. A second adjuvant, saponin, derived from the South American tree *Quillaja saponaria mollina*, is also incorporated, as well as a preservative such as merthiolate or chloroform.~~

Oil-adjuvanted vaccines are usually formulated as water-in-oil emulsion using mineral oils, such as Marcol and Drakeol ~~as adjuvants. These preparations offer a number of advantages over the standard aluminium hydroxide/saponin vaccine, not least of which is their efficacy in pigs. They are widely used for vaccinating cattle in South America because of the longer duration of immunity~~. The mineral oil is usually premixed with an emulsifying agent ~~such as mannide monooleate,~~ before the addition of a proportion, or all, of the aqueous phase of the vaccine, and emulsified by use of a colloid mill or continuous mechanical or flow ultrasonic emulsifier.

More complex double emulsions (water/oil/water) may be produced by emulsifying once more in an aqueous phase containing a small amount of detergent such as Tween 80 (Barnett *et al.,* 1996, Doel *et al.,* 1994; Herbert, 1965).

The aqueous vaccine is prepared by adsorbing the virus on to aluminium hydroxide gel, one of the adjuvant constituents of the final vaccine blend.

The final blend of the vaccine can include other components, such as antifoam, phenol red dye, lactalbumin hydrolysate, tryptose phosphate broth, amino acids, vitamins, buffer salts and other substances. An adjuvant, such as saponins, can also be incorporated, as well as preservatives.

~~The most commonly used preservatives are chloroform.~~ Preservatives may be used as long as their usefulness as a preservative and absence of interference with FMDV antigen has been properly demonstrated. ~~and merthiolate. The latter is used at a final concentration of 1/30,000 (w/v)~~.

~~A further alternative are the 'ready to use' oil adjuvants. Oils containing esters of octadecenoic acid and 2,5 anhydro-d mannitol, for example, readily form double or mixed emulsions (water/oil/water) that are both stable and of low viscosity, without the requirement of sophisticated emulsification equipment (Barnett *et al.,* 1996, Doel *et al.,* 1994).~~ When using novel components, including adjuvants or preservatives, in any vaccine it is important to take into account that its status with regard to residues in products derived from food-producing species must be assessed to ensure that adequate assurance can be giving to licensing authorities in relation to safety for consumers. This requirement limits considerably the choice of adjuvants and preservatives for use in food-producing species.

3. In-process control

In general, virus titres reach optimum levels within about 24 hours of the cell culture being infected. The time chosen to harvest the culture may be based on a number of assays; for instance cell death. Virus concentration may be assessed by an infectivity test, sucrose density gradient (Bartelling & Meloen, 1974; Fayet et al., 1971 ~~Doel *et al.,* 1982~~) or serological techniques. It is preferable to use a method for measuring antigenic mass, such as sucrose density gradient analysis, as well as one that measures infectivity, as the two properties do not necessarily coincide and the different methods may complement one another.

a) Inactivation kinetics

During inactivation of the virus, timed samples should be taken at regular intervals for the purpose of monitoring the rate and linearity of the inactivation process. Virus titres in the samples are determined by inoculation of cell cultures proven to be highly susceptible to FMDV, e.g. BHK ~~or bovine thyroid cells~~. Such cultures permit the testing of statistically meaningful samples under reproducible conditions. The $\log_{10}$ infectivities of the timed samples are plotted against time, and the inactivation procedure is not considered to be satisfactory unless at least the latter part of the slope of the line is linear and extrapolation indicates that there would be less than one infectious particle per 10^4 litres of liquid preparation at the end of the inactivation period.

~~**4. Tests on the final product**~~

b) Inactivation control

The test for innocuity is an in-process test that should be carried out for every batch of antigen. Cells used to test for absence of residual live virus are not suitable if use of an amount of virus corresponding to 1 µg of 146S antigen gives a titre of less than 10^6 $TCID_{50}$ (European Pharmacopoeia, 2008). Following inactivation, a sample of each batch of inactivated antigen representing at least 200 doses should be tested for freedom from infectious virus by inoculation of sensitive monolayer cell cultures, preferably of the same origin as those used for the production of antigen. It may be preferable to concentrate the antigen to do this, in which case it must be shown that the concentrated material does not interfere with the sensitivity or reading of the assay. The cell sheets are examined daily over a period of 3 days, after which the spent medium is transferred to fresh monolayers and the original monolayers are replenished with fresh medium. Using this method, traces of live virus can be amplified by the passage procedure and detected on the basis of CPE observed. Two to three passages of the original virus preparation are commonly used. A variant on this method is to freeze–thaw the old monolayers to release intracellular virus, which can be detected by further passage.

4. Final product batch tests

a) Sterility

The bulk inactivated antigen, concentrated antigen and the final formulated product should undergo sterility testing. The preferred method is to collect any contaminating microorganisms by membrane filtration of the material to be examined and to detect any organisms present by incubation of the membranes with culture media. This procedure allows the removal of preservatives, etc., which may inhibit the detection of microorganisms. Guidelines on techniques and culture media, which allow the detection of a wide range of organisms, are described in the European Pharmacopoeia (2008) (also refer to Chapter 1.1.9 Tests for sterility and freedom from contamination of biological materials).

b) Identity testing

The bulk inactivated antigen, concentrated antigen and the final formulated product should undergo identity testing to demonstrate that the relevant strains are present. No other FMD virus serotype registered on the manufacturing site should be present in the vaccine, to be assured by adequate tests.

c) Viral nonstructural protein testing

Non structural proteins refer to proteins not present in the FMD viral capsid. Only products claiming to be purified from NSPs have to demonstrate their level of purification. Unless consistency of purification is demonstrated and approved in the registration dossier, and the production process is approved for consistency in accordance with the standard requirements referred to in Chapter 1.1.8 of the *Terrestrial Manual,* NSP lack of reactivity has to be demonstrated in the final product (see Section C.5. Requirements for registration of vaccine).

Confirmation of vaccine purity may be shown by testing sera from animals vaccinated at least twice with the batch for absence of antibodies to non structural proteins.

d) Safety

Unless consistent safety of the product is demonstrated and approved in the registration dossier and the production process is approved for consistency in accordance with the standard requirements referred to in Chapter 1.1.8 of the *Terrestrial Manual*, batch safety testing is to be performed.

This final product batch safety test is conducted to detect any abnormal local or systemic adverse reactions.

For the purposes of batch release, each of at least two healthy sero-negative target animals is inoculated by the recommended route of administration with the recommended dose of vaccine. The animals are observed for local and systemic reactions to vaccination for no fewer than 14 days. Any undue reaction attributable to the vaccine should be assessed and may prevent acceptance of the batch. If the potency test is performed in the target species, observation of the safety during this test can also be considered as an alternative to the batch safety test described above.

e) Potency

Potency is examined on the final formulated product, or alternatively for antigen banks on a representative batch of vaccine prepared from the same bulk inactivated antigen.

The potency testing standard is the vaccination challenge test. However, for batch release indirect tests can also be used for practicability and animal welfare considerations, as long as correlation has been validated to percentage of protection in the target animal. Frequently indirect potency tests include antibody titration after vaccination of target species. Alternative methods could be used if suitably validated.

Ideally, indirect tests are carried out for each strain for one species and each formulation of vaccine to establish correlation between the indirect test results and the vaccine efficacy.

i) *Expected percentage of protection* (*EPP*)

The EPP estimates the likelihood that cattle would be protected against a challenge of 10,000 infective doses after a single vaccination.

- Individual sera collected 30–60 days post-vaccination using a full dose of the vaccine are required from a group of either 16 or 30 18–24 month-old cattle.
- This panel of sera and sera of two control cattle are tested for antibody titres to the homologous FMD vaccine strain a strongly correlated LPB-ELISA (see Sections B.2.a and B.2.c).
- The antigens used in the ELISA may be inactivated using BEI.
- The EPP is determined by reference to predetermined tables of correlation between serological titres and clinical protection.
- Batches with at least 75% EPP (with 16 vaccinated cattle) or at least 70% EPP (with 30 vaccinated cattle) are satisfactory for potency.

The presence of more than one serotype in a vaccine does not diminish the induction of antibodies against another serotype or the correlation of antibody titre with protection.

ii) *Other methods for evaluating protection*

Other tests were published using different ELISA methods and VNT methods to indirectly evaluate the protection given by vaccines. Their results could be accepted only if a strong correlation with protection in relation to the vaccine strain being tested and the serological method being used has been scientifically demonstrated and published in a peer reviewed journal (Ahl *et al.,* 1990)

5. Requirements for registration of vaccine

a) Manufacturing process

For registration of vaccine, all relevant details concerning manufacture of the vaccine and quality control testing (see Sections C.1–4) should be submitted to the authorities. This information shall be provided from three consecutive vaccine batches with a volume not less than 1/3 of the typical industrial batch volume.

~~a)~~b) Safety

~~Tests for innocuity (non-infectivity) are most effectively carried out on the bulk, concentrated, inactivated viral harvest (see Sections D.3 and D.5.b, below). Although it may be possible to confirm innocuity by testing virus eluted from the vaccine, this is not universally applicable to all formulations and is not as reliable as testing concentrated antigens. For example, saponin influences greatly the elution of FMDV from aluminium hydroxide/saponin vaccines (Doel & Staple, 1982). If the elution procedure is appropriate to a particular formulation, then it may be validated by seeding parallel samples of vaccine with trace amounts of live virus (Barteling & Vreeswijk, 1991).~~

For the purposes of gaining regulatory approval, a trial batch of vaccine should be tested for local and systemic toxicity by each recommended route of administration in an *in-vivo* test in ~~an appropriate number of~~ eight animals of each target species ~~cattle~~ (European Pharmacopoeia, 2008). ~~Double~~ Single dose and repeat dose tests using vaccines formulated to contain the maximum permitted ~~amount~~ payload and number of antigens should be conducted. The repeat dose test should correspond to the primary vaccination schedule (e.g. two injections) plus the first revaccination (i.e. a total of three injections). The animals are observed for local and systemic reactions to vaccination for no fewer than 14 days after each injection. Any undue reaction attributable to the vaccine should be assessed and may prevent acceptance of the vaccine.

~~b~~)c) ~~Potency~~ Efficacy

Vaccine ~~potency~~ efficacy is estimated in vaccinated animals ~~either~~ directly, by evaluating their resistance to live virus challenge, ~~or indirectly, by inference from the levels of specific antibody induced by vaccination~~. The uncertainty of measurement in ~~these~~ this test~~s~~ should be taken into account when interpreting ~~their~~ its significance (Goris *et al.,* 2007; 2008). Vaccine efficacy should be established for every strain to be authorised for use in the vaccine.

i) *PD_{50} test:*

The number of protective doses in a vaccine is estimated from the resistance to live virus challenge of animal groups receiving different amounts of vaccine. Cattle of at least 6 months of age, obtained from areas free from FMD that have not previously been vaccinated against FMD and are free from antibodies ~~to the different types of~~ to FMDV should be used. Three groups of no fewer than five cattle per group should be vaccinated by the route recommended by the manufacturer. The vaccine should be administered at different doses per group by injecting different volumes of the vaccine. For example, if the label states that the injection of 2 ml corresponds to the administration of 1 dose of vaccine, a 1/4 dose of vaccine would be obtained by injecting 0.5 ml, and a 1/10 dose would be obtained by injecting 0.2 ml. These animals and a control group of two non-vaccinated animals are challenged either 3 weeks (aqueous) or up to 4 weeks (oil) after vaccination with a suspension of bovine virus that is fully virulent and appropriate to the virus types in the vaccine under test by inoculating the equivalent of a total of 10,000 BID_{50} (50% bovine infectious dose) intradermally into two sites on the upper surface of the tongue (0.1 ml per site). Animals are observed for at least 8 days. Unprotected animals show lesions at sites other than the tongue. Control animals must develop lesions on at least three feet. From the number of animals protected in each group, the PD_{50} content of the vaccine is calculated. There are a variety of methods for calculating PD_{50} (FAO, 1997), but procedures based on the Kärber (1931) method are generally preferred when interpreting PD_{50} estimates calculated in this way. The vaccine should contain at least 3 PD_{50} per dose for cattle. ~~when employed for routine prophylactic use, although 6 PD_{50} per dose is more commonly preferred. In some cases, vaccine of high potency will prevent the development of local tongue lesions at the site of challenge.~~

ii) *PGP test* (*percentage of protection against generalised foot infection*)

For this method, a group of 16 FMD-seronegative cattle of at least 6 months of age, with the same characteristics described for the PD_{50} test, are vaccinated with a bovine dose by the route and in the volume recommended by the manufacturer. These animals and a control group of two non-vaccinated animals are challenged 4 weeks or more after vaccination with the challenge strain, which is a suspension of bovine virus that is fully virulent and appropriate to the virus types in the vaccine under test by inoculating a total of 10,000 BID_{50}, intradermally into at least two sites on the upper surface of the tongue. Unprotected animals show lesions ~~at sites other than the tongue~~ on the feet within 7 days after inoculation. Control animals must develop lesions on at least three feet; for routine prophylactic use, the vaccine should protect at least 12 animals out of 16 vaccinated. Animals are observed at 7–8 days after challenge (Vianna Filho *et al.,* 1993). This test does not provide an estimate of how many protective doses are in a single vaccine dose but gives a certain measure of the protection following the injection of single commercial bovine doses of vaccine in a limited cattle population (50–60).

iii) *Efficacy* in other species

~~Potency~~ Efficacy tests in other target species, such as sheep, goats, pigs or buffalo are either different or not yet standardised. In general, a successful test in cattle is considered to be sufficient evidence of the quality of a vaccine to endorse its use in other species. Under circumstances where a vaccine is produced for use primarily in a species other than cattle, it may be more appropriate to potency test the vaccine in that same species. With respect to sheep, goats and African (*Syncerus caffer*) or Asiatic buffalo (*Bubalus bubalis*) ~~and sheep~~, due to the often inapparent nature of the disease in these species, potency results from a cattle test may be a more reliable indicator of vaccine quality than attempting a potency test reliant on the detection of clinical signs in these other species.

~~A similar protocol to the cattle PD_{50} test can be adopted for potency testing FMD vaccines in pigs using three groups of five pigs, not less than 2 months old and free from antibodies neutralising the different serotypes of FMDV. One group is vaccinated with the full pig dose recommended by the manufacturer, one group receives a reduced dose, e.g. 1/4 dose, and a third group receives a further reduced dose, e.g. 1/16 dose of the vaccine. Traditionally, the response to oil vaccine is allowed longer to develop, and not until day 28 after vaccination are the three groups, plus two unvaccinated control pigs challenged. However, depending on the formulation, this interval could be reduced to that used in the cattle test. It is important that the different dose groups are individually separated from each other during the trial and that animals are removed as soon as they appear to be developing generalised FMD to avoid excessive challenge to those remaining. Challenge is by intradermal injection into the heel bulbs of one foot with~~

~~10,000 $TCID_{50}$ (0.2 ml), as calculated by growth in a suitable pig cell culture, of a virulent challenge virus homologous to a strain used in the vaccine and that normally results in generalised disease in the pig. Alternatively, the challenge virus may be administered into one site in the muscular part of the neck behind the ear, using a dose of virus known to cause generalised disease by this route. The animals are observed daily for 10 days after challenge for clinical signs of FMD. Both control animals should develop clinical signs on more than one foot. From the number of animals protected in each group, the PD_{50} content of the vaccine is calculated. There are a variety of methods for calculating PD_{50} (FAO, 1997), including procedures based on Kärber. The vaccine should contain at least 3 PD_{50} per dose for pigs. Likewise, a similar protocol to the PGP test in cattle can be adopted for pigs using a group of 16 animals vaccinated with a full vaccine dose and two non-vaccinated controls. Challenge is by intradermal injection into the heel bulb of one foot with 10,000 $TCID_{50}$ (0.2 ml) of a virulent challenge virus homologous to the strain used in the vaccine and that is known to induce clinical signs in pigs.~~

~~Indirect tests, including measurement following vaccination of virus-neutralising antibodies in cell culture, or ELISA, or LP-ELISA antibodies (Maradei *et al.*, 2008; Periolo *et al.*, 1993), or serum protecting antibodies in suckling mice, may be used to assess the potency of a vaccine provided that a statistical evaluation has established a satisfactory correlation between the results obtained by the test on the relevant vaccine serotype and the potency test in cattle (Neizert *et al.*, 1991). For example, the expected percentage of protection is used to analyse the sera of a group of at least 16 vaccinated cattle and to express the probability of an animal being protected by measuring neutralising, ELISA or LP-ELISA or the serum protecting antibodies in suckling mice. In a single group of animals given a full dose of vaccine, the mean individual expected percentage protection should be equal to or greater than 75% when 16 animals are used or 70% when 30 animals are used in the experimental group.~~

~~The presence of more than one serotype in a vaccine does not diminish the induction of antibodies against another serotype or the correlation of antibody titre with protection.~~

~~c~~)d) Purity: testing for antibody against nonstructural proteins

The OIE *Terrestrial Animal Health Code* stipulates that a criterion for regaining FMD free status following an outbreak, if vaccine is used, is to test the vaccinated animals for antibody against NSP. Likewise, countries wishing to be recognised as FMD free with vaccination must demonstrate the absence of virus circulation by showing that vaccinated animals are free from antibody to NSPs arising as a result of infection. Consequently, FMD antigens used to formulate vaccines that may be used in these circumstances should be purified to reduce the NSP content. With current manufacturing techniques it is possible to exclude the majority of NSPs so that they induce little, if any, NSP-specific antibody. Under these circumstances, detection of NSP antibodies can provide evidence that vaccinated animals have been exposed to FMDV. Vaccine manufacturers may wish to exploit this potential by including a claim that their vaccines do not induce antibody to one or more NSPs and can be used in conjunction with an appropriate diagnostic test. In addition to providing supporting documentation on the processes involved in such purification, manufacturers should demonstrate lack of immunogenicity against NSPs as part of the licensing procedure in order to make such a claim on their product literature. A recommended test method that can be used is to vaccinate ~~an appropriate number~~ not less than 8 ~~of calves~~ naïve cattle~~, preferably~~ with ~~at least a double dose of~~ a trial blend of the vaccine containing the maximum number of strains and amounts of antigen permitted on the authorisation (~~these calves may be the same as those used for the safety test described in Section D .4.a of this chapter~~). ~~Calves~~ Cattle should be vaccinated at least three times at 21- to 30-day intervals ~~over a period of 3–6 months~~ and then tested before each revaccination and 30–60 days after the last vaccination for the presence of antibody to NSPs using the tests described in Section B.2.d of this chapter. Negative results in ~~these~~ NSP assays support claims that the vaccine does not induce antibody to NSPs for the number of injections tested. These cattle may be the same as those used for the safety test described in Section C.5.b of this chapter

~~At the batch level, confirmation of vaccine purity can be shown by demonstrating a lack of increase in reactivity against NSPs of the sera from the animals used in the potency test obtained 30 days after primovaccination and before challenge, when compared with the sera of the same animals prior to vaccination.~~

~~d~~)e) Duration of immunity

The duration of immunity (D.O.I) of an FMD vaccine will depend on the efficacy (formulation and antigen payload). As part of the authorisation procedure the manufacturer should be required to demonstrate the D.O.I. of a given vaccine by either challenge or the use of a validated alternative test, such as serology at the end of the claimed period of protection, in compliance with Section 5.c. D.O.I. studies should be conducted in each species for which the vaccine is indicated or the manufacturer should indicate that the D.O.I. for that species is not known. Likewise, the manufacturer should demonstrate the effectiveness of the recommended booster regime in line with these guidelines, usually by measuring the magnitude and kinetics of the serological response observed.

~~In order to establish a satisfactory level of immunity~~ ~~It~~ In endemic or outbreak situations, vaccine is usually ~~to~~ given as a primary course consisting of one or two ~~inoculations~~ doses of vaccine 3-4 weeks apart (based on animal population immunological status, vaccine potency, virus-vaccine matching, virus challenge levels, and other factors), ~~2–4 weeks apart~~, followed by revaccination every ~~4~~ 6–12 months. The frequency of revaccination will depend on the epidemiological situation and the type and quality of vaccine used. ~~Where access to the animals is difficult, it is preferable to use oil adjuvanted vaccine at 4 months and 1 year of age, followed by annual revaccination. Wherever possible, vaccine manufacturers should demonstrate the duration of immunity for their specific formulation in each species for which it is indicated.~~

For target animals born to vaccinated dams, vaccination should be delayed to allow decline of maternally derived antibodies. Primary vaccination of offspring to non-vaccinated dams can occur as early as 1 week of age (Auge De Mello *et al.,* 1989). Pigs from vaccinated dams are usually vaccinated at 8–10 weeks of age. Calves are usually vaccinated at about 4 months of age.

Information should be provided by manufacturers to indicate the appropriate vaccination programme(s) to minimize interference with maternally derived antibodies in target species.

~~For calves born of vaccinated dams, the first vaccination should be delayed as long as possible to allow decline of maternal antibody. This period should not be extended beyond 4 months, as by this age a high proportion can be expected to respond effectively to vaccination. For calves born to non-vaccinated dams, the first vaccination may be administered from as early as 1 week of age for some vaccines (Auge De Mello *et al.,* 1989).~~

~~e)~~f) Stability

~~The shelf life of conventional FMD vaccines is usually 1–2 years at 4°C (maximum range 2–8°C), but they are temperature labile and should never be frozen or stored above a target temperature of 4°C.~~ The stability of all vaccines, ~~but particularly~~ including oil emulsion vaccines, should be demonstrated as part of the shelf-life determination studies for authorisation.

The shelf life of conventional FMD vaccines is usually 1–2 years at 2–8°C. Vaccines should never be frozen or stored above the target temperature.

~~f) Preservatives~~

~~The most commonly used preservatives are chloroform and merthiolate. The latter is used at a final concentration of 1/30,000 (w/v).~~

g) Precautions (hazards)

Current FMD vaccines are innocuous and present no toxic hazard to the ~~user~~ vaccinators. ~~Care must be taken to avoid self-injection with oil-emulsion vaccines~~. Manufacturers should provide adequate warnings that medical advice should be sought in case of self-injection of an oil-emulsion vaccine.

~~5. Batch control~~

~~a) Sterility~~

~~The bulk inactivated antigen, the adjuvants, the dilution buffers and the final formulated product should all undergo sterility testing. This may be carried out directly with components of the vaccine and the final product, but the preferred method is to collect any contaminating microorganisms by membrane filtration of the material to be examined and to detect any organisms present by incubation of the membranes with culture media. The latter procedure allows the removal of preservatives, etc., which may inhibit the detection of microorganisms. Guidelines on techniques and culture media, which allow the detection of a wide range of organisms, are described in the European Pharmacopoeia (2008) (see also refer to Chapter 1.1.9 Tests for sterility and freedom from contamination of biological materials).~~

~~b) Innocuity~~

~~The test for innocuity is an in-process test that should be carried out for every batch of antigen. Following inactivation, a sample of each batch of inactivated antigen representing at least 200 doses should be tested for freedom from infectious virus by inoculation of sensitive monolayer cell cultures, preferably of the same origin as those used for the production of antigen. It may be preferable to concentrate the antigen to do this, in which case it must be shown that the concentrated material does not interfere with the sensitivity or reading of the assay. The cell sheets are examined daily over a period of 3 days, after which the spent medium is transferred to fresh monolayers and the original monolayers are replenished with fresh medium. Using this method, traces of live virus can be amplified by the passage procedure and detected on the basis of CPE observed. Two to three passages of the original virus preparation are commonly used. A variant on this method is to freeze–thaw the old monolayers to release intracellular virus, which can be detected by further passage.~~

~~c) Safety~~

~~This final product batch safety test is conducted to detect any abnormal local or systemic adverse reactions. The test may also confirm innocuity but is not as sensitive as the *in-vitro* tests described above. For the purposes of batch release, each of at least two healthy seronegative cattle is inoculated by the recommended route of administration with double the recommended dose of vaccine. The animals are observed for local and systemic reactions to vaccination for no fewer than 14 days. Should any of the animals develop clinical signs of FMD, the vaccine fails the safety test. Equally, any undue toxicity attributable to the vaccine should be assessed and may prevent acceptance of the batch. Ideally, vaccines prepared for species other than cattle should also be safety tested in the species for which they are intended, administering a double dose of vaccine according to the manufacturer's recommended route and dose volume. The animals should be examined daily for a minimum of 14 days for evidence of toxicity or signs of FMD.~~

~~d) Potency~~

~~Potency is only examined on the final formulated product (see Section D.5.b). Antigen load can be used as an indirect indicator of potency, provided that~~

~~i) Good Manufacturing Practice ensures that the method of manufacture and formulation of the antigen/vaccine remains the same,~~

~~ii) A correlation has previously been established between antigen load, serological response and protection against challenge, and~~

~~iii) A suitable alternative test measuring the serological response to immunisation has been carried out with satisfactory results.~~

6. Storage and monitoring of concentrated antigens

Chapter 1.1.10 of the *Terrestrial Manual* provides guidelines for international standards for vaccine/antigen banks.

The process of storing concentrated antigens at ultra-low temperature for later formulation into FMD vaccine as described in Section C 2, is a well-established procedure for building stocks of immunogenic material ready to be formulated into vaccines in case of need. ~~becoming an increasingly popular option for vaccine manufacture~~. It not only forms the basis for the storage of antigens in a strategic reserve for emergency purposes ~~(see Chapter 1.1.10 Guidelines for International Standards for Vaccine Banks)~~, but allows the manufacturer immediate access to many different antigen strains that can be rapidly formulated and dispatched to the customer (Lombard & Fussel, 2007). Such stockpiling minimises delays subsequent to an order, particularly where a multivalent vaccine is requested. Another advantage of this procedure is that much of the quality testing can be completed well in advance of shipment. It is necessary to state that the concentrated antigens have to be controlled using standards indicated in Sections ~~D.1–4~~ C.1–4.

~~a) Prestorage criteria~~

~~Special attention should be paid to the following:~~

~~- freedom from extraneous agents;~~

~~antigens should be proven free from all extraneous agents listed by the appropriate licensing authorities.~~

~~- sensitivity of the cell line used to detect residual virus;~~

~~Cells used to test for absence of residual live virus are not suitable if use of an amount of virus corresponding to 1 µg of 146S antigen gives a titre of less than 10^6 $TCID_{50}$ (European Pharmacopoeia, 2008).~~

- ~~emergency procedures for provisional acceptance of new MSV, and subsequent release of formulated vaccines.~~

~~In the case of incursion in a region of a new strain that is antigenically distinct from existing vaccine strains, it may be necessary to develop a new vaccine strain from a representative field isolate. Before the new MSV can be accepted, full compliance should be demonstrated with the relevant guidelines to demonstrate freedom from all extraneous agents listed by the appropriate licensing authorities using both general and specific tests, and to establish homology to the original candidate isolates. The time taken to raise the specific antisera necessary to neutralise the new strain for use in the general tests for detection of extraneous agents and to conduct other specific tests that require specialised techniques may be lengthy. Therefore, in emergency situations where~~

~~there is insufficient time to complete full testing of the MSV, provisional acceptance of the new strain should be based on a risk analysis of the possibility of contamination of the antigen produced from the new MSV with extraneous agents. This risk assessment should take into account that a validated procedure to inactivate enveloped viruses must be used when establishing the MSV and that the virus is inactivated using a chemical inactivant with first order kinetics. Further assurance is provided by the requirement for the kinetics of inactivation to be monitored and recorded for each production batch.~~

~~In order to accelerate the release of batches of vaccine formulated to contain new vaccine strains, it may be acceptable for batch potency testing to be carried out using a vaccine formulated using an intermediate antigen lot pending production of all of the batches of antigen that are intended to constitute the final antigen lot. This will allow the potency of antigen derived from a new MSV to be determined whilst the manufacturer continues to build up stocks of this new antigen.~~

~~b)~~ a) Storage ~~criteria~~ conditions

- **Facilities**

It is important that all aspects of the storage of concentrated antigens conform fully to internationally accepted standards requirements such as those referred to in Chapter 1.1.8. of the *Terrestrial Manual.* ~~of Good Manufacturing Practice.~~ Housing, facilities and procedures should ensure the security of the stored antigen and prevent tampering, contamination or damage.

- **Containment of stored antigens**

The dose numbers or volumes stored are an important consideration, particularly where a reserve is shared between OIE Members and there is variation in number of doses perceived to be needed by each Member in an emergency. ~~It may be advisable to store antigen concentrates in user-friendly units to allow better use of storage space and capability in producing smaller vaccine batches. One to two litre sized containers can accommodate in excess of 30,000 bovine doses~~. Where the requirement is for a large stockpile of a particular vaccine strain that can only be produced from several separate production runs, vaccine bank managers must consider the need to either formulate each lot into a representative final blend for testing purposes or mixing the individual batches, at some convenient point, for ease of formulating and/or testing.

The type of container used to hold antigen concentrate is important. Under ultra-low temperature conditions it is important to use containers made from materials that do not become brittle ~~and~~ or fragile ~~a good example being fluoropolymer based moulded bottles. Polyfluoro alkoxy (PFA) based bottles, for example, have~~ at a temperature ~~resistance~~ range allowing for heat sterilisation and cold storage ~~of between -270°C and +250°C~~.

- **Labelling of stored antigens**

The concentrated antigens do not need to be labelled according to final or finished vaccine requirements and may be labelled as "in process" materials. ~~Although there are national and international guidelines on the required labelling of veterinary medical products, there are no such guidelines for emergency stored materials such as the antigen component of a vaccine, as these are essentially regarded in regulatory terms as 'in process' materials.~~ Under ultra-low temperature conditions, the method of labelling must be of a durable nature. From experience, wire tagging bottles is the most preferred option using a metal/plastic tag sizeable enough to allow the necessary detail. Such detail should include the antigen/vaccine strain, batch number, date received and should also include an individual container or stock number. This information should be clear to read and marked on the tag using an indelible marker pen. Storage records and positions of containers should be carefully maintained. ~~Aluminium metal tags have been used for such purpose and these can be obtained with different colour coatings to allow better identification and accessibility, particularly when different antigen strains are housed in the same container. Metal tags also allow information to be permanently engraved.~~

b) Monitoring of stored concentrated antigens

It is vitally important that antigen concentrates are optimally maintained and routinely monitored in order to have some assurance that they will be efficacious when needed. Therefore arrangements should be made to monitor these antigen concentrates on a routine basis and to include where necessary, and at appropriate time intervals, a testing regime to ensure integrity of the antigen component or acceptable potency of the final product. ~~For example, Storage temperature monitoring is normally undertaken and recorded in FMD vaccine banks. as well as periodic inspection of the bottles containing the antigen for cracks or leakage. Depending on type, volume and how they are stored, there may also be value in weighing antigen deposits annually to ensure they have not lyophilised. Some FMD vaccine banks have incorporated physico-chemical tests like sucrose density gradient analyses to monitor virus integrity and hence stability and some have also carried out *in-vivo* tests. However, as it has been shown that the shelf life of FMD antigen concentrates are likely to be well in excess of 15 years when stored at ultra-low temperature, a physico-chemical approach would appear sufficient (Barnett & Statham, 1990).~~

146S quantification, vaccination serology or vaccination challenge studies can be used for monitoring FMD antigen banks (Barnett & Statham, 1990; ~~Doel *et al.*, 1982;~~ Doel & Pullen, 1990; Bartelling & Meloen, 1974; Fayet et al., 1971). It is recommended to carry out these tests on receipt (year 0) and every 5 years thereafter.

~~The following timetable of tests is proposed as suitable for validation and revalidation of stored antigens.~~

~~Time~~	~~Test~~
~~On receipt (year 0) and every 5 years thereafter~~	~~146S quantificationPotency test in cattle that may rely on serological techniques where potency has been adequately correlated with immunogenicity for the antigen concerned or, at the discretion of the bank holder, may be a 'truncated' test** to demonstrate that the minimum potency of the vaccine remains greater than the minimum requirement; however, truncation may underestimate vaccine potency~~
~~Years 2 and 4, and Immediately before formulation if the need arises~~	~~146S quantification~~
~~Every 5 years~~	~~Evaluation of all data for the preceding 5 years to assess need to replace antigen~~

~~* Other physiochemical tests such as SDS-PAGE have been used to evaluate integrity of VP1 but are not sufficiently validated for routine use.~~

~~** In a truncated test all animals in the next lower volume group are assumed to have not been protected. The test therefore gives an artificially low PD_{50} value but reduces the number of animals required.~~

To support these testing requirements for depositories of antigen, concentrates should include a number of small samples that are representative of the larger stock. Small aliquots/stocks of FMD antigen have usually consisted of a volume representing approximately one milligram of antigen. These aliquots should be stored side by side with the bulk antigen,

7. Emergency release of vaccines prepared from concentrated antigens

In situations of extreme urgency and subject to agreement by the competent authority, a batch of vaccine may be released before completion of the tests and the determination of potency if a test for sterility has been carried out on the bulk inactivated antigen and all other components of the vaccine and if the test for safety and the determination of potency have been carried out on a representative batch of vaccine prepared from the same bulk inactivated antigen. In this context, a batch is not considered to be representative unless it has been prepared with not more than the amount of antigen or antigens and with the same formulation as the batch to be released (European Pharmacopoeia, 2008).

D ~~C.~~ VACCINE MATCHING TESTS

1. Introduction

~~The selection of vaccine strains has been reviewed (Paton *et al.*, 2005).~~

Appropriate vaccine strain selection is an important element in the control of FMD and is necessary for the application of vaccination programmes in FMD-affected regions as well as for the establishment and maintenance of vaccine antigen reserves to be used in the event of new FMD incursions.

Vaccination against one serotype of FMDV does not cross-protect against other serotypes and may also fail to protect fully or at all against other strains of the same serotype. The most direct and reliable method to measure cross-protection is to vaccinate relevant target species and then to challenge them by exposure to the virus isolate against which protection is required. This will take account of both potency and cross-reactivity.

However, such an approach requires the use of live FMDV and appropriate biosecurity procedures and practices must be used. The facility should meet the requirements for Containment Group 4 pathogens as outlined in Chapter 1.1.2 of this *Terrestrial Manual.* In addition to the safety concerns, this procedure is slow and expensive and requires specific expertise that is best available in OIE Reference laboratories. The use of animals for such studies should be avoided where possible by the use of *in vitro* alternatives.

A variety of *in vitro* serological methods can be used to quantify antigenic differences between FMDV strains and thereby estimate the likely cross-protection between a vaccine strain and a field isolate. Genetic characterisation and antigenic profiling can also reveal the emergence of new strains for which vaccine matching may be required and, conversely, may indicate that an isolate is similar to one for which vaccine matching information is already available. Such tests should be carried out in laboratories that work according to the standard specified in Chapters 1.1.2 and 1.1.3 of the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*, preferably OIE Reference Laboratories for FMD.

Shipping of samples should be in accordance with Chapter 1.1.2 Sections H and I and Chapter 1.1.1 of this *Terrestrial Manual*.

~~Appropriate vaccine strain selection is an important element in the control of FMD and is necessary for the application of vaccination programmes in FMD-affected regions as well as for the establishment and maintenance of vaccine antigen reserves to be used in the event of new FMD incursions.~~

Vaccine potency also contributes to the range of antigenic cover provided by a vaccine. A highly potent vaccine that stimulates a strong immune response may give greater protection against a heterologous virus than an equally cross-reactive vaccine that stimulates a weaker immune response (Brehm *et al.,* 2008). Furthermore, booster doses of vaccine can increase efficacy and the subsequent breadth of antigenic cover provided by a given vaccine, although the onset of full protection may be delayed (Pay, 1984).

2. Selection of field viruses for vaccine matching

For vaccine matching, preferably, more than one representative isolate should be evaluated from any outbreak.

Viruses should be selected based on epidemiological information, for instance isolation at different stages of an outbreak, from different geographical locations, or from different hosts (Alonso *et al.,* 1987). Field evidence for a suspected lack of vaccine efficacy, as shown by reduced apparent protection, is an important criterion for vaccine matching.

The serotype of the field isolate is usually determined by ELISA or CFT using type-specific serological reagents, although methods based on MAbs or genetic typing may also be used. If the number of viruses exceeds the capacity of the laboratory to carry out methods described in Section D.4-Vaccine matching tests, a pre-selection of isolates should be done.

In order to minimise the risk of missing a relevant sample, the pre-selection should be carried out using all the isolates received by the laboratory. The recommended approach is to engage in serological validated antigenic profiling methods using ELISA. The performance of VP1 sequencing could be used to verify the homogeneity of the virus isolate population.

Only isolates showing Important differences with vaccine strains are selected for vaccine matching tests.

~~Serological matching of field isolates to vaccine strains requires that isolates have been serotyped and adapted to growth in cell cultures. The serotype is usually determined by ELISA or CFT using type specific serological reagents, although methods based on MAbs or genetic typing may also be used. BHK or IB-RS-2 cell cultures are usually used for *in vitro* virus replication. For vaccine matching, preferably, at least two isolates should be evaluated from any outbreak and inconsistent results should be followed up to determine whether this is due to genuine antigenic differences or is an artefact of testing.~~

~~Viruses can be selected based on epidemiological information, for instance isolation at different stages of an epidemic, from different geographical locations or from different hosts (Alonso *et al.,* 1987). Field evidence for a suspected lack of vaccine efficacy, as shown by reduced apparent protection, is an important criterion for vaccine matching.~~

~~Antigenic profiling by CFT or ELISA, or sequence analysis of the VP1 gene, are suitable approaches for selecting representative virus isolates for vaccine matching. Antigenic profiling is performed by CFT using panels of hyperimmune guinea pig sera raised against epidemiologically relevant field isolates (Bergmann *et al.*, 1988) or by ELISA using panels of well-characterised MAbs (Alonso *et al.*, 1993).~~

3. Selection of vaccine strains to be matched

The serotype of the virus, the region of origin and any information on the characteristics of the field isolate and, as appropriate, the vaccine strain used in the region of origin, may give indications as to the vaccine strains to be selected for vaccine matching tests ~~most likely to provide an antigenic match~~. The availability of reagents for matching to particular vaccine strains may limit the extent of testing that is possible. Antigenic characterisation has two purposes; first, to choose the most effective vaccine strain for use in a particular circumstance and, second, to monitor, on an ongoing basis, the suitability of vaccine strains maintained in strategic antigen reserves.

4. ~~Choice of~~ Vaccine matching test

The serological relationship between a field isolate and a vaccine virus ('r' value) can be determined by ~~CFT~~, VNT or ELISA (Kitching *et al.,* 1988; Mattion *et al.,* 2009; Pereira, 1977; ~~Rweyemamu *et al.*, 1978~~). One way testing is recommended (r_1) with a vaccine antiserum, rather than two way testing (r_2), which also requires an antiserum against the field isolate to be matched. VNT using chequer-board titration method will give more accuracy to the results obtained. ~~Because of the inherently low repeatability of the assays used, tests need to be repeated to have confidence in the results (Rweyemamu *et al.*, 1978).~~ *In-vitro* neutralisation may be more relevant to predict *in-vivo* protection by the vaccine than other measures of virus-antibody interaction. ~~although non-neutralising antibodies may also contribute to protection (McCullough *et al.*, 1992). Advantages of ELISA are that the test is rapid and uses smaller volumes of post vaccination sera, which are often available in only limited quantities. ELISA and CFT are recommended to be used as screening methods,~~

VNT is the method of choice (Mattion *et al.,* 2009) compared with ELISA which can be used only as screening method for vaccine matching.

~~Whereas VNT or the expected percentage of protection (EPP) method provides more definitive results.~~ For either VNT or ELISA, post-vaccination sera should be derived from at least five cattle 21–30 days after ~~immunisation~~ inoculation. The titre of antibody to the vaccine strain is established for each serum. Sera are used individually or pooled, after excluding low responders. ~~The CFT method uses guinea pig sera raised against vaccine strains.~~

~~A more thorough evaluation is provided by the EPP method (Alonso *et al.,* 1987), which measures the reactivity of a panel of post-vaccination antisera using either VNT or ELISA and relates the serological titres to the probability of protection, established through correlation tables associating antibody titres with protection against the relevant vaccine strain. These correlation tables derive from previously performed vaccine-specific challenge tests. However, the requirement for a panel of antisera and accompanying challenge test data for the vaccine in question currently cannot be met for a wide range of vaccine strains.~~

~~a) Vaccine matching by ELISA~~

~~This test uses an antiserum raised against a vaccine strain. The blocking ELISA titres of this reference serum against antigens prepared from the homologous vaccine strain are compared with the corresponding titres of the serum against a field isolate to determine how antigenically 'similar' the field virus is to the vaccine virus.~~

~~The test procedure is similar to that of the liquid-phase blocking ELISA (see Section B.2.c). Additional biological reagents are: 21–30 day post vaccination bovine vaccine sera (inactivated at 56°C for 45–60 minutes); the homologous vaccine strain; and the test virus, a field isolate of the same serotype as the vaccine strain.~~

- ~~**Test procedure**~~

~~i) Grow the field isolate and the vaccine strain in BHK or IB-RS-2 cells. The number of cell culture passages should be kept to a minimum (normally less than four) to avoid selection of antigenic variants unrepresentative of those in the original material. A sufficient quantity of virus should be present if cell cultures show CPE within 24 hours of inoculation.~~

~~ii) Harvest and titrate the vaccine and field viruses using a panel of trapping rabbit antisera and detector guinea pig antisera raised against the same or closely related vaccine strains. If necessary, the virus antigens may be inactivated prior to use using binary ethyleneimine (BEI).~~

~~iii) Select the optimum trapper/detector combination and the working dilution of the field virus. This should not be less than 1/6. If there is no suitable trapper/detector combination then a back-titration of the antigen stock must be performed to confirm that sufficient virus is present. If it is confirmed that the field virus is present at high titre, this indicates that none of the available vaccine strains is suitable.~~

~~iv) Titrate 21–30 day post-vaccination serum of a chosen vaccine strain against the field isolate and the homologous vaccine strain. The titre against the vaccine strain should not fluctuate more than twofold either side of the running mean value for the virus stock.~~

~~v) To determine the serum titre, calculate the average optical density (OD) of 24 antigen control wells without blocking serum. This represents the maximum OD value for the test, i.e. the 100% control value. Divide this by 2 to determine the 50% inhibition value. Score wells with blocking serum positive if the OD is less than or equal to 50% and negative if the OD value is greater than this. The end-point is defined as the dilution at which half of the wells show 50% inhibition or more (i.e. identify the dilution at which one out of the two duplicate wells has an OD less than 50% of the antigen control). If the end-point falls between two dilutions, it is taken as the mid-point between these dilutions as estimated by the Spearman–Kärber method.~~

~~Derive the 'r' value, the relationship between the field and the vaccine strain, as:~~

~~r_1 = reciprocal arithmetic titre of reference serum against field virus / reciprocal arithmetic titre of reference serum against vaccine virus~~

~~At least two consistent results are needed for acceptance.~~

~~vi) *Interpretation of the results:* for r_1 values derived by ELISA the following guidelines are used for interpretation (Ferris & Donaldson, 1992):~~

~~0.4–1.0: Close relationship between field isolate and vaccine strain. A potent vaccine containing the vaccine strain is likely to confer protection.~~

~~0.2–0.39: The field isolate is antigenically related to the vaccine strain. The vaccine strain might be suitable for use if no closer match can be found provided that a potent vaccine is used and animals are preferably immunised more than once.~~

~~<0.2: The field isolate is only distantly related to the vaccine strain and the vaccine strain is unlikely to protect against challenge with the field isolate.~~

a) Relationship between the field isolate and the vaccine strain

The recommended standard test is the VNT. The ELISA can be used as a screening method.

~~b)~~i) *Vaccine matching by two-dimensional (chequerboard) neutralisation test*

This test uses antiserum raised against a vaccine strain. The titres of this serum against 100 $TCID_{50}$ of the homologous vaccine strain and the same dose of a field isolate are compared to determine how antigenically 'similar' the field virus is to the vaccine strain.

~~The procedure is similar to that of the microtitre plate VNT (see Section B.2.a). Additional biological reagents are: 21–30 day post-vaccination bovine vaccine sera (inactivated at 56°C for 45–60 minutes); the homologous vaccine strain; and the test virus, a field isolate of the same serotype as the vaccine strain.~~

- **Test procedure**

The procedure is similar to that of the VNT (see Section B.2.a).

Additional biological reagents are: monovalent 21–30 day post-vaccination bovine sera (inactivated at 56°C for 45–60 minutes), the homologous vaccine strain; and the test virus, a field isolate of the same serotype as the vaccine strain.

~~i)~~ a) Field isolates are passaged on cell cultures until adapted to give 100% CPE in 24 hours. Passages should be kept to a minimum. When adapted, determine the virus titre (log_{10} $TCID_{50}$/ml) by end-point titration.

~~ii)~~ b) For each test and vaccine virus a chequerboard titration is performed of virus against vaccine serum along with a back-titration of virus. Cells are added and incubated at 37°C for 48–72 hours after which time CPE is assessed.

~~iii)~~c) Antibody titres of the vaccine serum against the vaccine strain and field isolate for each virus dose used are calculated using the Spearman–Kärber method. The titre of the vaccine serum against 100 $TCID_{50}$ of each virus can then be estimated by regression. The relationship between the field isolate and the vaccine strain is then expressed as an 'r' value as ~~for vaccine matching by ELISA~~:

r_1 = reciprocal arithmetic titre of reference serum against field virus / reciprocal arithmetic titre of reference serum against vaccine virus

~~iv~~• **Interpretation**

Interpretation of the results: ~~in the case of neutralisation~~ It is generally accepted that in the case of neutralisation, r_1 values greater than 0.3 indicate that the field isolate is sufficiently similar to the vaccine strain that use of a vaccine based on this strain is likely to confer protection against challenge with the field isolate (Rweyemamu, 1984).

Conversely, values less than 0.3 suggest that the field isolate is sufficiently different from the vaccine strains tested that a vaccine based on these strains is less likely to protect. In this case, either the field isolate should be examined against other vaccine strains or the field isolate could be tested against existing vaccines in a heterologous cross protection challenge test. Alternatively, a suitable field isolate could be adapted to become a new vaccine strain.

~~v)~~ Tests should always be repeated more than once. The confidence with which 'r' values can be taken to indicate differences between strains is related to the number of times that the examination is repeated (Rweyemamu &Hingley, 1984). In practice, a minimum of at least three repetitions is advised.

ii) Vaccine matching by ELISA

The use of the Liquid-phase blocking ELISA for vaccine matching has been reported (Ferris & Donaldson, 1992).

b) Testing the fitness for purpose of a vaccine

Only when the r-value suggests an insufficient match of a certain vaccine strain, the suitability of a vaccine based on such a vaccine strain could be demonstrated by a heterologous cross-protection challenge test carried out as described in Section C.4.b. in animals vaccinated with a known vaccine and challenged with the (heterologous) field virus. If the r value is under 0.3, the following differences in the previously described test are recommended respecting the instructions for vaccination. Vaccinate at least seven cattle without FMD antibodies, with a commercial dose of the current vaccine to be used in the region. Between 28 to 30 days later, boost all these animals with a second commercial dose in the same conditions and vaccinate a second group of at least 7 animals with the same vaccine dosage and same route. 30 days later, challenge the two groups plus two control animals (not vaccinated) with the equivalent of a total of 10.000 BID 50% (50% bovine infective dose) of the new field strain duly titrated. The results are valid if each of the two control animal shows podal lesions. Final results are reported either as the number of protected animals (without podal lesion) over the total number of animal per group, or by percentage of protection where 100% is the total number of animals used per group. If results in the group of once vaccinated cattle indicate a protection level under 75%, and in the group of twice vaccinated cattle, protection under 100%, the change for a more appropriate vaccine strain is recommended (Henderson, 1949).

The use of the Expected Percentage of Protection (EPP) method (Alonso *et al.*, 1987) is not recommended under heterologous conditions. This method measures the reactivity of a panel of post-vaccination antisera using either VNT or ELISA and relates the serological titres to the probability of protection, established through correlation tables associating antibody titres with protection against the homologous vaccine strain. Consequently the correlation from the panels of antisera and accompanying challenge tests cannot be extrapolated to any other strain (Robiolo *et al.*, 2010).

~~c) Vaccine matching by complement fixation test~~

~~The relationship between a field isolate and a vaccine strain can also be determined by CFT using a guinea-pig antiserum raised against the relevant vaccine strain.~~

~~CFT 50% titres of this reference serum against antigens prepared from the homologous vaccine strain and a field isolate are compared to determine how antigenically 'similar' the field virus is to the homologous vaccine virus.~~

~~i) Field isolates are passaged on cell cultures until adapted to give 100% CPE in 24 hours. Passages should be kept to a minimum. When adapted, the virus titre that fixes 2.5 CFU_{50} (50% complement fixing units) is determined.~~

~~ii) A relationship is established by titration of the guinea-pig antisera through a twofold dilution series against 2.5 CFU_{50} of the homologous and heterologous antigens in veronal buffer diluent (VBD) or borate saline solution (BSS) placed in separate tubes. Four haemolysis units of complement are then added to each reaction.~~

~~iii) The test system is incubated at 37°C for 30 minutes prior to the addition of 2% of standardised sheep red blood cells (SRBC) in VBD or BSS sensitised with rabbit anti-SRBC. Reagents are incubated at 37°C for a further 30 minutes and the tubes are subsequently centrifuged and read.~~

iv) The CFT 50% titres are calculated by the Spearman–Kärber method and an 'r' value is derived from the relationship between the reactivity of the field isolate and the vaccine strain, as:

$$r_4 = \frac{\text{reciprocal arithmetic titre of hyperimmune serum against field virus}}{\text{reciprocal arithmetic titre of hyperimmune serum against vaccine virus}}$$

v) *Interpretation of the results:* in the case of CFT, r_4 values greater than 0.25 indicate that the field isolate is sufficiently similar to the vaccine strain and that use of the vaccine is likely to confer protection against challenge with the field strain (2).

d) Expected percentage of protection

The EPP estimates the likelihood that cattle would be protected against a challenge of 10,000 infective doses after a single or boosted vaccination.

i) Individual sera are required from 16 or 30 18–24 month old cattle at 30 days post vaccination and 30 days post-revaccination, using a full dose of the vaccine strain to be matched.

ii) This panel of sera is tested for antibody titres to the homologous FMD vaccine strain and the field isolate to be matched using VNT or LPB-ELISA (see Sections B.2.a and B.2.c).

iii) If necessary, the antigens used in the ELISA may be inactivated prior to using BEI.

iv) The EPP is determined from the serological titre obtained, for each individual serum, by reference to predetermined tables of correlation between serological titres and clinical protection. The mean EPP is then calculated from the EPP for each individual serum.

v) The clinical protection data are derived from previously performed experiments carried out on hundreds of cattle that have been immunised using the vaccine strain in question and challenged with a homologous virus (similar to the PGP potency tests described in Section D.4.b). Each animal is scored as protected or not, and tables of correlation based on logistic regression models are established between antibody titre and clinical protection.

vi) An EPP <75% (when sera from a group of 16 revaccinated animals are used) and <70% (when sera from a group of 30 revaccinated animals are used) is an indication that the vaccines will give a low protection against the field strain (56).

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NB: There are OIE Reference Laboratories for Foot and mouth disease (see Table in Part 3 of this *Terrestrial Manual* or consult the OIE Web site for the most up-to-date list: www.oie.int).

Appendix IV

Original: English
April 2011

REPORT OF THE MEETING OF THE OIE *AD HOC* GROUP ON VALIDATION OF DIAGNOSTIC TESTS FOR WILDLIFE

Paris, 27–29 April 2011

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The meeting of the OIE *ad hoc* Group on Validation of Diagnostic Tests for Wildlife was held at OIE Headquarters, Paris from 27 to 29 April 2011.

1. Opening and purpose of the meeting

Dr Kazuaki Miyagishima, Deputy Director General, welcomed the participants of the meeting on behalf of Dr Bernard Vallat, the Director General of the OIE. He presented the different activities of the OIE in the area of wildlife and explained briefly the objectives of the *ad hoc* Group based on the draft Terms of Reference.

2. Adoption of agenda and appointment of chairman and rapporteur

The meeting was chaired by Dr John Fischer, and Prof. Anita L. Michel was designated as rapporteur. Dr Fischer introduced the provisional agenda, which was subsequently adopted by the Group. The adopted agenda and list of participants are attached as Appendices I and II, respectively.

3. Adoption of draft Terms of Reference of the *ad hoc* Group

The draft Terms of Reference for this meeting, as provided by the OIE Biological Standards Commission, were accepted by the Group and are attached as Appendix III.

4. Information on existing documents and initiatives

Dr Fischer, as member of the OIE Working Group on Wildlife Diseases, presented the work and information collected on the validation of diagnostic tests for wildlife by this Working Group.

Prof. Ian Gardner, as participant in the *ad hoc* Group on Validation of Diagnostic Tests, which met three times between 2008 and 2010, presented briefly the drafts of the updated version of Chapter 1.1.4./5. of the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (*Terrestrial Manual)* and of the seven appendices developed by this *ad hoc* Group. This version of the chapter and the seven appendices had been forwarded for consideration to the Biological Standards Commission (BSC) Members at its meeting in February 2011. The BSC Members however could not review the texts in detail due to time limitation. The documents remained therefore drafts and would be discussed at the next meeting of the BSC in September 2011.

5. Information in the context of the Biological Standards Commission

Prof. Vincenzo Caporale, the representative of the Biological Standards Commission (BSC), further explained the Commission's request regarding validation of diagnostic tests and its application to wildlife.

The Group discussed in-depth the specific needs and difficulties related to validation of diagnostic tests in wildlife. The Group affirmed, in principle, that draft Chapter 1.1.4./5. was highly relevant and covered all aspects of test validation and therefore was an appropriate starting point for adapting the principles to a broad range of wildlife species. The Group started by identifying the challenges and needs specific to the validation and use of diagnostic tests in wildlife. These difficulties included: (i) Broad range of animal species; (ii) Different pathobiology/pathologies; (iii) Rare and endangered species (low animal numbers, ethical considerations); (iv) Small animals (small sample volume or weight); (v) Accessibility - Immobilisation or hunting required to collect samples; (vi) Cost implications from sampling and testing (no owners, in some cases); (vii) Epidemiology of the disease (Uneven distribution of disease in populations, seasonality, unknown epidemiology); (viii) Natural history of the host and vector; (ix) Limited resources for addressing wildlife diseases that are not zoonotic or do not occur in domestic animals; (x) Logistics: transport and conservation of samples from remote areas; (xi) Quality of samples including autolysis; and (xii) Lack/scarcity of reference samples.

The Group decided to draft a practical, stand-alone chapter to address issues identified, for inclusion in the *Terrestrial Manual*.

6. Drafting of guidelines according to the Terms of Reference

The Group felt that the step-wise approach taken in draft Chapter 1.1.4./5. would be suitable for wildlife, but with changes. In particular, the Group proposed changing "provisional recognition" at the end of stage 1 to the concept of "provisional acceptance", which included partial completion of stage 2. Stage 2 was split into two levels (2a and 2b) to accommodate this concept. Stage 1 and stage 2a needed to be completed for provisional acceptance. Provisional acceptance of a test provided the reassurance that the process up to this point had been based on scientifically sound principles, which supported its use to wildlife for a defined testing purpose in wildlife on a local or regional level.

In developing the guidelines the Group considered ways of making the whole approach for validation practical and feasible for those working in wildlife diagnostics. The Group constructed a flow chart to structure the thinking about this logical step-wise approach. The flow chart incorporated stages 2a and 2b to allow implementation of validation principles for wildlife, which were outlined in draft Chapter 1.1.4./5. In developing the flow chart, consideration was given to the availability of samples from rare and endangered species. For completion of stage 2 validation, the Group re-affirmed that the requirements in Table 1 of the draft Chapter 1.1.4./5. should be met.

During the discussion several questions were raised in relation to specific areas of draft Chapter 1.1.4/5 and appendices in their present format. Professor Caporale, representing the BSC, took note of the questions raised and suggested that the *ad hoc* Group should, at the end of their work, make a list of problem areas encountered. Such a list would be helpful for deciding if and what kind of revision to the chapter and appendices would be needed to finalise them.

Although the Group felt that the document covered the most common scenarios, because of limited time, all relevant details could not be adequately addressed and several outstanding issues were identified that should be considered in order to improve the quality of the draft guidelines:

- Relatedness of species in relation to type of test (direct vs indirect tests);
- Influence of type of test on the flow chart (parallel scenarios with modifications necessary?);
- Guidance on how to take into account autolysed samples in the validation process;
- Template for documenting the source and characteristics of reference samples;
- Lack of a standard test for comparison for some diseases;
- "Grandfathering" tests that have been historically used successfully by reference laboratories.

The draft guidelines were circulated to members for final review and are attached as Appendix IV.

7. Other matters

If these draft guidelines were to be accepted as a stand-alone chapter in the *Terrestrial Manual*, they would benefit from inclusion of essential material from draft Chapter 1.1.4./5. and some of the appendices. Likewise, there would be a need to show coherence between both documents, in particular between stage 2 and the concept of provisional acceptance (of validation).

The Group agreed that the finalisation of the draft guidelines document would be best achieved by an additional meeting of the *ad hoc* Group.

8. Adoption of report

The Group reviewed the preliminary outline of the draft report provided by the rapporteur, and revised and commented on the draft guidelines produced. The Group agreed that the report and the draft guidelines would be subject to a short period of circulation in the Group for minor comments and final adoption.

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…/Appendices

Appendix I

MEETING OF THE OIE *AD HOC* GROUP
ON VALIDATION OF DIAGNOSTIC TESTS FOR WILDLIFE
Paris, 27 - 29 April 2011

Agenda

1. Opening and purpose of the meeting.
2. Adoption of the agenda and appointment of chairman and rapporteur.
3. Adoption of the draft Terms of Reference of the *ad hoc* Group.
4. Information on existing documents and initiatives.
5. Draft of guidelines according to the Terms of Reference.
6. Other matters.
7. Adoption of the draft report.

Appendix II

MEETING OF THE OIE *AD HOC* GROUP
ON VALIDATION OF DIAGNOSTIC TESTS FOR WILDLIFE
Paris, 27 - 29 April 2011

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List of participants

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Appendix III

MEETING OF THE OIE AD HOC GROUP

ON VALIDATION OF DIAGNOSTIC TESTS FOR WILDLIFE

Paris, 27 - 29 April 2011

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Terms of Reference

- Draft guidelines on "Principles and methods for the validation of diagnostic tests for infectious diseases applicable to wildlife" taking into account the existing chapters of the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (in particular Chapter 1.1.4./5. "Principles and methods of validation of diagnostic assays for infectious diseases") and the documents provided by the OIE Working Group on Wildlife Diseases on the mentioned subject.

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Appendix IV

Draft Guidelines on "Principles and methods for the validation of diagnostic tests for infectious diseases applicable to wildlife"

1. Introduction

Diagnostic testing of wildlife for infectious diseases is becoming increasingly important as interest grows in diseases that occur in wildlife that may have an impact on wildlife populations and biodiversity, as well as the health of humans and domestic animals. For the purposes of this chapter, "wildlife" will be defined as animals belonging to one or more of the following groups:

- *Wild animals*: Those animals that do not live under human supervision or control and do not have their phenotype selected by humans.
- *Captive wild animals*: Those animals that live under human supervision or control but do not have their phenotype selected by humans.
- *Feral animals*: Those animals that do not live under human supervision or control but do have their phenotype selected by humans.

Wild animals generally are susceptible to infection with the same disease agents as domestic animals and in some cases, the tests developed and validated in other species may have utility for wildlife species. However, diagnostic testing of wildlife can be more challenging than in domestic animals for a variety of reasons that range from difficulties in accessibility and associated costs to poor sample quality or poor knowledge of pathogenesis/epidemiology of the disease in a particular species. Affordability of tests is a key consideration because wildlife do not have owners who pay for testing. Hence, low cost may be a critical factor in test selection for use for a designated purpose.

Many routine diagnostic tests that have been developed and are currently used for detecting or confirming diseases in domestic livestock have generally not been validated for wildlife. The question remains as to whether there are any essential differences in sensitivity or specificity of these tests when they are applied to wildlife samples.

Diagnostic tests can arbitrarily be divided into two categories which have some overlap: agent identification techniques, which include direct visual diagnostics, antigen detection and molecular techniques; and indirect identification techniques. Direct diagnostic techniques include macroscopic examination to identify macroparasites, disease vectors, and pathognomonic macroscopic lesions at necropsy. Microscopic examination can be employed to detect and identify microparasites in sample types ranging from body fluids or tissue smears/sections to fecal examinations. The typical light microscopic appearance or electron-microscopic features of some specific diseases in organ sections may be diagnostic. Use of fluorescent conjugates, special stains, and/or immunohistochemical techniques may be used to identify aetiological agents in tissue smears or sections.

There are various direct methods of detecting infectious agents and antigen in specimens. These include *In vitro* or *in vivo* culture – commonly used to isolate bacteria, viruses, fungi and some protozoa; and molecular techniques – including polymerase chain reaction (PCR) amplification of the agent's genetic material, and specific DNA probes to detect antigen. Importantly, these direct agent identification diagnostic techniques should theoretically not be affected by the species of the host, i.e. domestic livestock or wildlife. However, there may be some species variation in the proliferation rate or amplification of the agent, which may affect the amount and distribution of antigen in the tissues.

Validation of diagnostic tests for individual wildlife species presents challenges, including the accessibility of adequate sample numbers and volumes to be used in the validation process. The underlining principles and stepwise approach to the validation of a diagnostic test are outlined in the draft chapter 1.1.4./5. The purpose of this chapter is to present the information necessary to validate a diagnostic test for wildlife species that will be recognised by the OIE (completion of Stages 1, 2 and 3). However, recognizing that completion of this process may not be necessary, or even possible, in all circumstances, guidance is provided for following the validation pathway to a point where the test can be provisionally accepted to provide confidence in results and for use in specific applications in a regional or national context.

2. Test validation principles

Validation is a process that determines the fitness of an assay, which has been developed, optimized and standardised, for an intended purpose. Ideally, the validation process of tests for wildlife should be conducted in the same way as for tests for domestic animals (presented in draft chapter 1.1.4/5). However, as explained above, wildlife diagnostic testing often meets difficulties, which also place limitations on the prospects of full validation. Therefore, in cases in which an optimal full validation process is not feasible, the best possible alternative may be to evaluate the wildlife assay's fitness in a reduced number of reference samples. The preliminary estimates of a the test's performance may provide sufficient information for government authorities to agree that a test can be provisionally accepted for testing of animals being moved/translocated or for pathogen surveillance within a county.

In many cases, existing diagnostic tests validated for one species may be adapted to and evaluated in other species. In other cases, new tests for wildlife may need to be developed without the advantage of a pre-existing test which may require minimal or no modification. In all cases, the intended purpose(s) and application(s) of the test should be established / defined before it is developed and validated since this may impact selection of appropriate reference samples and ultimately, generalisability of the validation results.

1.1. Fitness for purpose

A list of purposes for diagnostic testing is provided in the draft chapter 1.1.4/5. More specifically for wildlife testing, the main purposes to develop and apply a diagnostic assay are:

1) Screening wildlife populations for the presence of infectious agents, for example:

    a) for surveillance (e.g. early detection, evaluation of trends in prevalence or incidence)
    b) to estimate prevalence of infection or exposure

2) Screening or testing vectors or environmental samples for the presence of infectious agents

3) Confirming a diagnosis of suspect or clinical cases (includes confirmation of positive results from a screening test)

4) Certifying freedom from infection or presence of the agent in individual animals or products, for

    a) movement or translocation
    b) human consumption

5) Monitoring of the geographical distribution and prevalence changes due to management interventions (including determining immune status of individual animals or populations)

6) Studying agent, host and environment factors associated with disease occurrence

2.2. Reference samples and sample quality

By definition, all reference samples should be well characterised in terms of the host and its source population, and the infectious agent involved. Although the same description detail would be desirable for reference samples from wildlife as compared with livestock, the relevant information is often not available. In such cases as many details as possible should be recorded. In general, reference samples should represent the target condition of interest e.g. clinically diseased, subclinically infected without overt signs of disease. Experience indicates that selection of inappropriate positive reference samples from clinically affected animals when the test will be used to detect subclinical infection results in overly optimistic estimates of sensitivity and specificity. Experimentally infected animals may be the only source of reference samples in some cases but their use should be supplemented with samples from naturally infected animals, wherever possible.

The following information was considered to be the minimum requirement to adequately characterise a reference sample: a) the precise host species, b) tests used for confirmation of the presence or absence of the pathogen, c) geographical location with reference to known disease free or infected areas/regions, and d) the date of sample collection. Wherever possible, information on sex and age category (juvenile, sub-adult, adult) will add value since some infections have a sex- or age-specific predilection.

a) *Pooling of reference samples*

Ideally, reference samples should be obtained from individual animals and aliquoted into smaller volumes (weights) for subsequent testing. However, when animals of small body mass are the source of the reference samples or when very few animals are infected with the particular agent of interest, pooling of samples is considered to be an acceptable approach to obtain a reference sample. Preferably the stage of infection of the individual animals should be known to estimate whether the pooled sample will contain a low, average, or high concentration of an analyte. A strongly positive sample of good quality can be diluted with the same sample matrix, for example faeces or serum, from the same host species to generate a series of samples with decreasing concentrations of the agent or products of the immune response. If certain stages of infection are not available, it should be documented.

In cases where only a limited volume of a suitable sample of good quality is available, it can be used as a reference sample in support of a well-planned set of test runs (e.g. for a repeatability study).

b) *Negative reference samples and samples of unknown infection status*

If no reference samples are available to determine diagnostic specificity in terms of certain agents known to cause cross-reactivity, this should be documented.

Latent-class statistical models for estimating diagnostic sensitivity and specificity in the absence of a perfect reference standard (sometimes termed a gold standard) are appealing for validating diagnostic assays for wildlife (see the draft chapter XXX and the statistical annex for details). However, these approaches are based on a thorough description of the reference population(s) which is very difficult or even impossible in the case of free-ranging wildlife.

c) *Sample quality*

The sampling environment for wildlife is often sub-optimal and may lend itself to cross-contamination. In addition, opportunistic sampling constitutes an important aspect in screening and monitoring wildlife populations for infectious agents. This often results in the collection of samples with compromised integrity (e.g. contamination, advanced autolysis etc.). Therefore, investigators are responsible to determine the suitability of such samples for test validation, but given the overall

scarcity of samples for certain conditions or from certain host species (e.g. endangered species), great care should be taken to ensure maximum utilisation of samples of sub-optimal quality. A qualitative assessment of sample quality (e.g. good, poor, autolysed) should be recorded in databases documenting the characteristics of reference samples.

Therefore it is deemed useful and necessary to validate appropriate tests for a range of sample condition criteria such as changes in detectability over time, under different storage temperatures, during autolysis, etc. However, this step in the validation process should be conducted after the test has been provisionally accepted.

3. Test validation pathways and stages for wildlife

The two most common scenarios considered were when there was (or was not) an existing validated test in another species for the same disease of interest. A flow chart (Figure 1) was developed to show the stages in the process. Corresponding requirements to meet validation criteria and estimate test performance characteristics are shown in Table 1. Taxonomic criteria would be the primary consideration in defining relatedness for purposes of parameters that need to be estimated and appropriate sample sizes.

Figure 1: Flow chart of possible pathways and stages of test validation in wildlife when a previously validated test exists or does not exist

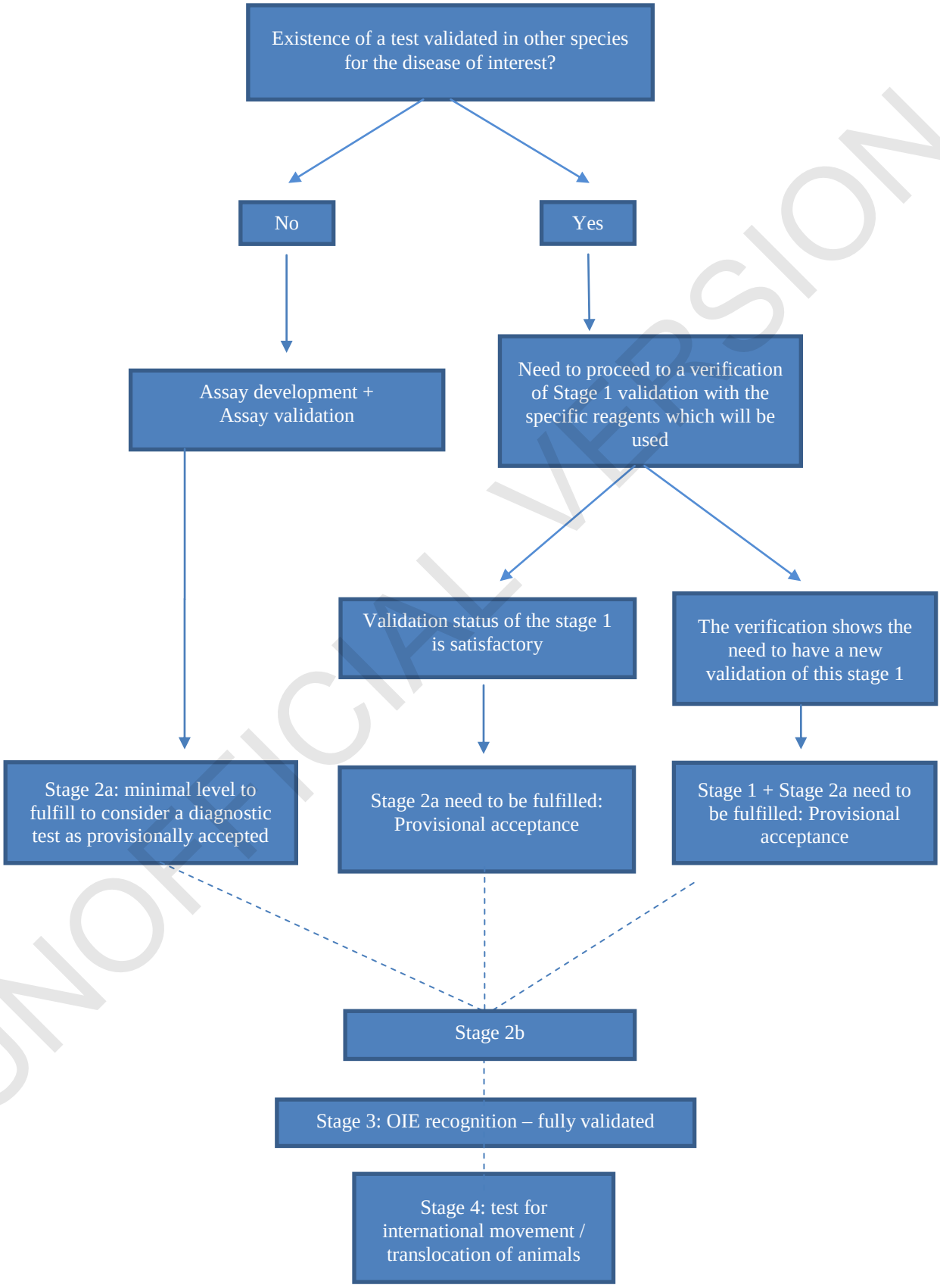

Table 1: Steps required to meet validation criteria describe in the draft chapter 1.1.4/5 and estimate test characteristics. Requirements in the different Stages need to be fulfilled with an acceptable outcome

Validation pathway Chapter 1.1.4/5	***Validated test in related species****	***No validated test in related species****
Stage 1	**Stage-1 verified in new target species**	**Stage-1 verified in new target species**
Analytical specificity	Yes	Yes
Analytical sensitivity	Yes	Yes
Repeatability	No	Yes
Reproducibility (preliminary)	No	Yes
Stage 2	**Stage 2a (Provisional acceptance)**	**Stage 2a (Provisional acceptance)**
Diagnostic sensitivity	Yes (minimum of 10 infected samples)	Yes (minimum of 30 infected samples)
Diagnostic specificity	Yes (minimum of 10 non-infected samples)	Yes (minimum of 30 non-infected samples)
Cut-off determination	Yes (total of 20 samples)	Yes (total of 60 samples)
Reference sample description	Yes	Yes
	Stage 2b	**Stage 2b**
Diagnostic sensitivity	Yes	Yes
Diagnostic specificity	Yes	Yes
Cut-off determination	Yes	Yes
Reference sample description	Yes	Yes
Stage 3	**Stage 3**	**Stage 3**
Reproducibility	Yes	Yes
Ruggedness	Yes	Yes
Repeatability (augmented)	Yes	Yes
Stage 4	**Stage 4**	**Stage 4**
Predictive values (populations)	Yes	Yes
Ruggedness (augmented)	Yes	Yes

* Taxonomic related species would be the most appropriate group to go down in the first column. Sub-families taxonomic level would be preferable but where it has been demonstrated effectiveness of reagents in two different species (e.g. peer reviewed publication), it would also be acceptable.

Number of field samples for stage-2 estimation of diagnostic characteristics

The selected sample size for completion of stage 2 should be based on expected values for diagnostic sensitivity (DSe) and specificity (DSp), the desired confidence level and error margin as shown in Table 1 in draft chapter 1.1.4/5. For example for an expected DSe of 90%, a sample size of 138 is required to yield an error margin of 5% with 95% confidence (see right panel of Table 1). However, it is acknowledged that this number of truly positive samples may be difficult to obtain for some wildlife species and can only be achieved when data from multiple testing laboratories using the same test in a standardized way are combined over time.

If numbers of reference samples (positive and negative) are lower than those listed in Table I, the calculated error margins on estimates (typically represented as 95% confidence intervals) for DSe and DSp, respectively, will be wider than those on which the table was based. Consequently, small sample sizes increase the uncertainty in test performance characteristics. Use of reference samples that are representative of the target condition is critical to achieve an unbiased (and practically useful) estimate of DSe and DSp that will stand up to scrutiny over time. This is often of greater concern than sample size.

For purposes of wildlife testing, stage 2 is proposed to be divided into stages 2a and stage 2b. Stage 2a needs to be completed for "provisional acceptance", as previously described. In stage 2a, the assumption is made that the pathway based on an existing validated test in a related livestock disease (compared with no validated test) was based on at least 20 positive reference samples and 20 negative reference samples and estimates of DSe and DSp are similar, if not identical, in the 2 species. These samples provide "credit" towards the reduced sample size (middle column vs. right column in table). Selection of the pathway with reduced sample size should be justified based on sample size and evidence of comparability (e.g. same test cutoff value and reagents) provided in peer-reviewed publications.

The net effect of a lower sample size is greater uncertainty in estimates unless the prior information about the DSe and DSP in the related species is formally incorporated through a Bayesian analysis. Table 2 shows the effect of use of 140 or fewer known positive samples when the DSe estimate (90%) was calculated after field samples were collected and tested.

Table 2: Approximate error margins and 95% confidence intervals for diagnostic sensitivity (DSe) for decreasing numbers of positive reference samples

No. positive reference samples	No. positive	DSe (%)	Approximate error margin on estimate of DSe	95% exact binomial confidence interval for DSe (%)
140	126	90	± 0.05	83.8 – 94.4
100	90	90	± 0.06	82.4 – 95.1
60	54	90	± 0.08	79.5 – 96.2
30	27	90	± 0.10	73.5 – 97.9
10	9	90	± 0.18	55.5 – 99.7

Calculations for 95% confidence intervals for DSp are affected similarly by the number of negative reference samples that are used.

Appendix V

Original English
September 2011

BRAINSTORMING MEETING FOR MODERNISING THE OIE *TERRESTRIAL MANUAL*

OIE Headquarters, Paris, 12–13 September 2011

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A Brainstorming Meeting for Modernising the OIE the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* was held at the OIE Headquarters from 12 to 13 September 2011. Dr Kazuaki Miyagishima, Head of the OIE Scientific and Technical Department, welcomed the participants on behalf of the Director General. He stated that this meeting was expected to provide new ideas for the OIE's future *Terrestrial Manual* and any improvements for the *Terrestrial Manual* could be proposed for the *Aquatic Manual* where appropriate, in order to keep consistency in purpose, style and content between the two *Manuals*. Dr Beverly Schmitt, Vice-President of the OIE Biological Standards Commission was elected chairperson of the Group and Dr Ana Nicola was appointed rapporteur. As the purpose of a brainstorming was to explore ideas, possibilities and directions on how to improve the *Terrestrial Manual*, with the ultimate aim of formulating actions, there was no formal agenda. This report therefore, is a record of the ideas and recommendations that were the outcome of the Group's discussions.

The List of Participants and of documents provided are given at Appendices I and II.

The invited group of laboratory experts identified the following problems and questions with the current edition of the *Terrestrial Manual:*

Problems/questions

1. Biosecurity definitions (including plasmids) should be included in every disease chapter.
2. Mention of the OIE Reference Laboratories should be an integral part of the chapters (left as an afterthought at the end of disease chapters).
3. The structure of *Manual*, the chapters and their content are not user friendly.
4. There is a need to identify the purpose of each of the diagnostic methods described in disease chapters.
5. The procedure for updating the *Manual* is not clear to Member Countries.
6. What diseases should be addressed in the *Manual*?
7. Perhaps more details on testing, including flowcharts and photographs could be added to the *Manual* or the website.
8. Is there a defined format for authors to follow? (Yes, but the authors do not always follow it)
9. Should the *Manual* updates be formatted in the same way as updates proposed by the Code Commission?
10. Who are the end-users of the *Manual*?
11. There is a need to include more information in Section C (vaccine evaluation and production) in disease chapters.
12. Not all introductory chapters are Standards (some are guidelines, some are reviews).

After extensive discussion, the group generated the following ideas/comments for *Manual* improvement:

Introductory chapters

1. The introductory chapters are generally useful and satisfactory; however, they need to be written as standards rather than guidelines.
2. Chapter 1.1.1 should be divided into two new chapters, one on transport of samples and one on sample collection.
3. There should be additional chapters on vaccine issues as there are some topic gaps in the vaccine chapters.
4. The order of the chapters should be reorganised such that the vaccine chapters are grouped together and the diagnostic chapters are grouped together.
5. It was suggested to take the guidelines from the OIE Quality Standard and Guidelines book (on proficiency testing, production of reference reagents, etc.) and put them in the *Manual* as standards

Disease chapters

1. It is important to have information such as epidemiology and disease control strategies in the introduction to the disease chapters. It was suggested to reiterate the current instruction to authors to keep chapter introductions to a maximum of 1 page or less.
2. The table in the *Aquatic Manual* chapters that lists the diagnostic methods alongside the purpose for which the assay has been validated could be usefully used. See number 10 below.
3. OIE Reference Laboratories could review the existing or develop new OIE disease cards.
4. Prophylactic vaccination of laboratory personnel for diseases such as Rift Valley fever was discussed. There was general agreement to include such information in the introduction to disease chapters where appropriate.
5. Information on biologicals such as tuberculin antigen should be left in the vaccine section of disease chapters.
6. It was recommended to leave the OIE Reference Laboratory list at the end of the *Manual* but to include a statement at the end of each disease chapter informing the reader that there is/are OIE Reference Laboratories for the disease in question and where to find the list. It was also suggested to add a link to the updated list of OIE Reference Laboratories in the online chapters of the *Manual* and to add instructions to contact the OIE Reference Laboratory for a list of kits, reagents, and/or vaccine manufacturers.
7. Harmonisation among the Reference Laboratories, where they exist, of test methods should be encouraged.
8. All assays described should include information on test performance.
9. Each chapter should be reviewed to identify assays that can be said to have historical validation.
10. Draft components for the table to be included in the chapters:
    - Method
    - Purpose
        - Population freedom from disease
        - Individual animal freedom from disease
        - Efficiency of eradication policies
        - Confirmation of clinical cases
        - Prevalence of infection - surveillance
        - Immune status in individual animals or populations post-vaccination
11. The need to fast track new assays, for example for emerging diseases, was discussed. It was recommended to refer these issues to the appropriate Reference Laboratories. It was felt that the OIE needs a fast track modification protocol, while retaining the requirement that all OIE Standard texts must ultimately be proposed for adoption by the Assembly.
12. The chapter revision procedure was discussed. It was noted that a second review by Member Countries before adoption is in place. It was suggested to place information on how the *Manual* is revised on the OIE website. The group suggested updating chapters only when there is documented scientific evidence for the need to update. Authors should be requested to provide supporting documents and rationale for all proposed amendments and these documents should be made available to Member Countries with the draft chapter. OIE Reference Laboratories should review and reach consensus regarding changes before the chapter is submitted to the OIE.

13. It was suggested to provide CD-ROMs with revised chapters to the Assembly each May as many laboratories use hard copy *Manuals* only.
14. It was suggested to mention in the chapter introduction the sentence from *Code* chapter 7.8 on limiting the use of animals in research and education.
15. The OIE should ask countries periodically which chapters need to be reviewed and should make available a page on their web site for comments.
16. The number of references in the chapters should be kept short and be limited to any assays described in the chapter and key review articles.
17. The OIE should ask authors to add a paragraph to each assay method with the description of cut-offs and interpretation as appropriate.
18. It was recommended to add a statement on biosafety and biosecurity level for each pathogen in the introduction of each disease chapter.
19. It was suggested to retain in the *Manual* the introductory chapters on vaccines and Section C of disease chapters. See suggested ordering of the introductory chapters below:

General standards

Chapter 1.1.1.	Sampling of diagnostic specimens
Chapter	Shipment of diagnostic specimens
Chapter 1.1.2.	Biosafety and biosecurity in the veterinary diagnostic microbiology laboratory and animal facilities
Chapter 1.1.3.	Quality management in veterinary testing laboratories
Chapter 1.1.4/5.	Principles of validation of diagnostic assays for infectious diseases
Chapter 1.1.8.	Principles of veterinary vaccine production (including diagnostic biologicals)
Chapter	Minimum requirements for vaccine production facilities
Chapter 1.1.9.	Quality control of vaccines
Chapter 1.1.10.	International standards for vaccine banks

At the end of *Manual* and clearly marked as guidelines and not standards

Chapter 1.1.6.	Laboratory methodologies for bacterial antimicrobial susceptibility testing
Chapter 1.1.7.	Biotechnology in the diagnosis of infectious diseases
Chapter 1.1.7a.	The application of biotechnology to the development of veterinary vaccines
Chapter 1.1.11.	The role of official bodies in the international regulation of veterinary biologicals

Summary

In general, the brainstorming group felt that the existing format and content of the *Manual* was good. They appreciated the opportunity to provide the Biological Standards Commission with input on potential improvements. The members of the BSC that attended the session expressed their appreciation for this group's efforts and looked forward to implementing many of the suggestions. The Group did not see an immediate need to conduct a wide questionnaire survey amongst the presumed users of the *Terrestrial Manual.*

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…/Appendices

Appendix I

BRAINSTORMING MEETING FOR MODERNISING THE OIE *TERRESTRIAL MANUAL*

Paris, 12–13 September 2011

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Appendix II

BRAINSTORMING MEETING FOR MODERNISING THE OIE *TERRESTRIAL MANUAL*

Paris, 12–13 September 2011

List of documents provided

1 User Questionnaire

2 Example questionnaire response 1: End-User

3 Example questionnaire response 2: Member Country

4 Example questionnaire response 3: Member Country

5 Summary of other good responses to the questionnaire

6 Report of the structure of chapters in the *Terrestrial Manual*, Consultant Editor

7 Instructions to authors

8 Example of problem chapter 1: Rabies + some Member Country comments

9 Example of problem chapter 2: Classical swine fever + expert comments

10 Example of problem chapter 3: African horse sickness

Work plan and activities (as of September 2011)

Topic	Progress made	Actions
1. Updating the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*	Brainstorming report endorsed by BSC	• Extend the next BSC meeting by 1 day • Brainstorming report to be used as guidance for future implementation of recommendations
2. *Manual* Chapters for adoption in May 2012	Chapters have been circulated once and amended according to comments received	• Circulation again to Member Countries as final texts to be proposed for adoption
3. *Manual* Chapters for adoption in May 2012 (maybe if enough time)	Updates of chapters that may be for adoption in May 2012 received	• Circulation of those already ready for Member comments
4. Designation of Reference Centres	On-going	
5. Survey Reference Labs about reagents		• Adapt annual report template or develop separate short questionnaire
6. Ref. Centres that do not reply to requests for advice, reagents, etc.	Identify the labs concened	• Draft a standard letter for Dr Vallat's signature reminding labs of their OIE obligations and asking is there is a particular problem
7. Keeping track of developments in new technologies and reaching a consenus to include in *Manual*		• Postponed to February 2012 meeting
8. How to include Genomic Sequencing in the OIE Information System	Liaise with OIE Information Department for opinion + Internal OIE discussions	

Ad hoc Groups

Existing Groups		
Title of *ad hoc* Group	**Progress by Sept. 2011 BSC meeting**	**Action by Feb. 2012 2011 BSC meeting**
Brainstorming for modernising the *Terrestrial Manual*	Report accepted by the BSC.	Recommendations will be further discussed and implemented on a step-by-step basis
Biosafety/Biocontainment Standards for Veterinary Laboratories	Draft ToRs approved. Meeting will take place 19–21 September 2011.	Report will be available for BSC in Feb. 2012
Future Groups		
Title of *ad hoc* Group	**Progress by Sept.. 2011 BSC meeting**	
Rift Valley Fever (Vaccines)	Need to draft ToRs. Dates: 6–8 December 2011	
Vaccine Quality related to Rabies	Need to draft ToRs. Dates: 10–12 January 2012	
Vaccine Quality related Classical swine feve	Need to draft ToRs. Dates: After February 2012	
Scientific Partnerships among Reference Centres – networking	Dates: 17–19 January 2012	