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REPORT OF THE MEETING OF THE OIE STANDARDS COMMISSION

Paris, 25 - 27 September 2001

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The OIE Standards Commission met at the OIE Headquarters from 25 to 27 September 2001.

Dr Bernard Vallat, Director General, sent his apologies for his absence – he was in Lebanon attending the OIE Regional Commission for the Middle East. Dr James Pearson welcomed the Members and participants to the meeting. Apologies were also received from Dr Beverly Schmitt, Secretary General, and Dr Peter Wright who were unable to travel due to recent disturbances in North America.

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

1. OIE Reference Laboratories

1.1. New applications for Collaborating Centre and Reference Laboratory status

The Commission discussed a modified application from the Centre for Animal Parasitology, Canadian Food Inspection Agency, Saskatchewan, Canada, to be designated as an OIE Collaborating Centre for Food-Borne Zoonotic Parasites. The Commission supports this application and referred it to the Regional Commission for the Americas for further comment.

The Commission recommends the following new application for OIE Reference Laboratory status:

Paratuberculosis

Veterinary Research Institute, Hudcova 70, 621 32 Brno, Czech Republic. Tel.: (420.5) 41.21.24.62; Fax: (420.5) 41.21.12.29; E-mail: vri@vri.cz
Designated Reference Expert: Dr I. Pavlik.

1.2. Updating the list of Reference Laboratories

The Commission approved a request by Dr S. Edwards, Veterinary Laboratories Agency, Weybridge, United Kingdom (UK) to be removed from the list of Reference Laboratories for transmissible gastroenteritis.

The OIE has been notified of the following changes to named experts at OIE Reference Laboratories. The Commission recommends their acceptance:

Foot and mouth disease and *Swine vesicular disease*

Dr A. Donaldson to replace Dr P. Kitching at the Institute for Animal Health, Pirbright, UK.

Lumpy skin disease and *Sheep pox and goat pox*

Dr P. Mellor to replace Dr P. Kitching at the Institute for Animal Health, Pirbright, UK.

Bluetongue

Dr P. Mellor to replace Dr J. Anderson at the Institute for Animal Health, Pirbright, UK.

Classical swine fever and *Equine viral arteritis*

Dr T. Drew to replace Dr D. Paton at the Veterinary Laboratories Agency, Weybridge, UK.

Contagious equine metritis

Dr E.M. Kamp to replace Dr J.H. Bongers at Institute for Animal Science and Health, Lelystad, Netherlands.

2. International standardisation of diagnostic tests and vaccines

2.1. OIE standardisation programmes for diagnostic tests

LIST A DISEASES

Contagious bovine pleuropneumonia – Coordinator Dr F.G. Santini

Dr F.G. Santini, OIE Reference Expert for contagious bovine pleuropneumonia (CBPP) at the OIE Reference Laboratory in Teramo, Italy, had responded to the Commission (report Feb. 2001) with regard to the preparation of reference sera for use in serological tests for CBPP. His proposal was greatly appreciated and will be pursued in correspondence.

Peste des petits ruminants – Coordinator Dr G. Libeau

Following earlier comments from the Standards Commission, (report Nov. 2000) Dr Libeau reported that new candidate weak positive standards are in preparation.

Classical swine fever – Coordinator Dr S. Edwards

Dr Edwards reported that technical difficulties had delayed the freeze-drying of the candidate standards.

LIST B DISEASES

Rabies serology – Coordinator Dr F. Cliquet

Dr Cliquet had reported that a negative reference standard prepared from a pool of dog serum of UK origin was available from the Reference Laboratory. There was not sufficient quantity to permit preparation of a weak positive. She commented that with the recent increase in testing laboratories, stocks of the OIE Standard positive dog serum were diminishing and plans are in hand to prepare a replacement. The Commission reiterated its standing recommendation that OIE International Standard Sera are intended principally for primary test standardisation at national laboratories. Secondary standards should be prepared, equivalent to the International Standard, for working test controls.

Equine influenza – Coordinator Dr J. Mumford

Dr Mumford had submitted a comprehensive report entitled 'Establishment of International Reference Standards for the Diagnosis of Equine Influenza'. The conclusions from the report submitted are listed in Appendix III. The full report may be obtained from the OIE. The Commission congratulated Dr Mumford and her co-workers, as well as the European Pharmacopoeia, for this significant step forward in international standardisation. The sera have already been adopted by the European

Pharmacopoeia as Biological Reference Preparations for standardisation of vaccines. The OIE should endorse their use for the same purpose internationally, and it is further recommended that they should be designated OIE International Standard Sera for diagnostic test standardisation. It was noted that, as advised previously by Dr Mumford, the single radial haemolysis test gave a more consistent performance than the haemagglutination inhibition test. The Commission also congratulated Dr Mumford and the European Directorate for the Quality of Medicines on organisation of a meeting, with the OIE as a co-sponsor, to address standards for equine influenza vaccines.

2.2. Stocks and supplies of OIE International Standard reagents

OIE Reference Laboratories had replied regarding the amounts in stock and the number of lots supplied of the different OIE International Standard Sera. Although a full response had not been received, the Commission was disappointed to note that some of the sera were in little demand. It wished to encourage national laboratories in Member Countries to make use of those sera that are available to further the work of international harmonisation of diagnostic test procedures. National laboratories are also encouraged to use the International Standards as a basis for preparing national standards for distribution to laboratories within the Member Country.

2.3. International standardisation of tests for dourine and surra

Following the meeting with Dr L. Touratier (report Feb. 2001) the Standards Commission had received further advice from a laboratory expert (Dr A.G. Luckins, Edinburgh University, UK). He confirmed that the complement fixation test for dourine had a number of deficiencies in terms of sensitivity, specificity and international standardisation. Although the ELISA[1] shows promise as a better test, it still lacks comprehensive validation data. The Commission urges Reference Laboratories and others to work towards resolving this problem.

Although surra is an international trade issue, and there is an identified need for improved international standardisation of diagnostic tests, there is no obvious candidate that could provide Reference Laboratory services.

3. List of prescribed and alternative tests

3.1. Glanders

A Member Country had submitted information regarding a plate agglutination test for glanders serology. The Commission will require further validation data before this method can be evaluated. It was noted that information generally on laboratory tests for glanders is hard to obtain, and any Member Country with information on the disease is encouraged to inform the OIE.

3.2. Caprine arthritis/encephalomyelitis and maedi-visna complex

A Member Country had suggested that ELISA should be adopted as a prescribed test for caprine arthritis/encephalomyelitis and maedi-visna. The Commission would in principle support this, but first needs to see the relevant validation data.

3.3. Bovine anaplasmosis

The Commission had been advised that the complement fixation test for anaplasmosis has a poor sensitivity and should be removed from the list of Alternative Tests for this disease. Views from Member Countries will be welcomed and the proposal will be finalised at the next meeting.

4. Questionnaire on bovine tuberculosis

The responses received from Member Countries will be analysed for discussion at the next meeting of the Commission.

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1 ELISA: Enzyme-linked immunosorbent assay

5. OIE *Manual of Standards of Diagnostic Tests and Vaccines*

5.1. Feedback from Member Countries on the fourth edition of the *Manual*

Many recipients of the *Manual* had kindly completed the questionnaire. The volume had been well-received and many supportive comments were noted. Some specific suggestions regarding improvements will be considered when preparing the next edition. These will be referred to the consultant editor for action. The Commission continues to have concerns about the content and format of the vaccine sections of disease chapters and will welcome any suggestions.

5.2. Planning the fifth edition of the *Manual*

The list of contributors and reviewers for individual chapters was checked and updated. Progress on first drafts is already being made. The Commission proposes to add further new chapters on *Yersinia enterocolitica* and *Listeria monocytogenes*. A suggestion of including a chapter on plague (caused by *Yersinia pestis)* will not be pursued. A possible chapter on virus diseases of bees will be considered. The Commission would like OIE to consider publishing the section on bee diseases as a separate *Manual* as it generally has a different target audience and is of little interest to most veterinary diagnostic laboratories.

One author had suggested tabulating values for sensitivity and specificity of different tests for a particular disease. This is in line with the desire of the Commission to improve the validation data for all tests, and should be encouraged, however it is recognised that for many diseases and tests such comprehensive information is still not available.

6. Preparation of booklet on guidelines

This Item was deferred until the next meeting.

7. Liaison with the International Animal Health Code Commission

7.1. Ovine pulmonary adenomatosis

A possible new chapter in the *Code* on ovine pulmonary adenomatosis was noted by the Commission. There is already a chapter in the *Manual* on this disease, and the author will be contacted to check the latest position on diagnostic tests.

7.2. Diagnostic tests for bovine spongiform encephalopathy

The Code Commission had asked for the latest information on sample preparation and validity of rapid diagnostic tests for bovine spongiform encephalopathy (BSE). Technical aspects of this were dealt with under Item 8.2. The Standards Commission advises that, when evaluating the results of laboratory test-based surveillance, OIE should seek assurances that the tests used, and the laboratories performing those tests, have reached internationally accepted standards of test performance, and that appropriate quality assurance is in place.

7.3. Intracerebral pathogenicity index for Newcastle disease vaccines

The Code Commission had asked for the recommended intracerebral pathogenicity index (ICPI) for Newcastle disease vaccines. This had been addressed at the November 2000 meeting and the following is the recommendation from that meeting:

The Ad hoc Group on Newcastle Disease (April 2000) had referred to the Standards Commission for an opinion on the selection of virus vaccine strains. After consultation with experts in the field, the Standards Commission took note of procedures in use in different regions. In principle it is recommended that vaccines should have an ICPI of less than 0.7. However, in order to account for interassay and interlaboratory variability, a safety margin should be allowed so that the working limit for ICPI in vaccine master seed virus strains should be 0.4. It is believed that this will not conflict with current practice in most Member Countries.

The author of the Newcastle disease chapter of the *Manual* will be asked to put the ICPI for vaccine

master seed virus into the next edition.

7.4. Removal of atrophic rhinitis of swine

There had as yet been no response from the Code Commission regarding the proposal to remove this disease from List B (report Feb. 2001).

8. Follow-up from the General Session in May 2001

8.1. Classical swine fever

Final Report of the General Session, May 2001: paragraph 330. The Commission will again seek advice from Reference Laboratories on the current status of marker vaccines for classical swine fever.

8.2. Bovine spongiform encephalopathy

For this session the Commission was joined by representatives of the OIE Reference Laboratories for BSE at VLA Weybridge, UK (Dr Kath A. Webster), and at the Institute of Animal Neurology, Bern, Switzerland (Dr Rudolf Meyer). There was also telephone contact with Dr Heinz Schimmel (Laboratory for Management of Reference Materials, Geel, Belgium). It was also noted that much useful information on BSE, including development and application of diagnostic tests, may be found in the June 2001 Progress Report on BSE in Great Britain (http://www.defra.gov.uk/animalh/bse/bse-publications/progress/jun01/report.pdf).

The Commission reviewed the current state of the art regarding sampling of bovine brains and testing of the samples by rapid immunoassays to detect the presence of PrP^{SC}. Some are based on monoclonal antibodies, and others on specific polyclonal antisera. None of the available tests is able to distinguish immunologically between PrP^{SC} and normal PrP. All methods are therefore critically dependent on the sampling and sample treatment procedures. There is a need to enlarge upon the procedures described in the *Manual* and this will be a continuing process as new methodologies are in a state of active development. Because of the rapid advances in this field, the OIE Reference Laboratories will be asked to provide updated information to the Standards Commission twice yearly in advance of its meetings. The gold standard against which other tests should be evaluated is immunohistochemistry. This is also recommended as a confirmatory test for samples that test positive by immunoassay.

In order to reduce hazard to the operators, bovine brains should be sampled without opening the cranium. This is readily achieved, even at abattoirs, following training of operators in the use of a specially designed spoon, which can be inserted through the foramen magnum of the severed head. A suitable protocol has been drafted by the OIE Reference Laboratory, Bern, Switzerland, and is included as Appendix IV. The brainstem sample collected by this method should be further sampled at the laboratory as also described in Appendix IV. The preferred sample for immunoassay should be at, or within 1.5 cm anterior to the obex. This is based on studies (Schimmel *et al*., 115th Annual Meeting of AOAC [Association of Official Agricultural Chemists], Kansas City, Missouri, USA, 9-13 September 2001) that showed that the distribution of PrP^{SC} in infected brains is heterogeneous, and variable between individual animals. The suggested sampling site gives the most consistent positive results. Because of the uneven distribution of PrP^{SC}, sample size should be as specified in the diagnostic kit or if not specified should be at least 0.5 g. Performance characteristics of all of the tests may be compromised by autolytic changes if brains are not sampled fresh.

For diagnostic and surveillance purposes on field material, further processing of the brain tissue should be carried out precisely as specified by the supplier or manufacturer of the test method or kit. Details of this procedure do vary from method to method and should not be varied without supportive validation data for the variant methodology.

None of the immunoassays has yet been fully validated. In particular the predictive value of negative results is uncertain and likely to remain so due to the difficulties of determining the true status. Nevertheless a number of methods have been shown to perform well for confirmation of clinical or late preclinical cases and these are being increasingly used by Member Countries for surveillance purposes. Through the OIE information networks, the Commission will seek to keep Member Countries abreast of current knowledge through the regular reports on diagnostic test methods from the Reference Laboratories.

Quality control (QC) and quality assessment (QA) should form an essential part of the testing procedures. The Commission recommends that OIE Reference Laboratories should develop and refine guidelines on QC and QA for PrP immunoassays and send this information to the Standards Commission. The OIE Reference Laboratories are encouraged to assist national laboratories in establishing QA for other laboratories within the country. Likewise OIE Delegates are encouraged to support their national laboratories in participating in interlaboratory comparisons at international level.

This is particularly important where laboratories are using immunoassays as part of a national surveillance programme.

For preparation of QA materials for either national or international ring trials of rapid tests, it is essential to produce a primary homogenate of brain so that all laboratories receive an identical sample, even though this may slightly compromise test performance due to commutability problems. This remains under study and the Commission urges the Reference Laboratories to address it as a matter of urgency. As an interim approach, the use of eight parts brain tissue homogenised in two parts of 5% sucrose may be recommended.

Known positive BSE brain material is not available in unlimited amounts. Available stocks need to be conserved for research purposes and for diagnostic applications where alternatives are not appropriate. Also handling the material could be a safety hazard, particularly in low prevalence countries where the risk is so low that routine testing can be done at containment level 2. For routine QC purposes, sheep brain confirmed as scrapie positive may be used. This has a lower risk category than BSE brain, is likely to be more readily available, and has the advantage over PrP peptides (used as controls for some kits) that it also controls the critical sample extraction procedures. The Commission suggests that the Reference Laboratories investigate further the use of sheep scrapie brain for QC and QA as a way of conserving precious BSE materials.

Detailed guidelines on laboratory safety procedures for handling BSE are available from the Reference Laboratories. In summary, each laboratory should carry out a local risk assessment. For BSE-infected countries it is advisable to do testing at containment level 3, at least for those samples considered higher risk. For BSE free countries, or where the likelihood of positives is very low, a containment level 2 facility may be adequate. However due to the aerosols produced, it is always advisable to carry out homogenisation in a safety cabinet.

8.3. Foot and mouth disease

For this session the Commission was joined by Dr Ingrid Bergmann (Centro Panamericano de Fiebre Aftosa, Rio de Janeiro, Brazil), Dr Emiliana Brocchi (Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy) and Dr Karl Sørensen (Danish Veterinary Institute, Lindholm, Denmark).

The rationale for use of nonstructural protein (NSP) serological assays is that the methods of vaccine production described in the *Manual* leave little or none of these proteins in the final product, and therefore animals vaccinated but not infected do not seroconvert to NSP. On subsequent infection with wild-type virus most animals seroconvert to NSP. The other advantage of NSP ELISAs is that they are independent of virus serotype. However, some vaccinated animals may develop very low antibody titres, which may not be detectable by some NSP tests. In addition, there are reports that not all animals that have been vaccinated and are subsequently infected, seroconvert to NSP. Because of these factors, the tests cannot be relied on for individual animal certification, and should always be applied on a herd or group basis. Also most of the validation work has been done in cattle and more information is needed on other species.

A number of different NSP test protocols have been described. It was noted that NSP 3D is found in vaccines and therefore vaccinated animals may be positive by the 3D assays. The reason for this is that the 3D protein is actually incorporated into the virion and consequently it will be present in the vaccine. The most reliable tests are those based on the 3ABC NSP complex, or components of it such as 3AB or 3B. It is essential that any NSP test used be fully validated, including determination of its diagnostic sensitivity and specificity. The sensitivity and specificity of NSP tests vary, and can to some extent be adjusted by the choice of cut-off value. As with many assays, baseline levels of activity in negative populations can vary with the population. Account should be taken of this in determining cut-off values. As a general principle, when the tests are used for surveillance screening,

the assay should be set to have a high sensitivity, with clarification of positive reactions by use of a confirmatory test, such as immunoblot ('EITB[2]' as described in the *Manual*) or ELISAs based on multiple NSPs.

Reference Laboratories are encouraged to develop a panel of sera that can be used to evaluate new NSP tests. It is understood that such a programme is being developed in conjunction with the OIE Collaborating Centre in Seibersdorf, Austria. The Standards Commission wishes to be kept appraised of progress on the project. The Commission will ensure that the next edition of the *Manual* includes the latest information on NSP test protocols.

It is important that any product development work on FMD vaccines, as well as quality control procedures during routine vaccine production, include procedures to check for the absence of residual NSP immunogenicity in the vaccine. This may conveniently be achieved by submitting sera collected for vaccine potency testing purposes to a NSP immunoassay.

It is acknowledged that many Member Countries will wish to use commercial NSP test kits for serosurveillance purposes. Before doing so they should seek information from the kit manufacturer, and in some countries the national licensing authorities, on the diagnostic performance characteristics of the assay, including detailed validation data, and if possible should seek independent confirmation from an OIE Reference Laboratory that the kit is suited to the proposed application. Indications for the use of NSP assays include :

- Evaluating countries or areas as 'FMD free with vaccination'. In this case it is important to agree with epidemiological experts on the minimum seroprevalence that the assay should detect to be sure that virus is not circulating subclinically. The OIE is developing an FMD surveillance Standard that will address this subject.

- Serosurveillance in countries 'free without vaccination' where a serotype-independent test is required to check for unexpected incursions of virus from outside the country.

- Serosurveillance in infected countries using vaccination, to assist in monitoring progress towards disease eradication and as an indication of the amount of subclinical circulation of wild-type virus.

The following additional points were discussed regarding FMD serology:

The VIAA[3] test lacks adequate sensitivity to detect persistently infected animals, particularly after prolonged infection or infection in some vaccinated animals. Also, it does not differentiate reliably between vaccinated and infected animals. Consequently, the VIAA should be deleted from the chapter in the *Manual* or else left in with a cautionary note as a test suitable only for general prevalence studies in endemic countries. The description of the EITB in the *Manual* should be clarified to indicate that it is an immunoblot test.

The Commission was informed that for structural protein-based ELISA, a solid-phase test has been developed[4] which has a higher specificity, similar sensitivity, and better reproducibility than the present prescribed test (liquid-phase blocking ELISA). The Standards Commission needs to be supplied with the relevant validation data in order to make a recommendation on this test.

8.4. Antimicrobial resistance

Resolution XXV (May 2001), paragraph 2a. The Commission will reconsider this topic at its next meeting. It noted that the proposed new chapter in the *Manual* on this subject will constitute a standard for laboratory procedures. No applications have yet been received for Reference Laboratory status.

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2 EITB: Enzyme-linked immuno-electrotransfer blot

3 VIAA: Virus infection-associated antigen

4 Mackay D.K.J., Bulut A.N., Rendle T., Davidson F. & Ferris N.P. (2001). *Journal of Virological Methods*, **97,** 33–48.

8.5. Emerging diseases

Resolution XX (May 2001). This will be considered at the next meeting of the Commission.

9. Any other business

9.1. OIE Biotechnology Group

The Commission commended the Biotechnology Working Group on the organisation of the joint OIE/WAVLD[5] Biotechnology Symposium in Salsamaggiore, Italy, on 4 July 2001. The Group has also provided expert advice to the Commission regarding preparation of the *Manual* and other specialist aspects of the Commission's work. Since the pioneering work of the Group, biotechnology has now become widely applied in many aspects of science and is integral to the work of veterinary laboratories world-wide and to the aims of the Standards Commission. It is difficult in this climate to identify a distinctive role for such a group. The Commission suggests that the Biotechnology Group should be disbanded, and that Ad hoc Groups should be convened to address specific biotechnological topics. Planning of the OIE/WAVLD Biotechnology Symposia could be taken forward by the Standards Commission, which has already been closely involved with this event.

The Commission also believes that the OIE Veterinary Biotechnology Reference Database has outlived its usefulness. When it was developed, the information that was in the database was very useful, but there are now other more effective sources. The material that is presently included has not been updated for almost five years and the Commission recommends that the Central Bureau immediately remove the Biotechnology Database from the OIE Web site

9.2. Agreement with World Bank

Dr Edwards had met with a representative of the World Bank, along with Dr Vallat and Dr Pearson, to discuss a proposal for funding under the CGIAR[6] Challenge Program. A contribution prepared by OIE had been incorporated into a formal Concept Paper submitted in the Animal Diseases, Food Safety and Trade section of the Program. The Commission took note of the final text of this submission.

The Standards Commission has the responsibility under the current OIE Work Plan to identify priorities for research in the field of animal diseases and zoonoses (Resolution XIX, May 2001). If the CGIAR proposal is successful, those priorities will be used to inform the allocation of funds under the project.

9.3. Swine vesicular disease

The Commission took note of the recommendation from the OIE Regional Commission for Europe (Sept 2000) to review the progress on new tests for this disease. This will be done as part of the preparation for the next edition of the *Manual.*

9.4. Meeting of presidents of the Specialist Commissions

It was noted that the Commission should meet three times during 2002, as a means of transition to the new timetable of meetings in June/July and December in subsequent years. The Commission had noted the improvements in the OIE Web site and will be considering at future meetings how to develop the Standards Commission pages.

9.5. Invitation to participate in COST Action on Foodborne Zoonoses

OIE has been invited to participate in the EU COST Action 920 'Foodborne Zoonoses – Foodchain Approach'. The Commission welcomes this opportunity and recommends that OIE should respond positively as it is very much in line with the current OIE Work Plan.

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5 WAVLD: World Association of Veterinary Laboratory Diagnosticians
6 CGIAR: Consultative Group on International Agricultural Research

9.6. Joint OIE/WAVLD Symposium in Thailand 2003

The Vice-President of the Commission had discussed with Dr J. Gorham, President of the OIE Working Group on Biotechnology, the content of the next OIE/WAVLD Biotechnology Symposium, to be organised in Thailand in 2003. It is proposed to focus on diagnostic methodologies that differentiate between vaccinated and infected animals. More detail of the content will be discussed at the next meeting of the Commission. It is hoped to find speakers from the Asia region.

9.7. Biosafety of bovine blood used to maintain fly colonies

The Commission had been asked for advice on sourcing and treatment of bovine blood and its international shipment for the purposes of maintaining tsetse fly colonies, which are essential for trypanosomosis research in Africa. The Commission recommends that specific risk assessments should be carried out on a case by case basis following the principles established in the *International Animal Health Code* (Section 1.5). These should take into account the prevalence of BSE in the country of origin, the low risk that bovine blood may be contaminated with BSE or other infectious agents, and the general rules on international shipment of biological products. Advice on laboratory safety procedures for handling potentially BSE-contaminated materials is available from the OIE Reference Laboratory for BSE at Weybridge, UK.

9.8. Dates of next Standards Commission meetings

29 January – 1 February 2002
The following tentative dates for future meetings were established: 12–14 June 2002 and 9-11 December 2002. An alternative that was proposed was: 17–19 September 2002 and early in January 2003. A decision will be made at the January 2002 meeting.

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.../Appendices

Appendix I

MEETING OF THE OIE STANDARDS COMMISSION

Paris, 25 - 27 September 2001

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Agenda

1. OIE Reference Laboratories
2. International standardisation of diagnostic tests and vaccines
3. List of prescribed and alternative tests
4. Questionnaire on bovine tuberculosis
5. OIE *Manual of Standards for Diagnostic Tests and Vaccines*
6. Preparation of booklet on guidelines
7. Liaison with the International Animal Health Code Commission
8. Follow-up from the General Session in May 2001
9. Any other business

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MEETING OF THE OIE STANDARDS COMMISSION
Paris, 25 - 27 September 2001

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CONCLUSIONS OF THE REPORT ENTITLED ESTABLISHMENT OF INTERNATIONAL REFERENCE STANDARDS FOR THE DIAGNOSIS OF EQUINE INFLUENZA

Three equine influenza horse antisera were evaluated in this international collaborative study to determine their suitability as international reference standards (IRSs) (diagnostic test) and biological reference preparations (BRPs) (vaccines immunogenicity control). Ten laboratories from seven countries assayed the candidate preparations together with three test samples with serological assays, i.e. SRH[7] and HI[8].

Due to the poor repeatability and reproducibility of the HI test, the HI titres determined for the sera should be in the range of 64 to 256 for serum A, and of 32 to 128 for both sera B and C.

For use in vaccines quality control, only the SRH results could be taken into account for the purpose of calibrating the candidate equine influenza horse antisera BRPs. Indeed, the experimental results of this study confirmed that the SRH test is less variable than the HI test and demonstrated that a variability of ± 20% is achievable for all laboratories and can therefore be used as suitability criteria.

As a consequence, each candidate reference material was calibrated by SRH and sera A, B and C were established as by the Ph. Eur. Commission in November 1999 BRPs for the assay of equine influenza vaccines (immunogenicity testing) through serological methods complying with the Ph. Eur. monograph *0248*.

Sample A was established as the first Equine influenza subtype 2 American-like horse antiserum BRP with an SRH antibody titre of 180 mm^2 ± 20%.

Sample B was established as the first Equine influenza subtype 2 European-like horse antiserum BRP with an SRH antibody titre of 155 mm^2 ± 20%.

Sample C was established as the first Equine influenza subtype 1 horse antiserum BRP with an SRH antibody titre of 125 mm^2 ± 20%.

In view of the results of the collaborative study, it is proposed to adopt the sera A, B, C and D as OIE IRS, i.e.:

- positive reference standards for A (A/2 American-like influenza horse antiserum), B (A/2 European-like influenza horse antiserum) and C (A/1 influenza horse antiserum)

- negative reference standard for D (A/influenza negative horse serum)

These reference standards to be used for the performance of serological tests for diagnostic purposes, i.e. SRH or HI test on paired sera to show a rise in antibody titre in contaminated horses.

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7 SRH: Single radial heamolysis
8 HI: Haemagglutination inhibition

Treatment of brain material for use in Bovine spongiform encephalopathy tests

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Special care must be taken to sample the correct anatomical brain areas for testing. In BSE, PrP^{Sc} accumulation may be confined to the tissue around the caudal end of the fourth ventricle (the so called obex), where the fourth ventricle closes and becomes the central canal (Fig. 2). To make sure that the test results are reliable it is compulsory to include the obex area in the material submitted for examination, no matter which test is used (Western blot, ELISA etc.).

1. Removal of the brainstem

After the head has been separated from the body between the atlas and foramen magnum, the head is put on a support with the frontal bone down; the caudal end of the brain stem is visible through the foramen magnum. The brainstem is dissected through the foramen magnum without opening the skull by means of a 'teaspoon' with sharp edges and a long handle (Fig. 1). The spoon is inserted into the foramen magnum between the brainstem and the bone and moved along the wall of the skull moving to the left and the right to severe the cranial nerves on both sides, while avoiding damage to the brain tissue by keeping close to the bone. The spoon is advanced for a distance of approx. 7 cm in this fashion and than bent downwards cutting and separating the caudal medulla oblongata (with some fragments of cerebellum) from the rest of the brain. The spoon – remaining in a bent downward position – is then pulled towards the operator. In this way the severed brainstem slips out of the skull through the foramen magnum.

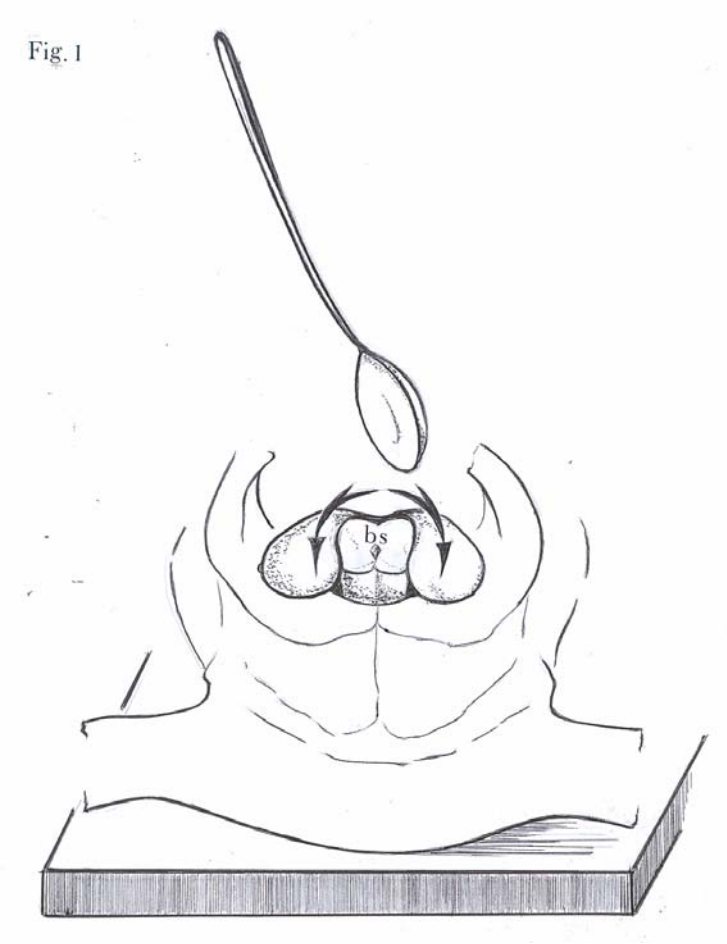

Fig. 1. The head is separated from the body and placed on a support upside down; the brainstem is (bs) is separated from the bone with cutting movements left and right (curved arrows) by means of a long-handled 'teaspoon' with sharp edges, inserted in the foramen magnum between bone and brain tissue.

2. Packaging, shipment

The brainstem tissue is put in a tightly closed plastic container and kept cool. Care should be taken that the each sample is properly labelled to insure that an animal can be identified at all stages of the process. The samples are shipped to the laboratory in solid closed containers. No liquid should leak out of the container at any time.

3. Selection of the correct anatomical area for testing

The obtained brainstem is depicted in Fig. 2. In the laboratory, it is divided in two halves by cutting along the midline with a sharp knife (Fig. 2). One half is refrigerated at 4°C until the result is known.

From the other half, a slice of the obex region is removed by cross-sectioning (Fig. 2). The dissected piece should weigh at least 1 g. The remaining material of this half of the brainstem is also refrigerated at 4°C.

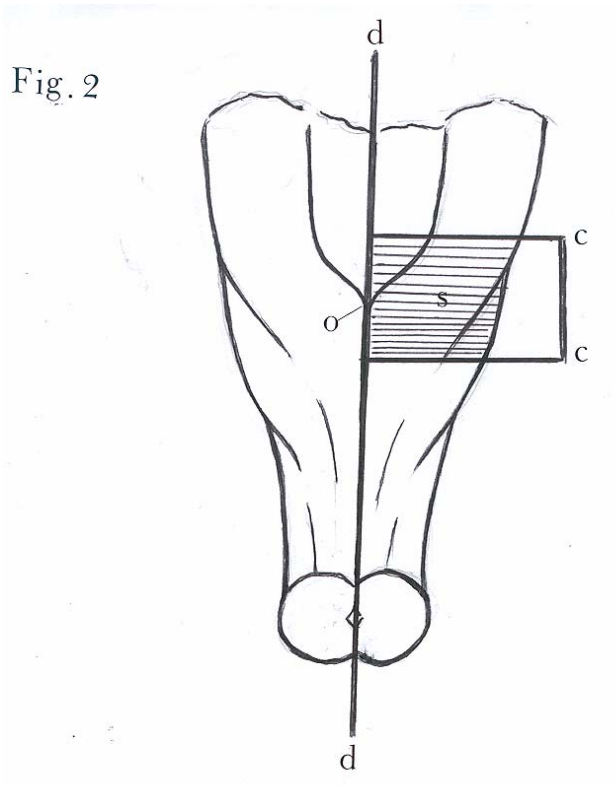

Fig. 2. Caudal medulla oblongata: obex (o), longitudinal division line (d), cross-sections (c), slice used for test (s).

4. Testing

Processing of the selected sample for PrP^{sc} testing according to the guidelines of the manufacturer.

5. Confirmation of positive test results

In case of a positive result the refrigerated half of the brainstem (Fig. 2, left) is put in a 50 ml plastic centrifugation tube with a screw cap containing 4% buffered formaldehyde. The top of the tube is further wrapped in paraffin film to prevent leakage. The remainder of the refrigerated brain tissue is put into another empty screw-capped tube. Both the fixed and fresh brain sample are packaged in a well sealed solid container and sent by express mail or other carrier to the reference laboratory for confirmation of the result by histology and immunocytochemistry for PrP^{sc}. If the material cannot be sent immediately, freeze the fresh tissue until shipment.