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REPORT OF THE MEETING OF THE OIE BIOLOGICAL STANDARDS COMMISSION

Paris, 25–27 September 2007

The OIE Biological Standards Commission met at the OIE Headquarters from 25 to 27 September 2007. Dr Gideon Brückner, Head, OIE Scientific and Technical Department, speaking on behalf of Dr Bernard Vallat, Director General of the OIE, welcomed the Members of the Commission, Professor Steven Edwards, President, Dr Beverly Schmitt, Vice-President, Dr Mehdi El Harrak, Secretary General and Dr Santanu K. Bandhopadhyay, member of the Commission as well as the other expert participant, Dr Peter Wright, Canada. Dr James Pearson, Consultant Editor of the *Terrestrial Manual*, also joined the meeting to present and discuss the latest changes to the *Terrestrial Manual,* which is scheduled for publication on 2008.

Dr Vallat later met with the Commission when he emphasised that the success of the first twinning projects will be critical in encouraging others to participate. The aim of the project is to improve the capacity of laboratories in developing countries, and it is not necessarily expected that all applicants will achieve full Reference Laboratory status. When accepting applications, a balance must be maintained between regions and diseases.

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

1. OIE Reference Laboratories and Collaborating Centres

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

The Commission reviewed the following applications for OIE Collaborating Centre status:

The Commission recommended the acceptance of the Ecole Inter-Etats des Sciences et Médecine Vétérinaires (EISMV) de Dakar, SENEGAL as the *OIE Collaborating Centre for Training veterinary officials, diagnosing infectious animal diseases and zoonoses in Tropical Africa*.
Tel.: (221) 865.10.08; Fax: (221) 825.42.83; E-mail: tekoagbo2001@yahoo.fr

The Commission agreed in principle to recommend the Centre d'Etudes et de Recherches Vétérinaires et Agrochimiques (CERVA) de Ukkel, BELGIUM as an OIE Collaborating Centre but suggested that a change in title. The suggested title would be the *OIE Collaborating Centre for Validation, Quality Assessment and Quality Control of Diagnostic Assays and Vaccine Testing for Vesicular Diseases in Europe*
Tel.: (+32-2) 379.04.00; Fax: (+32-2) 379.06.66; E-mail: kris.de.clercq@var.fgov.be. This proposal will be submitted to the Administrative Commission.

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

OIE Reference Laboratory for Enzootic bovine leukosis
National Veterinary Research Institute, POLAND
Tel.: (+48-81) 886.30.51); Fax: (+48-81) 886.25.95; E-mail: jkuzmak@piwet.pulawy.pl
Designated Reference Expert: Dr Jacek Kuzmak

OIE Reference Laboratory for American foulbrood
Laboratorio de Loque Americana de la Unidad de Bacteriología del Centro de Investigaciones en Fitopatología (CIDEFI), ARGENTINA
Tel.: (+54-221) 423 6758 ext. 423; Fax: (+54-221) 425.2346; E-mail: amalippi@netverk.com.ar
alippi@biol.unlp.edu.ar
Designated Reference Expert: Dr Adriana M. Alippi

OIE Reference Laboratory for Foot and mouth disease
Onderstepoort Veterinary Institute, Exotic Diseases Division, SOUTH AFRICA
Tel.: (+27-12) 529.95.92; Fax: (+27-12) 529.92.49; E-mail: vosloow@arc.agric.za
Designated Reference Expert: Dr Wilna Vosloo

OIE Reference Laboratory for Bovine viral diarrhoea
Elizabeth Macarthur Agriculture Institute (EMAI), AUSTRALIA
Tel.: (+61-2) 46.40.63.31; Fax: (+61-2) 46.40.64.29; E-mail: peter.kirkland@dpi.nsw.gov.au
Designated Reference Expert: Dr Peter D. Kirkland

Other applications received either required clarification or were deemed not to fulfil the requirements of an OIE Reference Laboratory. The Commission determined that Reference Laboratory designation should normally be limited to diseases on the OIE List, except for one or two specialised cases where the designation was topic-based. It also confirmed that the correct title should be "OIE Reference Laboratory for [name of disease]".

1.2. Updating the list of Reference Laboratories

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

Enzootic bovine leukosis
Dr Thomas Vahlenkamp to replace Dagmar Beier at the Friedrich-Loeffler-Institute, Wusterhausen/ Dosse, GERMANY.

Bovine spongiform encephalopathy and scrapie
Dr Marion Simmons to replace Dr Danny Matthews at the VLA Weybridge, UNITED KINGDOM.

Contagious equine metritis
Mr Paul Todd to replace Mr Peter Heath at the VLA Bury St Edmunds, UNITED KINGDOM.

1.3. Update on twinning – review of 'twinning handbook'

Mr Keith Hamilton submitted a draft manual on twinning of laboratories. There was discussion on the aims of the project, which are to build capacity in laboratories in developing countries, as well as to have more reference laboratories in those countries. It is important that the candidate laboratories can show evidence of sustainability and credibility. The twinning manual will be circulated electronically to the Commission members for comment. It will also be sent to the Aquatic Animal Health Standards Commission to ensure that aquatic animal disease laboratories are adequately covered. Collaborating Centres can apply for a twinning project if they are based in a laboratory of appropriate quality.

1.4. Review of twinning applications

An application had been received for a twinning project between the Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Italy and the Russian Federal Centre for Animal Health (FGI-ARRIAH), Russia for Avian Influenza and Newcastle disease. The Commission recommends acceptance of this application and referred the dossier to the OIE Director General for approval of the budget.

2. International Standardisation of diagnostic tests and vaccines

2.1. OIE standardisation programmes for diagnostic tests

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

Dr Selleck informed the Commission that work on the preparation of OIE international reference serum for the AI AGID[1] test is well advanced. The Commission encouraged Dr Selleck to continue with his efforts.

Enzootic bovine leukosis (*EBL*) *PCR*[2]

Following the withdrawal of the laboratory in Sweden from OIE Reference Laboratory status, the newly appointed expert in Germany and the expert in the proposed new Reference Laboratory in Poland will be asked to take forward the project to develop a standard protocol for EBL PCR.

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

Mrs J. Stack reported that progress is being made in the development of candidate sera. The Commission encourages Mrs Stack to continue this important work.

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

Dr Nielsen informed the Commission that progress continues with this project. The Commission wished to encourage the participating laboratories to send their results to Dr Nielsen.

2.2. Standardisation of tuberculin production

The Commission noted the initiative undertaken by laboratories in South America to attempt to standardise tuberculin production amongst themselves. The Commission believes that this issue is too complex to attempt global standardisation at the present time, but encourages the South Americans to continue with their efforts with the view to achieving regional harmonisation as a first step.

3. List of prescribed tests and substitution tests

3.1. Need for a PCR to differentiate African horse sickness from equine encephalosis viruses

The Commission received a report from the Onderstepoort Veterinary Institute that demonstrated cross-reactivity between African horse sickness and equine encephalosis viruses in the hemi-nested PCR for African horse sickness. A need was thus identified to develop PCR tests capable of differentiating between these viruses. Following consultation with the Code Commission, the Biological Standards Commission agreed to write to the scientists thanking them for this information and suggesting that they may wish to discuss with their OIE Delegate whether an application to have equine encephalosis listed would be warranted, as presently this disease does not appear on the OIE list.

3.2. A new CFT[3] for dourine

The Commission had received a dossier on a new method for CFT for dourine from the Kazakhstan National Veterinary Association of Horses and Camel Breeders. The OIE Reference Laboratory would be asked to provide an opinion. In the meantime, the developers of the test would be asked to provide information on the diagnostic sensitivity and specificity of the test.

3.3. Revision of the list of prescribed and alternative tests

The Commission reviewed the current list of prescribed and alternative tests in the light of newly adopted or proposed chapters in the *Terrestrial Code*.

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1 AGID: agar gel immunodiffusion
2 PCR: polymerase chain reaction
3 CFT: complement fixation test

For African horse sickness, tests for identification of the agent need to be validated so that tools are available in readiness for any new requirements in the *Terrestrial Code*.

For West Nile fever, the Biological Standards Commission discussed with the Code Commission the proposed requirement for testing of ducks and geese for the purpose of trade. While there is a validated PCR for diagnosis in horses, no PCR has yet been adapted or validated for ducks and geese, which are the species most likely to be implicated in transmission.

3.4. Follow-up from last meeting – rabies ELISA[4]

Dr F. Cliquet had informed the OIE that full results on the interlaboratory comparison using the ELISA kit on the OIE Register were not yet available. These studies are ongoing and the Commission was keen to see a full interlaboratory comparison using all the available kits.

4. *Ad hoc* Groups

4.1. *Ad hoc* Group on Biotechnology

Dr Tomoko Ishibashi updated the Commission on the work of the *ad hoc* Group on Biotechnology. The report of this meeting is included at Appendix III. The *ad hoc* Group had developed guidelines for somatic cell nuclear transfer (see Appendix III of the *ad hoc* Group report), which are proposed for eventual inclusion in the *Terrestrial Code*. Member Countries are requested to send comments on this draft by the end of December 2007 for examination by the Biological Standards Commission as well as the Code Commission. The *ad hoc* Group also developed draft guidelines for Veterinary Plasmid DNA Vaccines (see Appendix IV of the *ad hoc* Group report), which should be considered for inclusion in the *Terrestrial Manual*. It was agreed that genetically modified animals should fall within the remit of this *ad hoc* Group, but for aspects related to traceability, the Group should collaborate with the *ad hoc* Group on Traceability. The Commission suggested that an expert from the *ad hoc* Group on Biotechnology be present at the next meeting of the *ad hoc* Group on Traceability. The Biological Standards Commission endorsed the proposals of the *ad hoc* Group on Biotechnology to produce a series of background papers as listed in their report (Section 13, paragraph 2). Information on vaccine schedules should be incorporated into the other topics. The Commission requested in addition a background paper on nanotechnology. The Commission also endorses the proposals to develop new chapters for the *Terrestrial Manual* (Section 13, paragraph 3). The topic of DIVA vaccines and companion tests[5] should not be a separate chapter but should be incorporated as appropriate in individual disease chapters.

4.2. Update on Antimicrobial Resistance

Dr Ishibashi updated the Commission on a forthcoming meeting to be held in Rome, Italy in November, involving the OIE, WHO[6] and FAO[7] on critically important antimicrobials. The three organisations have selected 15 experts to participate in the meeting. The aim of the meeting is for the three organisations to agree a policy with regard to the two lists of critically important antimicrobials (for humans and for animals).

5. Review of OIE guidelines

The updated booklet "OIE Quality Standard and Guidelines for Veterinary Laboratories" was reviewed and finalised. It is envisaged to publish this second edition later this year.

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4 ELISA: immunoenzymatic method
5 DIVA vaccines and companion tests: vaccines and companion tests that allow vaccinated animal to be differentiated from infected one
6 WHO: World Health Organization
7 FAO: Food and Agriculture Organization of the United Nations

6. OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (*mammals, birds and bees*)

For this agenda item, the Commission was joined by the Consultant Editor, Dr James Pearson.

The sixth edition was adopted by the International Committee in May this year with the proviso that the Commission could make last minute changes. For most of the chapters, Dr Pearson has reviewed the Member Country comments and amended them accordingly; a small number of chapters remain to be reviewed. Once amended, the chapters are returned to the authors for final review and correction and to address any remaining queries. It is hoped to publish the next edition in the first quarter of 2008. Dr Pearson had singled out a number of chapters that had received a large amount of Member Country comment, and asked the Commission for advice on these issues.

Dr François Diaz informed the Commission that the French translation of the 2005 edition of the T*errestrial Manual* was now available electronically on the OIE web site. The Spanish edition will shortly be available on the web. For the 2008 edition, it is hoped to have the French and Spanish translations available within 6–8 months of publication of the English edition.

7. Register of OIE validated and certified diagnostic tests

7.1. OIE Procedure - Final assessment report(s) forwarded to the Commission for opinion

The Commission endorsed the report submitted by the experts who had reviewed the dossier for the Prionics® Check Western Blot for bovine spongiform encephalopathy (BSE) and recommends that the kit be approved as a test for BSE in the three categories for which they have requested. If adopted by the International Committee it will be placed on the OIE Register.

7.2. Questions raised on the OIE Register

A request had been received for the details of the validation data for the OIE certified rabies ELISA kit. The Commission recommended that the manufacturers be asked for authorisation to publish this information in summary form on the web page. Dr Diaz will prepare a draft of a summary template for this purpose.

A query had been received from a biotechnology company concerning a PCR kit for animal traceability. The Commission felt that this was not within the remit of the certification process, which was specifically designed for the evaluation of test methods for infectious disease diagnosis.

7.3. Meeting at the OIE with the AEFRV (European Association of Veterinary Diagnostic Manufacturers)

Dr Diaz presented the agenda of the meeting to be held on 28 September with the AEFRV to discuss the OIE procedure for validation and registration of diagnostic assays. A report will be submitted to the Commission.

8. Follow-up from the General Session

During the General Session in May, a request had been made by a Member Country for the Commission to convene an *ad hoc* Group to review the diagnosis of infectious diseases of dromedaries. The Commission asked OIE to approach relevant countries to ask if they would identify experts who could participate in such a group. Terms of Reference will need to be drafted and priority areas, diseases, suitability and validation of the available diagnostic kits for dromedaries identified.

At some point in the future, the Commission considers that a similar approach will be needed for diagnosis of diseases in water buffalo.

9. Liaison with the other Commissions

A joint meeting was held between the Biological Standards and the Code Commissions.

9.1. Equine rhinopneumonitis

It was agreed that the name of the OIE Listed disease should remain as Equine Rhinopneumonitis. However the wording of the *Terrestrial Code* chapter is such that only infections with EHV-1 are the subject of regulation for international trade or movement, whereas the chapter in the *Terrestrial Manual* covers clinical signs and diagnostic procedures for both EHV-1 and EHV-4 infections. Among the requirements in the *Terrestrial Code* is that horses should show "no clinical sign of equine herpes virus type 1 infection, on the day of shipment and during the 21 days prior to shipment". Advice would be sought from the Reference Laboratories to better define this terminology.

9.2. Proposal to move the information dealing with risk analysis for veterinary vaccines and biologicals from the *Terrestrial Code* to the *Terrestrial Manual*

The Commission agreed to append *Terrestrial Code* texts dealing with risk analysis for veterinary vaccines and biologicals to the introductory chapters in the *Terrestrial Manual* on vaccine production and tests for sterility respectively.

9.3. OIE PVS tool (OIE Tool for the Evaluation of Performance of Veterinary Services)

The Commission provided advice to the Code Commission on the elements of this tool that deals with evaluation of laboratories.

9.4. Bovine tuberculosis – prescribed tests

A Member Country had suggested that gamma interferon should be considered for designation as a Prescribed Test. Advice would be sought from the Reference Laboratories regarding the validation status of this test, and further consideration will be given at the next meeting.

9.5. DIVA tests for classical swine fever

Although the DIVA approach shows promise for control of classical swine fever, evidence is still lacking that it is fully effective. More comprehensive validation data are needed before it can be recommended. The Commissions discussed wording for the *Terrestrial Code* chapter that would address these concerns.

9.6. Rabies

A discussion was held regarding infections of bat lyssaviruses and the implications of such infections for country status. The Commission advised that all lyssavirus infections should be considered as rabies, but it should be recognised that in some countries lyssaviruses appear to circulate in bats with little or no spillover into domestic animal populations. The Code Commission would take this information forward in consideration for the wording of the relevant chapter.

10. Miscellaneous questions

10.1. Update on OFFLU[8]

Dr Edwards updated the Commission on OFFLU. The Steering Committee would meet in October to review progress in the network and identify priorities for further activity. A project meeting will be held in November on the results of vaccination programmes in Indonesia and the application of genetic and antigenic mapping tools. A post-doctorate scientist funded through OFFLU is in the process of being appointed to be based at VLA Weybridge. Mr Keith Hamilton was also working in support of OFFLU at the OIE Headquarters in liaison with the FAO, with a specific focus on laboratory twinning projects.

8 OFFLU: OIE/FAO Network on Avian Influenza

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

The Commission noted the final programme for the OIE Biotechnology Seminar to be held in Australia.

10.3. Iowa State University (an OIE Collaborating Centre) disease sheets

The Commission noted the initiative to replace the OIE disease sheets, which are very outdated, with a link to the disease sheets developed by the Iowa State University. The latter would also draft new disease sheets for those OIE listed diseases not currently covered by Iowa.

10.4. Laboratory biosecurity in the light of recent outbreaks of FMD in the UK

The Delegate for the United Kingdom had asked if any lessons had been learnt that could be shared with the international community in the light of the recent outbreaks of foot and mouth disease in her country. The Commission reviewed the current OIE Standards for biosafety and biosecurity and concluded that they were appropriate and fit for purpose. A number of points were identified that could merit stronger emphasis when the chapter is next reviewed:

- All contaminated effluent must be maintained under containment conditions, and effluent treatment plants should be as close to the source as possible.

- The question of decontamination methods for effluent treatment merits further expert attention. The suggestion of the Commission is that small volumes, arising for example from diagnostic testing activities, can be adequately dealt with by chemical methods but large volumes, arising from large scale culture of organisms as for example in vaccine production, should be subject to validated heat treatment.

- Facilities generating large quantities of pathogens at high titre for vaccine production must comply with the highest possible containment standards.

- High containment laboratories and facilities must have access controls on people and vehicles, including records of visitors.

- Documented operating procedures must be in place to cover all aspects of biosecurity. A biosecurity officer should be appointed to monitor compliance with the operating procedures. This officer should be free from conflicts of interest arising from other responsibilities within the facility.

- National authorities or owners of facilities should recognise that high containment laboratories have very high maintenance costs, and should make budgetary provision to ensure that sustainable operating procedures can continue.

10.5. Global inventory of laboratory quality standards and EQAS

The Commission noted a mission report by Ms Linnane and Dr Diaz concerning a joint initiative with WHO and FAO to collect information by online questionnaire about laboratory quality systems and external quality assessment schemes (EQAS). The survey will be advertised on the web sites of all participating agencies, and the online questionnaires will be hosted through WHO's data collection site. Potential outcomes could be produced via a database or report, with both quantitative and qualitative data, depending on the success of the survey and the data collected.

10.6. Proposed Guideline for the harmonisation of labelling systems used for veterinary medicines

A draft guideline had been received from the OIE Regional Representative for the Americas. While this is a praiseworthy concept, the Commission considered it would be unachievable at the present time, and did not propose to pursue it.

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9 WAVLD: World Association of Veterinary Laboratory Diagnosticians

10.7. World Bank's guidance for laboratory evaluation

The Commission expressed its willingness to assist the World Bank if required with the development of guidance for laboratory evaluation prior to funding requests.

10.8. Second Conference for OIE Reference Laboratories and Collaborating Centres

The Commission reiterated its recommendation that OIE should hold a second conference, and this should if possible be linked to the next WAVLD Conference in Spain in 2009.

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

The next meeting of the Commission is scheduled for 22 to 24 January 2008. The following meeting is scheduled for 23 to 25 September 2008 in Paris.

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.../Appendices

Appendix I

MEETING OF THE OIE BIOLOGICAL STANDARDS COMMISSION

Paris, 25–27 September 2007

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Agenda

1. OIE Reference Laboratories and Collaborating Centres
2. International Standardisation of Diagnostic Tests and Vaccines
3. List of Prescribed and Alternative Tests
4. *Ad hoc* Groups
5. Review of the OIE guidelines
6. *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*
7. OIE Register of diagnostic tests
8. Follow-up from the General Session
9. Liaison with other Commissions
10. Any other business

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MEETING OF THE OIE BIOLOGICAL STANDARDS COMMISSION
Paris, 25–27 September 2007

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Appendix III

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

Paris, 12–14 June 2007

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A meeting of the OIE *ad hoc* Group on Biotechnology was held at the OIE Headquarters in Paris from 12 to 14 June 2007. The meeting was chaired by Prof. Sándor Belak. Dr Cyril G. Gay acted as rapporteur. The Agenda and List of Participants are given at Appendices I and II, respectively. It was agreed that the next meeting of the *ad hoc* Group on Biotechnology would be 6 months from the date of this meeting.

1. Introduction

The *ad hoc* Group was welcomed by Dr Gideon Brückner, Head of the OIE Scientific and Technical Department, on behalf of Dr Bernard Vallat, Director General of the OIE. Dr Brückner introduced Dr Belak as new chairman of the *ad hoc* Group and indicated that as an interim arrangement, he will be assisted as co-chairman by Professor Paul-Pierre Pastoret.

Dr Brückner reviewed the purpose of the *ad hoc* Group, which is to address the scientific and technical aspects of biotechnology that impact on animal health. The OIE has separate expert groups that deal with animal welfare, traceability, and food safety. When animal biotechnology impacts on food safety or animal welfare, guidelines prepared by the *ad hoc* Group should identify that these issues need to be addressed by other existing OIE expert groups.

2. Review of the Terms of Reference

The *ad hoc* Group recommended adding RNA-based technologies as a new objective to the Terms of Reference. These new technologies are moving very fast with applications in pathogen control, biological control of insects, biotherapeutics, and drugs.

The Group also agreed to improve the Terms of Reference with the clear identification of the different categories of animal biotechnologies (e.g. transgenic *versus* cloned) and biological function (somatic *versus* germ line/heritability).

3. Report on the outcome of the 75th General Session – *Terrestrial Manual* adopted

No specific questions or issues relating to biotechnology were raised by Member Countries during the General Session and the report of the President of the Biological Standards Commission that covers the activities of the *ad hoc* Group was approved.

4. Report on the meeting of Codex ad hoc Intergovernmental Task Force on Food Derived from Biotechnology

A report was provided of the meeting of the Codex *ad hoc* Intergovernmental Task Force on Food Derived from Biotechnology, which was held in Chiba (Japan) in 2006. Key points discussed included: 1) the kind of animals to be included in the Codex and whether animal welfare should be considered; 2) safety of livestock not intended for non-food use; 3) dangers of allergenicity; 4) use of antibiotic resistance marker genes for selection (the consensus was that antibiotic resistance genes should not be used); 5) issues related to the cloning of genes in the germ line.

It was indicated that the issues discussed by this Codex *ad hoc* Intergovernmental Task Force address transgenic animals (animals carrying recombinant DNA). The *ad hoc* Group has focused to date on animal clones only. It was also noted that the FAO/WHO expert consultation suggested at its meeting held in February to March 2007 that the OIE address the technical and safety issues associated with transgenic animals.

5. Cloning in respect to animal health

5.1. Letter to the United States of America (USA)

The Group took note of a copy of a letter from Dr Vallat to the United States Secretary of Health and Human Services confirming that OIE is inherently involved in all aspects of biotechnology that impact on animal health and describing the work of the *ad hoc* Group including the guidelines the Group had produced on Somatic Cell Nuclear Transfer[1] technologies for animal clones (see Appendix III). This letter clarifies the important role of the *ad hoc* Group, specifically that the safety of human consumption of products derived from SCNT and their progeny is under the mandate of the Codex Alimentarius Committee.

It was emphasised that the Codex *ad hoc* Intergovernmental Task Force on Food Derived from Biotechnology is not addressing the food safety of animal clones and the expectation is that the OIE will address this issue through its relevant Specialist Commissions. However it was noted that this is currently not under the mandate of the OIE.

5.2. General discussion

The Group was informed of a guideline entitled "FAO/WHO Expert Consultation on Safety Assessment of Foods Derived from Recombinant DNA Animals" prepared during the meeting held 28 February to 2 March, 2007, in Geneva, Switzerland, and made the following recommendations:

1. In the executive summary of the report, it was mentioned that 'a suitable venue for developing guidelines for safe use of virally derived vectors in rDNA animals' would be the OIE.

2. In the scope section, it was mentioned that OIE should address the questions about the effects on health and welfare of rDNA animals through applications of marker and reporter genes and non-heritable genetic constructs.

3. In the recommendations sections, it was recommended that 'the animal health issues should form the basis for guidelines on health of rDNA animals similar to the one being developed for animal clones by OIE.

6. Discussion on Draft Guidelines for Somatic Cell Nuclear Transfer in Production Livestock and Horses

Dr Tomoko Ishibashi, Deputy Head, OIE Scientific and Technical Department, outlined future procedures for these draft guidelines. Once the decision is made as to whether they should be published in the *Terrestrial Animal Health Code* (*Terrestrial Code*) or the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (*Terrestrial Manual*), they will be appropriately reformatted. She requested that the *ad hoc* Group finalise the guidelines on SCNT.

The Group enquired whether there was a standard OIE format for guidelines, as this may have an impact on the issues addressed by the *ad hoc* Group as well as the technical information provided in the final guidelines and provided examples showing that the *Terrestrial Code* provides general information, while the *Terrestrial Manual* provides detailed technical information. It was decided that the Draft Guidelines for SCNT in Production Livestock and Horses should were more suitable for inclusion in the *Terrestrial Code* as they provide more general information.

1 SCNT: Somatic Cell Nuclear Transfer

The following issues in the draft guidelines that need to be addressed by the *ad hoc* Group were listed:

1. Generation of animals – should 'animals' be clarified? It was decided that the guidelines should be restricted at present to livestock and horses, but that poultry, fish, and insects should be addressed in the future when cloning of these species progresses.

2. On the issue of whether of the diagram produced for the guidelines was reflective of the discussion and input from the *ad hoc* Group, it was agreed that it did indeed reflect the Group's vision.

3. Can reference of the detailed document of the IETS[2] entitled "Health Assessment and Care for Animals Involved in the Cloning Process," be included to bring context to animal care? Dr Kochhar is currently the Chair of IETS working group that prepared this document and the document parallels the guidelines prepared by the *ad hoc* Group, i.e. developmental nodes based on life cycle. The *ad hoc* Group requested more time to review the document. It was recommended that, as with other official OIE documents that refer to IETS, the guidelines should cite the IETS document as a reference. In addition, all references will be removed from the guidelines as this will follow the *Terrestrial Code* format.

4. On the issue of re-cloning, it was decided to rephrase the statement "lack of information" to "information on re-cloning is only beginning to appear."

It was further recommended that the Draft Guidelines on SCNT in Production Livestock and Horses be presented to the *Terrestrial Animal Health Standards Commission* for consideration for inclusion in the *Terrestrial Code*. The *ad hoc* Group agreed with this recommendation.

7. Update on guidelines on biotechnology-derived vaccines

The *ad hoc* Group unanimously agreed to recommend to the Director General that Professor Paul-Pierre Pastoret continue to serve as a member of the Group. His expertise in vaccinology research and regulatory requirements will continue to be vital to the work of the *ad hoc* Group as it continues to develop chapters on new vaccine technologies.

The OIE will publish the sixth edition of the *Terrestrial Manual* in 2008. A fast track revision has been requested for the guidelines on biotechnology-derived vaccines. Documents should be submitted by December 2007 so that they can be reviewed when the Commission meets in January 2008.

7.1. Review of the sections relating to biotechnology-derived vaccines in chapter 1.1.7 of the *Terrestrial Manual* on Principles of Veterinary Vaccine Production

The *ad hoc* Group discussed the instructions provided by OIE and unanimously agreed that more precise instructions were needed in order for the Group to complete its tasks. It was agreed that the sections relating to biotechnology-derived vaccines of chapter 1.1.7 were very general in scope, and although sufficient at the time they were prepared, additional information could usefully be added, especially in the areas of the classification of the three categories of biotechnology-derived vaccines and the release of live rDNA products.

The following recommendations are submitted for consideration by the Commission:

1. It was agreed that the *ad hoc* Group could update the relevant sections relating to biotechnology-derived vaccines of chapter 1.1.7.

2. The Group further agreed that several new technologies have evolved or are currently being investigated or developed since the sections relating to biotechnology-derived vaccines were written; therefore, new chapters are needed on new trends in biotechnology-derived vaccines and the environmental release of these products.

3. In addition, specific detailed chapters should be written on new and emerging technologies, such as chapters on DNA vaccines, reverse genetics, cDNA clones, and plant-based vaccines, to facilitate the development of these new technologies.

2 IETS: International Embryo Transfer Society

The Group agreed to review the draft guidelines for DNA vaccines and submit them to the Commission for review at its September 2007 meeting (see Appendix IV).

7.2. Development of draft guidelines for DNA vaccines and plant-expressed antigens

Draft Guidelines on DNA Vaccines

The Group agreed to limit the scope of the guidelines to plasmid DNA vaccines, non-amplifiable in eukaryotic cells.

The recommendation was made that scientific papers should be referenced whenever possible to support recommendations.

The draft document was reviewed, modified and unanimously approved by the *ad hoc* Group and can be found at Appendix IV.

The next step will be to submit the final draft to the Biological Standards Commission.

Draft Guidelines on Plant-based Vaccines

The *ad hoc* Group resolved that insufficient time was given to develop a specific chapter on plant-based vaccines, but this will be prepared for future meetings to be incorporated in a new chapter on risk assessment of the release of biotechnology-derived vaccines (see item 13.3 below).

8. Follow-up on the recommendations by the ad hoc Group at October 2006 meeting on nanotechnology and animal health

Dr Anne MacKenzie was not able to attend the meeting and therefore no report was provided for this meeting.

The *ad hoc* Group agreed that the term 'Nanotechnology' is very broad and that not all nanotechnologies have relevance to animal health. The Group agreed that the most relevant applications to animal health in nanotechnology to date have been in the area of diagnostic discovery. The Group therefore agreed to focus in its future work on specific nanotechnologies that are relevant to animal health, such as new diagnostic platforms and drug delivery. The Group also agreed to address relevant safety issues such as toxicology.

9. Identification and tracing of animals and animal products that have resulted from biotechnological intervention – Cooperation with the OIE ad hoc Group on Traceability

A draft paper entitled "Current Options in Food Animal Traceability" was written and presented by Dr Kochhar for consideration by the *ad hoc* Group. The *ad hoc* Group agreed that the paper is a good starting point for discussion and commended Dr Kochhar for his proactive work on animal traceability. Due to time constraints the paper could not be reviewed in detail by the *ad hoc* Group but will be considered for future discussion in subsequent meetings.

Dr Ishibashi, stated that the paper is very informative and should be referred to the *ad hoc* Group on Animal Traceability. She requested that the *ad hoc* Group on Biotechnology identify objectives relevant to traceability of animals derived from biotechnological interventions for consideration by the *ad hoc* Group on Animal Traceability. The latter should then prepare a draft for the consideration of the *ad hoc* Group on Biotechnology.

It was also recommended that the Chairman of the *ad hoc* Group on Animal Traceability be invited to present what is expected from the *ad hoc* Group on Biotechnology.

The Group agreed to evaluate in the future efficient, cost-effective, genomic-based tools that could be used for traceability of animals as they relate to animal health and disease control. These tools may also be used to test their ability to detect cloned, genetically modified/transgenic, and somatic transgenic animals.

10. Follow-up to the discussion on OIE scope and definition of biotechnology

The *ad hoc* Group agreed to the following scope and definition:

Scope

Biotechnology draws upon scientific disciplines, such as genetics, molecular biology, biochemistry, microbiology, bioinformatics, embryology and cell biology, that are in turn linked to practical applications such as animal cloning, transgenesis, diagnostics, vaccines, biotherapeutics, and nanotechnology. For the purpose of the work of the *ad hoc* Group, it was agreed unanimously to focus on biotechnology as laboratory-based techniques being developed in biological research, such as recombinant DNA or cell culture-based processes, as they relate to animal health, but to define the term in a much broader sense to describe the whole range of methods, both conventional and current, used to engineer organisms to enhance animal health and production.

Definition

"Biotechnology is any technological use of biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific purposes. In the context of the World Organisation for Animal Health (*OIE*), *biotechnology refers to technologies relevant to animal health and production."*

11. Update on the organisation of the International Symposium 'Animal Genomics for Animal Health'

An update on the organisation of the International Symposium "Animal Genomics for Animal Health." was provided.

The scientific programme and invited speakers were reviewed. The call for abstracts, which ended May 2007, was implemented successfully with 108 abstracts received from 26 countries. Fifteen abstracts were selected for oral presentation, in addition to the 19 speakers already invited by the Scientific Committee. The scientific programme can be reviewed on the symposium Web site: http://www.ars.usda.gov/meetings/AGAH2007/. The majority of the remaining abstracts will be selected for poster presentations.

Manuscripts will be requested from the papers selected for oral presentation and for publication. The importance of ensuring concrete outcomes from the symposium was identified, such as next steps, key recommendations, and key points to be captured in the proceedings. As such, the round table discussion at the end of the programme will serve as the forum for achieving these outcomes. The following key areas were recommended by the *ad hoc* Group for discussion during the symposium round table discussion:

1. Which traits should be selected?
2. Phenotypic definition of diseases and identification of genes associated with variations in disease susceptibility/resistance;
3. Biodiversity of domestic species;
4. Collaboration with the private sector;
5. Forensic veterinary medicine and traceability;
6. Diagnosis of animal genetic defects and predictive medicine;
7. Impact on veterinary education.

In addition, the following key points are recommended for discussion:

1. Individual diseases:
    - Priorities;
    - Animal good *versus* poor responders to vaccination.

2. Population genetics to address animal health traits:

   - Pedigrees;
   - Experimental evidences;
   - Epigenetic control.

3. Animal breeding companies:

   - Pharmaceutical companies;
   - Regulatory agencies;
   - Experimental data on vaccines (genetic variation linked to efficacy/safety);
   - Experimental data on drugs (genetic variation linked to efficacy/safety);
   - Pharmaco/vaccinovigilance;
   - Confidentiality;
   - Contribution of veterinary practitioners.

The *ad hoc* Group commended the steering and scientific committees for their excellent work in organising this important meeting. The Group supports the objectives of this international symposium and agrees that the specific outcomes and next steps identified during the symposium will serve as an important roadmap for future research and work in the application of animal genomics to support animal health.

12. Update on the organisation of the 8th OIE/WAVLD Seminar on Biotechnology

The Group discussed the program of the upcoming 8th OIE/WAVLD Seminar on Biotechnology entitled "Applications of Biotechnology to the Diagnosis and Pathology of Animal Diseases" to be held in Melbourne, Australia, 13 November 2007.

The *ad hoc* Group supports and encourages participation in this seminar. Invited OIE experts will give scientific presentations at this seminar and could use this opportunity to enlighten the audience about the objectives/initiatives of the *ad hoc* Group.

13. Future work programme/schedule

The Group agreed that the following should be presented to the Biological Standards Commission as the proposed future work of the *ad hoc* Group:

1. Prepare background papers on new technologies (to be published by OIE), which will form the basis for the Commission to select new chapters/guidelines on subjects that will facilitate the development of new biotechnologies for animal health.

2. The following subjects were thought to warrant a background paper:

   - RNA-based technologies for the treatment and control of animal diseases;
   - Transgenic animal technology;
   - Reverse genetics as a new vaccine platform;
   - cDNA clones as a new vaccine delivery system;
   - Chimeric viruses for vaccine development;
   - Vaccination schedule(s).

3. The following subjects were suggested to be of high importance and recommended for development into chapters/guidelines:

   Proposal for incorporation in the *Terrestrial Manual*:

   - Plasmid DNA vaccines (completed, see Appendix IV);
   - Update the categories of biotechnology-derived vaccines in the sections relating to chapter 1.1.7, including the release of biotechnology-derived products;
   - A new chapter on the risk assessment of the release of biotechnology-derived vaccines, including plant-based vaccines;

- DIVA[3] vaccines and associated companion diagnostic tests.

Proposal for incorporation in the *Terrestrial Code*:

- Guidelines for Somatic Cell Nuclear Transfer in Production Livestock and Horses (completed see Appendix III).

4. Additional Issues

- The Group discussed the need for guidelines concerning the assessment of risk associated with biotechnology-derived vaccines in response to the numerous requests from the Codex *ad hoc* Intergovernmental Task Force on Food Derived from Biotechnology.

- The chairman raised the general question concerning the lack of veterinary scientists in research and diagnostic laboratories at present and in the future. The group discussed and agreed that the number of veterinarians in research and diagnostic laboratories is decreasing and that "molecular" scientists may not have the animal health/disease expertise of veterinary scientists. This observation is shared and was confirmed by the Group as applicable to most areas of the world. The Group supports the initiatives already taken by the OIE regarding veterinary education both through a special issue of the OIE *Scientific and Technical Review* on veterinary education and the proposed meeting of representatives of the Deans of Veterinary Schools worldwide.

- A separate chapter/guidelines on plant-based vaccines will only be prepared when this new technology achieves proof-of-concept as a direct vaccine delivery system.

14. Finalisation of the draft meeting report

The next meeting of the *ad hoc* Group is scheduled for 28–30 November 2007.

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…/Appendices

3 DIVA: Differentiating infected from vaccinated animals

Appendix I

MEETING OF THE OIE *AD HOC* GROUP ON BIOTECHNOLOGY

Paris, 12–14 June 2007

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Agenda

1. Introduction
2. Review of the Terms of Reference
3. Report on the outcome of the 75th General Session - *Terrestrial Manual* adopted
4. Report on the meeting of Codex *ad hoc* Intergovernmental Task Force on Food Derived from Biotechnology
5. Cloning in respect to animal health
   - 5.1. Letter to the United States of America
   - 5.2. General discussion
6. Discussion on draft guidelines for Somatic Cell Nuclear Transfer in Production Livestock and Horses
7. Update on guidelines on biotechnology-derived vaccines
   - 7.1. Review of the sections relating to biotechnology-derived vaccines in chapter 1.1.7 of the *Terrestrial Manual* on Principles of Veterinary Vaccine Production
   - 7.2. Development of draft guidelines on DNA vaccines and plant-expressed antigens
8. Follow-up on the recommendations by the *ad hoc* Group at October 2006 meeting on nanotechnology and animal health
9. Identification and tracing of animals and animal products that have resulted from biotechnological intervention – Cooperation with the OIE *ad hoc* Group on Traceability
10. Follow-up to the discussion on OIE scope and definition of biotechnology
11. Update on the organisation of the International Symposium 'Animal Genomics for Animal Health'
12. Update on the organisation of the 8th OIE/WAVLD Seminar on Biotechnology
13. Future work programme/schedule
14. Finalisation of the draft meeting report

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Appendix II

MEETING OF THE OIE *AD HOC* GROUP ON BIOTECTNOLOGY

Paris, 12–14 June 2007

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Appendix III

Draft Guidelines for Somatic Cell Nuclear Transfer in Production Livestock and Horses

PREFACE

Following the first meeting of the OIE ad hoc *Group on Biotechnology held from 3 to 5 April 2006, the Biological Standards Commission suggested restricting the mandate "to develop guidelines on the animal health risks arising from SCNT[1] cloning of production animals, including criteria for assessing the health of embryos and animals derived from such cloning." The following document is a starting point for identifying, characterising and providing a basis for discussion on the animal health risks associated with SCNT cloning technology.*

Overview

At the first meeting of the *ad hoc* Group on Biotechnology, it was recommended that the Subgroup on Reproductive Animal Biotechnologies should draft guidelines on risk analysis, based on the life-cycle approach, for biotechnology-derived animals. The definition of 'Reproductive Animal Biotechnology' was proposed as "the generation of animals through the use of ART[2], which range from artificial insemination through to technologies involving a significant *in-vitro* component, such as *in-vitro* fertilisation, embryo transfer, embryo splitting and including asexual reproduction such as nuclear transfer". The following draft is restricted to SCNT and is based on a risk analysis approach to biotechnology-derived animals categorised according to the life-cycle approach consisting of: i) embryos, ii) recipients, iii) offspring, and iv) progeny of animal clones.

Scope

These guidelines address animal health [~~and welfare related~~] aspects of production animals derived from some reproductive biotechnologies.

Recognising the mandate of the OIE and the suggestion of the Biological Standards Commission, it is the recommendation of the *ad hoc* Group on Biotechnology to identify risk analysis parameters for animal health and their implication for environmental safety and food and feed safety. These guidelines will focus initially on the scientific basis for the risk assessment aspects, prevention measures and guidance for production livestock and horses derived from ART. This is without prejudice to the addition of any relevant issue at a later stage. At present, these guidelines include the following:

- Identification of animal health risks and recommendations for management of those risks in embryos, recipients, animal clones and progeny of clones;
- Risk and prevention measures related with SCNT cloning technology;
- Some welfare issues.

Recognising further that the following issues have been discussed or may be addressed by other bodies or instruments, or that they may be addressed at a later stage by the OIE, the document does not address:

- Safety and nutritional aspects of food derived from ART, for example transgenics (addressed by Codex);
- Risks related to the environmental release of animal clones;
- Risks related to transgenic animals that have not involved SCNT or other cloning technology;

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1 SCNT: somatic cell nuclear transfer
2 ART: assisted reproductive technologies

- Non-reproductive animal biotechnologies;
- Risks related to animals produced for xenotransplantation or organ donors;
- Technologies related to stem cells;
- Risk related to aquatic animal health, including fish clones;
- Risks related to other terrestrial animals, such as wild mammals and non-mammals, including avian species and insects.

Background

Risk analysis– general principles

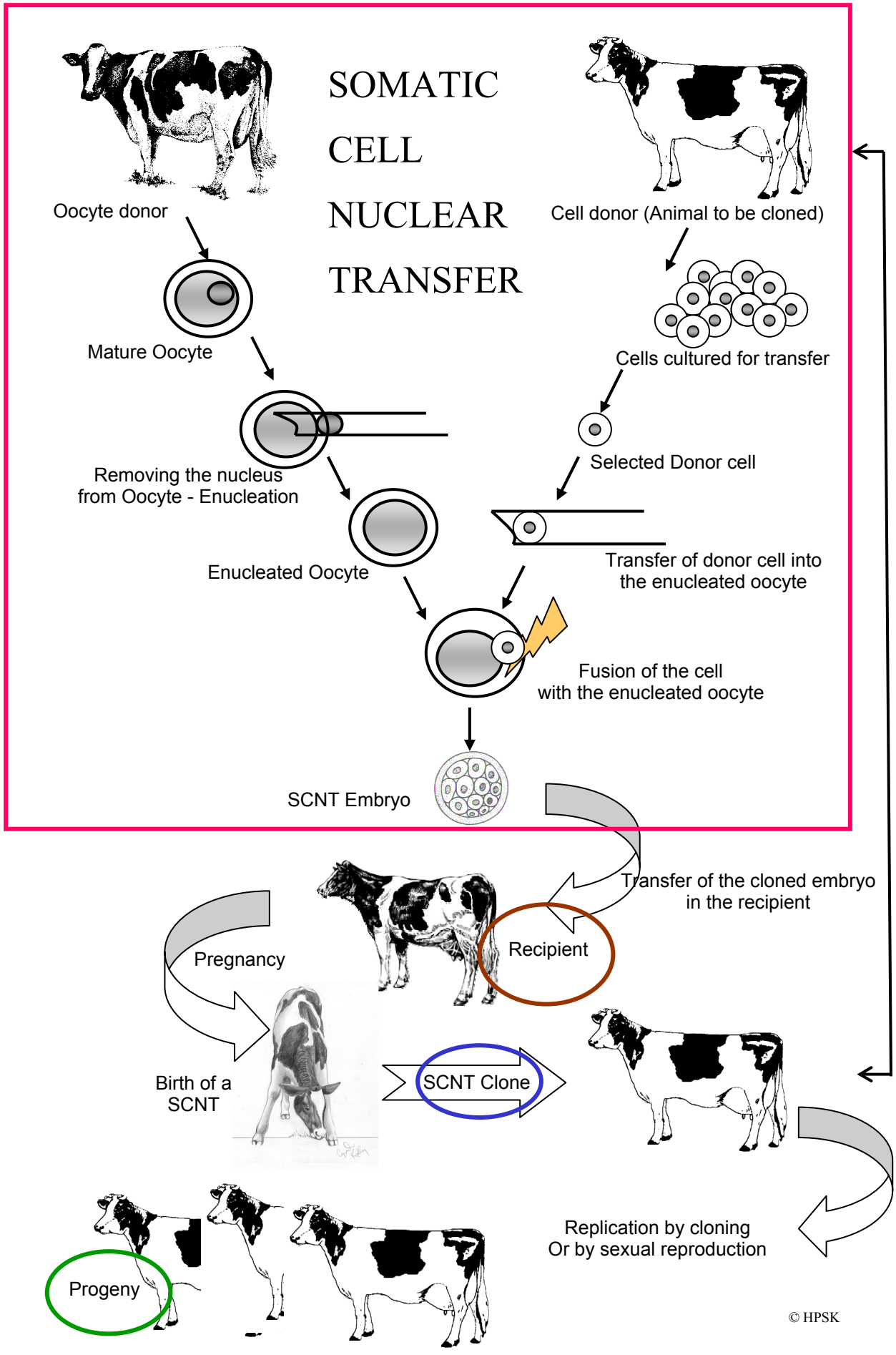
Risk analysis in general includes hazard identification, risk assessment, risk management and risk communication. The risk assessment is the component of the analysis that estimates the risks associated with a hazard (OIE *Terrestrial Animal Health Code* [*Terrestrial Code*], 2006, Chapter 1.3.1). These principles are routinely used by regulators in making decisions about experimental or commercial releases. These analyses can then be used to determine whether the outcomes require management or regulation. Risk management is the process by which risk managers evaluate alternative actions or policies in response to the result(s) of the risk assessment taking into consideration the various social, economic, and legal considerations that form the environment in which such activities occur.

For animal diseases, particularly those listed in the OIE *Terrestrial Code*, there is broad agreement concerning the likely risks and these risks can be qualitative or quantitative (OIE *Terrestrial Code*, Chapter 1.3.1). In disease scenarios it is more likely that a qualitative risk assessment is all that is required. Qualitative assessments do not require mathematical modelling to carry out routine decision-making. Quantitative or semi-quantitative risk assessments assign magnitudes to the risks in numerical (e.g. 1/1,000,000) or verbal (high/medium/low) terms.

In the context of animal cloning, two broad categories of risk assessments are considered: absolute risk assessment and comparative risk assessments. Absolute risk assessments characterise risk independent of a comparator (e.g. the likelihood of an animal transmitting a specific livestock disease). A comparative risk assessment (or relative risk assessment) puts the risk in the context of a comparator. For example the degree to which an animal produced by one reproductive technology can transmit a particular disease to another animal of the same species compared with the degree to which a similar animal produced by another reproductive technology transmits the same disease to another animal of same species.

Regardless of the methodology used, hazard identification is an early step in all science-based risk assessments. In the context of assessing the risks associated with animal cloning (SCNT) and starting with the embryo and moving on through animal clone development and subsequent progeny, it is important to be clear at this juncture that only a comparative semi-quantitative risk assessment can be completed. A systematic, absolute, quantitative risk assessment of potential risks is difficult, due to the relative newness of the technology, and the variability in outcomes among laboratories and species cloned. Furthermore, with the technology of SCNT there is no introduced hazard (which may potentially happen in transgenesis). Thus, to analyse what factors contribute to animal health risks, the existing baseline must be analysed.

In short, the specific points where the risk assessment needs to be focused need to be identified. As illustrated in the accompanying diagram – the focus is to look at the basics of creating an embryo – using current terminology, starting from the selection of donor of oocyte and the cells to the creation of an embryo by the cloning methodology. The second phase will focus on the recipient of the embryo clone and the animal health and care considerations for the animals. The actual embryo clone that is born as an offspring is the third part of the paradigm that needs clear guidelines for assessment, and the next generation, either the progeny of the animal clone (which is a result of normal sexual reproduction) or animals produced by recloning (clones of clones) is the fourth and final stage.


SOMATIC
CELL
NUCLEAR
TRANSFER
Oocyte donor
Cell donor (Animal to be cloned)
Mature Oocyte
Cells cultured for transfer
Removing the nucleus
from Oocyte - Enucleation
Selected Donor cell
Enucleated Oocyte
Transfer of donor cell into
the enucleated oocyte
Fusion of the cell
with the enucleated oocyte
SCNT Embryo
Transfer of the cloned embryo
in the recipient
Pregnancy
Recipient
Birth of a
SCNT
SCNT Clone
Replication by cloning
Or by sexual reproduction
Progeny
© HPSK

Managing Animal Health Risks associated with embryos

Embryo production by *in-vitro* techniques has been applied for many years. Although the additional steps involved in cloning add a new dimension to this procedure, many of the risks associated with SCNT have previously been identified for established ART (OIE *Terrestrial Code*, Appendix 3.3.2). An analysis of SCNT methodology allows the procedural details to be categorised into:

i) Oocytes (obtained from the abattoir, recovered from trans-vaginal ultrasound-guided procedures or by laparotomy procedures).

The primary risks are associated with the health status of the animal from which the ovaries are harvested and the quality of the oocytes.

ii) Donor cells (cells obtained from animals chosen to be cloned – by biopsy, harvesting at slaughter or after death).

Currently there are no specific new risks identified with SCNT cloning. There is a proposed risk related to activation of endogenous retroviruses during cell transfer procedures, however, this may be more theoretical than practical. In some current experimental procedures, the donor cell may be treated with chemicals to modify its composition, for example cell cycle inhibitors or chromatin modifiers.

iii) *In-vitro* culture of reconstructed embryos (procedure used to fuse the donor and recipient material and to culture the reconstructed embryo).

Risks associated with the method of fusing donor cells with enucleated recipient oocytes and with culture conditions.

In addition, the practitioner should ensure that the clone pregnancy is compatible to the surrogate dam's breed, anatomy and physiology.

Oocytes

- The laboratory or the producer should establish a detailed record of ovaries – their origin, health of the animal from which the ovaries are obtained, details of any systemic lesion on the animal and proper herd data. This is particularly useful where the pooling of ovaries may provide cross-contamination of ovarian tissue.
- Follicular fluids may carry various infectious agents like bovine viral diarrhoea virus (BVDV) and can contaminate pooled follicular fluid from healthy animals. Furthermore, the technique for collecting oocytes, such as aspiration or slicing of the ovarian follicles, determines the extent of blood contamination or extraneous material. A representative sample to demonstrate the absence of infectious biological material should be done with each pooled batch.
- Oocytes are matured as cumulus oocyte complexes (COCs) and then matured in most instances in the culture/maturation media. Care and efforts should be taken to carefully select and mature the oocytes from the pools that are morphologically good; also the media used should have been quality tested. Use of serum or protein components from an undefined or untested source should be avoided. Addition of proper and safe antibiotics in the culture media to control opportunistic bacteria should be encouraged.
- Use of proper sanitary and disinfection procedures is of utmost importance and should be emphasised in any *in-vitro* fertilisation (IVF) laboratory. Proper handling and following sanitary protocols during the maturation and further culture of embryos should be encouraged.

Donor cells

In order to minimise risks

- Donor cells should be properly harvested from the animal and cultured under proper sanitary conditions using good laboratory practices.
- When applicable, the passaging of the cells used for the cloning procedure should be documented and at different stage sampling may be warranted to look at the chromosomal component of the cell lines. If possible, procedures should be in place for regular sampling of the cells for morphological and other characteristics.

- Master cell lines (to be used for cloning at a later stage) should be stored under conditions found to be optimal for maintaining viability. Freedom from extraneous agents should be established by testing for bacteria, fungi, mycoplasmas or viruses, using appropriate tests (IETS[3] Manual, 1998).

Cloning procedures/reconstruction

- The cloning procedure that employs the use of chemicals or other reagents should be carefully evaluated, in terms of the quality of embryos and overall efficiency.

- During the fusion of recipient and donor material by chemical or physical means care and control should be employed. The optimisation of the procedure based on the laboratory protocols or published reports should be determined to avoid early embryonic mortalities.
- If co-culture of the cell is used for the culture procedure after reconstruction of embryos, proper screening of the co-culture cells should be done. A sample of each batch may be tested for the bacterial, fungal, mycoplasmal or viral component.
- Embryos should be cultured and harvested for an appropriate time and stage to transfer them or to cryo-preserve them for later use. Proper procedures based on the international standards (IETS Codes of Practice) for washing and preservation of the embryos should be followed.
- Care should be taken with regard to grading the embryos before transfer (OIE *Terrestrial Code*, Appendices 3.3.1 and 3.3.2).

Managing animal health risks related to the recipients (surrogate dams)

1. Animal health risks to the surrogate dams

Currently, when compared with *in-vitro* produced embryos, SCNT has a higher rate of pregnancy failure and, in some species, placental abnormalities. Loss due to defects in the embryo or failure to implant in the uterus of the surrogate dam does not pose a hazard to the dam. Rather, the surrogate dam simply resorbs any embryonic tissue and returns to cycling. Mid- and late-term spontaneous abortions may be hazardous to surrogates if they are unable to expel the fetus and its associated membranes. Most abortions in natural service and artificial insemination (AI) pregnancies in cattle remain undiagnosed due to the expense of laboratory work and the low profit margin in both the beef and dairy industry. Producers and veterinarians become concerned when the rate of abortion exceeds 3–5% in a herd. The same potential impact of external influences should be considered with pregnancy evaluation with SCNT and other reproductive technologies. Disease, under-nutrition, and severe environmental conditions are stressors known to interfere with animal fertility and embryo survival. Under these circumstances, the risk to the pregnancy is directly related to stress factors and not to the technology used.

To date, a species-specific effect has been seen. Abnormalities in clones may result from incomplete reprogramming of the donor nucleus. Epigenetic reprogramming occurs at different times in embryos in different species. Many of the abnormalities reported in cattle and sheep pregnancies have not been noted in goats or swine carrying SCNT clones. The amount of *in-vitro* manipulation of an embryo inversely correlates to the chances for successful pregnancy outcomes. This has been observed in both SCNT embryos and *in-vitro* produced fertilised embryos. Unlike other forms of other reproductive technologies SCNT pregnancy losses occur at all stages of gestation in cattle. Clone pregnancies have been lost during the second and third trimesters and have been accompanied by reports of hydrops, enlarged umbilicus, and abnormal placentation.

2. Animal health risks posed by the surrogate dam to the clone embryos

No new animal health risks have been identified for the developing clone fetus from the surrogate dam compared with conventional pregnancies. The latter include vertically transmitted diseases and abnormalities due to metabolic or physiological stress.

[3] IETS: International Embryo Transfer Society

With respect to the animal health risks associated with the surrogate dam, it is difficult to document the relative frequency of early stage losses of SCNT embryos compared with early stage losses of other pregnancies as these abortions are not typically diagnosed with other reproductive technologies. Additionally, external stressors will similarly impact SCNT pregnancies.

Veterinarians should monitor the progress of pregnancy as the common gestational anomalies seen in other assisted reproductive technologies may be exhibited and diagnosed during the physical examination. A database of commonly encountered problems in clone pregnancies would be useful if available to animal health experts.

- Care should be taken to assess the general health of the recipient dam before selection to carry the embryo clones. The general health status of the recipient should be determined in terms of freedom from infection and disease, proper vaccination and follow up, and, if applicable, proof of earlier uneventful pregnancies, absence of birthing problems, and proper post-pregnancy recovery.
- Pregnancy loss is greatest with SCNT embryos prior to 60 days' gestation in cattle. This is similar to the pattern seen with other reproductive technologies. However, in clones, high pregnancy losses during this time of placental formation (between 45–60 days) suggest that embryonic death may be a consequence of faulty placentation. Abnormal placentation may lead to a build up of wastes in the fetus and associated membranes, or inadequate transfer of nutrients and oxygen from the dam to the fetus. Care should be taken to monitor the recipient dam during pregnancy. Once the pregnancy is established and confirmed, regular veterinary assessments and monitoring of animal health status is desirable up to the birth of the offspring.
- To ensure that the recipient is pregnant and to monitor its health during the first trimester, it is useful to perform ultrasonographic assessments, determine hormonal profiles and assess the general physiological parameters. Based on these profiles, proper attention should be paid to aid in the proper establishment of pregnancy by providing proper husbandry conditions and nutrition.
- The animals should be observed carefully for the signs of labour nearing the time of birth. In some species, one of the more common problems is uterine inertia and the absence of contractions. The absence of contractions may result in prolonged pregnancies with associated sequellae that may require assistance with deliveries.
- A surgical intervention should be decided and should be available for the near term animal if the situation so warrants. Proper procedures should be employed to ascertain the proper handling of the offspring and the surrogate dam.
- Health concerns may arise as a result of surgical procedures, excessive traction, or other complications such as retained fetal membranes. In these cases *post-partum* care may be necessary.

Managing animal health risks of animal clones

The health problems of individual clones can be observed *in utero* and *post-partum*. These appear to be the same as observed in other ART, but they may be more common in clones. It is important to determine whether the abnormalities are of genetic or epigenetic origin. LOS[4] and placental abnormalities are particularly observed in sheep and cattle.

- Appropriate husbandry practices are important to the health of animal clones. Care should be taken to provide colostrums and a clean and hygienic environment, supervision for the first few weeks after birth should be practiced.
- The animal clones must be checked routinely for the most common phenotypic anomalies, such as atresia anii, umblical hernia, flexor muscle contractions, respiratory or cardiac insufficiency, and failure to suckle. This will allow proper treatment and care of the newborn and increase the survival of the young one.

[4] LOS: large offspring syndrome

- To consolidate current understanding of the health status of animal clones, a comprehensive veterinary examination should be performed to monitor the progress of the clone, as unexplained fatalities or fatalities arising from systemic complications have been reported. It is encouraged to follow the health profile of the animals to at least the reproductive maturity stage, and to record the ability to reproduce (fertility index).
- Animal welfare concerns ranging from LOS to serious abnormalities are notable in the debates pertaining to cloning technology. Proper research and peer-reviewed data should be generated. The animal clones should undergo species-specific basic welfare assessments. If welfare concerns are detected at initial screening, a more extensive characterisation of that phenotype should be performed to document the animal welfare concerns.
- Proper monitoring of the animal population during different stages of life from birth to puberty should be documented to address and validate the genomic potential of the animal clones.

Managing animal health risks related to sexually reproduced progeny of clones

Presently there is no evidence of an increased health risk if sexual reproduction is used for obtaining progeny. Some data indicate that the reprogramming errors during the cloning process may actually be corrected during the natural mating and reproduction process.

- Characterisation of the health profile, including health status and data on animal welfare, would consolidate the knowledge of sexually reproduced progeny.
- Monitoring the reproductive performance of sexually reproduced progeny of clones would be useful to assess their reproductive capacity in comparison with their conventional counterparts.

Managing animal health risks associated with re-cloning/clones of clones

[~~There is a lack of information on recloning.~~] Information on recloning is only beginning to appear. It is therefore necessary to follow the approach below:

- The health profile (health status and data on animal welfare) should be characterised to consolidate the knowledge.
- The reproductive performance of clones of clones should be monitored to assess the capacity of the animals to perform in comparison with their conventional counterparts.

Breeding regimes should consider genetic diversity effects in relation to the desired use of SCNT technology.

Review of guidelines

The goal of these guidelines is to provide a scientific basis and recommendations on animal health and welfare risks to animals involved in SCNT cloning compared with other ART. These guidelines will focus initially on the scientific basis for the risk assessment aspects, prevention measures and guidance for production livestock and horses derived from ART and should be reviewed in light of new scientific information.

Glossary:

Hazard: (as defined in OIE)

Hazard means a biological, chemical or physical agent, or a condition of, an animal or animal product with the potential to cause an adverse health effect.

A hazard is an element or event that poses potential harm; an adverse event or adverse outcome. A hazard is identified by describing what might go wrong and how that might happen [~~(2)~~]. Covello and Merkhofer [~~(11)~~] defined a hazard as a (potential) source of risk that does not necessarily produce risk. A hazard produces risk only if an exposure pathway exists and if exposures create that possibility of adverse consequences. Hazard identification is the process of identifying new agents in sources of risk. Risk sources may release risk agents into the environment.

Risk:

Risk means the likelihood of the occurrence and likely the magnitude of consequences of an adverse event to animal or human health during a specified time period, as a result of hazard.

The likelihood of the occurrence and the magnitude of the consequences of an adverse event; a measure of the probability of harm and the severity of impact of a hazard. Objective measurement and scientific repeatability are hallmarks of risk. In risk studies it is common, especially in oral communication, to use "risk" synonymously with the likelihood (probability or frequency) of occurrence of a hazardous event. In such instances, the magnitude of the event is assumed to be significant [~~(2, 4~~]

Risk analysis:

Risk analysis means the process composed of hazard identification, risk assessment, risk management and risk communication.

The process of risk analysis includes risk assessment, risk management and risk communication [~~(11, 4)~~].

Risk Assessment:

Risk assessment means the evaluation of the likelihood and biological and economic consequences of entry, establishment, or spread of a pathogenic agent.

The process of identifying a hazard and evaluating the risk of a specific hazard, either in absolute or relative terms. The risk assessment process involves four interrelated assessment steps: release assessment, exposure assessment, consequence assessment and risk estimation. It includes estimates of uncertainty in process, and is an objective, repeatable, scientific process. Quantitative risk assessment characterises the risk in numerical representations [~~(2, 4)~~]. Qualitative risk assessment characterises the outputs on the likelihood of the outcome or the magnitude of the consequences in qualitative terms such as “high”, “medium”, “low” or “negligible” [~~(17)~~].

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

Draft Guidelines for Veterinary Plasmid DNA Vaccines

Preface

The following document is the compilation of the scientific expertise and regulatory experience that follow the discovery that naked DNA could induce a protective immune response. Gary Rhodes of Vical Inc., San Diego, first reported in the early 1990s that mice inoculated with a naked plasmid DNA construct containing the influenza virus haemagglutinin gene resulted in an antigen-specific humoral immune response (x, x). Although this novel discovery showed great promise in the laboratory and a potential new measure to prevent some of the most challenging emerging diseases of the 20th century (AIDS[1], influenza, malaria), not a single human vaccine has made it through the pipeline to date. In anticipation of significant breakthroughs in this new technology, the medical community drafted the first regulatory guidelines in 1996 (x). However, these guidelines were written prior to the availability of significant scientific information on mechanisms of action and safety. With the progress made by research, other guidelines (x, x, x) have since been drafted to facilitate the development of this promising technology. Nevertheless, these guidelines were written prior to any significant advancement in establishing efficacy in relevant animal species other than mice or the implementation of full vaccine development plans by industry. This situation has recently changed with the discovery and recent development of DNA vaccines with veterinary applications, the first against West Nile virus for use in horses that was licensed in the USA on 13 July 2005.

This document provides the starting point for discussing the development of the World Organisation for Animal Health (OIE) guidelines for DNA vaccines that benefit from the great work completed to date by various expert groups but also capitalises on the animal health experience and the recent scientific and regulatory findings that have facilitated the successful testing and marketing of the first DNA vaccines. These guidelines follow the mandate and expected output defined by Dr Bernard Vallat on 3 April 2006, for the OIE *ad hoc* Group on Biotechnology, which includes guidelines to facilitate the development of new technologies that are likely to benefit the animal health community. The *ad hoc* Group on Biotechnology's Subgroup 2 on Vaccines respectfully submits the following draft guidelines for consideration by the OIE Biological Standards Commission.

I. Introduction

The use of plasmid DNA as a platform for veterinary vaccine delivery has progressed significantly in the past few years with two products licensed in USA (13 July 2005, for Fort Dodge Animal Health's West Nile Virus Vaccine and 22 March 2007 for Merial's [Conditional] Canine Melanoma Vaccine) and several clinical trials completed or in progress (x, x, x). DNA vaccination involves the inoculation of a gene(s) encoding a relevant immunogen against which an immune response is desired, under the control of a promoter that will permit its expression in the vaccinated animal. This gene construct is contained, for the manipulation and for manufacturing purposes, within a bacterial plasmid DNA molecule. This type of vaccine has potentially important advantages over the more traditional approaches, including the stimulation of both B and T cell responses, improved stability of the vaccine, absence of any infectious agents, the ability to express an immunogen that cannot be produced by conventional methods (e.g. cell culture), the absence of an immune response to the backbone sometimes observed with recombinant-vectored vaccines, and the relative ease of large-scale manufacture. It can also have safety advantages over live attenuated vaccine strains by eliminating concerns with reversion to virulence or inadequate inactivation of killed vaccine preparations. In addition, the analytic dossier of a plasmid DNA vaccine may be simpler to develop as the components responsible for immunogenicity are known, manufacturing practices do not need to be altered when the transgene is replaced with an alternate immunogen, and DNA itself is expected to be very stable in a final formulation when compared with whole microorganisms or subunit preparations of microorganisms.

[1] AIDS: Auto-immune deficiency syndrome

II. Scope of the Document

This document is intended to provide guidance to manufacturers seeking to develop a DNA vaccine for use in animals when the vaccine consists of a bacterial DNA plasmid. It is applicable to vaccines consisting of plasmid DNA, non-amplifiable in eukaryotic cells. New developments involving plasmid DNA delivered by live vectors or capable of amplification in the vaccinated animal by any mechanisms are not within the scope of this document.

As final formulated DNA vaccines may be composed of a mixture of plasmids encoding for different immunogens isolated from a single pathogen (virus, bacteria or parasites) or from different pathogens (mono- and multivalent DNA vaccines), Final Formulated Vaccine, as defined in this document, should be understood as a single or range of plasmids destined for use in a given species to induce an immune response. Some vaccines may carry gene(s) encoding 'non-antigenic' molecules with biological activities such as cytokines, in order to enhance efficacy.

DNA vaccines are not only being developed for prophylaxis but also for therapeutic use, either against infectious diseases or other purposes such as metabolic disorders and cancer. It is clear that the manufacture and quality control of plasmid DNA for any of the above indications will be essentially identical and as a result, these guidelines are applicable to DNA vaccines for therapeutic as well as prophylactic use.

Many aspects of these guidelines may be applicable to vaccines based on RNA, although different requirements are likely to apply, especially for safety testing for these types of vaccine. Plasmid DNA vaccines for use in gene therapy, DNA vaccines derived in eukaryotic cells, vaccines in which a bacterial cell acts as a carrier for a plasmid DNA encoding a relevant immunogen and nucleic acid vaccines made entirely by chemical means such as synthetic oligonucleotides, are all outside the scope of this document.

This document should be read in conjunction with other related OIE *Terrestrial Manual* chapters or other specialised guidelines, as all appropriate standard requirements for veterinary vaccines are also applicable to the products within the scope of this document. The production of other biological products can provide the necessary experience on which a plasmid DNA biological product should be controlled. Therefore, these guidelines will focus on particular aspects relevant to this novel form of vaccination, developmental testing and control of DNA vaccines bearing in mind that each vaccine should be considered on a case-by-case basis. It is intended as guidance to help manufacturers define studies and tests to carry out the development of veterinary DNA vaccines. Regulatory authorities may wish to use this document to develop their own guidelines for DNA vaccines.

III. Special Issues

One of the central issues for DNA vaccines is the source of the DNA incorporated into the vector, including eukaryotic promoters and enhancers; termination/polyadenylation addition sites; antibiotic resistance markers; and other selection markers. In order to minimise the risk of chromosomal integration, homology of plasmid DNA sequences to known sequences in the genome of target animal species should be examined and assessed. In this context, the current knowledge of recombination is limited and the decision to exclude sequences should be assessed experimentally. Viral promoters and mammalian and viral termination and polyadenylation signals are frequently used, however, the results of expression and safety studies should dictate the choice of regulatory control sequences used in the plasmid DNA vaccine.

The following four issues should be addressed prior to conducting field safety studies:

1. The plasmid DNA that is internalised by the cells of the vaccinated animal may integrate into its chromosome(s) and disrupt normal cellular homeostasis, possibly leading to diseases such as cancer. After injection of DNA into an animal, a small proportion of the DNA molecules enter cells, while the remainder are confined to the interstitial spaces and destroyed. Tissue distribution studies indicate that the plasmid sequence may be detected by PCR[2] amplification of DNA purified from internal organs and peripheral tissues but in nearly all cases the signal diminishes to undetectable levels within a few weeks at all sites except the site of inoculation. The probability of any internalised plasmid DNA molecule integrating into the chromosome is also low, and given that

[2] PCR: Polymerase chain reaction

oncogenesis and other pathological processes are multi-factorial events, the risk of insertional mutagenesis is exceedingly low. To date, the integration of plasmid DNA into chromosomal DNA of a vaccinated animal has not been observed. However, the probability of integration events occurring may differ according to the DNA sequence of the plasmid DNA vaccine, animal species, tissue type, the route of administration, the amount of plasmid administered and the age of the vaccinated animal.

2. Plasmid DNA vaccines may result in undesirable immune reactions:

   The mechanism of the immune response to an immunogen, which is expressed due to injected DNA, is poorly understood. This raises concerns about the possible adverse effects on the immune system, including auto-immune reactions.

   Although DNA can have a very low immunogenic potential, certain bacterial DNA sequences are known to have a mitogenic or immunostimulatory effect (x). This property may be advantageous in some DNA vaccines and is under active investigation. To date, human trials with DNA vaccines have not demonstrated the induction of anti-double -stranded DNA immune responses.

3. The additional use of genes encoding cytokines or co-stimulatory molecules may pose additional risks:

   There is considerable interest in the co-administration of a gene encoding a cytokine in order to direct a specific type of immune response. However, this could have detrimental effects, especially if the cytokine has been introduced on an expression plasmid the expression of which cannot be terminated. Furthermore, it will be important to avoid the induction of an immune response towards an encoded cytokine, which could have untoward and undesirable consequences for a vaccine recipient.

4. The expressed immunogen may itself have undesirable biological activity:

   An encoded immunogen may exhibit undesirable biological activity and if this is the case appropriate steps may have to be taken, such as deletion mutagenesis, to eliminate the undesirable biological activity while retaining the capacity to induce the desired immune response. The fact that the expressed gene product has not had deleterious effects when administered as a recombinant protein does not necessarily eliminate the risk of toxicity associated with the expressed transgene, because plasmid DNA vaccine expression is intracellular.

IV. Points to be Addressed for a Plasmid DNA Vaccine

As indicated above, the standard data requirements for veterinary vaccines also have to be addressed when developing a plasmid DNA vaccine. The information must be presented in accordance with the format set out in the country in which the plasmid DNA vaccine is to be marketed. The following are given as examples of the level of detail and points to address in a registration dossier.

A. Analytical Section:

A detailed description of the development of the vaccine plasmid should be provided. This should include details of the gene encoding the protein against which an immune response is sought, information on the construction of the entire plasmid and the host bacterial cell. The origin of the gene of interest should be described in detail, such as the name of the microorganism or cell from which the gene was derived, the origin of the source, its species, passage history, the subtype and isolation strategy used. The rationale for the use of the gene(s) should be discussed and the sequence of the wild-type gene and the immunogenic properties of the encoded protein in its natural state should be provided.

The steps in the construction of the entire vaccine plasmid should be described, including the source of the plasmid(s) used and subclones generated during the cloning procedure. Functional components such as regulatory sequences (origins of replication, viral/eukaryotic promoters, introns, termination sequences) and selection markers should be clearly indicated and the information on the source and function of these elements should be provided. Sequence data on the entire plasmid, including an informative map of restriction enzyme sites, should be provided and the use of all specific elements or regions of DNA should be justified. DNA sequence homology checks of the plasmid DNA vaccine with all

published DNA sequence data of the target animal species should be performed and documented in the application dossier. Special attention should be given to the nature of a selection marker. The use of certain selection markers such as resistance to therapeutics as well as certain sequences, such as retroviral-like long terminal repeats (LTRs) and oncogenes, should be avoided. The rationale for the choice of the host bacterial cell used for the production of the plasmid should be provided along with a description of its source, phenotype and genotype. It should be demonstrated that the host cell is free from bacteriophage viruses and other adventitious agent contamination.

The identity of the vaccine plasmid after transfection into the bacterial cell to be used for production and the phenotype of the transfected cell should be confirmed. As rearrangements of the plasmid are unacceptable, data on the stability of the plasmid within the bacterial cell should be provided. The expression of the prokaryotic genes, such as a selection marker, in a eukaryotic cell line should be investigated.

a) Master Cell Seed

The production of plasmid DNA vaccines should be based on a well defined MCS[3] and WCS[4] system. The cloning and culturing procedures used for the establishment of the MCS should be described. The origin, form, storage, use, and expected duration at the anticipated rate of use should be described in full for all cell seeds. The MCS should be fully characterised and specific phenotypic features that form a basis for identification should be described. The sequence of the entire plasmid DNA vaccine should be established at the stage of the MCS. WCSs should be adequately characterised and meet established acceptance criteria. The viability of the host–plasmid system in the MCS and WCS under storage and recovery conditions should be determined. It should be demonstrated that the MCS and WCS are free from extraneous microbial agents.

b) Manufacture, validation, and in-process testing

Plasmid DNA vaccines should be considered similar to bacterial and viral vaccines produced by traditional methods, where adequate control of the starting materials and manufacturing process is just as important as that of the product. Considerable emphasis should be given to 'in-process' controls for assuring the safety and effectiveness of the vaccine as well as the comprehensive characterisation of the vaccine itself. Appropriate attention should be given to the quality of all reagents used in production, including the components of fermentation media. Many of the general requirements for the quality control of biological products, such as tests for potency, endotoxins, stability and sterility, also apply to DNA vaccines.

Changes made to the product composition (adjuvant, preservatives) or manufacture (process, site or scale) during the development of clinical and post-approval manufacturing lots may have a significant impact on quality, safety and/or efficacy. Any change in the production of a plasmid DNA vaccine places responsibility on the manufacturer to show that the product is equivalent to that used in preclinical studies or earlier stage clinical trials. Such changes should be evaluated on a case-by case basis to determine what supporting data should be provided to show comparability of the modified version to the previous one.

Procedures and materials used during fermentation and harvesting should be described in detail. Data on consistency of fermentation and harvesting conditions, culture growth and plasmid yield should be presented. Relevant in-process controls should be identified and rejection criteria during fermentation and harvesting should be established.

The minimum and maximum level of cell growth and scale to be permitted during production should be specified, based on information on the stability of the host-cell/plasmid system up to and beyond the level of fermentation used in production by the time of application for marketing authorisation. Host–plasmid characteristics at the end of fermentation should be investigated. This should include, as a minimum, plasmid copy number and restriction enzyme mapping, and the yield of both host cells and plasmid DNA vaccine.

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3 MSC: Master cell seed

4 WSC: Working cell seed

Any methods used to extract the plasmid DNA vaccine and remove and/or reduce the concentration of unwanted materials should be described in detail and the process explained and validated.

Clearance capacity for the removal of contaminants should be established for the purification process by the difference in contaminating levels before and after each purification step. Batch acceptance should be established on the basis of compliance with the upper acceptance limits defined for each contaminant. Special attention should be given to the removal of bacterial genomic DNA and endotoxins.

Validated in-process controls for any potential contaminants of concern should be developed and routine batch test upper acceptance limits established, based on data from tests showing the safety of that concentration.

c) Routine control of bulk vaccine and finished product

Identity

Each batch should be subjected to an appropriate selection of the tests used to characterise the purified plasmid DNA vaccine in order to confirm its identity. The specific tests that adequately characterise any particular plasmid DNA vaccine on a lot-to-lot basis, however, may depend on both the nature of the plasmid DNA vaccine and its method of production and purification. Typically, the full sequence of the plasmid DNA vaccine should be determined and a restriction analysis should be the primary approach to confirm identity; however, *in vitro* or *in vivo* expression of the plasmid DNA vaccine accompanied by confirmation of the identity of the expressed immunogen should also be considered.

Other tests may be required depending on the method of purification and production.

Biological activity

Plasmid DNA vaccines may also contain genes in the same or different plasmids that encode molecules other than the selected immunogen, such as cytokines.

For plasmid DNA vaccines that include such biologically active molecules, these molecules should also be expressed *in vitro* and expression assessed with an appropriate bioassay. The biological activity of each batch should be determined using a suitable well characterised assay together with an appropriate in-house reference preparation. The biologically active molecule should be expressed *in vitro* by transfection of a suitable cell line and the expressed protein characterised; for example, by immunofluorescence or by Western blot. The *in vitro* assay should be shown to correlate with the biological activity or efficacy in the target animal species.

DNA content

Quantification of the plasmid DNA vaccine is usually determined using absorbance at 260 nm. A quantitative test for total DNA content per ml or per dose should be carried out on each batch of finished product.

Tests for contaminants

The purity of each batch of vaccine should be assessed and the level of contaminants should be within specified limits to be set for any identified contaminant of bacterial cell origin. Each batch of product must also be tested for endotoxins, unless it is demonstrated that the bacterial host is endotoxin negative and that there is no endotoxins identified in three successive batches, or shown to be within the limit established as safe for the product. The degree of contamination with chromosomal DNA, RNA and proteins should be assessed and limits established, and criteria for rejection should be established and specified. Additional tests should be considered depending on the production process used, and the results obtained from purification, validation, and safety studies in the target host.

Integrity

The structural integrity of the plasmid DNA vaccine product with which efficacy is demonstrated should be determined; for example, the plasmid DNA vaccine should be evaluated using the ratio of denatured to supercoiled DNA in gel electrophoresis or an equivalent test and the per cent circular DNA should be determined, as nicking greatly diminishes the likelihood of cellular uptake and thus immunogenicity. The acceptance limit should be determined, specified, and consistent with the preparation used to determine efficacy.

Stability

Genotypic and phenotypic stability is evaluated through sequence and expression analysis of the MCS and the highest passage used in production.

Batch potency test

An appropriate assay for the potency of the plasmid DNA vaccine should be required. The most appropriate approach will vary depending on the composition of the vaccine, the nature of the disease, the expressed immunogen(s) and the immune response being sought. Assays that measure relevant biomarkers and correlate to efficacy should be encouraged but the design of the potency test must be assessed on a case-by-case basis. Whatever the assay, an approved in-house reference preparation should be required and should be established from an appropriately characterised batch of vaccine. Quantification of expression of the correct immunogen *in vitro* should be performed using qualitative and quantitative test methods such as ELISA[5].

Fully validated *in-vitro* expression assays should be considered sufficient for establishing batch potency and should be preferred to avoid the use of animals in batch testing, provided that correlation has been established for the reference preparation between immunogen expression *in vitro* and potency in the target animal host species.

Batch safety tests

The routine batch safety tests should be conducted with a minimum of 10 doses of product administered to a suitable laboratory animal species.

B. Safety Testing

Safety testing should be carried out in accordance with OIE standards for vaccines. Batches with maximal DNA content and potency should be used in these studies. The following headings identify specific points that should be addressed.

a) Overdose studies

The overdose studies should be conducted using a minimum of 10 times the recommended dose of the finished product in laboratory animals and the target host animal species for which the product is recommended.

b) Biodistribution studies

Tissue distribution data in the target animal species should be derived for plasmid DNA vaccines. Distribution data obtained with one type of plasmid should be applicable to all other plasmids sharing the same backbone and differing only by the cloned gene encoding the immunogen provided that the Foreign Genetic Inserts are approximately the same size.

The amount of plasmid DNA vaccine administered and the route of DNA inoculation may influence the distribution of the DNA in the target animal species. Localisation studies should therefore include a batch of production at maximum potency and be designed to determine the distribution of the DNA after administration via the route indicated on the product label. Using the most sensitive

[5] ELISA: Enzyme-linked immnuosorbent assay

methods available, the extent of DNA distribution and cellular uptake by the target and surrounding tissues, including the draining lymph nodes, should be analysed at various time points (e.g. Day 1, Day 7, and 1 month after vaccination). The timing of sampling should take into account information on the duration of gene expression and the persistence of the DNA in the body of the vaccinated animal.

c) Residue clearance

The persistence of the plasmid DNA vaccine should be within acceptable limits for the product or $\leq$ 30 copies of plasmid per 10^5 host cells persisting at the site of injection after 60 days.

d) Recombination

Plasmid DNA vaccine integration in the chromosome(s) of the target animal species should be avoided.

Products encoding 'non-attenuated' virulence factors should be assessed for recombination with wild-type and vaccine strains of the target pathogen(s).

e) Integration and tumourigenesis

These studies should be conducted using the Final Formulated Vaccine, and in the case of a multivalent vaccine, not with each individual plasmid DNA vaccine when more than one DNA fraction is combined in the final product.

A step-by-step analysis should be carried out. The first step should be to test for persistence of plasmid DNA in the target tissue and draining lymph node(s). If plasmid DNA is detected, the most sensitive methods available should be used to investigate their integration. For DNA plasmids with backbones similar to those previously tested, integration studies are not generally required if tissue distribution studies show that plasmid levels in peripheral tissues are similar. If integration is detected or suspected, tests for tumourigenesis in a susceptible laboratory animal system should be carried out. The incidence of tumours in the target animal species, particularly at the site of injection and the target tissue, should be monitored and recorded at the end of each experiment in the safety and efficacy studies.

Consideration should be given to include tumourigencity assessments in the duration of immunity studies (see efficacy studies below). Any increase in insertional mutations or tumour development should be noted. Tumours should be examined for plasmid DNA.

As plasmid DNA vaccine integration could potentially perturb any gene activity, post-vaccination monitoring for any clinical signs of disease in the target animal species should be conducted and recorded as part of vaccinovigilance.

f) Reproductive toxicity

Standard studies on impact on reproductive performance should be conducted for plasmid DNA vaccines as with other types of vaccines.

The possibility of migration of DNA to gonadal tissues and potential DNA transfer into germ-line cells of vaccinated male and female animals should be evaluated. The distribution studies discussed above should be extended to provide the necessary data.

g) Examination of immunological functions

Specific studies should be conducted to address the possibility of adverse effects on the immune system, especially if cytokine genes are used as molecular adjuvants.

h) Field safety studies

Field safety studies should be conducted in the target animal species. The number of adverse events observed should be recorded by personnel not aware of treatment group assignment and group membership. Attempt to identify plasmid DNA in lesions associated with a disease event as a consequence of vaccination should be considered. All tumours should be examined for plasmid DNA.

C. Efficacy Studies

Efficacy studies should be carried out in accordance with OIE guidelines for veterinary vaccines.

The standard requirements for efficacy testing of veterinary vaccines are applicable to plasmid DNA vaccines. Tests should be conducted on batches with minimum DNA content and potency. Dose–response studies should be conducted to establish a minimum protective dose. Potency tests should be correlated to host animal efficacy at the time of the efficacy trials. Studies should be conducted to determine the correlation between immunological parameters or biomarkers and efficacy in the target animal species. Information on the duration of protection should be provided. The data generated should be used to determine the vaccination schedule recommended on the label.

D. Risk Assessment

The determination of the need for an environmental risk assessment for a plasmid DNA vaccine should be assessed on a case-by-case basis and may not be required taking into account that plasmid DNA vaccines are non-replicating in eukaryotic cells. However, it may be required to assess the potential of recombination that may lead to a deleterious event on the host or changes in the genotype/phenotype of the target pathogen. The procedures for the assessment should follow the guidance provided in the sections relating to biotechnology derived vaccines of chapter 1.1.7 of the *Terrestrial Manual*.

V. Definitions

The definitions given below apply to the terms used in these guidelines only. They may have different meanings in other contexts.

Plasmid DNA Vaccine

A plasmid is a circular, extra chromosomal bacterial DNA element that undergoes autonomous replication in bacterial cells. It usually carries a few genes, some of which confer resistance to various antibiotics; such resistance is often used to discriminate between organisms that may contain the plasmid and those that do not. Plasmid DNA can be found in various physical states, including supercoiled, nicked, relaxed, or linear.

For the purposes of this document, plasmid DNA vaccines are defined as purified preparations of plasmid DNA designed to contain a gene or genes for the intended vaccine immunogen as well as genes incorporated into the construct to allow for production in a suitable host system. For the purpose of this document, the vaccine consists of plasmid DNA that is non-amplifiable in eukaryotic cells.

Foreign Genetic Insert

The inserted genes that may include genes encoding for protective immunogens and molecular adjuvants that will induce a protective immune response in the target animal host.

Bacterial Host Cell

The Bacterial Host Cell is the bacterium to be used for plasmid amplification. It does not contain the plasmid.

Master Cell Seed (MCS)

A homogeneous suspension of bacterial cells, already transformed by the plasmid DNA vaccine, dispensed in aliquots into individual containers for storage.

All containers are treated identically during storage and, once removed from it and used, they can no longer be considered part of the MCS.

Working Cell Seed (WCS)

A homogeneous suspension of bacterial cells derived from a single vial of the master cell seed dispensed in aliquots into individual containers for storage. All containers are treated identically and, once removed from storage, are not returned to the WCS.

Typically, a single or a defined number of aliquots is used to manufacture a batch of vaccine. In some cases, a WCS may not be established and vaccine manufacturers may begin from an aliquot of the MCS.

Bulk Purified Plasmid

The Bulk Purified Plasmid is purified plasmid DNA vaccine before final formulation. It is obtained from one or more bulk harvests, and is kept in one or more containers designated as a single homogeneous production lot and used in the preparation of the final dosage form.

Final Formulated Vaccine

The finished formulated vaccine product may contain a single or range of plasmids formulated in final form. The DNA vaccine may be freeze-dried and/or contain excipients and/or adjuvants.

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