ANNEX 3

EU COMMENTS
ON THE PROPOSED CHANGES TO THE
WOAH MANUAL OF DIAGNOSTIC TESTS AND VACCINES
FOR TERRESTRIAL ANIMALS
PRESENTED FOR COMMENTS IN OCTOBER 2022[1]

[1] The draft chapters can be downloaded from the WOAH website at the following address: http://web.oie.int/downld/Terr_Manual/Mailing_Oct_2022.zip

EU COMMENTS on the proposed changes to the WOAH Manual of Diagnostic Tests and Vaccines for Terrestrial Animals

SUMMARY

EU COMMENTS on the proposed changes to the WOAH Manual of Diagnostic Tests and Vaccines for Terrestrial Animals

Glossary

General comment:

The EU can support the revised glossary.

Specific comments:

None

1.1.6. Principles and methods of validation of diagnostic assays for infectious diseases

General comment:

The EU can in general support this revised chapter. Specific comments are provided below.

Specific comments:

LINES 39-43; LINES 105-106; LINES 197-207; LINES 528-529:

There is a need to be consistent about the test purposes and the number of these – are there six or seven? Both six and seven test purposes are mentioned in different parts of the document. Table 1 lists six purposes including purpose 1(a) and 1(b). Lines 528 and 529 refer to seven purposes. There is need to decide on the number of test purposes and to remain constant. In lines 39-43 one could combine "contribute to eradication" with "identifying of infected animals".

LINE 63: Notes - repeatability of results of operators in different labs is likely to be greater. Suggest adding "(higher SD, higher Cv)" in parentheses after repeatability to clarify that actual repeatability would be lower between laboratories, as reflected by higher SD or Cv.

LINE 557: Correct "PPV V" to "PPV".

1.1.10. Vaccine banks

General comment:

The EU can in general support this revised chapter. Specific comments are provided below.

Specific comments:

LINES 23-24: The text is not accurate. Indeed, it is the pathogenic agent (e.g. a virus) that shows variation, not the disease. Furthermore, even within a serotype there can be extensive antigenic diversity. Thus, we suggest the following wording:

"Also, for ~~diseases~~ pathogenic agents that have several serotypes and/or show ~~broad strain~~ extensive variation in their antigenic characteristics, the fixed formulation may not sufficiently protect against the strain involved in a given outbreak."

LINE 29: Please add the following at the end of the sentence: "unless shelf life can be extended after appropriate testing".

LINE 74: Since these animals are not supposed to enter the food chain, please replace the word "slaughter" with "stamping out", for clarity.

LINES 106-108: Same issue as with the summary (LINES 23-24). We suggest the following wording:

"Another inconvenience of ready-to-use formulated vaccines for ~~diseases~~ pathogenic agents that have several serotypes and/or exhibit ~~broad strain~~ extensive variation in their antigenic characteristics is that the fixed formulation may not sufficiently protect against the strain involved in a given outbreak.

LINES 139-141: In line with the above comments, we suggest the text be changed as follows:

"The value of any vaccine bank is dependent upon the appropriateness of what it holds for field application, particularly in respect of ~~diseases~~ pathogenic agents that have several serotypes and/or exhibit extensive variation in their antigenic characteristics."

LINES 158- 160: It is not only knowledge about what strains are circulating that is important but also knowledge about the extent of antigenic match between these strains and the virus antigens stored in the vaccine bank. This should be added to the text.

LINES 205 and 206: Please delete the parentheses "(e.g. Chapters 1.1.8, 2.2.3, 2.2.4, 2.3.2)" and "(such as 3.1.8 Foot and mouth disease)" as they are superfluous.

LINE 211: Please add "or products authorised in another country" after "unauthorised products".

LINE 214: Please replace the word "Licencing" with the words "regulatory approval".

LINES 237-238 and 243, 257: Please replace "product licence (authorisation or registration)" with "regulatory approval".

LINES 307-316: Please delete the new text proposed, as it is out of the remit of this chapter and of the Manual. Indeed, questions of international trade and country disease status are dealt with in the Code.

LINE 341: Please change ending to: "…..are numerous and seek to: "

LINE 355: Please change "the same standards" to "the same or equivalent standards"

Minor corrections:

LINE 191: delete "a", thus "…demand at national, regional and global levels".

LINE 267: add "a", to make "…exposure to a relevant infection…".

LINE 339: "diseases" should be "disease".

3.1.1. Anthrax

General comment:

The EU can in general support this revised chapter. Specific comments are provided below.

Specific comments:

LINE 15: Please consider replacing "Identification of the agent" with "Detection of the agent" and adapting the rest of the text accordingly (Table 1; title of Section 1).

LINES 157-159: We agree in principle with the change proposed, however would like to be able to use the methylene blue stain when having a good quality batch (from a commercial supplier). We therefore suggest adding the following at the end of the sentence: "or polychrome methylene blue (M'Fadyean)."

LINE 258 *et seqq.*: We would like to see this reference included in Section 1.3.:

"ÅGREN, J., HAMIDJAJA, R. A., HANSEN, T., RUULS, R., THIERRY, S., VIGRE, H., ... & DERZELLE, S. (2013). In silico and in vitro evaluation of PCR-based assays for the detection of Bacillus anthracis chromosomal signature sequences. ***Virulence***, *4(8)*, 671-685." The current overview of PCRs is relatively old. According the mentioned article some of the PCRs also give bad results in in-silico analyses (such as Jackson *et al*. and Ramisse *et al*.).

LINE 62; LINES 103-104:

We have found some discrepancie among the recommended diagnostic techniques:

On the one hand, in Table 1, page 4, line 62, PCR is indicated with the sign (+) for confirmation of clinical cases, which means that it is considered adequate in very few situations. However, capsule demonstration, absence of motility, lysis by phage gamma and penicillin susceptibility are considered recommended methods but with limitations (++).

On the other hand, in the part of "Identification of the agent", in section "1.1 Culture and identification of Bacillus anthracis", and subsection "1.1.1 Fresh specimens", in lines 103-104, it is recommended the confirmation by PCR of isolates in which resistance to phage or penicillin is suspected.

Therefore, it is understood that traditional microbiological techniques have greater limitations than PCR, since the latter could be used as the final confirmation tool.

The following change is proposed in Table 1, page 4, line 62.

Method	Purpose

	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Identification of the agent[(a)]						
Demonstration of capsule	–	–	–	++	–	–
Demonstration of lack of motility	–	–	–	++	–	–
-Gamma phage lysis	–	–	–	++	–	–
Penicillin susceptibility	–	–	–	++	–	–
~~Real-time~~ PCR	–	–	–	++	+++/++	–

Key: +++ = recommended for this purpose; ++ recommended but has limitations; + = suitable in very limited circumstances; – = not appropriate for this purpose. PCR = polymerase chain reaction.
[(a)]A combination of agent identification methods applied on the same clinical sample is recommended.

LINE 205*:* Following should be added:
For combined selection and differentiation, blood agar-based media can provide advantages. In addition, PLET agar contains the toxic thallium acetate. An alternative for this is the so-called TSPB agar, a blood agar that has high selectivity against Gram-negative bacteria due to the addition of trimethoprim, sulfamethoxazole and polymyxin B (Beyer *et al*., 2008). TSPB agar is prepared by dissolving 40 g/ l nutrient agar base (e.g. SIFIN). The mixture is autoclaved and uniformly cooled to 45°C before adding 50 ml/ l sterile sheep blood (5%), trimethoprim (13.1 mg/l), sulfamethoxazole (20 mg/l) and polymyxin B (30,000 IU/l). After mixing thoroughly, the agar is dispensed into Petri dishes.

LINE 281: Following should be added:
Agren *et al*. 2013 published a very comprehensive study on the in silico and in vitro evaluation of 35 PCR-based methods for 20 chromosomal markers of *B*. *anthracis*. The PL3 (target: part of pro-phage type 3) assay (Wielinga *et al*., 2011) was identified as one of the best performing assays in this study and could be used for routine diagnostics.

LINE 287: Following should be added:
Molecular typing techniques for *B*. *anthracis* like canonical single-nucleotide polymorphisms typing (e.g. Van Ert *et al*., 2007; Girault *et al*., 2014) and variable number tandem repeat analysis (e.g. Keim *et al*., 2000; Lista *et al*., 2006; Thierry *et al*., 2014) are appropriate for use in specialised laboratories. Molecular typing based on whole genome sequencing e.g. core genome multilocus sequence typing may be useful to elucidate the diversity of *B*. *anthracis* genotypes circulating, to determine connections between outbreak events and supports infection chain tracing (Abdel-Glil *et al*., 2021).

References to be added: alphabetic

ABDEL-GLIL M.Y., CHIAVERINI A., GAROFOLO G., FASANELLA A., PARISI A., HARMSEN D., JOLLEY K.A., ELSCHNER M.C., TOMASO H., LINDE J. & GALANTE D. (2021). A Whole-Genome-Based Gene-by-Gene Typing System for Standardized High-Resolution Strain Typing of Bacillus anthracis. *J Clin Microbiol*, **59**(7):e0288920. doi: 10.1128/JCM.02889-20

ÅGREN J., HAMIDJAJA R.A., HANSEN T., RUULS R., THIERRY S., VIGRE H., JANSE I., SUNDSTRÖM A., SEGERMAN B., KOENE M., LÖFSTRÖM C., VAN ROTTERDAM B. & DERZELLE S. (2013). In silico and in vitro evaluation of PCR-based assays for the detection of *Bacillus anthracis* chromosomal signature sequences. *Virulence*, **15**, 671-685. doi: 10.4161/viru.26288

BEYER W., BARTLING C. & NEUBAUER H., (2008). *Bacillus anthracis* (Milzbrand). In: Qualitätsstandards in der mikrobiologisch-infektiologischen Diagnostik: Hochpathogene Erreger/Biologische Kampfstoffe. *ELSEVIER*, 38–55.

GIRAULT G., THIERRY S., CHERCHAME E. & DERZELLE S. (2014). Application of High-Throughput Sequencing: Discovery of Informative SNPs to Subtype *Bacillus anthracis*. *J Advances in Bioscience and Biotechnology*, **5**, 7-9. doi: 10.4236/abb.2014.57079.

KEIM P., PRICE L.B., KLEVYTSKA A.M., SMITH K.L., SCHUPP J.M., OKINAKA R., ET AL. (2000). Multiple-locus variable-number tandem repeat analysis reveals genetic relationships within *Bacillus anthracis*. *J Bacteriol*, **182**(10), 2928–36.

LISTA F., FAGGIONI G., VALJEVAC S., CIAMMARUCONI A., VAISSAIRE J., LE DOUJET C., GORGÉ O., DE SANTIS R., CARATTOLI A., CIERVO A., FASANELLA A., ORSINI F., D'AMELIO R., POURCEL C., CASSONE A. & VERGNAUD G. (2006). Genotyping of *Bacillus anthracis* strains based on automated capillary 25-loci multiple locus variable-number tandem repeats analysis. *BMC Microbiol*, **6**, 33.

MARSTON C.K., ALLEN C.A., BEAUDRY J., PRICE E.P., WOLKEN S.R., PEARSON T., KEIM P. & HOFFMASTER A.R. (2011). Molecular epidemiology of anthrax cases associated with recreational use of animal hides and yarn in the United States. *PLoS One*, **6**(12):e28274 10.1371/journal.pone.0028274

WIELINGA P.R., HAMIDJAJA R.A., AGREN J., KNUTSSON R., SEGERMAN B., FRICKER M., EHLING-SCHULZ M., DE GROOT A., BURTON J., BROOKS T., JANSE I. & VAN ROTTERDAM B. (2011). A multiplex real-time PCR for identifying and differentiating *B. anthracis* virulent types. *Int J Food Microbiol*, **145**, Suppl 1:S137-44. doi: 10.1016/j.ijfoodmicro.2010.07.039. Epub 2010 Aug 10. PMID: 20826037.

THIERRY S., TOURTEREL C., LE FLÈCHE P., DERZELLE S., DEKHIL N., MENDY C., COLANERI C., VERGNAUD G. & MADANI N. (2014). Genotyping of French *Bacillus anthracis* Strains Based on 31-Loci Multi Locus VNTR Analysis: Epidemiology, Marker Evaluation, and Update of the Internet Genotype Database. *PLoS One*, **9**(6): e95131 10.1371/journal.pone.0095131

VAN ERT M.N., EASTERDAY W.R., HUYNH L.Y., OKINAKA R.T., HUGH-JONES M.E., RAVEL J., ZANECKI S.R., PEARSON T., SIMONSON T.S., U'REN J.M., KACHUR S.M., LEADEM-DOUGHERTY R.R., RHOTON S.D., ZINSER G., FARLOW J., COKER P.R, SMITH K.L., WANG B., KENEFIC LG., FRASER-LIGGETT C.M., WAGNER D.M. & KEIM P. (2007). Global genetic population structure of *Bacillus anthracis*. *PLoS One*, **2**(5):e461. Epub 2007/05/24. doi: 10.1371/journal.pone.0000461.

3.1.5. Crimean–Congo haemorrhagic fever

General comment:

The EU can in general support this revised chapter. The Summary sections seems a bit long, and could be shortened (especially the first three paragraphs). Specific comments are provided below.

In addition, the fact that Part A of this chapter has been updated should be brought to the attention of the Terrestrial Animal Health Standards Commission, so that the disease and/or case definition in the Terrestrial Code be developed or updated. (This comment is valid for all chapters after such update.)

Finally, regarding the comment below on line 32, and in parallel with comments on the same issue in other chapters, consistency should be sought throughout the Manual (and the Code) in the terminology referring to identification or detection. Indeed, in some chapters of the Manual, it is "Identification of the agent", while in others it is "Detection of the agent" or even "Identification and detection of the agent". In general, identification would be of the agent, while detection would be of antigens, genetic material or antibodies.

Specific comments:

LINES 16-18: This level of detail is not necessary in the Manual. Indeed, such references are quickly outdated, and naming of individual countries should in general be avoided. Instead, a reference to WAHIS could be considered as is the case in other chapters (e.g. "For current information on distribution consult WAHIS").

LINE 32: Please consider replacing "Identification of the agent" with "Detection of the agent" and adapting the rest of the text accordingly (Table 1; title of Section 1).

LINE 50: "order *Bunyvirales*" should be "order *Bunyavirales*" according to the ICTV nomenclature.

LINES 200-202: The sentence starting with "Members of the ……." should be updated according to the ICTV nomenclature.

3.1.18. Rabies (infection with rabies virus and other lyssaviruses)

General comment:

The EU can in general support this revised chapter. However, it considers it inappropriate to already refer in an international standard to tools that are under development, have shown important limitations or need proper validation before being used for a given objective. Such references may indeed be understood as definitive recommendations by WOAH, especially if included in Table 1 with indication of "++ = recommended but has limitations" and "+ = suitable in very limited circumstances". Therefore, the EU suggests that the text on such tools be either deleted or carefully reformulated with the awareness of and clear reference to their limitations.

This is particularly relevant for the messages conveyed on Lateral Flow Devices (LFDs) appear to be confusing and should be clarified to avoid any misuse of these tests. Without a clear mention of a prior compulsory brand/batch validation (whose validation process has been detailed by WOAH), LFDs cannot be recommended as tools able to contribute to eradication policies, confirmation of clinical cases and prevalence of infection – surveillance. This is further detailed in the specific comments below together with a few additional comments.

Specific comments:

LINE 12: Please consider replacing "Identification of the agent" with "Detection of the agent" and adapting the rest of the text accordingly (Table 1; title of Section 1).

LINES 34-35: Please delete the sentence "while non-replicating, nonviable biological or replication-restricted rabies vaccines (for dogs and cats) are still under development". Indeed, while it may be suitable for a review article, it is not appropriate for an international standard (especially in the summary section).

LINE 48: Please provide a reference for this statement ("60,000 human fatalities annually").

LINE 68 (Table 1) and LINES 354-367: It seems that there are some contradictions between the information contained in Table 1 and the text in Section 1.3.3. as regards LFDs. While the text indicates that "Improvement (..) are still required before LFDs can be recommended by the OIE", Table 1 presents LFDs as appropriate methods to contribute to eradication policies, confirmation of clinical cases and prevalence of infection – surveillance.

The indication of "+" ("= suitable in very limited circumstances) or even "++" ("= recommended but has limitations") for these tests in Table 1 could encourage the use of LFDs while many reservations have been expressed in the text about them. At least 13 LFD kits from different brands have been evaluated by the scientific community and presented highly variable performances, as indicated in the text of

Section 1.3.3. While specificity is generally equal to 100% (except 4 studies showing specificity between 93% and 99%), sensitivity is highly heterogeneous depending on the kits. Based on these references, 77% of kit's brands presented sensitivity below 70% and more dramatically, 62% of kit's brands presented sensitivity below 20%. This drastic lack of sensitivity for many brands constitutes a strong risk of misdiagnosis and underestimation of the health situation, or even non-management of bite cases (if considering use for confirmation of clinical cases as indicated in Table 1) which could lead to human deaths.

As rabies is a fatal disease, a false negative result constitutes a greater harm than a false positive result. By consequence, lack of sensitivity in a method should be unacceptable in the risk/benefit assessment of rabies surveillance. For this reason, the EU does not support the inclusion of LFDs in Table 1 at this stage and requests that the corresponding entry be removed. The use of these tests should not be recommended in the OIE Manual before they have undergone appropriate validation in accordance with the Manual standards.

Furthermore, the reference to the OIE Register of diagnostic kits (**LINES 362-363**) seems premature, especially given the current limitations of LFDs described above and therefore inappropriate in the Manual at this stage.

References:

EGGERBAUER E., DE BENEDICTIS P., HOFFMANN B., METTENLEITER T. C., SCHLOTTAU K., NGOEPE E. C., SABETA C. T., FREULING C. M. & MULLER T. (2016). Evaluation of Six Commercially Available Rapid Immunochromatographic Tests for the Diagnosis of Rabies in Brain Material. *PLoS Negl Trop Dis*,10(6): e0004776.

SERVAT A., ROBARDET E. & CLIQUET F. (2019). An inter-laboratory comparison to evaluate the technical performance of rabies diagnosis lateral flow assays. *Journal of Virological Methods*, 272.

KIMITSUKI K., SAITO N., YAMADA K., PARK C. H., INOUE S., SUZUKI M., SAITO-OBATA M., KAMIYA Y., MANALO D. L., DEMETRIA C. S., MANANGGIT M. R., QUIAMBAO B. P. & NISHIZONO A. (2020). Evaluation of the diagnostic accuracy of lateral flow devices as a tool to diagnose rabies in post-mortem animals. *PLoS neglected tropical diseases* 14(11): e0008844.

KLEIN A., FAHRION A., FINKE S., EYNGOR M., NOVAK S., YAKOBSON B., NGOEPE E., PHAHLADIRA B., SABETA C., DE BENEDICTIS P., GOURLAOUEN M., ORCIARI A., YAGER P. A., GIGANTE C. M., KIMBERLY KNOWLES M., FEHLNER-GARDINER C., SERVAT A., CLIQUET F., MARSTON D., MCELHINNEY L. M., JOHNSON T., FOOKS A. R., MÜLLER T. & FREULING C. M. (2020). Further evidence of inadequate quality in lateral flow devices commercially offered for the diagnosis of rabies. *Tropical Medicine and Infectious Disease* 5(1).

SERVAT A. & ROBARDET E. (2021). Technical evaluation of two rapid kits for rabies diagnosis, *European Union Reference Laboratory for Rabies*: 9.

LINES 908-913: The new text proposed should be deleted, as it is not appropriate for the Manual. Indeed, there is no need to refer to vaccines under development and potentially available in the future in an international standard (this is not a review article). Furthermore, recommendations for international trade of animals is the remit of the Code, not the Manual.

LINE 1410: It is suggested to update the reference to "EUROPEAN PHARMACOPOEIA 7.0. (2013a). Monograph 0451: Rabies vaccine (inactivated) for veterinary use. European Directorate for the Quality of Medicines and HealthCare (EDQM), Council of Europe, Strasbourg, France" by a reference to "EUROPEAN PHARMACOPOEIA 10.4. (2021). Monograph 0451: Rabies vaccine (inactivated) for veterinary use. European Directorate for the Quality of Medicines and HealthCare (EDQM), Council of Europe, Strasbourg, France."

LINE 1412: It is suggested to update the reference to "EUROPEAN PHARMACOPOEIA 7.0. (2013b). Monograph 0746: Rabies vaccine (live, oral) for foxes. European Directorate for the Quality of Medicines and HealthCare (EDQM), Council of Europe, Strasbourg, France" by a reference to "EUROPEAN PHARMACOPOEIA 10.2. (2020). Monograph 0746: Rabies vaccine (live, oral) for foxes and raccoon dogs. European Directorate for the Quality of Medicines and HealthCare (EDQM), Council of Europe, Strasbourg, France".

3.1.19. Rift Valley fever (infection with Rift Valley fever virus)

General comment:

The EU can in general support this revised chapter. Specific comments are provided below.

Specific comments:

LINE 128: Please replace “identification” with “detection” in the footnote (a) for consistency with the rest of the text.

LINE 158: ‘Confirmation of virus isolation should be performed preferably by using immunostaining or RT-PCR.’ Sentence can be deleted as it is already mentioned in the section before.

LINES 182 and 204: Instead of detailing the amounts of µl etc., a remark should be added that changes in the PCR program might be necessary or that the program, amount of µl etc. will have to be adjusted according to the manufacturer’s recommendation for the enzyme mix used.

LINES 186, 209 and 234: Please replace ‘to be assayed followed’ by ‘and’.

LINE 244: An alternative qRT-PCR protocol should be added, which targets the L-segment of RVFV in contrast to the protocol adapted from Drosten *et al*., which targets the M-segment. Both assays can be used individually or in combination. Please add the following (avoiding reference to commercial kits and manufacturers name if possible):

Protocol 2 is adapted from Bird *et al*. 2007 (doi: 10.1128/JCM.00936-07) using the Quanti Tect Probe RT-PCR Kit (Qiagen®) test procedure:

a) Prepare a Mix consisting of 12,5 µl 2x QuantiTect Probe RT-PCR Master Mix, 0,25µl QuantiTect RT-Mix and Primer/probe mix and 5 pmol forward primer (5'-TGAAAATTCCTGAGACACATGG-3'), 5 pmol reverse primer (5'-ACTTCCTTGCATCATCTGATG-3') and 0,625 pmol probe (5'-FAM-CAATGTAAGG GGCCTGTGTGGACTTG TG-BHQ1'). Add H_2O bidest to a total volume of 20 µl. Add 5.0µl extracted RNA. In addition to the extracted field samples, a positive control (PC) RNA and a "no template control" (NTC) were added and amplified. After addition of the template RNA and sealing of the reaction tubes, the PCR preparations are briefly centrifuged.

b) Place the plate in a real-time PCR machine for PCR amplification and run the following programme: 50°C for 30 minutes: 1 cycle; 95°C for 15 minutes: 1 cycle; 95°C for 15 seconds, 55°C for 30 seconds, 72°C for 30 seconds: 45 cycles.

A ct-value should be detectable for the PC. The PC ensures the function of the RVFV genome detection system. If no ct-value is detectable for the PC, repeat the

qRT-PCR with a new RVFV mix FAM and/or a new PC. No ct(FAM) value should be detectable for the NTC. If all controls used react in the correct manner, an evaluation of the field sample assays is possible. A suspect sample for RVFV is considered positive in RT-PCR if a ct(FAM) value is detected for the corresponding sample and/or a significant increase in FAM fluorescence above the baseline level is observed.

Reference:

BIRD BH, BAWIEC DA, KSIAZEK TG, SHOEMAKER TR, NICHOL ST. (2007). Highly sensitive and broadly reactive quantitative reverse transcription-PCR assay for high-throughput detection of Rift Valley fever virus. *J Clin Microbiol*, 45(11):3506-13. doi: 10.1128/JCM.00936-07.

LINE 253: This sentence is not very clear. Does this mean that one can be sure that commercially available antisera and controls are properly inactivated or that even in these controls small amounts of infectious viral particles can be expected and therefore it is recommended to inactivate the controls before usage? It should be stated by the manufacturer if the controls are already inactivated or not, shouldn't it?

LINE 257: protocols for virus inactivation should be described in the chapter since it is a necessary step before some of the diagnostic test listed in the chapter, thus the inactivation process should be considered as part of the diagnostic activities.

LINE 270: Polyclonal hyperimmune mouse ascitic fluid (HMAF) or monoclonal antibodies specific for RVF?

LINE 274: "successively down" (please delete the comma).

LINE 291: Stop solution, measurement and wavelength is missing.

LINES 391 and 421: "washed, as in step b above" should be "washed as in step ii) above".

LINE 397: 450 nm or 650nm?

LINE 448: Please delete 'in tubes'.

LINE 475: Since virus neutralization test (VNT) is cited in Table 1 as an alternative to PRNT, its protocol should be described in the chapter.

LINES 496-497: In addition to ruminants and non-human primates, the vaccine has also been successfully tested in alpacas. Alpacas belong to the Camelidae family, which is a highly susceptible group to RVFV and should be included in the text, as follows:

"Master seed and vaccine lots of the MP-12 strain have been generated, and their safety and efficacy have been evaluated in ruminants (Morrill *et al*., 1997), camelidae

(Rissmann *et al*., 2017) and nonhuman primates (Morrill *et al.*, 2003; 2011a; 2011b).”

Reference:

RISSMANN M, ULRICH R, SCHRÖDER C, HAMMERSCHMIDT B, HANKE D, MROZ C, GROSCHUP MH, EIDEN M. (2017) Vaccination of alpacas against Rift Valley fever virus: Safety, immunogenicity and pathogenicity of MP-12 vaccine. *Vaccine*, 35(4):655-662. doi:10.1016/j.vaccine.2016.12.003

3.1.22. Trichinellosis (infection with Trichinella spp.)

General comment:

The EU can in general support this revised chapter. In general, it would be good to include the scientific names for wildlife species in this chapter. Specific comments are provided below.

Specific comments:

LINES 5-7: Suggestion that the first two sentences are changed to: *Eating raw or undercooked meat of domestic animals or game containing infective* Trichinella spp. *larvae can cause trichinellosis in humans. Animals can become infected by feeding on Trichinella-infected tissues.*

LINE 9: Suggestion to change "flesh-eating" to *"*not stricktly herbivorous*"*.

LINE 11: Please consider replacing "Identification of the agent" with "Detection of the agent" and adapting the rest of the text accordingly.

LINE 14: Add spp.: exposure to, Trichinella spp. by tests for specific antibodies.

LINE 16: Suggestion to change "and these muscles" to "which*"*.

LINES 18-19: Suggestion to add "in order of preference*"* after the mentioned predilection sites: "example, in pigs, the diaphragm (crus), masseter and tongue muscles are the two most preferred sites (in order of preference)".

LINES 23-24: Suggestion to add "tissue*"* after muscle: "sedimentation procedures to recover and concentrate any larva that are released from the muscle tissue during digestion."

LINES 26-27: Change "comprimised" to "not optimal": (as well as reduced digestibility of some tissues and frozen or otherwise not optimal wildlife samples).

LINES 27-28: Change "is" to "can be": This can be compensated for by testing larger samples per carcass.

LINE 45: Suggestion to edit the sentence. It reads: **Requirements for vaccines:** There are no suitable vaccines for Trichinella infection in food animals. We suggest the following instead: **Requirements for vaccines:** There are no available vaccines for *Trichinella* infection.

LINE 47: Suggestion to edit the sentence "Trichinellosis in humans is caused by eating raw or undercooked meat from *Trichinella*-infected food animals or game." to "Eating raw or undercooked meat of domestic animals or game containing infective *Trichinella* spp. larvae can cause trichinellosis in humans."

LINE 50: Change "flesh-eating" to "not strictly herbivorous".

LINE 51: Change "infected tissue" to "tissue with infective larvae".

LINE 53: Omit "rapidly".

LINE 54: Omit “During this time, copulation takes place and” so that the sentence reads “The ovo-viviparous females release new-born larvae (NBL)…".

LINE 55: Add “The” in front of “NBL” to not begin a sentence with an abreviation.

LINES 55-57: Suggestion to edit the sentence to: The NBL are distributed throughout the body where they invade striated muscle cells and develop into infective first-stage larvae, with a predilection for specific muscle groups, which vary by host species.

LINE 60: Edit “general” to “most host species” as for some marine mammals, for example, the diaphragm is not believed to be a predilection site.

LINES 63-64: Omit “most” so that the sentence read: In cases of severe infection, voluntary striated muscles contain high numbers of larvae.

LINES 69-70: The origin and the reason for being widespread are detailed information and would require references. Perhaps it can be omitted – key point is that T. spiralis is widespread. The sentence may instead read: *Trichinella spiralis* (T1) has a widespread distribution and is commonly associated with domestic pigs.

LINE 71: Omit “other” as swine, mice or rats are not considered as carnivores.

LINE 72: Suggestion to insert “commonly” and add the reference:

OKSANEN A., KÄRSSIN A., BERG R., KOCH A., JOKELAINEN P., SHARMA R., JENKINS E. & LOGINOVA, O. (2022). Epidemiology of Trichinella in the Arctic and subarctic: A review. *Food and waterborne parasitology*, 28, e00167. https://doi.org/10.1016/j.fawpar.2022.e00167 .

So that the sentence reads: *Trichinella nativa* (T2) occurs commonly in mammalian carnivores of arctic and sub-arctic regions of North America, Europe and Asia (Oksanen *et al*. 2022).

Reasoning to add suggested reference: New reference summarising the epidemiology across Arctic areas, where trichinellosis is of substantial public health importance.

LINES 73-74: Correction needed, this must be removed as this is not true anymore: has been found in an outdoor-reared Mangalica pig in Finland in 2021 (mentioned and original ref. in Oksanen *et al*. 2022). Furthermore, it is not that uncommon in wild boar that it is only “sometimes”: e.g. in Estonia it is found in 0.1% of wild boars.

REF: KÄRSSIN A., HÄKKINEN L., VILEM A., JOKELAINEN P. & LASSEN B. (2021). *Trichinella* spp. in Wild Boars (*Sus scrofa*), Brown Bears (*Ursus arctos*), Eurasian Lynxes (*Lynx lynx*) and Badgers (*Meles meles*) in Estonia, 2007-2014. *Animals*, *11*(1), 183. https://doi.org/10.3390/ani11010183

No need to add this specific reference, it is covered in Oksanen *et al*. 2022 “Infections with *T. nativa,* including mixed infections with *T. nativa* and *T. britovi,* were detected in 0.13% of wild boars in the mainland of the country (Kärssin *et al*., 2021). ”

LINES 72-73: Suggestion to edit the sentence so that it reads: It is highly resistant to freezing, and has been experimentally shown to have poor infectivity for pigs and horses, however it has been found in wild boar from sub-arctic regions.

LINES 99-100: Suggestion to edit sentence so it reads: The disease is transmitted primarily by eating meat of infected pigs or game that has not been sufficiently cooked or otherwise treated to inactivate the parasite.

LINE 105: Change “food animals” to “animals raised for human consumption” and “meat” to “tissues”.

LINE 106: Wild boar meat is a very relevant source, and wild boars are not carnivores, they are omnivores. Beavers have appeared as a potential source in Europe; apparently they are not strict herbivores. Therefore, suggestion to edit sentence to: Meats from not strictly herbivorous game species should always be considered a potential source of infection, and should be tested or properly cooked.

LINES 109-112: Suggestion to edit sentence to: Overall, *Trichinella* parasites circulate mainly among wild animals; among domestic animals, they typically infect free-ranging and backyard pigs and rarely horses; meat of Trichinella-infected horses and game have been implicated also in outbreaks linked to international trade.

LINE 122: Add “or other suitable process” so that the sentence reads: ... should be cleaned with water at ≥85°C or other suitable processes to lyse and remove all larvae.

LINE 127: In table 1: 1) suggestion to edit “identification of the agent” to “Detection and identification of the agent”, 2) change “Enzymatic digestion” to “Artificial digestion” as the ICT uses the expression “Artificial digestion method” it would be good to use the same method in this document – also generally in the text, 3) suggest to only have “PCR” instead of “Multiplex PCR”, because it can also be e.g. PCR and sequencing; multiplex is often used for species-level determination. Add an extra + to the PCR suitability for “Prevalence of infection – surveillance" as species-level information is relevant for surveillance purposes, 4) edit the text for (b) to: (b) PCR is used as confirmatory test and species-determination.

LINE 148: Change “direct testing method” to “artificial digestion method”. Reason for edit suggestion: It appears that this text is about artificial digestion method(s), and it would be good to specify it already here.

LINE 149: Edit suggestion: change “will” to “can”; “Direct methods can identify...”.

LINE 161: Omit “of this test” so it reads; “...the sensitivity is further increased.”

LINE 174: Suggestion to edit “For food safety testing...” to “For testing for food safety purposes...”.

LINE 183: Omit “infected” as “infected meat” is not a good expression, because a piece of meat cannot really acquire the infection actively itself. Better expression is e.g. meat from/of infected animal, or meat with infective larvae.

LINE 194: Tap water preheated to 46 - 48°C.

LINES 224 and 227: Digestion mix should operate at 44 - 46°C.

LINE 240: Sieve aperture size 180µm.

LINE 203: We propose to include the following in Section 1.1.4. iii):

"Remove as much fat and fascia as possible from each sample of meat, as well as the epithelium of the tongue when that sample is used."

LINE 255: Suggestion to edit sentence to: "Gentle tapping of the funnel wall [e.g. every 10 minutes] can be used to facilitate the larvae settling to the bottom of the funnel.

LINE 266: 15 -20x for microscopic examination.

LINE 269: 60 – 100x for microscopic examination.

LINES 314-327: Overall quite a lot of details; text can be shortened. Edit suggestion: This assay is ~~recommended as~~ an alternative to the commonly used digestion procedure described above~~, and is approved by the EU for export use~~. The method has been validated for use on pork and horsemeat (Forbes & Gajadhar, 1999; Forbes *et al.*, 2008). It includes a spin-bar digestion technique and sequential separatory funnels for sedimentation of the larvae. An incubation chamber equipped with transparent glass doors and set at 45°C is used to perform the digestion. The digestion is conducted in 3 litres of digest fluid on a magnetic stirrer. Following digestion, the suspension is poured into a 4 litre separatory funnel through a 177–180 µm sieve, which is rinsed thoroughly into the separatory funnel with tap water. The suspension is allowed to settle for 30 minutes, and the bottom 125 ml ~~is drained~~ then dispensed directly into a 500 ml separatory funnel. The volume in this smaller separatory funnel is increased to 500 ml by adding tap water, and the resultant suspension is~~is~~ allowed to settle for ~~an additional~~ 10 minutes. Finally, 22–27 ml of sediment is ~~drained~~ dispensed into a Petri dish and observed for larvae as described for artificial digestion method.

LINE 340: "not recommended" in bold font, because bold font is used for a similar statement later.

LINES 364-370: This part is best without details, as this method should not be used. Edit suggestion: This method ~~involves~~ entails the compression of pieces of muscle tissue between two glass plates until they become translucent, followed by examination using a microscopic technique. There are good comparative data available indicating that trichinoscopy is not as sensitive as digestion assays (~~Forbes *et al.*, 2003;~~ Gajadhar *et al.,* 2009). Trichinoscopy is **not recommended** by the ICT, EU or WOAH for the routine examination of carcasses (European Commission, 2015/1375; http://www.trichinellosis.org/).

LINES 409-410: Add "and moose (*Alces alces*) and reference to the list: ...for wild boar, dogs, and moose (*Alces alces*) (Bruschi *et al*., 2019; Kärssin *et al*., 2021).

KÄRSSIN A., REMES N., KORGE K., VIIGIPUU M., STENSVOLD C. R., GÓMEZ-MORALES M. A., LUDOVISI A., JOKELAINEN P. & LASSEN B. (2021). Herbivores as accidental hosts for trichinella: search for evidence of trichinella infection and exposure in free-ranging

moose (*Alces alces*) in a highly endemic setting. *Journal of wildlife diseases*, *57*(1), 116–124. https://doi.org/10.7589/JWD-D-19-00011

Reasoning for suggested new reference to include: not covered in Bruschi *et al*., 2019.

LINE 413: The word “slaughter” is not suitable for hunted game, therefore it is suggested to omit “collected before or after slaughter”.

LINE 489: Suggestion to edit sentence to: There are no vaccines available for prevention of *Trichinella* infections.

LINE 605: Update website to: https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3

3.2.2. American foulbrood of honey bees (infection of honey bees with *Paenibacillus larvae*)

General comment:

The EU can in general support this revised chapter. Specific comments are provided below.

Specific comments:

LINES 42-44: Some comments as to where the new ERIC V belongs, *P. l. larvae* or the former subspecies *P. l. pulvifaciens*. If that is not known then which ERIC type that ERIC V it is mostly related to.

LINES 109-111: Here the virulence of ERIC V type should be mentioned (Beims *et al*., 2020).

LINE 54: Delete "," and "was": "Genotype ERIC V was recently was isolated from a Spanish multi-flower honey sample."

LINE 116 (Table 1): Please replace "Identification of the agent" with "Detection of the agent", for consistency with line 20 and consider adapting the rest of the text accordingly (e.g. footnote (a) in **LINE 119**; title of Section 1 in **LINE 121**)

LINE 134: Add "," and add "of": "Alternatively, the brood may be sampled by cutting out a piece of comb of about 20 cm^2 in size,…"

LINE 184: Change "comb" to plural: "Larval/pupal remains from brood combs are collected with…"

LINES 188-189: Temperature is missing "Plates are incubated in 5–7% CO2 and examined daily for up to seven days.":

Comment: As mentioned in the manuscript, *P. larvae* is grown at 37±1°C. Growth can be enhanced by cultivation in atmosphere with 5–10% CO2.

LINE 202: Replace "+" with "±": "…at 37±1°C for…"

LINE 237: Consider rewriting "forms" into "is formed": After 2 minutes standing time, a deposit of a watery solution containing *P. larvae* spores is formed (Ritter, 2003).

LINES 250 and 255: Consider adding "the": "While warming the sealed tube in the water bath,…" "…at the bottom of the tubes."

LINE 306: Consider rephrasing "is as above" to "as mentioned above": "Addition of nalidixic acid and pipemidic acid as mentioned above.

LINE 312: Comment: To our knowledge not only MYPGP agar is routinely used to cultivate *P. larvae*. Also the other mentioned agars are used routinely, especially CSA.

LINE 321: Replace “+” with “±”: “…at 37±1°C for…”

LINE 471: Delete “space” in between value and unit: “…flood with 0.5% safranin…”

LINES 535-536: Table in-between LINE 535 and 536: Why the PCR product size from Dobbelaere *et al*., 2001 is changed from 1106 bp to 1096 bp? 1106 bp is exactly what is written in the respective reference.

LINE 551: Comment: Recently it has been reported that the offspring of *P. larvae* bacterin treated honey bee queens were less susceptible to an infection of American Foulbrood (Dickel *et al*. 2022, https://doi.org/10.3389/fvets.2022.946237). This might be considered a first step for an oral vaccination method of honeybees against *P. larvae*.

LINES 606-608: We would prefer not to delete the reference “Genersch *et al*. 2006” (here and everywhere it is mentioned in the text), as ERIC genotyping of *P. larvae* was initiated on the basis of this paper.

3.2.3. European foulbrood of honey bees (infection of honey bees with *Melissococcus plutonius*)

General comment:

The EU can in general support this revised chapter. Specific comments are provided below.

Specific comments:

LINE 40: Please replace “few” with “little”.

LINE 45: Consider replacing “Epizootiology” with “Epidemiology”.

LINES 59-60: Check for the right tense in text of Fig. 2: “become” instead of “became”; “transform” instead of “transformed”.

LINE 82 (Table 1): Please replace “Identification of the agent” with “Detection of the agent”, for consistency with line 13 and consider adapting the rest of the text accordingly (e.g. footnote (a) in **LINE 85**; title of Section 1 in **LINE 86**)

LINES 144-146: We would suggest writing the declaration of weight before the ingredient name.

LINE 248: The publication of Dainat *et al*. (2018) could be added as well as in the references: Dainat B., Grossar D., Ecoffey B., Haldemann C.2018. Triplex real-time PCR method for the qualitative detection of European and American foulbrood in honeybee. J. Microbiol. Methods 146: 61–63. doi:10.1016/j.mimet.2018.01.018

3.3.10. Fowlpox

General comment:

The EU can in general support this revised chapter. A specific comment is provided below.

Specific comments:

LINES 127-128: “Diagnostic PCR primers used for detection and phylogenetic analyses of fowlpox virus DNA target conserved regions of the major core protein, P4b”: Verb is missing.

3.3.13. Marek's disease

General comment:

The EU can in general support this revised chapter. Specific comments are provided below.

Specific comments:

Introduction: No mentioning of the acute cytolytic form (early mortality syndrome) in very young birds (10-14 days of age).

LINE 64: It could be useful to add the age group of this manifestation form (5-18 weeks of age).

LINE 126: Peripheral neuropathy – a reference for the increasing incidence in Europe would be appropriated.

LINE 139: Please replace "identification" with "detection" in the footnote (a) for consistency with the rest of the text.

LINE 465: This information is not relevant for an international standard. Consider deleting the whole Section 2.3.4. if this is the only information to be provided in it.

LINE 476: Please replace "registration/licensing procedure" with "regulatory approval" (for consistency).

3.4.12. Lumpy skin disease

General comment:

The EU can in general support this revised chapter. Specific comments are provided below.

Specific comments:

LINE 17: Literal mistake: plural of sequestrum is sequestra, not sequestrae

LINE 43: "associated with an outbreak of sheep pox": associated implies that LSD and SP have some connection; we would prefer: "at the same time as an outbreak"

LINE 130: Correct: "10% neutral buffered formaldehyde", instead of "formal saline".

LINES 138-152: Furthermore, two techniques are always mixed: histology and virus isolation. This should be sorted, because someone could think that biorisk requirements are not necessary in virus isolation technique.

LINES 220 and 222: Suggest putting quotation marks for DIVA here as well, same as in line 218.

LINE 221: Please insert "recently" before "isolated from disease outbreaks in Asia"

LINES 242 and 250: "virions of Capripoxvirus" or "Capripoxvirus virions"? – to use "Capripox virions" would be enough, or "Capripoxvirus particles".

3.6.11. Glanders and melioidosis

General comment:

The EU can in general support this revised chapter.

Multiple lines: Refer to *Burkholderia mallei* and *Burkholderia pseudomallei* fully only once in the introduction and main text, then follow though as *B. mallei* and *B. pseudomallei*.

Multiple lines: Spell all numbers one to ten, use numbers for anything >=11, and insert a hyphen after use of 'non' as a prefix, for example non-motile.

Multiple lines: PCR is proposed as a "+++" test method, with isolation as a "+" test method, for the confirmation of clinical cases (line 116). However, this hierarchy is not reflected further in the text, where it would follow that PCR should therefore be mentioned first as the more preferable option (line 126), with isolation ideally presented as the second-choice option. In the PCR method section (line 226), the limitations of molecular assays are discussed- including the need for further validation and possibility of false negatives- which would not seem to support the "+++" test method designation, rather a "++" designation.

Specific comments are provided below.

Specific comments:

LINE 6: The full stop is missing after "for spread".

LINE 7: Clearly state glanders is a zoonosis in the introduction.

LINES 8-14: Divide into two paragraphs- one being the meilodosis introduction, the other being the chapter scope.

LINE 10: Unlike B.mallei, B.pseudomallei -> B. mallei, B. pseudomallei (spaces between "." and "mallei/pseudomallei" missing)

LINE 11: Please add the word "on" (This chapter focuses on ...).

LINE 30: Typographical error : "disagnosis"

LINE 43: Clearly state that glanders 'primarily' (i.e. not solely) affects perissodactyls or odd-toed ungulates, as the other species in which it may cause disease are also listed below.

LINES 102: Dodin (1992) is a difficult to access text, and we were unable to cross-check the reference.

LINE 104: Sprague and Neubauer (2004) did not only base their recommendations on Dodin (1992), therefore removal of the subsequent 'consequently' would clarify.

LINE 136: Please change title to "2.1.Morphology of *Burkholderia mallei* and *B. pseudomallei*".

LINE 179: Full stop is missing at the end of sentence (after "(Sprague & Neubauer, 2004)").

LINE 242: Typographical error ("Fresh tissues can be ...)

LINES 252-255: Please add either manufacturer to the master mix or add a remark that the PCR program depends on the kit's manufacturer and that the program might need adaptation when kits from another manufacturer than stated are used. Or keep the whole protocol more general (amount of master mix/denaturation step/..depends on the polymerase used) or add citation (Scholz *et al*. 2006) after protocol/PCR conditions.

LINES 270-272: Same as above.

LINE 317: Please include camels in the title of the section ("... in horses, donkeys, camels and mules").

LINES 309-311: We suggest replacing the last sentence of Section 2.4. with the following:

"Molecular typing based on whole genome sequencing contributes to elucidate the diversity of *B. mallei* genotypes circulating, to determine connections between outbreak events and supports infection chain tracing. (Apelt *et al*., 2022; Brangsch *et al*., 2022; Gilling *et al*., 2014; McRobb *et al*., 2015; Price *et al*., 2015)."

Reference to be added:

BRANGSCH H., SAQIB M., SIAL A.U.R., MELZER F., LINDE J. & ELSCHNER M.C. (2022). Sequencing-Based Genotyping of Pakistani *Burkholderia mallei* Strains: A Useful Way for Investigating Glanders Outbreaks. *Pathogens*, **11**, 614. https://doi.org/10.3390/pathogens11060614

3.7.2. Rabbit haemorrhagic disease

General comment:

The EU can in general support this revised chapter.

We thank WOAH for the successful revision of the chapter and acknowledge that the revision has increased the clarity of the chapter, which is now up to date and well adapted to the current epidemic situation.

The nomenclature should be adapted to the proposed ICTV taxonomic profile (e.g. GI.1 and GI.2 or GII.1).

Specific comments are provided below.

Specific comments:

LINE 36: Please replace "available on the market" by "commercially available".

LINE 65: As it is the only reported case in large mammals, we would suggest rephrasing: "the only reported case in large mammals has been in alpine musk deer (*Moschus sifanicus*) in China (People's Rep. of)…"

LINE 70: Please add "infection" in: "RHDV2 infection has also been reported in badgers (*Meles meles*) in Spain."

LINE 99 (Table 1): Please take the following comments into account:

- HA: replace "+" by "++"
- Detection of immune response is not suitable for confirmation of clinical cases

LINE 194: It should be clearly stated that real time RT-PCR is the first choice for PCR-diagnostics to manage the high contamination risk

LINE 286: It should be noted that the diagnostic ELISAs are VP60 based and are therefore not able to discriminate vaccinated from infected animals without definition of Ig classes and titers.

LINES 732-733: The sentence is poorly written and is thus unclear. Please consider revision by a native speaker.

LINE 752: Recombinant Myxoma and Baculovirus vaccines which can be produced in a way that increases animal welfare, have been approved and are commercially distributed:…..

LINE 757: Suggestion to replace "2-3 weeks" by "approximately 3 weeks".

3.9.7. Influenza A virus of swine

General comment:

The EU can in general support this revised chapter.

We do not support changing the abbreviation from "IAV-S" to "swIAV-s", as this changes the connotation: indeed, there is no "swine influenza" or "swine influenza viruses", only "influenza of swine" and "influenza viruses of swine" (as implied by the title of the chapter). Furthermore, use of the abbreviation "swIAV-s" seems overly complex and is highly confusing.

Furthermore, we do not support changing to the name to "swine influenza A viruses" within the text. Indeed, "influenza A viruses of swine" should be kept throughout the chapter, for consistency with the title of the chapter and to avoid confusion.

Specific comments are provided below.

Specific comments:

LINE 12: Please consider replacing "Identification of the agent" with "Detection of the agent" and adapting the rest of the text accordingly (Table 1; title of Section 1).

LINE 61: rectal temperature – 'rectal' can be deleted.

LINE 81: anthropozoonoses – we're not sure if this wording exists in the English language – if so, please ignore this comment, if not: probably use 'zoonotic disease with two-way (or reverse) transmission' instead.

LINE 89: simultaneously and the virus: 'and' should be deleted.

LINE 114: add e.g. because it is not always possible to ensure the medium used for sampling; ie or "e.g. phosphate-buffered saline (PBS), supplemented with antibiotics".

LINE 121: add e.g.: are centrifuged e.g. at 1500–1900 g for 15–30 minutes at 4°C.

LINE 186: delete "at 1500–1900 g for 10–20 minutes at 4°C", eggs fluids are harvested by a syringe, we never centrifuge afterwards, so I wonder if it a mistake in the text that centrifugation is mentioned here.

LINE 221: We do not support the change of the title of Section 1.2. which would be very confusing. This needs to be reverted to "influenza A viruses of swine", for consistency with the title of the chapter.

LINE 310: superior to lysine-coated slides by some): delete 'by some'.

LINES 336, 341 and 343: Replace "swine influenza A viruses" with "influenza A viruses of swine".

LINE 361: Replace "cDNA product" with "cDNA or PCR product".

LINES 372-373 and 416-419: It is our experience that due to the high diversity of swine HA and NA gene sequences, it can be difficult to use real-time RT-PCR to discriminate between subtypes and especially to discriminate between different lineages within a subtype. Therefore, sequencing is often more precise. This issue should be made clear in these text sections.

3.10.1. Bunyaviral diseases of animals (excluding RVF fever and Crimean–Congo haemorrhagic fever)

General comment:

The EU can in general support this revised chapter. Specific comments are provided below.

Specific comments:

LINE 29: Replace “polymerase chain reaction (PCR)” with “reverse-transcription polymerase chain reaction (RT-PCR)”.

LINE 32: Replace “real-time reverse-transcription PCR (RT-PCR)” with “real-time RT-PCR”.

LINE 141: Use “detected” rather than “isolated”.

LINE 143: Replace “real-time reverse-transcription polymerase chain reaction (RT-PCR)” with “real-time RT-PCR”.

LINE 204 (Tables): Please replace “PCR” with “RT-PCR” (reverse-transcription polymerase chain reaction) in Tables 1.2. and 1.3. (incl. in the relevant footnotes).

LINES 304-305: The sentence “There are currently no available kits for detecting the virus” should be modified to ”There are currently no commercial kits available for detecting the virus”.