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REPORT OF THE MEETING OF THE OIE STANDARDS COMMISSION

Paris, 14-17 January 2003

The OIE Standards Commission met at the OIE Headquarters from 14 to 17 January 2003. Prof. Marian Truszczynski welcomed Dr Alejandro Schudel, and Members of the Commission. Dr Bernard Vallat had sent his regrets that he would be unable to attend the Commission meeting due to an official trip to the United States of America. Dr Vallat had written a letter to the Members in which he encouraged the Commission to strengthen the process for validating diagnostic kits. It was noted that this is Prof. Truszczynski's final Commission meeting and he was commended for his 30 years of service to the OIE.

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

OIE Collaborating Centre for New and Emerging Diseases

The Commission received an application for an OIE Collaborating Centre for New and Emerging Diseases. It questioned the need for such a centre and requested more information.

OIE Collaborating Centre for Animal Welfare

An incomplete application had been received for an OIE Collaborating Centre for Animal Welfare. The Commission considered the possibility of designating a Collaborating Centre for this area, but will await the report of the OIE Working Group on Animal Welfare. If a formal application is submitted to the OIE, it should be referred initially to this Working Group.

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

Heartwater

CIRAD-EMVT¹ Guadaloupe Designated Reference Expert: Dr Dominique Martinez

¹ Centre de coopération internationale en recherche agronomique pour le développement - Département d'élevage et de médecine vétérinaire du CIRAD

Porcine circovirus 2 and post-weaning and multisystemic wasting syndrome

The Commission discussed the potential need for a Reference Laboratory for porcine circovirus 2 and post-weaning and multisystemic wasting syndrome and determined it does not require one at this time.

1.2. Updating the list of Reference Laboratories

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

Rabies

Dr A. Liebenberg to replace Dr C. De Mattos at the Onderstepoort Veterinary Institute, South Africa.

Echinococcosis/Hydatidosis

Dr G. Christofi to replace Dr P. Economides at the Central Veterinary Laboratory, Cyprus.

Bovine tuberculosis

Dr A. Bernardelli to replace Dr A. Reniero at Gerencia de Laboratorios (GELAB) del Servicio Nacional de Sanidad y Calidad, Agroalimentaria (SENASA), Argentina.

1.3. Annual Reference Laboratories report for 2003

Reports had been received from 114/119 Reference Laboratories and 8/9 Collaborating Centres for terrestrial animals. The Commission commented once again on the impressive range of activities by the Reference Laboratories towards the objectives of the OIE, and the continuing support provided by individual experts to the work of the Standards Commission. The full set of reports will be supplied to Member Countries and to all the Reference Laboratories and Collaborating Centres. The international activities relevant to the work of the OIE are summarised below:

	General activities	Percentage of Laboratories carrying out these activities	Percentage of Collaborating Centres carrying out these activities
1a)	Diagnostic tests performed	109 (96%)	3 (38%)
1b)	Agent identification performed	100 (88%)	3 (38%)
2	Production, testing and distribution of diagnostic reagents	88 (77%)	1 (13%)
3	Research	92 (81%)	2 (25%)
	Specific OIE activities		
1	International harmonisation/standardisation of methods	53 (46%)	5 (63%)
2	Preparation and supply of international reference standards	57 (50%)	4 (50%)
3	Collection, analysis and dissemination of epizootiological data	44 (39%)	5 (63%)
4	Provision of consultant expertise	61 (54%)	6 (75%)
5	Provision of scientific and technical training	60 (53%)	8 (100%)
6	Organisation of international scientific meetings	29 (25%)	6 (75%)
7	Participation in international scientific collaborative studies	61 (54%)	6 (75%)
8	Publications	92 (81%)	6 (75%)

The Commission reviewed the list of Reference Laboratories that did not submit an annual report for 2001. The Commission recommended removal of one laboratory (Aujeszky's disease in Hungary) as it had not submitted an annual report for the previous two years.

2. International standardisation of diagnostic tests and vaccines

2.1. OIE standardisation programmes for diagnostic tests

LIST A DISEASES

Peste des petits ruminants – Coordinator Dr G. Libeau, CIRAD-EMVT Montpellier, France

The Commission has not yet received a data sheet for the weak positive OIE International Standard Serum for use in the competitive ELISA².

Contagious bovine pleuropneumonia – Coordinator Dr A. Pini, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale', Teramo, Italy

Dr A. Pini reported that irradiation was not a favourable method of treating serum for CF^3 testing because of the significant decrease in antibody titre. The Commission will ask Dr Pini to irradiate a portion of the reference sera for use in the competitive ELISA and to safety test a portion of non-irradiated serum for use in the CF test.

LIST B DISEASES

Rabies – Coordinator Dr F. Cliquet, AFSSA⁴ Nancy, France

The Commission feels there is still a need for a weak positive OIE International Standard Serum and will assist Dr Cliquet in obtaining rabies antibody negative dog serum from Mauritius.

Enzootic bovine leukosis – Co-ordinator Dr L. Renström, National Veterinary Institute (SVA), Uppsala, Sweden

A candidate serum has been identified and is performing well in the AGID⁵ test. A comparison of the serum by three different laboratories will be complete in March 2003.

Equine rhinopneumonitis – Coordinator Dr J. Mumford, Animal Health Trust, Newmarket, United Kingdom

The Commission supports Dr Mumford suggestion to approach the European Pharmacopoeia (EP) about their willingness to adopt the proposed equine herpesvirus standards. It was also suggested that the EP contact the OIE Reference Laboratory for equine rhinopneumonitis in the United States of America for input.

The Commission discussed the potential need for other reference sera, particularly for List A diseases. It will contact the OIE Reference Laboratories for highly pathogenic avian influenza regarding the need for reference sera for ELISA and/or AGID assays.

3. List of prescribed and alternative tests

3.1. ELISA for rabies serology

The Commission met with Dr Tony Fooks (Veterinary Laboratory Agencies [VLA], United Kingdom), Dr Serge Leterme (Synbiotics, France) and Dr Florence Cliquet (AFFSA Nancy, France) regarding the use of the rabies ELISA for determination of vaccine-induced titres. Comparison with the FAVN⁶ indicates that the ELISA has a lower sensitivity. The Commission will propose to the International Committee that the rabies ELISA be added to the list as an alternative test. Text will be added to the chapter on rabies in the *Manual of Standards for Diagnostic Tests and Vaccines* (the *Manual*) stating that the ELISA should be used as a screening test with confirmation of negatives by

² ELISA: enzyme-linked immunosorbent assay

³ CF: complement fixation

⁴ AFFSA: Agence française de sécurité sanitaire des aliments

⁵ AGID: agar gel immunodiffusion

⁶ FAVN: fluorescent antibody virus neutralisation

either the FAVN assay or RFFIT⁷. The Commission will propose that the Code Commission revisit the resolution on the rabies chapter in the *Animal Health Code* (the *Code*) adopted last year with the view to removing the reference to the neutralising antibody titration test from the *Code* chapter and replacing it with a reference to the use of tests outlined in the *Manual*.

3.2. Nonstructural protein tests for foot and mouth disease

An Ad hoc Group on the Evaluation of Nonstructural Protein (NSP) Tests for Foot and Mouth Disease (FMD) Diagnosis met at the OIE headquarters from 2 to 4 October 2002. The Commission reviewed the report and noted the Group's concerns regarding variability between diagnostic kits, lack of standard reference sera, and the need for test validation in species other than cattle (see <u>Appendix III</u> for the full report of the Ad hoc Group). The Standards Commission and the Ad hoc Group are working hard to complete all the information necessary to make final recommendations on this topic in as timely a manner as possible.

3.3. N1-N3 discriminatory test for avian influenza

The Commission discussed the potential introduction of the N1-N3 assay into the *Manual* chapter on highly pathogenic avian influenza. The Commission decided to add a short descriptive paragraph at the end of the diagnostic portion of the chapter and to provide a cross-reference to the description of the use of different neuraminidase subtypes as vaccines.

4. Questionnaire on bovine tuberculosis

The Commission reviewed the analysis of tuberculin manufacturing protocols carried out by the OIE Reference Laboratory for tuberculosis at VLA Weybridge, United Kingdom. It was noted that a wide variety of media are used. Methodology to determine potency was also observed to be inconsistent among manufacturers.

The Commission endorses the recommendations of the OIE Reference Laboratory. These are as follows: 1) all manufacturers should produce tuberculin using good manufacturing practice (GMP), 2) all manufacturers should follow standard methods for tuberculin production found in the *Manual*, and 3) a meeting of tuberculin producers should be convened to discuss specific, problematic technical issues

5. OIE Manual of Standards of Diagnostic Tests and Vaccines

For this section of the agenda the Commission was joined by the consultant editor, Dr J.E. Pearson.

The Commission discussed Member Country comments on several chapters for the fifth edition of the *Manual*, which is scheduled to be published in early 2004. Some of the specific items discussed were as follows: it was decided to list companies that manufacture diagnostic kits as a footnote in some chapters; it was agreed that the liquid-phase blocking ELISA for FMD diagnosis will not be designated as a prescribed test for international trade due to lack of use; in the chapter on FMD, text stating that live virus for FMD vaccines should be produced in biocontainment level 4 facilities as defined in the *Code* will be added; a note at the end of each chapter for which there is a Reference Laboratory will be added referring the reader to the list of Reference Laboratories; new information on bovine spongiform encephalopathy (BSE) diagnostic kits will be added; text stating that it is strongly suggested that Newcastle disease virus and avian influenza vaccine production and efficacy testing also be performed at *Code* level 4 biocontainment will be added to these chapters.

Experts on ovine pulmonary adenomatosis met in 2002 and decided to change the name of the disease to ovine pulmonary adenocarcinoma. This change is published in: Jaagsiekte Sheep Retrovirus and Lung Cancer. Current Topics in Microbiology and Immunology (2002), vol. 275, ed. H.Y. Fan. The Commission recommends this change be referred to the Code Commission for consideration.

⁷ RFFIT: rapid fluorescent focus inhibition test

6. Standards Commission Guidelines

6.1. Guidelines on antimicrobial resistance

The Commission reviewed the draft guidelines on antimicrobial resistance that had been prepared by the Ad hoc Group on Antimicrobial Resistance. The Commission agreed that three of the four guidelines should be passed to the Code Commission with a recommendation for adoption by the International Committee by Resolution during the General Session in May 2003 and eventual inclusion in the *Code* (see <u>Appendix IV</u>). Guideline 2 should be incorporated into the *Manual* chapter on antimicrobial resistance. Guideline 5 on Risk analysis for antimicrobial resistance has been passed directly to the Code Commission for consideration at its next meeting.

6.2. ILAC⁸ Guidelines for the Requirements for the Competence of Providers of Proficiency Testing Schemes

The Commission reviewed the ILAC guidelines and determined the technical requirements in its own guidelines are in line with the ILAC document.

7. Report of the meeting of the Ad hoc Group on Avian Influenza

The Commission reviewed the report of the Ad hoc Group on Avian Influenza. It was determined not to change the *Manual* chapter until the International Committee has adopted the proposed changes to the *Code* chapter on highly pathogenic avian influenza by Resolution during the General Session in May 2003. If the proposed changes are adopted, the new definition of avian influenza should be published in the *Manual* (fifth edition 2004) rather than in the *Code*. The new definition would be as follows: 'an influenza of poultry caused by either any influenza A virus which has an IVPI (intravenous pathogenicity index) in 6-week-old chickens greater than 1.2 or an influenza A virus of H5 or H7 subtype.'

8. Liaison with other Commissions

CODE COMMISSION

8.1. Report of the meeting of the Ad hoc Group on BSE

The Commission reviewed the report of the Ad hoc Group on BSE. Updated information on rapid tests will be added to the *Manual* as noted in point 5 above.

9. Any other business

9.1. Joint OIE/WAVLD⁹ Symposium in Thailand 2003

The Commission reviewed and approved the agenda and speakers for the Joint OIE/WAVLD Biotechnology Seminar in Thailand.

9.2. Letter on BSE testing

The Commission reviewed the letter from the German Delegate regarding the need to include a standardised protocol for BSE immunoblotting in the *Manual* chapter. The Commission will consult with the Reference Laboratory in the United Kingdom.

9.3. Report to OIE on the WHO¹⁰ consultation on campylobacteriosis

The Commission reviewed and recommended the report of the WHO expert advisory group on campylobacteriosis be forwarded the OIE Working Group on Food Safety.

⁸ International Laboratory Accreditation Conference

⁹ WAVLD: World Association of Veterinary Laboratory Diagnosticians

¹⁰ WHO: World Health Organization

9.4. Update on the European Union Committee on Diagnostic techniques and Vaccines for FMD, classical swine fever and avian influenza

Dr Steve Edwards updated the Commission on progress by the European Union Working Group of the Scientific Committee on Animal Health and Animal Welfare (SCAHAW) on Diagnostic Techniques and Vaccines for FMD, classical swine fever and avian influenza. He had represented the OIE on the subgroup dealing with diagnostic tests, while Dr Schudel had provided inputs to the subgroup on vaccines. The group had reviewed the current state of the art with regard to diagnostic tests and their validation, including an assessment of the potential contribution from new technologies. Specific areas needing further research and development had been identified. The group would be making recommendations to the EU Scientific Committee on Animal Health and Welfare, which would hopefully help steer future funding towards the areas of the highest priority.

9.5. FAO/IAEA¹¹ Consultants Meeting on 'OIE Validation and Certification of Diagnostic Assays for Infectious Animal Diseases'

The Commission accepted the report of the FAO/IAEA meeting and recommends to the OIE International Committee the following: 1) fitness for purpose should be used as a criteria for validation, 2) the OIE Collaborating Centre at the FAO/IAEA should develop a validation template to serve as a guideline for assay submission to the OIE, 3) the OIE should establish a registry for tests with levels of validation specified, 4) there is a need for establishing reference collections to be used for validation, 5) OIE Reference Laboratories should be intimately involved with validation efforts, 6) a funding source needs to be identified to implement these suggestions, 7) the OIE should review the current procedures in place to approve assays and 8) give the Standards Commission more authority to approve assays in a timely fashion. A summary of the report is included as <u>Appendix V</u>.

9.6. Report to the OIE Standards Commission from the Equine Influenza Surveillance Panel

The Commission reviewed the report and noted its recommendations (see Appendix VI).

9.7. Dates of next Standards Commission meetings

The next meeting of the Standards Commission will be held from 17 to 19 September 2003.

 \dots /Appendices

¹¹ FAO/IAEA: Food and Agriculture Organization of the United Nations/International Atomic Energy Agency

Appendix I

MEETING OF THE OIE STANDARDS COMMISSION

Paris, 14–17 January 2003

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. Standards Commission guidelines
- 7. Report of the Ad hoc Group on Avian Influenza
- 8. Liaison with the other Commissions
- 9. Any other business

MEETING OF THE OIE STANDARDS COMMISSION Paris, 14-17 January 2003

List of participants

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Appendix III

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

Paris, 2–4 October 2002

A meeting of the OIE Ad hoc Group on Evaluation of Nonstructural Protein Tests for Foot and Mouth Disease Diagnosis was held at the OIE Headquarters in Paris from 2 to 4 October 2002. The meeting was chaired by Dr Peter Wright, who acted as rapporteur.

The List of Participants is given at Appendix I.

1. General discussion

The following text highlights the salient points established by the experts as having an impact on standardisation, validation, application and interpretation of the nonstructural protein (NSP) tests for foot and mouth disease (FMD) diagnosis.

NSP tests for FMD have potential application in the differentiation of vaccinated animals from those that have experienced virus replication. The basis of this differentiation lies in the detection, in serum, of antibodies to the NSPs of FMD virus (FMDV). The detection of these antibodies is an indicator that viral replication, even if limited, has occurred and has been detected by the host immune system.

A number of NSPs associated with FMDV replication have been identified and include: L, 3A, 3B, 2C, 3D, 3AB and 3ABC. These proteins have recently been used as antigens, either individually or in various combinations, in a number of ELISA¹² and western blot techniques for the detection of antibody.

Vaccines to FMD are inactivated and adjuvanted whole virus particles. In the production of vaccines, the resulting NSPs associated with virus replication are removed by various purification procedures. Vaccine purity is most often tested by repeated inoculation of the target species and testing for the development of antibody to the NSPs. Antibody to NSP 3D may be expected as this protein is incorporated into the FMD virion.

In general terms, standard doses of vaccine are protective in that they prevent the development of clinical disease on subsequent exposure to FMDV. However, limited virus replication in the pharyngeal region of the exposed animal can occur. If detectable virus persists in oesophageal–pharyngeal (OP) secretions beyond 28 days, the animal is classified as a carrier. The duration of the carrier state is limited and varies by host species. The threat posed by carriers with respect to virus transmission is the subject of debate.

Most of the data accumulated to date, with respect to vaccinated animals that have been subsequently exposed to live virus, have been derived from cattle. The majority of vaccinated cattle subsequently exposed to live virus experience limited virus replication, but do not become carriers. The proportion of animals that become carriers under experimental conditions may be high, but in the field this proportion may be lower. Among carriers, and depending on the circumstances (immunological status of the population, pathogenicity, etc.), the majority, but not all, develop a readily detectable anti-NSP humoral response. However, as population-based strategies are recommended, the risk of missing individual non-responders is overcome.

¹² ELISA: Enzyme-linked immmunosorbent assay

2. Current test methods

There are a number of ELISAs currently available or soon to be available commercially. The descriptions given below are based on information from the experts and not direct product information.

- UBI (United Biomedical Inc.) markets an indirect ELISA based on a synthetic 3B peptide. The peptide is absorbed directly to the plate. The detection system employs a Protein A conjugate for ruminants and an anti-swine immunoglobulin for pigs.
- Bommeli Diagnostics/Intervet markets an indirect ELISA based on a recombinant 3ABC expressed in *Escherichia coli*. The 3ABC polypeptide is affinity purified and absorbed directly to the plate. The detection system employs a monoclonal anti-IgG₁ for ruminants and a polyclonal anti-swine immunoglobulin for pigs.
- Embrabio markets an indirect ELISA based on a recombinant 3ABC expressed in *E. coli*. The polypeptide is purified and absorbed directly to the plate. Species-specific polyclonal antiimmunoglobulins are used for detection. This ELISA is applied as a screening test with positive reactors confirmed in the EITB¹³ test (western blot).
- Bayer is in the process of developing an indirect ELISA based on two synthetic polypeptides from 3B. This test is meant to be a companion test to their vaccine.

There are also a number of ELISAs that have been developed in various national or international laboratories.

- The Pan American Foot-and-Mouth Disease Center, PAHO/WHO (Panaftosa) has an indirect ELISA based on a recombinant 3ABC expressed in *E. coli*. The polypeptide is purified by preparative electrophoresis and absorbed directly to the plate. Species-specific polyclonal anti-immunoglobulins are used for detection. This ELISA is applied as a screening test with positive reactors confirmed in the EITB test. A similar test has been used in Spain.
- ¹ The Brescia laboratory (IZSLER, Italy) has an indirect ELISA based on Panaftosa's recombinant 3ABC expressed in *E. coli*. However, the polypeptide is not purified, but is captured by a monoclonal antibody absorbed to the plate. The detection system employs a monoclonal anti-IgG₁ for ruminants and a polyclonal anti-swine immunoglobulin for pigs.
- ¹ The Pirbright laboratory (IAH, United Kingdom) has an indirect ELISA based on a recombinant similar to Panaftosa's recombinant 3ABC expressed in *E. coli*. The polypeptide is not purified, but is captured by a polyclonal antibody absorbed to the plate. Both polyclonal and monoclonal anti-species detection systems have been used.
- ¹ The Lindholm laboratory (DVIVR, Denmark) has a competitive ELISA based on a recombinant 3ABC expressed in baculovirus. The polypeptide is not purified, but is captured by a monoclonal antibody absorbed to the plate. The same monoclonal antibody is used as the competing antibody in this assay.

As may be discerned from the foregoing, there are a number of commonalties amongst these tests. However, it is also obvious that there are differences that may affect diagnostic performance characteristics.

All of the above assays have reportedly been validated, some more extensively than others. Without a standard of comparison, there is really no way of comparing these assays.

3. Index test method

At present, there is no index (or reference) test method that has been agreed by the Experts. An index test method is needed as a point of reference for the comparison of other tests. It was agreed at this meeting that the indirect ELISA from Panaftosa (briefly described above) would become the index method. This method

¹³ EITB: Enzyme-linked immuno-electrotransfer blot

is currently described in the OIE Manual of Standards for Diagnostic Tests and Vaccines (2000 edition)

and was reviewed and updated for the 2004 edition of the *Manual* at this meeting. The original *E. coli* clone has been distributed to a number of laboratories and is in current use for the production of recombinant 3ABC polyprotein. The recombinant 3ABC polyprotein would be made available as a reference reagent for standardisation and comparative purposes by selected reference laboratories (Panaftosa, INIA [Madrid, Spain], IZSLER).

4. Reference standard sera

At present, there are no reference standard sera for the calibration of NSP test methods or the production of national or working standards. It was agreed at this meeting that these sera are essential and should be prepared for cattle, sheep and swine. Three sera are to be prepared according to the OIE Guidelines for International Reference Standards for Antibody Assays. These include strong positive, weak positive and negative standard sera as defined by a typical dose–response curve in the index test method. It was emphasised that these sera are necessary to set minimum limits for analytical sensitivity and to establish uniform process control for these ELISAs. Three Experts were identified at this meeting who would be willing to prepare reference standard sera for cattle, sheep and swine, respectively. Arrangements will also be made to have these sera gamma irradiated in order to facilitate distribution worldwide.

5. Threshold/cut-off serum

Setting a threshold or cut-off that is universally applicable for all test methods is not possible. However, based on dilution of the reference standard weak positive serum (above), a method for defining a threshold/cut-off from an analytical standpoint has been defined and agreed by the Experts at this meeting. This method will be explained in the 2004 edition of the OIE *Manual*. Individual laboratories may set their own threshold by other means depending on the specified application of their test, but the above method may be used as a reference point for comparison.

6. Inter-laboratory check panels

At some point in the future, it will be necessary to collaborate on the development of panels of sera suitable for inter-laboratory comparisons of diagnostic performance, as well as proficiency testing. Until there are standardised, calibrated and harmonised tests, creation of these panels will remain on hold.

7. Diagnostic validation

Diagnostic validation data are scattered throughout the literature. For the most part, these data are from cattle, but there are some data from sheep and pigs. The largest volumes of data available, with respect to estimates of diagnostic specificity (DSp) and sensitivity (DSn), have been derived from experimental and field studies conducted by Panaftosa.

Description		ELISA		EITB	
	n	DSp%	DSn%	DSp%	DSn%
Naïve cattle	12,804	99.05	_	100	_
Single vaccination	3,500	98.49	_	100	_
Multiple vaccinations, age > 2 years	2,517	95.20	_	99.68	_
Multiple vaccinations, age < 2years*	79,649	97.90	_	99.97	_
Natural infection, non-vaccination	1,000	_	98.80	-	98.80

Panaftosa results compiled from data on cattle are summarised below.

* data compiled from multiple seroepidemiological surveys throughout South America

The above estimates of DSp and DSn are presented as a comparison of the diagnostic performance characteristics as experienced by Panaftosa. These estimates demonstrate the potential of these assays in the hands of the laboratory that adapted these reagents and assay formats.

Panaftosa has observed similar results in sheep and pigs, however the number of samples analysed is very small compared with those for cattle.

Experimental data were also presented by Panaftosa on paired samples of OP secretions (probang) and blood taken from 78 cattle (34 non-vaccinated and 44 vaccinated) infected with or exposed to the FMDV under controlled conditions. Irrespective of vaccination status, the data suggest that the DSn of the ELISA/EITB combination <u>relative</u> to OP positive samples (n = 428) is 100%, OP positive samples being those from which virus was successfully isolated. On the other hand, the DSn of OP isolation <u>relative</u> to ELISA/EITB positive results (n = 861) in these cattle is only 46.9%.

The table below compares estimates of DSn, for the various other ELISA techniques, under experimental conditions in which cattle were vaccinated and subsequently infected with FMDV.

Description	ELISA technique – DSn%					
	Α	В	C	D	Е	F
Vaccinated, infected, ~ 30 days post- infection	90.0	85.7	93.3	70.0	100	68.6
Vaccinated, infected, ~ 30–180 days post- infection	92.6	78.6	90.3	80.2	100	75.5

The above data are for comparative purposes only. The numbers of cattle involved in these various experiments ranges from 15 to 75. In some cases the same cattle were tested with more than one of the ELISA techniques. The second set of data represents sequential bleeds beyond 30 days post-infection. The variability demonstrated, in addition to different experimental conditions, probably represents inherent differences in reagents and assay techniques and the fact that none of these techniques is calibrated against reference standard sera.

8. Conventional versus high potency vaccines

The available data with respect to induction of carrier states and seroconversion are based on conventional doses of vaccine. Work needs to be done to determine whether or not the use of high potency vaccines will alter carrier states and DSn estimates in vaccinated animals.

9. Screening versus confirmatory testing

It has been proposed that the ELISAs be used as screening tests. The DSn's of these tests need to be maximised and harmonised through calibration against international reference standard sera. It would then be expected that there would be a predictable false-negative rates. Confirmation of true positive status would then require re-testing using a test of higher DSp. At present, the only confirmatory test proposed is the EITB, a western blot technique developed at Panaftosa and described in the 2000 edition of the OIE *Manual*. The description of this technique has been reviewed by the Expert group and will be updated for the 2004 edition of the *Manual*. This will include more definitive criteria for interpretation of results when used as a confirmatory test. Background activity in the EITB is currently being evaluated in various regions other than South America, specifically to assess DSp estimates under different husbandry conditions.

It was also agreed that the EITB will require defined reference standard sera and that these may very well be the same as those used for the ELISA. The definition and use of these sera (including a threshold/cut-off serum) will be described in the 2004 edition of the *Manual*.

Other potential screening or confirmatory tests should be evaluated as they become available.

10. Application and interpretation of tests

Based on the foregoing, there is good evidence that the NSP ELISAs and the EITB have definite potential in the monitoring of free status and during recovery from an FMD outbreak in both regularly vaccinated populations and naïve populations vaccinated in the face of an outbreak, including with high potency vaccines. These tests are better indicators of previous infection in vaccinated animals compared with the currently available test methods. Current data would suggest that these tests are superior to virus isolation from OP secretions. It is not presently known how these tests would compare with genome detection in OP samples. The index test methods, developed and applied by Panaftosa, have been successfully used to evaluate epidemiological risk to cattle in South America.

All of the above tests have application in the detection of carrier states in vaccinated cattle. However, based on the variability in diagnostic performance estimates, in particular DSn, no single sampling strategy can be recommended. Calibration against reference standard sera and harmonisation through inter-laboratory comparisons against the index methods should improve this situation. Once this achieved, sampling strategies may be established for the various ELISAs that would provide uniform confidence in providing evidence that virus is not circulating in vaccinated animals.

Considerably more data will be required on sheep and pigs before application of these serological techniques can be considered in these species.

It is proposed that the above Ad hoc Group meet in mid-2003 to review progress on all of the above and report to the OIE Standards Commission.

Appendix I

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

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Appendix IV

OIE GUIDELINE ON ANTIMICROBIAL RESISTANCE 1:

HARMONISATION OF NATIONAL ANTIMICROBIAL RESISTANCE MONITORING AND SURVEILLANCE PROGRAMMES IN ANIMALS AND IN ANIMAL-DERIVED FOOD

1. Purpose of the document

This document provides criteria for the:

- i) development of national antimicrobial resistance monitoring and surveillance programmes,
- ii) harmonisation of existing national monitoring and surveillance programmes.

2. Purpose and definition of monitoring and surveillance

Surveillance and monitoring of antimicrobial resistance is necessary to:

- i) follow trends in antimicrobial resistance in bacteria,
- ii) detect the emergence of new antimicrobial resistance mechanisms,
- iii) provide the data necessary for conducting risk analyses with relevance for human and animal health,
- iv) provide a basis for policy recommendations for animal and public health,
- v) Provide information for prescribing practices and prudent use recommendations.

Antimicrobial resistance monitoring and surveillance programmes may include the following components:

- i) scientifically based surveys (including statistically based programmes),
- ii) routine sampling and testing of animals on the farm, at market or at slaughter,
- iii) an organised sentinel programme, sampling animals, herds, flocks, and vectors,
- iv) analysis of veterinary practice and diagnostic laboratory records.

Countries should conduct active surveillance and monitoring. Passive surveillance and monitoring may offer additional information.

Targeted surveillance is conducted through an active sampling scheme designed to meet programme objectives. Passive surveillance is conducted when samples are submitted to a laboratory for testing from sources outside the programme.

3. Steps to be taken for the development of antimicrobial resistance surveillance and monitoring programmes

3.1. General aspects

Surveillance of antimicrobial resistance at regular intervals or ongoing monitoring of prevalence changes of resistant bacteria of animal, food, environmental and human origin, constitutes a critical part of a strategy aimed at limiting the spread of antimicrobial resistance and optimising the choice of antimicrobials used in therapy.

Monitoring of bacteria from animal-derived food collected at different steps of the food chain, including processing, packing and retailing, should also be considered.

3.2. Sampling strategies

3.2.1. General

Sampling should be conducted on a statistical basis. The sampling strategy should assure:

- i) the sample representativeness of the population of interest,
- ii) the robustness of the sampling method.

The following criteria are to be considered:

- i) sample size,
- ii) sample source (animal, food),
- iii) animal species,
- iv) category of animal within species (age group, production type),
- v) stratification within category,
- vi) health status of the animals (healthy, diseased),
- vii) random sample (targeted, systematic),
- viii) sample specimens (faecal, carcass, processed food).

3.2.2. Sample size

The sample size should be

- i) large enough to allow detection of existing resistance,
- ii) not excessively large to avoid waste of resources.

Details are provided in Appendix A. Sampling shall follow Standard Operating Procedures.

3.3. Sample sources

3.3.1. Animals

Each Member Country should examine its livestock production systems and decide, after risk analysis, the relative importance of antimicrobial resistance and its impact on animal and human health.

Categories of livestock that should be considered for sampling include cattle and calves, slaughter pigs, broiler chickens, layer hens and/or other poultry and farmed fish.

3.3.2. Food and animal feed

Contaminated food is commonly considered to be the principal route for the transfer of antimicrobial resistance from animals to humans. Plants and vegetables of different types may be exposed to manure or sewage from livestock and may thereby become contaminated with resistant bacteria of animal origin. Animal feed, including imported feed, may also be considered in monitoring and surveillance programmes.

Source	Sample type	Outcome	Additional information required/additional stratification
Herd of origin		Prevalence of resistance in bacteria originating from animal populations (of different production types) Relationship resistance – antibiotic use	Per age categories, production types, etc. Antibiotic use over time
Abattoir	Faecal	Prevalence of resistance in bacterial populations originating from animals at slaughter age	
	Intestine	As above	
	Carcass	Hygiene, contamination during slaughter	
Processing, packing	Meat products	Hygiene, contamination during processing and handling	
Retail	Meat products	Prevalence of resistance in bacteria originating from food, exposure data for consumers	
	Vegetables	Prevalence of resistance in bacteria originating from vegetables, exposure data for consumers	
Various origin	Animal feed	Prevalence of resistance in bacteria originating from animal feed, exposure data for animals	

Table 1. Examples of sampling sources, sample types and outcome of monitoring

3.4. Sample specimens to be collected

Faecal samples should be collected from livestock, and whole caeca should be collected from poultry. In cattle and pigs, a faecal sample size at least of 5 g provides a sufficient sample for isolation of the bacteria of concern.

Sampling of the carcasses at the abattoir provides information on slaughter practices, slaughter hygiene and the level of faecal contamination of meat during the slaughter process. Further sampling from the retail chain provides information on prevalence changes before the food reaches the consumer.

Existing food processing microbiological monitoring and 'hazard analysis and critical control points' (HACCP) programmes may provide useful samples for monitoring and surveillance of resistance in the food chain after slaughter.

3.5. Bacterial isolates

The following categories of bacteria could be monitored:

- i) animal bacterial pathogens,
- ii) zoonotic bacteria,
- iii) commensal bacteria.

3.6. Animal bacterial pathogens

Monitoring of antimicrobial resistance in animal pathogens is important, both to:

- i) detect emerging resistance that may pose a concern for human and animal health,
- ii) guide veterinarians in their prescribing decisions.

Information on the occurrence of antimicrobial resistance in animal pathogens is in general derived from routine clinical material sent to veterinary diagnostic laboratories. These samples, often derived from severe or recurrent clinical cases including therapy failures, may provide biased information.

Target animals	Respiratory pathogens	Enteric pathogens	Udder pathogens	Other pathogens
Cattle	Pasteurella spp.	Escherichia coli	Staphylococcus aureus	
	Haemophilus somnus	Salmonella spp.	Streptococcus spp.	
Pigs	Actinobacillus pleuropneumoniae	Escherichia coli		Streptococcus suis
		Brachyspira spp.		
		Salmonella spp.		
Poultry				Escherichia coli
Fish				<i>Vibrio</i> spp.
				Aeromonas spp.

Table 2. Examples of animal bacterial pathogens that may be included in resistance surveillance and monitoring

3.7. Zoonotic bacteria

3.7.1. Salmonella

Salmonella should be sampled from cattle, pigs, broilers and other poultry. For the purpose of facilitating sampling and reducing the concurrent costs, samples should preferably be taken at the abattoir. Monitoring and surveillance programmes may also use bacterial isolates from designated national laboratories originating from other sources.

Isolation and identification of bacteria and bacterial strains should follow internationally accepted procedures.

Serovars of epidemiological importance such as *S*. Typhimurium and *S*. Enteritidis should be included. The selection of other relevant serovars will depend on the epidemiological situation in each country.

All *Salmonella* isolates should be serotyped and, when appropriate, phage-typed according to standard methods used at the nationally designated laboratories.

Validated methods should be used.

3.7.2. Campylobacter

Campylobacter jejuni and *C. coli* can be isolated from the same samples as commensal bacteria. Isolation and identification of these bacteria should follow internationally accepted procedures. *Campylobacter* isolates should be identified to the species level.

Agar or broth micro-dilution methods are recommended for *Campylobacter* susceptibility testing. Internal and external quality control programmes should be strictly adhered to.

Validated methods with appropriate reference strains are expected to become available in the near future.

3.7.3. Enterohaemorrhagic Escherichia coli (EHEC)

Enterohaemorrhagic *E. coli*, such as the serotype O157, which is pathogenic to humans but not to animals, may be included in resistance monitoring and surveillance programmes.

3.8. Commensal bacteria

Escherichia coli and enterococci are common commensal bacteria. These bacteria are considered to constitute a reservoir of antimicrobial resistance genes, which may be transferred to pathogenic bacteria causing disease in animals or humans. It is considered that these bacteria should be isolated from healthy animals, preferably at the abattoir, and be monitored for antimicrobial resistance.

Validated methods should be used.

3.9. Storage of bacterial strains

If possible, isolates should be preserved at least until reporting is completed. Preferably, isolates should be permanently stored. Bacterial strain collections, established by storage of all isolates from certain years, will provide the possibility of conducting retrospective studies.

3.10. Antimicrobials to be used in susceptibility testing

Clinically important antimicrobial classes used in human and veterinary medicine should be monitored. However, the number of tested antimicrobials may have to be limited according to the financial resources of the country.

3.11. Type of data to be recorded and stored

Data on antimicrobial susceptibility should be reported quantitatively.

Appropriate validated methods should be used in accordance with chapter I.1.10. Laboratory methodologies for bacterial antimicrobial susceptibility testing from the OIE *Manual of Standards for Diagnostic Tests and Vaccine*, fifth edition.

3.12. Recording, storage and interpretation of results

Because of the volume and complexity of the information to be stored and the need to keep these data available for an undetermined period of time, careful consideration should be given to database design.

The storage of raw (primary, non-interpreted) data is essential to allow the evaluation of the data in response to various kinds of questions, including those arising in the future.

Consideration should be given to the technical requirements of computer systems when an exchange of data between different systems (comparability of automatic recording of laboratory data and transfer of these data to resistance monitoring programmes) is envisaged. Results should be collected in a suitable national database. They shall be recorded quantitatively:

- i) as distribution of minimum inhibitory concentrations (MICs) in milligrams per litre,
- ii) or inhibition zone diameters in millimetres.

The information to be recorded should include at least the following aspects:

- i) sampling programme,
- ii) sampling date,
- iii) animal species/livestock category,
- iv) type of sample,
- v) purpose of sampling,
- vi) geographical origin of herd, flock or animal,
- vii) age of animal.

The reporting of laboratory data should include the following information:

- i) identity of laboratory,
- ii) isolation date,
- iii) reporting date,
- iv) bacterial species,

and, where relevant, other typing characteristics, such as:

- v) serovar,
- vi) phage-type,
- vii) antimicrobial susceptibility result/resistance phenotype.

The proportion of isolates regarded as resistant should be reported, including the defined breakpoints.

In the clinical setting, breakpoints are used to categorise bacterial strains as susceptible, intermediate susceptible or resistant. These breakpoints, often referred to as clinical or pharmacological breakpoints, are elaborated on a national basis and vary between countries.

The system of reference used should be recorded.

For surveillance purposes, the microbiological breakpoint, which is based on the distribution of MICs or inhibition zone diameters of the specific bacterial species tested, is preferred. When using microbiological breakpoints, only the bacterial population with acquired resistance that clearly deviates from the distribution of the normal susceptible population will be designated as resistant.

If available, the phenotype of the isolates (resistance pattern) should be recorded.

3.13. Reference laboratory and annual reports

Countries should designate a national reference centre that assumes the responsibility to:

- i) coordinate the activities related to the resistance surveillance and monitoring programmes,
- ii) collect information at a central location within the country,
- ii) produce an annual report on the resistance situation of the country.

The national reference centre should have access to the

- i) raw data,
- ii) complete results of quality assurance and inter-laboratory calibration activities,
- iii) proficiency testing results,
- iv) information on the structure of the monitoring system,
- v) information on the chosen laboratory methods.

Appendix A.

			Level of co	onfidence		
Expected prevalence	90% desired precision			95% desired precision		
	10%	5%	1%	10%	5%	1%
10%	24	97	2,429	35	138	3,445
20%	43	173	4,310	61	246	6,109
30%	57	227	5,650	81	323	8,003
40%	65	260	6,451	92	369	9,135
50%	68	270	6,718	96	384	9,512
60%	65	260	6,451	92	369	9,135
70%	57	227	5,650	81	323	8,003
80%	43	173	4,310	61	246	6,109
90%	24	97	2,429	35	138	3,445

Calculations based on Epi Info v6.04b to c Upgrade, October 1997, Centers for Disease Control (public domain software available at http://www.cdc.gov/epo/epi/epiinfo.htm)

OIE GUIDELINE ON ANTIMICROBIAL RESISTANCE 3:

MONITORING THE QUANTITIES OF ANTIMICROBIALS USED IN ANIMAL HUSBANDRY

1. Introduction

1.1. Purpose

The purpose of this document is to describe an approach to the monitoring of quantities of antimicrobials used in animal husbandry.

1.2. Scope

This guideline is intended for use by OIE Member Countries to collect objective and quantitative information to evaluate usage patterns by animal species, antimicrobial class, potency and type of use in order to evaluate antimicrobial exposure.

1.3. Objectives

These data are essential for risk analyses and planning, can be helpful in interpreting resistance surveillance data and can assist in the ability to respond to problems of antimicrobial resistance in a precise and targeted way. The data may also assist in evaluating the effectiveness of efforts to ensure prudent use and mitigation strategies (for example, by identifying changes in prescribing practices for veterinarians) and to indicate where alteration of antimicrobial prescribing practices might be appropriate, or if changes in prescription practice have altered the pattern of antimicrobial use.

The continued collection of this basic data will also help give an indication of trends in the use of animal antimicrobials over time and the role of these trends in the development of antimicrobial resistance in animals.

For all OIE Member Countries, the minimum basic information collected should be the annual weight in kilograms of the active ingredient of the antimicrobial(s) used in food animal production. In addition, the type of use (therapeutic or growth promotion) and route of administration (parenteral or oral administration) should be recorded.

Member Countries may wish to consider, for reasons of cost and administrative efficiency, collecting medical, food animal, agricultural and other antimicrobial use data in a single programme. A consolidated programme would also facilitate comparisons of animal use with human use data for relative risk analysis and help to promote optimal usage of antimicrobials.

1.4. Development and standardisation

Systems to monitor antimicrobial usage consist of the following elements:

- i) Sources of antimicrobial data,
- ii) Categories of data,
- iii) Other important information.

2. Sources of antimicrobial use data

2.1. Basic sources

Sources of data will vary from country to country. Such sources may include customs, import and export data, manufacturing and manufacturing sales data.

2.2. Direct sources

Data from animal drug registration, wholesalers, retailers, pharmacists, veterinarians, feed stores, feed mills and organised industry associations in these countries might be efficient and practical sources. A possible mechanism for the collection of this information is to make the provision of appropriate information by manufacturers to the regulatory authority one of the requirements of antimicrobial registration.

2.3. End-use sources (veterinarians and food animal producers)

This may be appropriate when basic or direct sources cannot be used for the routine collection of this information and when more accurate and locally specific information is required.

Periodic collection of this type of information may be sufficient.

It may be important when writing recommendations on antimicrobial resistance to take into account factors such as seasonality and disease conditions, species affected, agricultural systems (e.g. extensive range conditions and feedlots), dose rate, duration and length of treatment with antimicrobials.

Collection, storage and processing of data from end-use sources are likely to be inefficient and expensive processes unless carefully designed and well managed, but should have the advantage of producing accurate and targeted information.

3. Categories of data

3.1. Requirements for data on antimicrobial use

The **minimal data** collected should be the annual weight in kilograms of the active ingredient of the antimicrobial(s) used in food animal production. This should be related to the scale of production (see section 3.4 below).

- For active ingredients present in the form of compounds or derivatives, the mass of active entity of the molecule should be recorded.
- For antibiotics expressed in International Units, the calculation required to convert these units to mass of active entity should be stated.

If a Member Country has the infrastructure for capturing basic animal antimicrobial use data for a specific antimicrobial, then **additional information** can be considered to cascade from this in a series of subdivisions or levels of detail. Such a cascade of levels should include the following:

- i) The absolute amount in kilograms of active antimicrobial used per antimicrobial family per year, or for a specific antimicrobial chemical entity when this information is required.
- ii) Therapeutic and growth promotion use in kilograms of the specific active antimicrobial.
- iii) Subdivision of antimicrobial use into therapeutic and growth promotion use by animal species.
- iv) Subdivision of the data into the route of administration, specifically in-feed, in-water, injectable, oral, intramammary, intra-uterine and topical.
- v) Further subdivision of these figures by season and region by a Member Country may be useful (note: this may be especially management conditions, or where animals are moved from one locality to another during production).

vi) Further breakdown of data for analysis of antimicrobial use at the regional, local, herd and individual veterinarian level may be possible using veterinary practice computer management software as part of specific targeted surveys or audits. Analysis of this information within the local or regional context could be useful for individual practitioners and practices where specific antimicrobial resistance has been identified and feedback is required.

3.2. Classes of antimicrobials

Nomenclature of antimicrobials should comply with international standards where available.

Decisions need to be made on what classes of antimicrobials should be considered and what members of various antimicrobial classes should be included in the data collection programme. These decisions should be based on currently known mechanisms of antimicrobial activity and resistance of the particular antimicrobial and its relative potency

3.3. Species and production systems

Countries should keep a register of all animal use of antimicrobials for individual food animal species (cattle, sheep, goats, pigs, poultry, horses and fish) and for specific diseases. This will help to identify possible nonauthorised usage.

3.4. Other important information

Breakdown of farm livestock into species and production categories, including total live weights, would be most useful in any risk analysis or for comparison of animal antimicrobial use with human medical use within and between countries. For example, the total number of food animals by category and their weight in kilograms for food production per year (meat, dairy and draught cattle, and meat, fibre, poultry and dairy sheep) in the country would be essential basic information.

4. Conclusion

Data on the use of antimicrobials in animals are essential for risk analysis and the design and planning of antimicrobial resistance monitoring and surveillance programmes, as well as for the ongoing management of antimicrobial resistance at the individual farm, district, regional, national and international levels.

OIE GUIDELINE ON ANTIMICROBIAL RESISTANCE 4:

RESPONSIBLE AND PRUDENT USE OF ANTIMICROBIAL AGENTS IN VETERINARY MEDICINE

1. Introduction

1.1. Purpose

This document provides guidance for the responsible and prudent use of antimicrobials in veterinary medicine, with the aim of protecting both animal and human health. The competent authorities responsible for the registration and control of all groups involved in the production, distribution and use of veterinary antimicrobials have specific obligations.

Prudent use is principally determined by the outcome of the marketing authorisation procedure and by the implementation of specifications when antimicrobials are administered to animals.

1.2. Principles of prudent use

Prudent use includes a set of practical measures and recommendations intended to prevent and/or reduce the selection of antimicrobial-resistant bacteria in animals to:

- i) maintain the efficacy of antimicrobial agents and to ensure the rational use of antimicrobials in animals with the purpose of optimising both their efficacy and safety in animals,
- ii) comply with the ethical obligation and economic need to keep animals in good health,
- iii) prevent, or reduce, as far as possible, the transfer of bacteria (with their resistance determinants) within animal populations,
- iv) maintain the efficacy of antimicrobial agents used in livestock,
- v) prevent or reduce the transfer of resistant bacteria or resistance determinants from animals to humans,
- vi) maintain the efficacy of antimicrobial agents used in human medicine and prolong the usefulness of the antimicrobials,
- vii) prevent the contamination of animal-derived food with antimicrobial residues that exceed the established maximum residue limit (MRL),
- viii) protect consumer health by ensuring the safety of food of animal origin.

1.3. Responsibilities

These include the responsibilities of:

- i) the regulatory authorities,
- ii) the veterinary pharmaceutical industry,
- iii) pharmacists,
- iv) veterinarians,
- v) livestock producers.

2. Responsibilities of the regulatory authorities

2.1. The national regulatory authorities

The national regulatory authorities are responsible for granting the marketing authorisation. They have a significant role in specifying the terms of this authorisation and in providing the appropriate information to the veterinarian.

2.2 The pharmaceutical industry

The pharmaceutical industry has to submit the data requested for the granting of the marketing authorisation. The marketing authorisation is granted only if the criteria of safety, quality and efficacy are met. An assessment of the potential risk to both the animal and the consumer resulting from the use of antimicrobial agents in food-producing animals must be carried out. The evaluation should focus on each individual antimicrobial product and not be generalised to the class of antimicrobials to which the particular active principle belongs. If dose ranges or different durations of treatment are suggested, guidance on the usage should be provided.

2.3. Market approval

Regulatory authorities should attempt to expedite the market approval process of a new antimicrobial in order to address a specific need for the treatment of disease.

2.4. Registration procedures

Countries lacking the necessary resources to implement an efficient registration procedure for veterinary medicinal products (VMPs), and whose supply principally depends on imports from foreign countries, must undertake the following measures:

- i) check the efficacy of administrative controls on the import of these VMPs,
- ii) check the validity of the registration procedures of the exporting country,
- iii) develop the necessary technical co-operation with experienced authorities to check the quality of imported VMPs as well as the validity of the recommended conditions of use.

Regulatory authorities of importing countries should request the pharmaceutical industry to provide quality certificates prepared by the competent authority of the exporting country. All countries should make every effort to actively combat the trade, distribution and use of unlicensed and counterfeit products.

2.5. Quality control of antimicrobial agents

Quality controls should be performed:

- i) in compliance with the provisions of good manufacturing practices,
- ii) to ensure that analysis specifications of antimicrobial agents used as active ingredients comply with the provisions of approved monographs,
- iii) to ensure that the quality and concentration (stability) of antimicrobial agents in the marketed dosage form(s) are maintained until the expiry date, established under the recommended storage conditions,
- iv) to ensure the stability of antimicrobials when mixed with feed or drinking water,
- v) to ensure that all antimicrobials are manufactured to the appropriate quality and purity in order to guarantee their safety and efficacy.

2.6. Control of therapeutic efficacy

2.6.1. Preclinical trials

Preclinical trials should:

- i) establish the range of activity of antimicrobial agents on both pathogens and non-pathogens (commensals),
- ii) assess the ability of the antimicrobial agent to select for resistant bacteria *in vitro* and *in vivo*, taking into consideration pre-existing resistant strains,
- iii) establish an appropriate dosage regimen necessary to ensure the therapeutic efficacy of the antimicrobial agent and limit the selection of antimicrobial-resistant bacteria.

2.6.1.1. Pharmacodynamics and the establishment of the activity of antimicrobial agents towards the targeted bacteria

The following criteria should be taken into account:

- i) mode of action,
- ii) minimum inhibitory and bactericidal concentrations,
- iii) time- or concentration-dependent activity,
- iv) activity at the site of infection.
- 2.6.1.2. Pharmacokinetics and the establishment of the dosage regimens allowing maintenance of effective antimicrobial levels

The following criteria should be taken into account:

- i) bio-availability according to the route of administration,
- ii) concentration of the antimicrobial at the site of infection and its distribution in the treated animal,
- iii) metabolism that may lead to the inactivation of antimicrobials,
- iv) excretion routes,
- v) The use of combinations of antimicrobial agents should be justified.

2.6.2. Clinical trials

Clinical trials should be performed to confirm the validity of the claimed therapeutic indications and dosage regimens established during the preclinical phase.

The following criteria should be taken into account:

- i) diversity of the clinical cases encountered when performing multi-centre trials,
- ii) compliance of protocols with good clinical practice,
- iii) eligibility of studied clinical cases, based on appropriate criteria of clinical and bacteriological diagnoses,
- iv) parameters for qualitatively and quantitatively assessing the efficacy of the treatment.

2.7. Assessment of the potential of antimicrobials to select for resistant bacteria

The party applying for market authorisation should, where possible, supply data derived in target animal species under the intended conditions of use.

Considerations may include:

- i) the concentration of active compound in the gut of the animal (where the majority of potential food-borne pathogens reside) at the defined dosage level,
- ii) the level of human exposure to food-borne resistant bacteria,
- iii) the degree of cross-resistance within the class of antimicrobials and between classes of antimicrobials,
- iv) the pre-existing level of resistance in the pathogens of human health concern (baseline determination).

Other studies may be requested in support of the assessment of the potential of antimicrobials to select for resistant bacteria. The interpretation of their results should be undertaken with great caution.

2.8. Establishment of acceptable daily intake, maximum residue level and withdrawal periods for antimicrobial compounds

- i) When setting the acceptable daily intake (ADI) and MRL for an antimicrobial substance, the safety evaluation should also include the potential biological effects on the intestinal flora of humans.
- ii) The establishment of an ADI for each antimicrobial agent, and an MRL for each animal-derived food, should be undertaken.
- iii) For each VMP containing antimicrobial agents, withdrawal periods should be established in order to produce food in compliance with the MRL, taking into account:
 - a) the MRL established for the antimicrobial agent under consideration,
 - b) the composition of the product and the pharmaceutical form,
 - c) the target animal species,
 - d) the dosage regimen and the duration of treatment,
 - e) the route of administration.

The applicant should provide methods for regulatory testing of residues in food.

2.9. Protection of the environment

An assessment of the impact of the proposed antimicrobial use on the environment should be conducted. Efforts should be made to ensure that environmental contamination with antimicrobials is restricted to a minimum.

2.10. Establishment of a summary of product characteristics for each veterinary medicinal product

The summary of product characteristics contains the information necessary for the appropriate use of VMPs and constitutes the official reference for their labelling and package insert. This summary always contains the following items:

- i) pharmacological properties,
- ii) target animal species,
- iii) therapeutic indications,
- iv) target bacteria,
- v) dosage and administration route,
- vi) withdrawal periods,
- vii) incompatibilities,
- viii) expiry date,
- ix) operator safety,
- x) particular precautions before use,
- xi) particular precautions for the proper disposal of un-used products.

Antimicrobials that are considered to be important in treating critical diseases in humans should only be used in animals when alternatives are either unavailable or inappropriate.

Consideration should be given to providing such guidance by means of the product label and data sheet.

The oral route should be used with caution.

2.11. Post-marketing antimicrobial surveillance

The information collected through pharmacovigilance programmes, including lack of efficacy, should form part of the comprehensive strategy to minimise antimicrobial resistance.

2.11.1. Specific surveillance

Specific surveillance to assess the impact of the use of a specific antimicrobial may be implemented after the granting of the marketing authorisation The surveillance programme should evaluate not only resistance development in target animal pathogens, but also in food-borne pathogens and/or commensals. Such surveillance will also contribute to general epidemiological surveillance of antimicrobial resistance.

2.11.2. General epidemiological surveillance

The surveillance of animal bacteria resistant to antimicrobial agents is essential. The relevant authorities should implement a programme according to the OIE *International Animal Health Code*.

2.12. Distribution of the antimicrobial agents used in veterinary medicine

The relevant authorities should ensure that all the antimicrobial agents used in animals are:

- i) prescribed by a veterinarian or other suitably trained and authorised person,
- ii) delivered by an authorised animal health professional,
- iii) supplied only through licensed/authorised distribution systems,
- iv) administered to animals by a veterinarian or under the supervision of a veterinarian or by other authorised persons.

2.13. Control of advertising

All advertising of antimicrobials should be controlled by a code of advertising standards, and the relevant authorities must ensure that the advertising of antimicrobial products:

- i) complies with the marketing authorisation granted, in particular regarding the content of the summary of product characteristics,
- ii) is restricted to authorised professionals, according to national legislation in each country.

2.14. Training of antibiotic users

Training of antibiotic users should focus on:

- i) information on disease prevention and management strategies,
- ii) the ability of antimicrobials to select for resistant bacteria in food-producing animals,
- iii) the need to observe responsible use recommendations for the use of antimicrobial agents in animal husbandry in agreement with the provisions of the marketing authorisations

2.15. Research

The relevant authorities should encourage public- and industry-funded research in accordance with the recommendations of the OIE (*Rev. sci. tech. Off. int. Epiz.* [2001], **20** [3], 829–839).

3. Responsibilities of the veterinary pharmaceutical industry

3.1. Marketing authorisation of VMPs

The veterinary pharmaceutical industry has responsibilities to:

- i) supply all the information requested by the national regulatory authorities,
- ii) guarantee the quality of this information in compliance with the provisions of good manufacturing, laboratory and clinical practices,
- iii) implement a pharmacovigilance programme and on request, specific surveillance for bacterial susceptibility and resistance.

3.2. Marketing and export of VMPS

For the marketing and export of VMPs:

- i) only licensed and officially approved VMPs should be sold and supplied, and then only through licensed/authorised distribution systems,
- ii) only VMPs that have been authorised in the (exporting) country in which the product(s) is approved for sale or the quality of which is certified by a regulatory authority should be exported,
- iii) the national regulatory authority should be provided with the information necessary to evaluate the amount of antimicrobial agents marketed.

3.3. Advertising

The veterinary pharmaceutical industry should:

- i) disseminate information in compliance with the provisions of the granted authorisation,
- ii) ensure that the advertising of antimicrobials directly to the livestock producer is discouraged.

3.4. Training

The veterinary pharmaceutical industry should participate in training programmes as defined in section 2.14.

3.5. Research

The veterinary pharmaceutical industry should contribute to research as defined in section 2.15. above.

4. Responsibilities of pharmacists

Pharmacists should only distribute veterinary antimicrobials on prescription. All products should be appropriately labelled (see section 5.5.).

The guidelines on the responsible use of antimicrobials should be reinforced by pharmacists who should keep detailed records of:

- i) date of supply,
- ii) name of prescriber,
- iii) name of user,
- iv) name of product,
- v) batch number,
- vi) quantity supplied.

Pharmacists should also be involved in training programmes on the responsible use of antimicrobials, as defined in section 2.14.

5. Responsibilities of veterinarians

The prime concern of the veterinarian is to encourage good farming practice in order to minimise the need for antimicrobial use in livestock.

Veterinarians should only prescribe antimicrobials for animals under their care.

5.1. Use of antimicrobial agents when necessary

The responsibilities of veterinarians in this area are to carry out a proper clinical examination of the animal(s) and then:

- i) only prescribe antimicrobials when necessary,
- ii) make an appropriate choice of the antimicrobial based on experience of the efficacy of treatment.

On certain occasions, a group of animals that may have been exposed to pathogenic bacteria may need to be treated without recourse to an accurate diagnosis and antimicrobial susceptibility testing to prevent the development of clinical disease and for reasons of animal welfare.

5.2. Determination of the choice of an antimicrobial

5.2.1. The expected efficacy of the treatment is based on:

- i) the clinical experience of the veterinarian,
- ii) the activity towards the pathogenic bacteria involved,
- iii) the appropriate route of administration,
- iv) known pharmacokinetics/tissue distribution to ensure that the selected therapeutic agent is active at the site of infection,
- v) the epidemiological history of the rearing unit, particularly in relation to the antimicrobial resistance profiles of the pathogenic bacteria involved,
- vi) Should a first line antibiotic treatment fail or should the disease recur, a second line treatment should ideally be based on the results of diagnostic tests.

To minimise the likelihood of antimicrobial resistance developing, it is recommended that antimicrobials be targeted to bacteria likely to be the cause of infection.

5.2.2. Combinations of antimicrobials

Combinations of antimicrobials are used for their synergistic effect to increase therapeutic efficacy or to broaden the spectrum of activity. Furthermore, the use of combinations of antimicrobials can be protective against the selection of resistance in cases in which bacteria exhibit a high mutation rate against a given antimicrobial.

Some combinations of antimicrobials may, in certain cases, lead to an increase in the selection of resistance.

5.3. Appropriate use of the antimicrobial agent chosen

A prescription for antimicrobial agents must indicate precisely the treatment regime, the dose, the dosage intervals, the duration of the treatment, the withdrawal period and the amount of drug to be delivered, depending on the dosage and the number of animals to be treated.

5.3.1. 'Off label use' (extra-label use) of veterinary medicinal products

Although all medicinal products should be prescribed and used in accordance with the specifications of the marketing authorisation, the prescriber should have the discretion to adapt these in exceptional circumstances.

5.4. Recording

All available information should be consolidated into one form or database. This information should:

- i) allow monitoring of the quantities of medication used,
- ii) contain a list of all medicines supplied to each livestock holding,
- ii) contain a list of medicine withdrawal periods and a system for allowing information to be updated,
- iv) contain a record of antimicrobial susceptibilities,
- v) provide comments concerning the response of animals to medication,
- vi) allow the investigation of adverse reactions to antimicrobial treatment, including lack of response due to antimicrobial resistance. Suspected adverse reactions should be reported to the appropriate regulatory authorities.

5.5. Labelling

All medicines supplied by a veterinarian should be adequately labelled with the following minimum information:

- i) the name of the owner/keeper or person who has control of the animal(s),
- ii) the address of the premises where the animal(s) is kept,
- ii) the name and address of the prescribing veterinarian,
- iv) identification of the animal or group of animals to which the antimicrobial agent was administered,
- v) the date of supply,
- vi) the indication 'For animal treatment only',
- vii) the warning 'Keep out of the reach of children',
- viii) the relevant withdrawal period, even if this is nil.

The label should not obscure the expiry date of the preparation, batch number or other important information supplied by the manufacturer.

5.6. Training

Veterinary professional organisations should participate in the training programmes as defined in section 2.14.

6. Responsibilities of livestock producers

Livestock producers with the assistance of a veterinarian, where possible, are responsible for preventing outbreaks of disease and implementing health and welfare programmes on their farms.

Livestock producers have to:

- i) draw up a health plan with the veterinarian in charge of the animals that outlines preventative measures (mastitis plan, worming and vaccination programmes, etc.),
- ii) use antimicrobial agents only on prescription, and according to the provisions of the prescription,
- ii) use antimicrobial agents in the species, for the uses and at the doses on the approved/registered labels and in accordance with product label instructions or the advice of a veterinarian familiar with the animals and the production site,
- iv) isolate sick animals, when appropriate, to avoid the transfer of resistant bacteria,
- v) comply with the storage conditions of antimicrobials in the rearing unit, according to the provisions of the leaflet and package insert,

- vi) address hygienic conditions regarding contacts between people (veterinarians, breeders, owners, children) and the animals treated,
- vii) comply with the recommended withdrawal periods to ensure that residue levels in animal-derived food do not present a risk for the consumer,
- viii) dispose of surplus antimicrobials under safe conditions for the environment. Partially-used medicines should only be used within the expiry date, for the condition for which they were prescribed and, if possible, in consultation with the prescribing veterinarian,
- ix) maintain all the laboratory records of bacteriological and susceptibility tests. These data should be made available to the veterinarian responsible for treating the animals,
- x) keep adequate records of all medicines used, including the following:
 - a) name of the product/active substance and batch number,
 - b) name of supplier,
 - c) date of administration,
 - d) identification of the animal or group of animals to which the antimicrobial agent was administered,
 - e) diagnosis/clinical conditions treated,
 - f) quantity of the antimicrobial agent administered,
 - g) withdrawal periods,
 - h) result of laboratory tests,
 - i) effectiveness of therapy,
- xi) inform the responsible veterinarian of recurrent disease problems.

FAO/IAEA Consultants Meeting on 'OIE Guidelines for Validation and certification of Diagnostic Assays for Animal Infectious Diseases'

18-20 November 2002, Vienna, Austria

1. Background

One of the main objectives of the Office International des Epizooties (OIE) is to provide guidelines for the regulation of trade in animals and animal products with regard to infectious diseases. Guidelines and standards are published in the International Animal Health Code (the Code), in which the most important infectious animal diseases are divided into List A and List B diseases and the requirements necessary to minimise the risk for importing countries are defined. The OIE Manual of Standards for Diagnostic Tests and Vaccines (the Manual) specifically describes the diagnostic techniques and associated tests for each of these diseases and for the relevant vaccines. In the Manual, the OIE provides a list of diagnostic tests for List A and B diseases that are designated as prescribed or alternative tests for international trade. Prescribed tests are those that are required by the *Code* for the testing of animals before they are moved internationally. Alternative tests are those that are suitable for the diagnosis of disease within a local setting, and can also be used in the import/export of animals after bilateral agreement. There are often other tests described in the chapters, which may also be of some practical value in local situations or which may still be under development. All the tests described are 'validated' tests, i.e. the results that arise from their implementation can be taken with confidence for the prediction of the infection status of the animal to which the tests are applied, e.g. identification of the animal as being positive or negative for the analyte/process the test is measuring.

Validation is the evaluation of a process to determine its fitness for a particular use, e.g. identification of a product or a reaction. It quantifies the performance of the assay, the possible errors and the likelihood of their occurrence. Overall, it should give criteria of reliability (specificity, sensitivity, accuracy, etc.), reproducibility (importance of internal quality control, intra- and inter-laboratory variability, etc.), and relevance (relationship to biological status for decision making). Therefore many variables are to be addressed before an assay can be considered to be validated.

In Chapter I.1.3 of the 2000 edition of the *Manual* 'The Principles of Validation of Diagnostic Assays for Infectious Diseases' are given. These are, in fact, a summary of the development of an assay, the feasibility studies, the optimisation and standardisation of the reagents, the characterisation of the assay performance (sensitivity and specificity) and the interpretation of the assay results. Because the *Manual* provides the principles of assay validation but not standards for assay validation, the term 'validated assay' elicits various interpretations, e.g. many consider that validation of an assay is a time-limited process and not an on-going assessment of assay performance for as long as it is used as is clearly stated in these principles. Many points therefore need to be clarified for the 'harmonisation' of assay validation and use:

- Identification of the purpose for which the assay will be used
- The validation process

The validation process is meant to determine the usefulness/relevance of an assay to a defined problem by providing performance characteristics. Which samples and which players are involved in that process?

• Assay recognition procedures (certification of a validated assay)

Currently the adoption of an assay as a prescribed or alternative test is obtained from the OIE International Committee during the annual OIE General Session following a recommendation from the OIE Standards Commission. It is unclear what data are needed for this evaluation by the Standards Commission. Normally the data submission and request for an assay classification are made by the Delegates of the Member Countries to the OIE.

The list of prescribed and alternative diagnostic tests in the OIE *Manual* is limited to their application in international trade and there is no obligation on testing laboratories to adhere to those assays for other purposes.

It has become obvious that it is necessary to improve the current system for the qualification and certification of diagnostic assays for infectious animal diseases. This is why the Animal Production and Health Sub-Programme of the International Atomic Energy Agency (IAEA) and its laboratory, the OIE Collaborating Centre for ELISA¹⁴ and Molecular Techniques in Animal Disease Diagnosis, have convened a consultants meeting on the validation and certification of diagnostic assays for infectious animal diseases. This meeting was meant to elicit discussions on two mains areas:

- Validation with respect to 'fitness for purpose',
- The process by which the assay (kits/reagents) can be certified by the OIE for a purpose.

The conclusions and recommendations of the meeting will be sent to the Director General of the OIE as proposals for the improvement of animal health management in terms of risk assessment.

2. Conclusions and Recommendations

Animal disease management is carried out for economic, public health, and environmental reasons. Risk assessment is the key component in disease management. An important factor in risk assessment is evaluation of animals and their products. Diagnostic testing is an important activity in this process and is useful only if it is applied within specific contexts. Therefore, testing can be classified as to its fitness for purpose. The purposes can be classified into six broad categories.

Fitness for Purpose

Purposes

- 1) Demonstrate population 'freedom' from infection (prevalence apparently zero)*
 - a) 'free' with and/or without vaccination
 - b) historical 'freedom'
 - c) re-establishment of 'freedom' following outbreaks

*Note: Apparent freedom – absolute proof of freedom from infection in populations is not possible

- 2) Demonstrate freedom from infection or agent in individual animals or products for trade purposes
- 3) Eradication of infection from defined populations
- 4) Confirmatory diagnosis of clinical cases
- 5) Estimate prevalence of infection to facilitate risk analysis (surveys, classification of herd health status, implementation of disease control measures)
- 6) Determine immune status in individual animals or populations (post-vaccination)

The importance of analytical sensitivity and analytical specificity will vary depending on the purpose of use of the assay, but repeatability and reproducibility are always important factors to be considered. In addition to these performance characteristics some other factors need to be taken into account, such as sampling strategy, estimated disease prevalence, population characteristics, clinical evaluation, feasibility (including cost), efficacy of veterinary services, and host response to organisms and their variants.

¹⁴ ELISA: enzyme-linked immunosorbent assay

Recommendation:

Recognising that the OIE has made considerable progress in the application of prescribed tests for purposes of international trade, it is recommended that in the future the OIE considers the adoption of the following more broad-based approach to the application of tests.

- It is recommended that the OIE gives top priority to adopting a process for the evaluation of diagnostic tests for specific purposes. The six purposes identified above should be the basis for test classification and validation. For each disease described in the *Manual* tests should be classified according to their fitness for purpose.
- Currently, there is no guideline for submission of an assay following initial validation. It is recommended to develop a standard template. Its purpose is to standardise validation methods, provide guidance through the validation process, promote quality in diagnostic assays, support the incremental process of validation, and aid in the establishment of a registration process. For that, a registry of test methods would be created and managed by the OIE with the support of other organisations (FAO, IAEA, WHO) and possibly independent experts. This registry would have different levels of validation to be followed successively by the developers. These will be defined in the template (to be written and proposed by a group of experts).
- It is recommended that serum/sample collections be established by the OIE Reference Laboratories to provide analytical references, evaluation panels, and proficiency panels. Funding is a serious constraint to progress in this area, and it is recommended that the OIE should emphasise to international and national funders the importance of providing adequate resources on an ongoing basis.
- It is recommended that the OIE review the procedure for test validation and data submission. This should be based on the template and the fitness for purpose:
 - 1) Assay developer applies standard template requirements towards validation of new test.
 - 2) Total validation package is evaluated by other laboratories (they should not have been involved in the original validation).
 - 3) Evaluating laboratories must have established records in working with assays for the disease in question (at least one OIE Reference Laboratory if possible).
 - 4) The template with supporting documents are submitted to the OIE for evaluation.
 - 5) The OIE will accept the assay after a positive and independent peer review of results. The OIE provides an independent opinion on the purpose(s) for which the assay is deemed to be fit at the time of the OIE evaluation. Any subsequent changes need re-evaluation and demonstration of equivalency or improvement.

It is also recommended that OIE and the Collaborating Centre at the FAO/IAEA hold a meeting in 2003 with stakeholders (users, assays developers, private firms, donors, etc.) with the objective of providing the standard template and addressing the problems linked to references materials.

Conclusions and recommendations for 2003 from the meeting of the Expert Surveillance Panel on Equine Influenza Vaccines

These recommendations were made following a meeting of the Expert Surveillance Panel held on 13 January 2003 and relate to the composition of vaccines for the forthcoming year (2003).

Influenza activity, January 2002 – December 2002

Outbreaks of equine influenza in the Benelux, Canada, France, Germany, Israel, Italy, Sweden, the United Kingdom and the United States of America (USA) were reported. Activity was mainly sporadic with no epizootics and no reports of international transmission of equine influenza into countries previously free of the disease.

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

Characteristics of recent isolates

In haemagglutination inhibition (HI) tests with post-infection ferret sera, the European isolates from 2002 were antigenically related to the European lineage prototype strains A/eq/Newmarket/2/93 and A/eq/Suffolk/89, although there was some heterogeneity among the isolates. Isolates from the USA and Canada were all antigenically similar to the American lineage prototype strains A/eq/Newmarket/1/93 and A/eq/Kentucky/94.

The haemagglutinin (HA1) sequences of most European viruses from 2002 (including direct sequencing from additional specimens) were similar to the virus circulating in the previous year, although a few were genetically more related to viruses circulating from 1991 to 1995. The USA 2002 isolates were genetically similar to other recent American isolates.

The Expert Panel was pleased that there has been additional input to the surveillance effort, but urges the need for increased effort to isolate more viruses from a greater geographical area in order to increase the likelihood of early detection of emerging antigenic variants.

Recommendations for the composition of equine influenza vaccines

During the period January 2002 to December 2002, viruses of the American and European lineages of the H3N8 continued to cause sporadic outbreaks on the American and European continents, respectively.

It is recommended that vaccines to be used in 2003 contain the following:

- an A/eq/Newmarket/1/93 (H3N8) or A/eq/Kentucky/94 (H3N8)-like virus
- an A/eq/Newmarket/2/93 (H3N8)-like virus*

*A/eq/Suffolk/89 and A/eq/Borlänge/91, currently used in vaccine strains, continue to be acceptable.

Reference reagents

Reference reagents specific for the recommended vaccine strains are available for standardisation of vaccine content by single radial diffusion (SRD) assay and can be obtained from the National Institute for Biological Standards and Control (NIBSC).

Three equine influenza horse antisera, including antisera to the recommended vaccine strains, are available as European Pharmacopoeia Biological Reference Preparations (EP BRPs) for serological testing of equine influenza vaccines by the single radial haemolysis assay. These antisera are also available from the OIE Reference Laboratory in Newmarket (UK) for use as primary standards in diagnostic serological testing.

SRD reference reagents	EP BRPs for serological testing of equine influenza vaccines	OIE primary standards for diagnostic serological testing
NIBSC Blanche Lane South Mimms Potters Bar Herts EN6 3QG United Kingdom Fax: (+44-1707) 64.67.30 e-mail: enquiries@nibsc.ac.uk	European Directorate for the Quality of Medicines BP907 F-67029 Strasbourg Cedex France Website: http://www.pheur.org	Animal Health Trust Lanwades Park Kentford Newmarket Suffolk, CB8 7UU United Kingdom Fax: (+44-8700) 50.24.61 e-mail: info@aht.org.uk

${\rm \@Colored blue}$ Office International des Epizooties (OIE), 2003

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