# ANNEX 3

## EU COMMENTS

## ON THE PROPOSED CHANGES TO THE

## OIE MANUAL OF DIAGNOSTIC TESTS AND VACCINES FOR TERRESTRIAL ANIMALS

## PRESENTED FOR COMMENTS IN OCTOBER 2011

**EU COMMENTS**
**On the proposed changes to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals**

**SUMMARY:**

**Chapters**

**EU COMMENTS**
**On the proposed changes to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals**

## CHAPTER 1.1.4/5.: Principles and methods of validation of diagnostic assays for infectious diseases

General comments

**The EU can support the proposed changes.**

Specific comments

**None**

**EU COMMENTS**
**On the proposed changes to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals**

**CHAPTER Appendix 1.1.4.1.: Development and optimisation of antibody detection assays**

General comments

**The EU can support the proposed changes and has one specific comment.**

Specific comments

**LINE 6:** The word "constitute" should be replaced by "constitutes" (typographical error).

**EU COMMENTS**
**On the proposed changes to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals**

**CHAPTER Appendix 1.1.4.2.: Development and optimisation of antigen detection assays**

General comments

**The EU can in general support the proposed changes but has one specific comment.**

Specific comments

**LINE 324 - 343:** The section on reference animal populations describes that negative refers to non-exposed animals or animals from non endemic regions. However, if using such a population care must be taken to ensure that it is still representative and matched to the positive ref population. Therefore, the following should be added after **LINE 343**:

"The negative reference population should be chosen with care so as to ensure that it is representative and matches the positive reference population (e.g. as to the breed and exposure to environmental challenge."

**EU COMMENTS**
**On the proposed changes to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals**

**CHAPTER Appendix 1.1.4.3.: Development and optimisation of nucleic acid detection assays**

General comments

**The EU can support the proposed changes.**

Specific comments

**None**

**EU COMMENTS**
**On the proposed changes to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals**

**CHAPTER Appendix 1.1.4.4.: Measurement uncertainty**

General comments

**The EU can support the proposed changes.**

Specific comments

**None**

**EU COMMENTS**
**On the proposed changes to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals**

**CHAPTER Appendix 1.1.4.5.: Statistical approaches to validation**

General comments

**The EU can support the proposed changes.**

Specific comments

**None**

**EU COMMENTS**
**On the proposed changes to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals**

**CHAPTER Appendix 1.1.4.6.: Comparability of assays after minor changes in a validated test method**

General comments

**The EU can in general support the proposed changes but has some specific comments.**

Specific comments

**LINE 23:** As it is unclear what is meant by "not conducted" in this context, the EU suggests replacing it by "not tested".

**LINES 75 – 85:** It is unclear what point this example is trying to make. The EU would be grateful for a clarification and would suggest the author to reword the paragraph for more clarify.

**EU COMMENTS**
**On the proposed changes to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals**

**CHAPTER Appendix 1.1.4.7.: Selection and use of reference samples and panels**

General comments

**The EU can in general support the proposed changes.**

**However, it would be helpful to explain what exactly a group A or group B reference panel should consist of.**

Specific comments

**None**

**EU COMMENTS**
**On the proposed changes to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals**

**CHAPTER 1.1.7: Application of biotechnology to the development of new and emerging diagnostic technologies**

General comments

**The EU can in general support the proposed changes but has some specific comments.**

**As this Chapter is of a more informative nature for readers not familiar with these technologies and contains rather general information, the OIE should consider whether this Chapter should be part of the *Terrestrial Manual* and consequently be considered as an International Standard or whether it would be more appropriate to publish it elsewhere (i.e. on the OIE website).**

**Moreover, it would be helpful if this chapter highlighted the really useful and frequently used approaches, such as real-time PCR, LAMP and sequencing that are used a lot compared to PCR and gel based detection, while currently RFLP are infrequently used.**

**There is a section on new approaches for detection of proteins (B.3.6), and a similar section could be added for nucleic acid detection.**

Specific comments

**LINE 18:** Please insert ", validated in accordance with Chapter 1.1.4/5" before "and eventually automated".

**LINE 31:** Please replace the words "isothermal PCR" by the words "isothermal amplification", as this is the usual term.

**LINE 74:** Please add the following sentence:

"Assays increasingly use an internal control to demonstrate that PCR inhibitors are not present (see Chapter 1.1.4.3 for more details on inhibition controls)."

**LINES 88 – 89:** Since both the sensitivity and the specificity are enhanced in a nested PCR when compared to a conventional PCR, the beginning of the sentence should be amended as follows:

"Both the specificity and the sensitivity of a PCR may be enhanced [...]"

**LINE 92:** An explanation should be provided as to why the contamination risk is higher in a nested PCR, as the target readers may not be familiar with the technique. Thus, the following should be added at the end of the sentence:

", because the second reaction usually necessitates the transfer of an aliquot from the first reaction into a separate reaction tube."

**LINE 106:** Please add the following at the end of the sentence:

"in order to identify the pathogen species or genotype".

**LINE 107:** Please insert the word "targeted at" before the word "conserved" (clarity).

**LINE 108:** As the target readers may not be familiar with the term, please add the following parenthesis after the words "degenerate primers":

"(i.e. a mixture of similar primers with different bases in some of its positions)"

**LINE 113:** Please add the following to the sentence:

"and evaluated during assay development and optimization".

**LINE 137:** Please replace "PCR" by "assay" (in consistency with **LINE 31**).

**LINE 138:** Please delete the word "PCR".

**LINES 191 – 192:** A DNA based approach may offer such discrimination depending on how the assay has been designed. RFLP will only offer discrimination if the assay targets a variable region. Therefore, the following additional wording is suggested:

"Depending on the assay design, a DNA-based procedure will offer the higher degree of discrimination that is often required. ~~and a~~An appropriate starting point may be analyses for restriction fragment length polymorphisms (RFLP) targeting a variable genome region."

**LINE 199:** As the target readers may not be familiar with this term, please add the following parenthesis after the words "restriction enzymes":

"(i.e. endonucleases that recognise and cleave double stranded DNA at specific nucleotide sequences called restriction sites)."

**LINE 236:** Please insert the words "the four" before the word "individual" (clarity).

**LINE 239:** Please replace the word "than" by "then" (typographical error).

**LINE 248:** Please add the following sentence:

"Whilst genome sequencing is becoming less expensive, there are still significant challenges in the bioinformatic approaches needed to analyse the vast quantities of data created in order to answer specific biological questions."

**LINE 249:** Please replace the word "has" by "have" (clarity).

**LINE 316:** Please replace the words "to even" by "for even" (clarity).

**EU COMMENTS**
**On the proposed changes to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals**

**LINE 363:** Please add the following after "equine infectious anaemia":

"(Coggins test)".

**LINE 381:** Please delete the word "of" (clarity).

**LINES 383 – 385:** Immunoblotting for TSE was used on a large scale but has largely been replaced as a screening test by AgELISA or LFD based methods. It is still an important confirmatory test and integral to the differentiation of TSE strains (into typical and atypical BSE and scrapie). Therefore, the following amended wording is suggested:

"As an example of antigen detection, immunoblotting ~~is~~ has been used on a large scale as a major screening method for bovine spongiform encephalopathy (BSE) and scrapie; it has been used on millions of brain stem samples in Europe and elsewhere for the detection of prion protein (Schaller *et al.,* 1999). It has now largely been replaced as a screening test by AgELISA or LFD based methods but is still an important confirmatory test and integral to the differentiation of TSE strains (into typical and atypical BSE and scrapie)."

**LINE 425:** Please insert the words "competitive ELISA" before "cELISA" and put the latter in parenthesis, as it is the first time this term is used in the text.

**LINE 445:** Please insert the following before the sentence beginning with "The limiting step":

"As formalin fixation can denature the antigenic epitopes (i.e. the three dimensional structure which some antibodies recognize), the limiting step […]".

**LINE 496:** The EU suggests adding further subsections in section B3. to describe the frequently used FATs to detect antigens as well as the use of the MALDI-Tof analysis for determinative bacteriology.

**LINE 521:** Please replace the word "Anti-imunoglubulin" by "Anti-immunoglubulin" (typographical error).

**LINE 555:** Please delete the word "them" (clarity).

**LINE 574:** Please replace the word "disease" by "pathogen" (clarity).

**LINE 582:** Please replace the words "an expression system" by "a protein expression system" (clarity).

**EU COMMENTS**
**On the proposed changes to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals**

**CHAPTER 2.1.2.: Anthrax**

General comments

**The EU can in general support the proposed changes but has some specific comments.**

Specific comments

**LINE 57:** For reasons of consistency (cf. Chapter 2.8.2. "Atrophic rhinitis of swine"), the following should precede the paragraph:

***"Zoonotic risk and biosafety requirements:"***

Furthermore, a paragraph should be added after LINE 61 recommending a biosafety level and the vaccination of laboratory personnel, e.g. as follows:

"Containment level 3 practices are recommended for handling clinical specimens and cultures of *B. anthracis*. Vaccination of laboratory personnel is highly recommended."

**LINES 60 – 61:** The EU supports the addition of this new sentence; however for reasons of clarity it is suggested to reword is as follows:

"It is important that industrial workers use appropriate personal protective clothing and equipment~~gear~~ and follow standard operating procedures which minimise the risk of transmission occuring."

**LINE 557:** The EU suggests adding the following sentence to the note:

"The OIE Reference Laboratories can be contacted for a list of kits, reagents, and/or vaccine manufacturers".

**EU COMMENTS**
**On the proposed changes to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals**

**CHAPTER 2.1.17.: *Trypanosoma evansi* infection (surra)**

General comments

**The EU can in general support the proposed changes but has some specific comments.**

**The protocols proposed for addition under section B.3 "test applications" for the different host species are a sensible approach for laboratory diagnosis of *T. evansi* and go some way to differentiate between *T. evansi* and *T. equiperdum* infections; but due to the largely unknown provenance of most *T. equiperdum* laboratory strains held in labs worldwide, validation of test methods to distinguish between the two species is made very difficult indeed.**

Specific comments

**LINE 86:** For animal welfare reasons, the sentence should be modified as follows:

"Where ~~For~~ a definitive confirmation of the infection in suspected animals is needed (e.g. for importation into a disease-free area), mouse inoculation is the best test to apply. However, animal testing should be limited and used only if fully justified".

**LINE 94:** The fact that surra is not a zoonosis should be stated at the end of section A, e.g. as follows:

"***Zoonotic risk and biosafety requirements:*** *T. evansi* is not known to have a zoonotic potential."

**LINE 224:** For animal welfare reasons, the following sentence should be inserted at the beginning of the paragraph:

"Due to the increasing concern to eliminate the use of animals for biological testing, animal inoculation should be limited as far as possible and only used if fully justified."

**LINE 483:** The word "Equines" should be replaced by "Equids".

**LINE 484:** The word "equine" should be replaced by "equid".

**LINE 508:** The word "remind" should be replaced by "remain" (typographical error?).

**LINE 510:** The word "Fulfil" should be deleted.

**LINE 571:** For reasons of consistency, the EU suggests adding the following note:

"**NB:** There are OIE Reference Laboratories for surra (see Table in Part 3 of this *Terrestrial Manual* or consult the OIE Web site for the most up-to-date list: www.oie.int).The OIE Reference Laboratories can be contacted for a list of kits, reagents, and/or vaccine manufacturers".

**EU COMMENTS**
**On the proposed changes to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals**

**CHAPTER 2.3.1.: Avian chlamydiosis**

General comments

**The EU can in general support the proposed changes and has some specific comments.**

Specific comments

**LINE 21**: The parenthesis ("biosafety level 3") should be inserted before and not after the word "is".

**LINE 126:** For reasons of consistency (cf. Chapter 2.8.2. "Atrophic rhinitis of swine"), the following should precede the paragraph:

***"Zoonotic risk and biosafety requirements:"***

**LINE 268:** The word "a" should be inserted before the word "rare" (clarity).

**LINE 320:** For reasons of clarity, the word "that" should be deleted, as well as the repetition of the words "for use".

**LINE 595:** The EU suggests adding the following sentence to the note:

"The OIE Reference Laboratory can be contacted for a list of kits, reagents, and/or vaccine manufacturers".

**EU COMMENTS**
**On the proposed changes to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals**

**CHAPTER 2.3.11.: Fowl typhoid and Pullorum disease**

General comments

**The EU can in general support the proposed changes but has some specific comments.**

Specific comments

**LINES 7 – 8:** The words "In its acute form" should be deleted or moved to the beginning of the next paragraph (**LINE 9**) (typesetting error).

**LINES 57– 62:** This second part of the first paragraph ("*Salmonella* Gallinarum […] processed at CL3") pertains to zoonotic potential and biosafety in the laboratory. For consistency with other chapters (e.g. Atrophic rhinitis of sine), it is suggested to move it to the end of section A (i.e. after **LINE 86**) and to precede it by the following:

"**Zoonotic risk and biosafety requirements**: "*Salmonella* Gallinarum […] processed at CL3".

**LINES 63 – 64:** This sentence is already in the summary in **LINES 23 – 24** and while it contains important information, it should not be repeated here, and thus be deleted.

**EU COMMENTS**
**On the proposed changes to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals**

**CHAPTER 2.5.2.: Contagious equine metritis**

General comments

**The EU can in general support the proposed changes but has some specific comments.**

Specific comments

**LINE 14:** The word "asymptomatic" has been substituted for "sub clinical". Whilst there is a fine distinction in these definitions in the case of carrier stallions, they do not show clinical signs at any stage. Strictly speaking "sub clinical" describes a condition prior to the development or below the level of clinical signs. Therefore, "asymptomatic" is more appropriate in this instance and should be reinstated in the text.

**LINE 102:** The word "the code" should be replaced by the words "that Code of Practice", to avoid any possible misunderstanding and confusion with the OIE *Terrestrial Code*.

**LINE 106 and 114:** The words "in the Code of Practice" should be inserted between the words "are defined" and the word "as".

**LINES 111 – 113:** By referring to countries "currently listed in the guidelines as higher risk", that external document (i.e. UK's Horserace Betting Levy Board's Code of Practice) becomes part of the OIE Standard.

While this may be acceptable in this specific instance, the document referred to should be quoted in an unambiguous way, e.g. by replacing the word "guidelines" by the words "Code of Practice" in **LINES 111 and 113**.

**LINES 234 – 236:** The text should be replaced by the following:

"The specificity of the immunofluorescence test can be improved by the use of monoclonal antibodies that are now available. Nevertheless polyclonal (Breuil *et al.*, 2010) and monoclonal antibodies can be~~en~~ used in the differentiation of *T. equigenitalis* and *T. asinigenitalis.*"

Rationale: Breuil *et al*. (2010) have used polyclonal serum, whereas the Pourquier institute provides monoclonal serum. The immunofluorescent specificity is actually increased with monoclonal serum, but both types, monoclonal and polyclonal, can be used to differentiate *T. equigenitalis* from *T. asinigenitalis*. Consequently, the monoclonal serum can be used for screening of field samples whereas both types (monoclonal and polyclonal) can be used for immunofluorescence to confirm of a positive bacteriological result in order to differenciate *T. equigenitalis* from *T. asinigenitalis*.

**LINES 246 – 247:** The text should be replaced by the following:

"~~Recently a~~Another species of *Taylorella*, *T. asinigenitalis* (Jang *et al*., 2001), has been isolated from ~~male~~ donkey~~s~~ jacks and horse mares in the United

States of America (~~Jang et al., 2001~~ Katz *et al*., 2000) and from a horse stallion in Europe (Baverud *et al*., 2006; Breuil *et al*., 2011)."

Consequently, the following references should be added to the reference section:

"Katz J.B., Evans L.E., Hutto D.L., Schroeder-Tucker L.C., Carew A.M., Donahue J.M. & Hirsh D.C. (2000). Clinical, bacteriologic, serologic and pathologic features of infections with atypical *Taylorella equigenitalis* in mares. J. Am. Vet. Med. Assoc., **216**(12), 1945-1948.

Breuil M.F., Duquesne F., Laugier C. & Petry S. (2011). Phenotypic and 16S ribosomal RNA gene diversity of *Taylorella asinigenitalis* strains between 1995 to 2008. Vet. Microbiol., **148**, 260-266."

Rationale: The literature reference as to the first isolation of *T. asinigenitalis* in the USA end 1997 and beginning of 1998 in donkeys, but also in horse mares, is Katz *et al*. (2000), whereas Jang *et al*. (2001) pertains to the characterisation of the species *T. asinigenitalis*. The reference Baverud *et al*. (2006) historically is the first publication of a case of a stallion infected with *T. asinigenitalis* in Europe in 2004, however the retrospective study by Breuil *et al*. published in 2011 pertains to the first case of a stallion infected by *T. asinigenitalis* in Europe in 1995.

**LINE 148:** The words "newly described" should be deleted, as that bacterium will have been described more than 10 years ago at the time of possible adoption of this draft revised Chapter in May 2012 (2000/2001).

**LINE 371:** The EU suggests adding the following sentence to the note:

"The OIE Reference Laboratories can be contacted for a list of kits, reagents, and/or vaccine manufacturers".

**EU COMMENTS**
**On the proposed changes to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals**

**CHAPTER 2.7.7.: Enzootic abortion of ewes (ovine chlamydiosis)**

General comments

**The EU can in general support the proposed changes but has some specific comments.**

Specific comments

**LINES 30 and 34:** "*C. abortus*" should be used here after the first use of the name in full in line 6. On the other hand, in **LINE 34**, "*C. pecorum*" should be used in full as it is not referred to previously in the text.

**LINES 70 – 85:** This paragraph describes the taxonomy of the pathogen and is not directly related to the clinical and epidemiological description in the other paragraphs of section A. Thus it should be moved to the end of the section, i.e. after the paragraph in **LINES 86 – 96**.

**LINE 74:** "*C. pneumonia*" should be replaced by "*C. pneumoniae*" (typographical error).

**LINES 90 – 96:** For consistency with other Chapters (e.g. Atrophic rhinitis of sine), the second part of the paragraph (starting with "Human infections […]") should become a separate paragraph, and a laboratory biosafety level should be recommended here as well. Thus, the following wording is proposed:

"**Zoonotic risk and biosafety requirements**: Human infection may be acquired from infected products of abortion or parturition or from carelessly handled laboratory cultures of the organism, with effects that range from subclinical infection to acute influenza-like illness. Appropriate precautions should be taken and containment level 2 practices are recommended when handling cultures and potentially infected tissues (see Chapter 1.1.2 Biosafety and biosecurity in the veterinary microbiology laboratory and animal facilities). Authenticated cases of human placentitis and abortion caused by *C. abortus* of ovine origin indicate that pregnant women are at special risk and should not be exposed to sources of infection (Caul & Sillis, 1998; Longbottom *et al*., 2002)."

**LINE 149:** The word "chlamydosis" should be replaced by "chlamydiosis" (typographical error).

**LINE 150 – 151:** A concrete biosafety level should be specified here. Moreover, reference to Chapter 1.1.2 is redundant (already referred to in LINES 93 – 94. Thus, the following wording is suggested:

"[…] and thus isolation and identification procedures should ~~must~~ be carried out under ~~the appropriate~~ containment level 2 conditions ~~as described in Chapter 1.1.2~~."

**LINES 227 – 228:** The second sentence of this paragraph ("A multi-component […]") could be moved to section C 3 a.

**EU COMMENTS**
**On the proposed changes to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals**

**LINE 238:** The word "teracyclines" should be replaced by "tetracyclines" (typographical error).

**LINES 244 – 246:** The first sentence ("Vaccine stored […] withdrawn") should be deleted, as this type of information seems out of place in an OIE Standard and would rather be included in the vaccine manufacturer's information and direction for use.

**LINE 419:** The EU suggests adding the following sentence to the note:

"The OIE Reference Laboratories can be contacted for a list of kits, reagents, and/or vaccine manufacturers".

**EU COMMENTS**
**On the proposed changes to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals**

**CHAPTER 2.7.11.: *Peste des petits ruminants***

General comments

**The EU can in general support the proposed changes but has some comments that should be taken into account by the OIE.**

**Section C appears to be greatly out of date, and needs to be generalised to include the homologous vaccines developed in India, and how appropriate seed stocks can be obtained by those wishing to set up vaccine preparation. Given that there are at least three attenuated strains of PPRV in field use as vaccines, it would be appropriate to try to define the required characteristics of such a vaccine (e.g. potency testing) rather than focus on detailed production techniques for one particular vaccine strain, as is the case currently. Furthermore, for reasons of consistency, the section should be rewritten to match the new structure foreseen for section c (cf. draft revised Chapter 2.8.2. on Atrophic rhinitis of swine).**

**Given the desire to keep the manual to the essentials, it seems unnecessary to describe in some detail the protocols for tests that are carried out using commercial or fully packaged kits, such as the cELISA kit for PPRV, now marketed by IDVET, especially as there are other published and even commercially available kits that carry out the same function, but which will have different protocols. The Manual should focus on detailed instructions for preparation of materials or carrying out assays for which local diagnostic laboratories may need to develop their own reagents. Hence it makes sense to give instructions for carrying out a VNT (cf. section B.2.a), AGID or CIE (cf. sections B.1.b and B.1.c), which will often, if not always, be carried out using locally produced reagents, but not for carrying out the icELISA (cf. section B.1.d) as this test requires getting a kit from CIRAD, which will come with the instructions.**

**Consideration should be given to the acceptance of cELISA kits as alternatives for VNTs, as they provide as good a test for anti-PPRV antibodies. Initially it may be necessary to allow only kits that are commercially available and/or meet certain quality standards. It should be noted that the VNT "prescribed test" provides little in the way of specification as to the quality of how the test is carried out, or the necessary controls, while a cELISA may detect PPRV-specific antibodies that would be inhibitory in vivo (e.g. through complement recruitment) while not being neutralising in cell culture.**

**Some of the proposed new text is unclear, and needs updating to reflect our current state of knowledge, and the full spectrum of tools available to those working on this disease. Detailed comments are placed below.**

**EU COMMENTS**
**On the proposed changes to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals**

Specific comments

**LINES 22 – 23:** This sentence is ambiguous and refers only to samples from live animals. It should be replaced by the following:

"Specimens from live animals may be anticoagulant-treated blood or swabs of conjunctival discharges, nasal secretions, buccal mucosae or rectal mucosae."

**LINES 36 – 37:** The sentence does not take into account the eradication of rinderpest, so distinguishing the two viruses is not relevant anymore. Therefore the sentence should be moved to the end of the paragraph and replaced by the following:

"Infected animals present clinical signs similar to those historically seen with rinderpest in cattle, although the diseases are caused by distinct virus species".

**LINES 59 – 66:** These sentences ("The virus that was […] but no live virus was isolated") are hard to follow, and have some inaccuracies. Describing the 1971 outbreak of disease in cattle in Sudan as "natural rinderpest", when the virus was later identified as PPRV, is confusing. Moreover, the only published isolation of PPRV in camels is the paper by Khalafalla *et al*. 2010, where they did isolate live virus, contrary to the text. Therefore, the

"The virus that was responsible for a disease outbreak, originally described as rinderpest, in cattle, sheep and goats in Sudan in 1971 was, in fact, PPRV (Kwiatek *et al*., 2011). Moreover, PPRV was isolated from an outbreak of rinderpest-like disease in buffaloes in India in 1995 (Govindarajan *et al*., 1997). Camels appear to be susceptible to PPR disease, as not only have antibodies to PPRV been detected in camels in Egypt and Ethiopia (Abraham *et al*., 2005; Ismail *et al*., 1992; Kwiatek *et al*., 2011; Roger *et al*., 2000; 2001), but also the virus itself was detected (as antigen and nucleic acid, and by virus isolation) in samples from animals affected by a PPR-like epizootic disease that affected camels in Sudan in 2004 (Khalafalla *et al*. 2010)."

**LINE 81:** Please insert the word "that" between "except" and "prominent".

**LINE 92:** At the start of the paragraph, please insert the following sentence:

"For virus isolation, samples must be kept chilled after collection until delivered to the laboratory".

**LINES 139 – 168**: The detailed protocol is not required and should be replaced by the following:

**"d) Immunocapture enzyme-linked immunosorbent assay**

The most common immunocapture enzyme-linked immunosorbent assay (ELISA) (Libeau *et al.*, 1994) using ~~three~~ two anti-N protein monoclonal antibodies (MAb) ~~anti-N protein~~, allows a rapid ~~differential~~ identification of PPRV ~~or rinderpest viruses, and this is of great importance as the two~~

~~diseases had until recently a similar geographical distribution and may affect the same animal species~~. The kit to perform the assay, with detailed instructions of use, is obtainable from the OIE and FAO Reference Laboratory for PPR in France

i) ~~Microtitre ELISA plates (e.g. high adsorption capacity Nunc Maxisorb~~ Maxisorp~~) are coated with 100 µl of a capture MAb solution (diluted according to the instructions of the Reference Laboratory providing the kit). This MAb reacts with both rinderpest virus and PPRV.~~

ii) ~~After washing, 50 µl of the sample suspension is added to four~~ two ~~wells, and control wells are filled with buffer.~~

iii) ~~Immediately, 25 µl of a detection biotinylated MAb for PPR and 25 µl of streptavidin/peroxidase are added to two wells. and 25 µl of a detection MAb for rinderpest and 25 µl of streptavidin/peroxidase are added to the two other wells.~~

iv) ~~The plates are incubated at 37°C for 1 hour with constant agitation.~~

v) ~~After three vigorous washes, 100 µl of ortho-phenylenediamine (OPD) in hydrogen peroxide is added, and the plates are incubated for 10 more minutes at room temperature.~~

vi) ~~The reaction is stopped by the addition of 100 µl of 1 N sulphuric acid, and the absorbance is measured at 492 nm on a spectrophotometer/ELISA reader.~~

~~The cut-off above which samples are considered to be positive is calculated from~~ the ~~each blank (PPR blank and rinderpest blank) as three times the mean absorbance values.~~

~~A sandwich ELISA can also be performed: the sample is first allowed to react with the detection MAb and the immunocomplex is then captured by the second MAb adsorbed on to the ELISA plate.~~

The test is very specific and sensitive (it can detect $10^{0.6}$ $TCID_{50}$/well ~~for the PPRV and $10^{2.2}$ $TCID_{50}$ for the rinderpest virus~~). The results are obtained in 2 hours.

Another immunocapture test, based on the use of a single anti-H MAb ~~anti-H~~, has been described (Saliki *et al.*, 1994).

A sandwich form of the icELISA can also be performed (Singh *et al.*, 2004a): the sample is first allowed to react with the detection MAb and the immunocomplex is then captured by the MAb or polyclonal antibody adsorbed on to the ELISA plate. The assay shows high correlation to the cell infectivity assay ($TCID_{50}$) with a minimum detection limit of $10^3$ $TCID_{50}$/ml (Saravanan *et al.*, 2008)."

**LINES 170 – 172:** As rinderpest is now eradicated from the globe, this paragraph should be deleted or amended as follows:

"Historically, cDNA $^{32}P$ labelled clones have been used to differentiate PPR and rinderpest (Diallo *et al.*, 1989a)~~, but their use in routine diagnosis is not recommended due to the short half-life of the $^{32}P$ and the need for special equipment to protect the users.~~"

**LINES 173 – 185:** This paragraph needs updating and clarifying in some places. The following text is suggested:

"~~A r~~Reverse transcription PCR (RT-PCR) techniques based on the amplification of parts of the N~~p~~ and F protein genes has~~s~~ve been developed for the specific diagnosis of PPR (Couacy-Hymann *et al.*, 2002; Forsyth & Barrett, 1995~~; Saravanan *et al.*, 2004~~). This technique is very sensitive compared with other tests and results are obtained in 5 hours, including the RNA extraction. The OIE and FAO Reference Laboratory~~y~~ies for PPR in France or the UK (see Table given in Part 3 of this *Terrestrial Manual*) can advise on the use of this technique. A multiplex RT-PCR, based on the amplification of fragments of N and M protein genes, has been reported (George *et al.*, 2006). Another format of the N gene-based RT-PCR has also been described (Saravanan *et al.*, 2004; Kumar *et al.*, 2007). Instead of analysing the amplified product – the amplicon – by agarose gel electrophoresis, it is detected on a plate by ELISA through the use of a labelled probe. This ~~new~~ alternate format, RT-PCR-ELISA, is more than ten times ~~for~~ as sensitive ~~that~~ as the classical RT-PCR. In recent years, nucleic acid amplification methods for PPR diagnosis have been significantly improved with quantitative (real time) RT-PCR (Adombi *et al.*, 2011; Balamurugan *et al.*, 2010; Bao *et al.,* 2008; Batten *et al.*, 2011; Kwiatek *et al.,* 2010; Li *et al.*, 2010). ~~The nucleic acid isothermal amplification for PPR diagnosis~~ A linear (isothermal) amplification assay for detection of PPRV nucleic acid has also been described (Li *et al.*, 2010)."

Consequently, the following reference should be added to the reference section:

"Batten C. A., Banyard A. C., King D. P., Henstock M. R., Edwards L., Sanders A., Buczkowski H., Oura C. A. L. & Barrett T. (2011). A real-time PCR assay for the specific detection of Peste des Petits Ruminants Virus. *J. Virol. Methods.*, **171**(2), 401-404."

**LINES 189 – 197:** This paragraph needs further information added on the use of SLAM-expressing cell line derivatives, since there are several, all of which have proven superior to Vero cells for PPRV isolation. As the CV1-goat SLAM has not been found as sensitive for virus isolation as Vero-dog SLAM, for example, multiple options need to be provided until experience provides a clear ideal procedure. Therefore, the paragraph should be amended as follows:

"PPRV may be isolated in primary lamb kidney/lung cells and some cell lines (Vero, B95a). Unfortunately, PPRV isolation using ~~Vero~~ such cells is not always successful and may require multiple blind passages. Recently, ~~monkey CV1~~ derivatives of cell lines (Vero, CV1) expressing the ~~PPRV~~ morbillivirus receptor, the signalling lymphocyte activation molecule (SLAM, or CD150), have been developed ~~for quick~~ which can enable isolation of field viruses from pathological specimens in less than 1 week, without the requirement ~~of~~ for blind passages. These include a derivative of the monkey cell line CV1 expressing goat SLAM (Adombi *et al.*, 2011) and derivatives of Vero cells expressing human or dog SLAM (Ono *et al.*, 2001; Seki *et al.*, 2003). Monolayer cultures are inoculated with suspect material (swab material, buffy coat or 10% tissue suspensions) and examined daily for evidence of cytopathic effect (CPE). The CPE produced by PPRV can

develop within 5 days and consists of cell rounding and aggregation culminating in syncytia formation in lamb kidney cells and ~~the~~ cell lines~~, CV1~~ expressing SLAM."

Consequently, the following references should be added to the reference section:

"Ono N., Tatsuo H., Tanaka K., Minagawa H. & Yanagi Y. (2001). V domain of human SLAM (CDw150) is essential for its function as a measles virus receptor. *J. Virol.*, **75**, 1594-1600.

Seki F., Ono N., Yamaguchi R. & Yanagi Y. (2003). Efficient isolation of wild strains of canine distemper virus in Vero cells expressing canine SLAM (CD150) and their adaptability to marmoset B95a cells. *J. Virol.*, **77**, 9943-9950."

**LINES 230 – 231:** As rinderpest is now eradicated, this paragraph should be deleted.

**LINES 234 – 255:** This section contains a detailed description of how to use a commercial kit, despite this being only one of at least four published systems, and one of two commercially available kits, to carry out PPRV cELISAs. It is therefore suggested to shorten it and to replace it by the following text:

"**b) Competitive enzyme-linked immunosorbent assay**
Several ~~C~~competitive ELISAs (cELISAs) based on the use of Mabs that recognise viral proteins have been described. These can be divided into two types, those where the Mab recognises the N ~~anti-nucleo~~protein and ~~a~~ use recombinant N protein produced in baculovirus as the antigen (Libeau *et al*., 1995; Choi *et al*., 2003) and those where the ~~has been described. other competitive ELISA techniques, based on the use of monoclonal~~ MAb recognises the ~~anti-haemagglutinin~~ viral attachment protein (H) and the antigen consists of purified or partially-purified PPRV (normally a vaccine strain) (Anderson & McKay, 1994; Saliki *et al*., 1993; Singh *et al*., 2004b). One assay is commercially available (from IDVET), that of Libeau *et al* 1995. All assays work on the principle that anti-PPRV antibodies in test sera can block the binding of the MAb to the antigen.

~~i)Coat microtitre plates (e.g. high adsorption capacity Nunc Maxisorb Maxisorp) with 50 µl of a predetermined dilution of N-PPR protein (produced by a recombinant baculovirus) for 1 hour at 37°C with constant agitation.~~
~~ii)Wash the plates three times and blot dry.~~
~~iii) Distribute 45 µl of blocking buffer (PBS + 0.5% Tween 20 + 0.5 fetal calf serum) to all wells, and then add 5 µl of test sera to test wells (at a final dilution of 1/20) and 5 µl of the different control sera (strong positive, weak positive and negative serum) to control wells.~~
~~iv) Add 50 µl of MAb diluted 1/100 in blocking buffer, and incubate at 37°C for 1 hour.~~
~~v) Wash the plates three times and blot dry.~~
~~vi) Add 50 µl of anti-mouse conjugate diluted 1/1000, and incubate at 37°C for 1 hour.~~

~~vii) Wash the plates three times.~~
~~viii) Prepare OPD in hydrogen peroxide solution. Add 50 µl of substrate/conjugate mixture to each well. Stop the reaction after 10 minutes with 50 µl of 1 M sulphuric acid.~~
~~ix) Read on an ELISA reader at 492 nm.~~
~~The absorbance is converted to percentage inhibition (PI) using the formula: PI = 100 – (absorbance of the test wells/absorbance of the MAb control wells) × 100~~
~~Sera showing PI greater than 50% are positive.~~
~~Two Three other competitive ELISA techniques, based on the use of monoclonal MAb anti-haemagglutinin (H), have also been described (Andersen & McKay, 1994; Saliki et al., 1993; Singh et al., 2004b).”~~

Consequently, the following reference should be added to the reference section:

"Choi K.S., Nah J.J., Ko Y.J., Kang S.Y. & Jo N.I. (2005). Rapid competitive enzyme-linked immunosorbent assay for detection of antibodies to peste des petits ruminants virus. *Clin. Diagn. Lab. Immunol.*, **12**, 542-547."

**LINES 257 – 264:** This paragraph needs updating to include other homologous vaccines that have been developed. It should be replaced by the following:

"Sheep and goats that recover from PPR develop an active life-long immunity against the disease. ~~As antibodies have been demonstrated 4 years after infection, this immunity is probably life-long~~ (Durojaiye, 1982). Several h~~H~~omologous PPR vaccines, being tissue culture-attenuated strains of natural PPRV, are ~~is~~ available (Sen *et al*., 2010). In 1998, the OIE International Committee endorsed the use of such a vaccine in countries that have decided to follow the ‘OIE pathway’ for epidemiological surveillance for rinderpest in order to avoid confusion when serological surveys are performed. There have also been three ~~two~~ published reports on the preliminary results ~~of the development of~~ from recombinant capripox-based PPR vaccines which are able to protect against both capripox and PPR (Berhe *et al.*, 2003; Diallo *et al.*, 2002; Chen *et al*., 2010). The production and validation of ~~the commercially available attenuated PPRV~~ vaccine (seed stocks and production batches) from the commercially available attenuated PPRV is described here."

Consequently, the following references should be added to the reference section:

"Chen W., Hu S., Qu L., Hu Q., Zhang Q., Zhi H., Huang K. & Bu, Z. (2010). A goat poxvirus-vectored peste-des-petits-ruminants vaccine induces long-lasting neutralization antibody to high levels in goats and sheep. *Vaccine*, **28**(30), 4742-4750.

Sen A., Saravanan P., Balamurugan V., Rajak K. K., Sudhakar S. B., Bhanuprakash V., Parida S. & Singh R. K. (2010). Vaccines against peste des petits ruminants virus. *Expert Rev. Vaccines*, **9**(7), 785-796."

**EU COMMENTS**
**On the proposed changes to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals**

**LINE 284:** Please delete first sentence (it repeats what is said in **LINES 269 – 270**).

**LINES 287 and 289:** Please replace the word "dish" by the word "flask".

**LINE 347:** Please replace the word "innocuousness" by the word "innocuity", which is more usual for vaccine tests.

**LINE 398 – 413:** This section covers what is essentially a virus neutralisation test, which is already covered in section B.2.a (**LINES 217 – 233**). It would seem reasonable to refer back to this description and define a minimum titre for passing the test.

**LINE 571:** The EU suggests adding the following sentence to the note:

"The OIE Reference Laboratories can be contacted for a list of kits, reagents, and/or vaccine manufacturers".

**EU COMMENTS**
**On the proposed changes to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals**

**CHAPTER 2.8.2.: Atrophic rhinitis of swine**

General comments

**The EU can in general support the proposed changes and has one specific comment.**

Specific comments

**LINES 77-79:** As *P. multocida* is a very common secondary invader in many species and is also commonly found in humans with no contact with pigs, the EU proposes to amend the sentence as follows:

*"Pasteurella multocida* is an important and common secondary invader that can affect people that never have been in contact with pigs, but *P. multocida* has also frequently been isolated from healthy human carriers working in swine production sites or living nearby and has additionally been associated with chronic or acute respiratory disease in such individuals (Donnio *et al.,* 1999; Marois *et al.,* 2009)."