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 2015

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

SUMMARY:

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

CHAPTER 1.1.7.: Tests of biological materials for sterility and freedom from contamination

General comments

The EU cannot at this stage support this revised chapter. Indeed, although covering general procedures reasonably comprehensively, we find the inclusion of only a small number of specific examples (e.g. *Brucella*, *Mycoplasma mycoides*, *Babesia*, *Theileria*, PEDV) rather strange because it may give the impression that these are the only organisms that need special consideration.

Furthermore, general tests for viral contaminants are described in some detail but only one example of a specific test is given, whereas many other viruses require specific tests for their detection. This is in no way a comprehensive document and in view of the general nature of this chapter it might be best to avoid detailed descriptions of specific methods and give more emphasis to the different approaches needed for different types of materials and different agents.

For example, more attention could be given to the choice of appropriate cell lines for general viral tests and also to the application of immunochemical staining or other methods to detect specific viruses in cell cultures. The cross-reference to other chapters in the manual would be more valuable that the limited specific examples given.

In addition, PCR methods are increasingly being used to test seed lots for viral contamination. While mentioned in passing, it is surprising that the benefits and risks associated with these are not covered in more detail.

Specific comments

LINE 6: There is no definition of biological material in this chapter, nor in the glossary of the Terrestrial Manual. The EU therefore suggests elaborating such a definition, to prevent possible confusion as to the scope of this chapter (i.e. overlap with Terrestrial Code and animal origin commodities traded internationally as food).

LINES 17-18: The EU would prefer a reference to disease agents included on the OIE list of diseases, instead of "agents recognised in this Terrestrial Manual".

LINE 19: The definition of sterility should be aligned to the one included in the glossary of the Terrestrial Manual.

LINES 43-46: The EU requests the reference to the US Code of Federal Regulations be deleted, as those are national rules that are neither necessary nor relevant in an international OIE standard.

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LINES 46-47: The reference to the further reading should be moved to the end of the paragraph, i.e. after the references to Article 1.1.1. and 1.1.2.

LINE 55: The word "vaccine" should be deleted, as biological materials other than vaccines would also need to be covered by this recommendation.

LINE 69: It should be made clear in the text (e.g. by adding the words "for example") that the reference to the US Code of Federal Regulations is only given as an example.

LINES 88-90: The sentence is problematic, as in an international OIE standard there should not be recommendations on the procedure of how to agree on replacing old tests with new ones (especially the words "regulators and industry need to agree" should be deleted).

LINES 122-123: Inactivation studies on representative extraneous agents may not be necessary if the virus seed has been tested and shown to be free from extraneous agents.

LINES 124-125: Virus titration tests may not have sufficient sensitivity to ensure complete inactivation. Therefore a specific innocuity test, developed and validated for increased sensitivity, may be required. Ideally a second passage should be used to increase sensitivity.

LINES 141-142: The sentence referring to the US CFR should be deleted, as such a reference to a national regulation is inappropriate in an international OIE standard.

LINE 146: reference to section D is simplistic. It should not be necessary to test for extraneous viruses which would not grow in bacteriological culture media as long as freedom from contamination of all starting materials can be assured. Complete inactivation of the vaccinal bacteria should be demonstrated by means of titration and innocuity tests – in some cases general bacterial sterility testing (section 2.1) may suffice.

LINE 212: Use of the word "recently" seems inappropriate here, as the reference is from 2005.

LINE 232: Please add the following: "If the material being tested renders the medium turbid so that the presence or absence of microbial growth cannot be readily determined by visual examination, 14 days after the beginning of incubation transfer portions (each not less than 1 mL) of the medium to fresh vessels of the same medium and then incubate the original and transfer vessels for not less than 4 days."

LINES 275-277: This should only apply to frozen or freeze-dried vaccines. Liquid vaccines should be sterile because contaminating organisms could multiply during storage, thereby exceeding the limits indicated for batch release.

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LINES 278-279: Each batch of final container biological should have an average contamination of not more than one non-pathogenic bacterial or fungal colony per dose.

LINES 279-280 and 291-292: The maximum of one non-pathogenic organism per dose should apply for all species.

LINES 352-388: Reference could be made to VICH GL34.

LINES 425-428: It should be made clear in the text that the Australian procedures referenced here are to be regarded as an example only.

LINES 520-522: Reference to a commercial product is inappropriate in the OIE Terrestrial Manual, and should therefore be deleted.

LINES 621-680: The appendix is confusing. Indeed, it seems to indicate that Chapter 1.1.7. concerns vaccines only, as it starts by defining "biologicals" for the purposes of this chapter which exclude vaccines. Furthermore, the purpose of the categorisation section is unclear. Indeed, it neither says how to categorise products, nor for what purpose. Finally, it is very general and therefore of little added value. The EU thus suggests deleting it altogether.

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CHAPTER 1.1.8.: Minimum requirements for the organisational management of a vaccine manufacturing facility

General comments

The EU can in general support this new chapter. However, the scope of this chapter is not very clear. The title suggests "organisational management", whereas section 2 concerns requirements for premises and equipment. In addition, the title in LINES 2-4 does not correspond to the title as given in the header of the chapter. There also seem to be overlaps with Chapter 1.1.6. "*Principles of veterinary vaccine production*", which includes sections on production Facilities, documentation, process validation, which are also covered in this draft chapter. These overlaps need to be addressed before the adoption of this new chapter can be considered, by revising Chapter 1.1.6. which could be simplified accordingly. In addition, the new Chapters 1.1.8. and 1.1.9., which both address vaccine production, should be better coordinated.

Furthermore, the summary is very poor and should be expanded to adequately describe the scope and content of the chapter. The sentence "*National authorities should ensure that the standard is applied by manufacturers in their country*." (LINE 7) should be deleted, as issues of implementation of and compliance with OIE standards by national authorities should not be addressed within the Terrestrial Manual.

Specific comments

LINES 281-283: Ideally the sentence "It may be acceptable for connections [...] and there is no risk of leakage" should be deleted as this represents an old approach which is no longer an industry standard practice. It is acknowledged that this statement is taken from the current PIC/S GMP guide referenced in the document; however, it is expected that it will be removed at the next revision of the relevant section. No additional wording needs to be substituted.

LINE 480: It is unclear what is meant by "as interpreted in this chapter" and should therefore preferably be deleted.

LINES 564-565: It is suggested that the statement in parentheses regarding addresses, phone and/or fax numbers also makes reference to email as this may be a valid means of communication under these circumstances.

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CHAPTER 1.1.9.: Minimum requirements for the production and quality control of vaccines

General comments

The EU can in general support this new chapter. However, the scope of this chapter is not very clear and there seem to be significant overlaps with Chapter 1.1.6. "*Principles of veterinary vaccine production*", which includes sections on quality controls such as batch/serial testing that are also covered in this new draft chapter. These overlaps need to be addressed before the adoption of this new chapter can be considered, by revising Chapter 1.1.6. which could be simplified accordingly. In addition, the new Chapters 1.1.8. and 1.1.9., which both address vaccine production, should be better coordinated.

Furthermore, the summary is very poor and should be expanded to adequately describe the scope and content of the chapter. The sentence "*National authorities should ensure that the standard is applied by manufacturers in their country*." (LINE 7) should be deleted, as issues of implementation of and compliance with OIE standards by national authorities should not be addressed within the Terrestrial Manual.

Finally, the Appendix 1.1.9.1. is very detailed; it should be considered to turn it into a separate chapter.

Specific comments

LINES 176-178: The statement regarding embryonated eggs that "In almost all cases they should be derived from SPF chicken flocks [...]" is not accurate as a significant number of inactivated vaccine antigens are produced in eggs from healthy flocks. The EU therefore suggests amending the sentence to read as follows: "They should be derived from SPF chicken flocks [...] not been vaccinated or where justified (e.g. for use in production of some inactivated vaccines) and in line with the Marketing authorisation, from healthy chicken flocks".

LINES 199-200: The sentence "In other circumstances, chemical disinfection [...]" should be deleted as this is not representative of current practice / expectations. No additional text is required at this point.

LINE 359: The current expectation is that "Reserve" samples should be kept for a minimum of 12 months after the expiry date of the product and not 6 months as stated. This is in line with requirements in the PIC/S GMP Guide.

LINES 363-369: This paragraph is a repeat of the previous one (LINES 356-362) and can therefore be deleted.

LINES 398-408: This section repeats verbatim text in LINES 288-295 and 370-371 and therefore can be deleted.

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LINE 410: The statement that each batch/serial must be tested for safety is incorrect. For most veterinary vaccines the European Pharmacopoeia requirement for batch safety testing has been removed. As this document presents minimum standards, the EU suggests removing "safety" from the list.

LINES 446-456: As per comment above the statements regarding batch safety testing should be removed or at least the fact that they are not a requirement in some regions should be highlighted.

LINE 868: There has been a minor error in transcription of this paragraph from the PIC/S GMP guide. Where it is stated that "*Filled containers of parenteral products could be* [...].", the word "could" should be replaced by the word "should".

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CHAPTER 1.1.10.: Vaccine banks

General comments

The EU can in general support this revised chapter, however has some important comments that would need to be addressed before adoption of this modified chapter.

It seems unclear why the introduction section includes detailed definitions of the types of vaccination and recommendations on disease control strategies, which are clearly not in the scope of this chapter on vaccine banks. Indeed, the introduction consists mostly of definitions of vaccination strategies, which are rather out of place in this Manual chapter. Furthermore, it is understood that the OIE Scientific, Code and Biological Standards Commissions have jointly started working on a horizontal chapter on vaccination and disease control, including definitions of types of vaccination, to be included in the Terrestrial Code. Therefore, LINES 9 to 49 of the present draft revised chapter seem to anticipate that work. As they are not necessary for the purposes of the Manual, the EU therefore requests them to be deleted. Especially the text in LINES 16-25 is unacceptable in the context of the OIE Manual (starting with "*Emergency vaccination is one of several measures that may be deployed to control outbreaks* [...]"), as these are disease control recommendations which are out of the scope of the Manual.

Furthermore, instead of starting with an "introduction", the first section of the chapter should be a "summary" summarising the scope and contents of the chapter, in line with established Terrestrial Manual practice. Instead of focusing on types of vaccination or vaccination strategies, that summary should focus on vaccine banks.

Specific comments

LINE 7: The parenthesis" (defined in Section A)" is confusing, as it is not clear to what it refers. Indeed, Section A defines "vaccine banks", not "the context of this chapter", nor "vaccination strategies". Therefore, the parenthesis should be deleted.

LINES 9-49: For the reasons stated above, the text starting from "Types of vaccination" should be deleted, and replaced by a summary of the chapter focusing on types of vaccine banks.

LINES 51-52: The definition of vaccine bank as proposed is odd. Instead of defining vaccine bank, the terms "antigen or vaccine reserves" are defined. Furthermore, it does not seem necessary to restrict the definition to this chapter (i.e. the words "For the purposes of this chapter" should be deleted). The following alternative is suggested: "Vaccine banks are defined as antigen or vaccine reserves, which can be of different types".

LINES 55-56: The EU suggests deleting the last part of the sentence (", which will be described in [...]"), as this internal cross reference is not necessary.

LINE 59: The EU suggests replacing the word "members" by the word "countries", as it unclear what is meant by members in this context. Similarly, in LINE 63, the word “members” should be replaced by "owners".

LINES 66-67: The sentence starting with "However, [...]" should be deleted, as this information does not accurately reflect the situation in all countries. Indeed, in many countries derogations exist for emergency vaccination. Furthermore, the previous sentence (starting with "In the latter case, [...]") adequately covers the intended reservation (licensing and pharmaceutical responsibility of the dedicated facility of the vaccine bank).

LINE 69: The word "usually" seems superfluous in the context of this sentence.

LINE 81: Please remove comma between "antigens" and "are".

LINES 82-83: Please replace the words "long storage time" by the words "long shelf-life".

LINES 84-85: It is unclear what is meant by "the flexibility in antigen payload". It is not a common term and needs to be revised.

LINE 87: It is not clear what emergency situation is described in the Terrestrial Manual chapter 2.1.5. The EU suggests replacing the reference to that Manual chapter by the following example: "(for example an outbreak of Foot-and-Mouth disease in a country previously free of that disease)".

LINE 88-89: Similarly, the reference to Terrestrial Code Chapter 3.4. seems unclear, and should thus be deleted. The reference to the relevant horizontal Manual standard on vaccines (Chapter 1.1.6.) seems adequate in this context.

LINE 104: The EU suggests adding the following sentence at the end of the paragraph: "Alternatively, stocks of antigen or ready-to-use formulated vaccines could be rotated and replenished to ensure that there is a continuous supply of product within shelf-life".

LINE 119: The EU suggests replacing "The world as [...]" by "The world is [...]".

LINE 137: The words "(as defined in the introduction)" should be deleted.

LINE 175: It is unclear what is meant by "members" in this context. This should be explained (e.g. by replacing "members" by "members of a vaccine bank consortium" or simply by "countries").

LINE 182: The reference to the Code Chapter 3.4. seems odd. Indeed, that chapter deals with standards for national veterinary legislation. Here what is intended is to say that vaccines should be licenced according to national legislation, which in turn is compliant with the minimum standards of the Code

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Chapter 3.4. In general, references to Code chapters should be by title and not by number, as these are likely to change in the future. The EU therefore suggests the following alternative wording:
"Vaccines also should be appropriately licenced according to national veterinary legislation, in consideration of OIE standards such as the Terrestrial Code chapter on veterinary legislation".

LINES 188-189: This sentence is poorly drafted. The following alternative wording is suggested:
"For diseases for which there is official recognition of disease status by the OIE, vaccines used in the member countries concerned need to comply with the standards of the OIE Terrestrial Manual".

LINE 247: To avoid unnecessary repetition, we suggest deleting the words ", such as the OIE" from the title. Indeed, it is not usual to include such examples in the title, and these exact words are already included in the first sentence of the first paragraph of that section (LINE 248). For the same reason, repeating the mention of OIE in line 254 is equally unnecessary. Thus, the words ", including the OIE" should be deleted from **LINE 254**.

LINE 259: the words "for the purposes of this chapter" should be deleted, as explained in the EU comment above re. LINES 51-52.

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CHAPTER 1.1.11.: Standards for high throughput sequencing, bioinformatics and computational genomics

General comments

The EU can in general support this new chapter and has a few specific comments.
In general terms, the use of "must" should be reviewed throughout this chapter, and be replaced by "should" as appropriate (see specific comments below).

Specific comments

LINE 7: The EU suggests replacing the word "must" by the word "should", as is established practice in OIE standards.

LINE 21: Please replace the word "just" by "only" (style).

LINE 22: Please add "after suitable bioinformatics procedures have been developed" since the sequencing now already enables the generation of sufficient datasets for bacterial/parasitic whole genome analysis but sufficient data analysis tools and strategies are not available.

LINE 34: Please replace the word "normal" by "standard" (style).

LINE 75: Please add to the given reference a more recent overview on the available systems since the information given in the cited review is in part outdated. For instance, the 454 platform will be no longer supported from mid-2016 and the Minion is now available, although currently only in an early access program. Moreover, Illumina, Life Technologies, and Pacific Biosciences introduced new machines with different specifications.

LINES 101-102: Please add a precautionary note on target enrichment. In the article "Metagenomic approaches to identify infectious agents" to appear in Vol. 35 (1), April 2016 of the special issue of the OIE Scientific and Technical Review it is pointed out that host depletion and target enrichment strategies bear a high risk of information loss.

LINES 117-121: The definitive requirement for controls in this section needs tempering. Given that the sensitivities for these methods are continually improving, finding appropriate negative controls may become challenging. The issue of cross-contamination (possible linked to multiplexing suggests that inclusion of a positive control in every run could confound this problem. Therefore the appropriate controls for these methods will only become apparent after further testing. Thus, and in line with established practice in OIE standards, the word "must" should be replaced by the word "should" throughout this paragraph.
In addition, it would be worth adding some thoughts on what are appropriate controls for NGS since it is not straightforward as for other molecular techniques. For instance, since it doesn't make sense to prepare and sequence a library from a sample containing no DNA (like, for instance, the no

template control in qPCR) because the output will be too little as to sequence the library and draw conclusions from the results, a suitable negative control could be a sample of a closely related individual. However, even if the control individual might be healthy, i.e. free of detectable symptoms, it may nevertheless be a carrier of the pathogen in quest.

LINES 124-140: In "Section 4 Bioinformatics", it is necessary to add some thoughts on bioinformatics for metagenomics analyses (related to Section B, numbers i and iii when dealing with unknown agents) since this strongly differs from the assembly-based analysis described in the second paragraph of section 4. A major difference is that it frequently happens that the available data are not sufficient to assemble a whole genome or even not for the assembly of partial sequences but rather individual reads have to be classified.

LINE 185: Please replace "HTS-BGC" by "HTS-BCG".

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CHAPTER 2.1.7.: Japanese encephalitis

General comments

The EU can in general support this revised chapter. However, we do not agree on putting section C under study. Indeed, that section has been adopted in 2010 and should remain in place until revised in the future. Marking it as "under study" could be misunderstood as suspending it, i.e. for it to no longer be applicable, in analogy to the meaning of "under study" in the OIE Terrestrial Code.

Reference is made to the meaning of "under study" when used in the Code: "*The term '(under study)' is found in some rare instances, with reference to an article or part of an article. This means that this part of the text has not been adopted by the World Assembly of OIE Delegates and the particular provisions are thus not part of the Terrestrial Code.*" (Extract of the Code's User's Guide, point B2).

This comment is valid for all chapters, whenever a section is proposed to be put under study.

Specific comments

None.

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CHAPTER 2.1.14.: Rift Valley fever

General comments

The EU can in general support this revised chapter and has a few specific comments.

Specific comments

LINE 4: Please add "**Description of the disease:**" at the beginning of this paragraph (consistency with other Manual chapters).

LINES 417-418: As this Manual chapter already includes the table on test methods available for diagnosis of RVF and their purposes (new "fit for purpose" approach of the OIE Terrestrial Manual), deletion of the words "(the prescribed test for international trade)" should be considered. (This comment would be valid for all disease specific chapters of the Manual where a "fit for purpose" table has already been included.)

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CHAPTER 2.1.15.: Rinderpest

General comments

The EU can in general support this revised chapter. However, we do not agree on putting section B under study. Reference is made to the general EU comment on Chapter 2.1.7. Japanese encephalitis.

Specific comments

LINE 402: English usage: please replace the word "emergent" by the word "emergency".

LINES 424-425: English usage: please replace "It is to note that the virus however induces marked enlargement of spleen" by "Note that this strain induces marked enlargement of the spleen".

LINES 463-465: English usage: please replace "The batch of a final bulk must contain virus which are maintained by up to ten serial subcultures from the vaccine seed." by "A batch may be prepared from a bulk suspension containing virus which has been maintained by up to ten serial subcultures from the vaccine seed."

LINE 484: spelling: please replace ").5%" by "0.5%".

LINE 523: English usage: please insert "suspension" after "bulk".

LINE 526: English usage: please insert "suspension" after "bulk".

LINE 528: English usage: please replace "of a final bulk sample" by "of a sample from a final bulk suspension".

LINE 530: English usage: please replace "which stay alive" by "which are still alive".

LINE 531: English usage: please remove comma after "collected".

LINE 531: spelling: please replace "mG" with "mg".

LINES 536-537: English usage: please replace "On the day of virus harvesting the control cultures should be examined for the haemadsorption activity." By "On the day of virus harvesting, the control cultures should be examined for haemadsorption activity."

LINES 540-541: English usage: please replace "RBCs of those species origin." by "RBCs from either of those species".

LINES 542, 543, 554, 555: English usage: please insert "suspension" after "bulk".

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LINES 542, 543, 549: spelling: please remove hyphens in "in vitro" and "in vivo".

LINE 549: for consistency, please replace "final batch" by "final bulk suspension" (all other samples have been taken from the "final bulk (suspension)").

LINE 554: spelling: "dispensed".

LINE 607: English usage: please replace "rinderpest susceptible two Japanese" with "two rinderpest-susceptible Japanese".

LINE 609: English usage: please replace "doses" with "dose".

LINE 616: English usage: please replace "should be x10" by "are 10"

LINE 671-2: English usage: please replace "filled with nitrogen gas in the vial" with "filling the vial with nitrogen gas".

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CHAPTER 2.1.18.: Tularemia

General comments

The EU can in general support this revised chapter and has a few specific comments.

Specific comments

LINE 4: Please add "**Description of the disease:**" at the beginning of this paragraph (consistency with other Manual chapters).

LINES 211-215: An animal welfare indication should be added to this section, in line with established Terrestrial Manual practice, saying that animal inoculation should only be done to the extent necessary and if there are no alternative tests available.

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CHAPTER 2.1.X.: Infection with *Brucella abortus*, *B. melitensis* and *B. suis*

General comments

The EU can in general support this new merged chapter and has a few specific comments.

Specific comments

LINE 4: Please add "**Description of the disease:**" at the beginning of this paragraph (consistency with other Manual chapters).

LINE 49: Please delete the words "**Definition of the disease:**", as this may be confused and be in inconsistent with the case definition which is included in the Terrestrial Code and is relevant for the notification obligations of OIE member countries. By default, case definitions are included in the Code, not in the Manual.

LINES 263-267: Under this paragraph, the EU suggests including a paragraph describing the preferred transport method to the laboratory for samples intended for culturing, i.e. preferred transport medium for swabs, tissues, liquids and preferred temperature during transportation, as well as limitation of time before streaked onto agar. Indeed, the general section for 1.1 does not cover the special requirements of Brucella to survive transport. For example, in the current version of the Bovine Brucellosis chapter (Chapter 2.4.3.), the following paragraph is included: "*All samples should be cooled immediately after they are taken, and transported to the laboratory in the most rapid way. On arrival at the laboratory, milk and tissue samples should be frozen if they are not to be cultured immediately*."
It would be advisable to include further information. At present other sources, sometimes rather old, are sought to find out more specifics about transport and handling before processing in the laboratory. For example, in Alton *et al*. 1988, a time limit for transport is mentioned to be 12 hours and should be frozen if they spend more time in transit, and that the number of viable *Brucella* cells remains constant in tissues when stored frozen at -20 °C for at least 18 months. For those counties where the disease in not endemic, the quality of each individual sample is very important to rule out disease in suspected cases.

LINE 903: Please change the wording at the start from "I-ELISAs using rough LPS (R-LPS).'" to "'I-ELISAs using extracts from rough strains of Brucella (J. McGiven, G. Jungersen and B. Garin-Bastuji, personal results)."
The rationale for this change is that this wording is a more accurate description of the antigen. This wording is supported by Gregers Jungersen and Bruno Garin-Bastuji (the co-ordinating author of the chapter).

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CHAPTER 2.2.2.: American foulbrood of honey bees

General comments

The EU can in general support this revised chapter and has a few specific comments.

Specific comments

LINE 53-54: Both *P. larvae* genotypes (ERIC I and II) were identifies in samples from Germany, Sweden, Greece, France, Russia, Canada and New Zealand. Samples from Asian countries and Arabia contained *P. larvae* ERIC II while *P. larvae* ERIC I could be isolated from samples from Israel, Bangladesh and Cuba (Schäfer *et al*., 2014). These results suggest a worldwide distribution of both genotypes ERIC I and II.

LINE 58: A recent study uses a new multilocus sequence typing scheme to determine the distribution and biogeography of 294 samples of *P. larvae* from across six continents.
Reference:
Morrissey *et al*., 2015. Biogeography of *Paenibacillus larvae*, the causative agent of American foulbrood using a new multilocus sequence typing scheme. Environmental Microbiology, 17, 1414-1424.

LINE 188: Please add: "Direct plating of larval remains on the agar for cultivation without heat treatment is possible".
Reference:
Plagemann (1985). *Eine einfache Kulturmethode zur bakteriologischen Identifizierung von Bacillus larvae mit Columbia-Blut-Schrägagar*. Berl. Münch. Tierärztl. Wschr. 98, 061-062.

LINE195: Please replace the word "must" by "should".

LINES 207-208: Please replace the words "Incubate the plates at 37°C for 2–3 days" by the words "Incubate the plates at 37±1°C for 2–4 days".

LINE 314: Please add the following on culture media: "Cultivation on Columbia sheep-blood slant agar induces sporulation and allows the microscopic diagnosis of the flagellar bundles."
Reference:
Plagemann (1985). *Eine einfache Kulturmethode zur bakteriologischen Identifizierung von Bacillus larvae mit Columbia-Blut-Schrägagar*. Berl. Münch. Tierärztl. Wschr. 98, 061-062.

LINE 331: There is some contradiction concerning the mentioned temperature of "34-37 °C" to **LINE 207** "37 °C"; we suggest in both lines "37±1°C".

LINE 333: According to our experience we question the necessity of "daily checks" and the length of the incubation period; we recommend "incubation of 6 days and check after 3 and 6 days".

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LINE 345: According to our experience, the colonies on CSA are usually "rough" and not "glossy".

LINE 350: This statement is not correct. In the cited studies, it was described that both ERIC II and III are pigmented. This is correctly reflected in table 1 of this draft chapter.

LINE 417: Please note that "v = variable" is not described in Genersch *et al.* 2005.

LINE 504: Please add the following staining method:
"d) Nigrosin negative staining
Take a drop of the fluid phase of the Columbia sheep-blood slant agar, mix it on the slide with 5% aqueous Nigrosin solution, air dry and then heat-fix by passing three times through the flame of a Bunsen burner."
Referance:
Ritter (1996). *Diagnostik und Bekämpfung der Bienenkrankheiten*. Gustaf Fischer Verlag, Jena. page 37.

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CHAPTER 2.2.3.: European foulbrood of honey bees

General comments

The EU can in general support this revised chapter and has one specific comment.

Specific comments

LINE 227, Table of PCR-primers: An additional entry in the table is suggested as follows:

Roetschi A., Berthoud H., Kuhn R., Imdorf A. (2008): *Infection rate based on quantitative real-time PCR of* Melissococcus plutonius*, the causal agent of European foulbrood, in honeybee colonies before and after apiary sanitation*. Apidologie 39, 362-371.

Name and sequence of primers:
MelissoF: 5'-CAG CTA GTC GGT TTG GTT CC-3';
MelissoR: 5'-TTG GCT GTA GAT AGA ATT GACAAT-3';
Taqman_MGB (minor groove binding) probe: 6'FAM-CTT GGT TGG TCG TTG ACMBGNFQ-3'.

PCR product size: 79-bp.

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CHAPTER 2.3.10: Fowl pox

General comments

The EU can in general support this revised chapter and has a few specific comments.

Specific comments

LINE 4: Please add "**Description of the disease:**" at the beginning of this paragraph (consistency with other Manual chapters).

LINE 61: In Table 1, method Real-time RT-PCR: reverse transcription is not needed for detection of viral DNA by PCR, this is probably an editorial error (*Poxviridae* are DNA viruses).

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CHAPTER 2.3.12.: Infectious bursal disease (Gumboro disease)

General comments

The EU can in general support this revised chapter and has one specific comment.

Specific comments

LINE 4: Please add "**Description of the disease:**" at the beginning of this paragraph (consistency with other Manual chapters).

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CHAPTER 2.4.6.: Bovine spongiform encephalopathy

General comments

The EU can in general support this revised chapter and has some specific comments.

In general terms, since the BSE and Scrapie Manual chapters are updated in parallel, the OIE should consider more consistency between both chapters as regards the wording related to the prion hypothesis and in particular whether PrP^{sc} are to be considered the "agent" or not. Indeed, there are slight differences between the chapters, with the scrapie chapter stating clearer that PrP^{sc} are widely regarded as causative agent while the BSE chapter states that the nature of the agent is hypothetical.

Furthermore, the EU suggests making a distinction between classical and atypical BSE also in the beginning of the summary of the text, i.e. in the description of the disease, as is the case in the scrapie chapter. Indeed, it is somewhat confusing when this distinction is first made in line 44 of the BSE chapter, while the text in lines 4-44 is all about classical BSE. An alternative would be to clearly state that these first paragraphs relate to classical BSE only.

Specific comments

LINE 10: Please include "and milk replacers" after "meat-and-bone meal".

LINE 20: Please delete "the" between "and" and "variant".

LINE 34: "agents" should be reinstated, and "disease" deleted. A disease does not cause TSE.

LINE 45: After the sentence "Atypical forms of BSE have been detected in many countries", the EU proposes adding "Atypical BSE is a condition believed to occur spontaneously in all cattle populations at a very low rate". Indeed, this would ensure consistency with Terrestrial Code Chapter 11.4., as revised in May 2015.

LINES 60-61: Please add "and animal feedstuffs containing meat-and-bone meal" after "meat-and-bone meal".

LINES 70-71: Please delete the sentence "The onset of clinical signs is not associated with season or stage of breeding cycle". Indeed, BSE cases have been observed more often around calving, probably due to stress.

LINES 88 and 90: As BSE is a zoonosis, the word "slaughter" should be replaced by the word "culling". Indeed, BSE infected animals should not enter the food or feed chain.

LINE 100: Please add "(H- and L-type BSE)" after the words "atypical BSE".

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LINES 100-101: Please delete "or fallen stock" after "cattle" in line 100-101, and insert "fallen stock or" after "active surveillance of" in line 100. This reduces the current potential for misinterpretation that fallen stock are apparently healthy.

LINES 189, 426, 499, 506: For consistency, please delete the references to "SAF-immunoblot(ting)", as that method has been replaced by "Western Blot" according to Regulation (EC) No 999/2001 of the European Parliament and of the Council as amended by Commission Regulation (EC) No 162/2009 of 26 February 2009, and has been deleted in LINES 182, 212-2013 and 521-527.

LINE 193: Please delete the comma after "rapid".

LINE 199: Please add hyphens in the parenthesis, for it to read "(H- and L-type BSE)", for consistency with LINES 103 and 443.

LINE 162: "Table 2" should be "Table 1".

LINE 163: In the table, Histopathology is not included. It should be included, however showing its limitations (only valuable for confirmation but not for exclusion of BSE in suspect results).

LINE 212: Please delete "using immunohistochemistry", since Western blot can also be applied.

LINE 238: Please delete "to" and replace with "at", for it to read "medulla at the level of the obex".

LINE 240: Please reorder the text "reserved for the PrP^{Sc} detection by" to "reserved for the detection of PrP^{Sc} by".

LINE 258: Please delete the apostrophe from "weeks' ".

LINES 274-278: The expulsion of the brainstem with water or air pressure has been removed from most recommendations for the collection of brainstem for BSE testing, therefore it should be deleted in the Manual as well.

LINE 373: Please change "whole brains in active surveillance to increase" to "whole brains through active surveillance programmes to increase".

LINE 390: Please move the Casalone reference from its current position after "immunolabelling" to the end of the sentence after "target areas". The reference relates very specifically to target area associations and not just the immunolabelling.

LINE 403: Section 1.2.3. should be renumbered 1.2.2.1.

LINE 424: Section 1.2.4. should be renumbered 1.2.2.2.

LINE 425: Please delete the comma after techniques.

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LINE 447: Section 1.2.5 should be renumbered 1.2.2.3.

LINE 462: There appears to be a formatting error. Please delete the colon after "website" and replace it with "(".

Page 10 footnote 4: There is a redundant bracket at the end which can be deleted.

LINES 481-482: Please move the text "independent of the system of notification of suspect cases" from its current position in line 481 to line 482 after "the prevalence of BSE is considered necessary".

LINE 557: Please delete the word "currently", for consistency with LINE 54.

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

CHAPTER 2.4.14.: Lumpy skin disease

General comments

The EU can in general support this revised chapter and has some specific comments.

Specific comments

LINE 4: Please add "**Description of the disease:**" at the beginning of this paragraph (consistency with other Manual chapters).

LINES 14-15: This information needs to be updated. Indeed, the most recent outbreaks outside of Africa occurred in Israel (2012), Turkey (2013), Azerbaijan (July 2014), Russia (July 2015) and Greece (August 2015). The following alternative wording is suggested:
"Lumpy skin disease has traditionally occurred in sub-Saharan Africa however in 2006 it spread into the Middle East and then into Eastern Mediterranean and Western Asian countries. In 2012 it occurred in Israel, in 2013 in Turkey, in 2014 in Azerbaijan followed by Russia in 2015, and in 2015 it entered Europe (Greece) for the first time".

LINE 37: The phrase "but because immunity to LSD infection is predominantly cell mediated" should be deleted for two reasons – it has not been shown to be true, and that's not the reason why some animals have low antibody levels.

LINE 58: The specifier "western" should be included before "Asian", i.e.: "LSD occurrences were reported in the African and western Asian regions".

LINE 60: Please remove Vietnam from the list of countries, as it has never had an outbreak of LSDV.

LINE 63: please insert the words ", followed by the Russian Federation in 2015" after the words "Azerbaijan reported several outbreaks in 2014". Indeed, LSD was introduced in Russia in July 2015.

LINE 64: Please replace DEFRA references with the direct OIE ones (see also comment on line 665-669 below).

LINES 67-69: Please remove the sentence "The principal method of transmission is mechanical by arthropod vector" as this is speculative and not supported in any way by the reference given (Tuppurainen *et al*., 2015).

LINES 70-71: This sentence is inaccurate and should be replaced by "The severity of clinical signs of LSD depend on the strain of capripoxvirus and the age, immunological status and breed of host".

LINE 90: We have never come across a report of an aborted fetus covered with nodules. This statement requires a reference or it should be deleted.

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LINE 97: In Table 1, method Real-time RT-PCR: reverse transcription is not needed for detection of viral DNA by PCR, this is probably an editorial error (*Poxviridae* are DNA viruses).

LINE 157: After "stained using H&E", PCR should be added as an alternative detection method to H&E staining.

LINES 159-162: Poorly worded sentence should be replaced by "The CPE can be prevented or delayed by adding specific anti-LSD serum in the medium. In contrast to LSD, the herpesvirus which causes pseudo-LSD produces a Cowdry type A intranuclear inclusion body. It also forms syncytia which are not usually a feature of capripoxvirus infection (although they may be seen in Madin–Darby bovine kidney [MDBK] cells)".

LINE 168, section 1.3. Electron microscopy: This method is considered not to work correctly. Indeed, a 1 minute contact with the sample suspension is too short, and it is important to also place the grids on the ground of the drop, since poxviruses are relatively large and heavy. Furthermore, "pileoform carbon substrate" cannot be correct, as it does not exist.

LINES 196-207: Due to the low specificity of the AGID test it should not be recommended and this section (1.5) deleted from the document.

LINE 242: Please remove the sentence "The LSDV genome contains 156 putative genes (Tulman et al., 2001)."

LINE 391: Due to problems with the ELISA test it is not currently recommended for use to assess the immune status of animals with respect to capripoxvirus infection. This should be made clear by adding the following: "Attempts to develop an ELISA for the detection of capripoxviral antibodies have been made but the technique is not currently recommended for use".

LINES 418-419: "This has discouraged the use of vaccine even though the consequences of an outbreak of LSD are invariably more severe". Please remove the last half of this sentence as it is not correct.

LINES 668-677: The hyperlinks to these references do not work and the documents cannot be found directly through the DEFRA website. We thus suggest removing the DEFRA references and using the direct OIE references to the Turkish Thrace and Greece outbreaks.

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

CHAPTER 2.5.7.: Equine influenza

General comments

The EU can in general support this revised chapter and has a few specific comments.

Specific comments

LINE 4: Please add "**Description of the disease:**" at the beginning of this paragraph (consistency with other Manual chapters).

LINE 451: In the box, the final part of the last sentence seems to be missing.

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CHAPTER 2.6.2.: Rabbit haemorrhagic disease

General comments

The EU can in general support this revised chapter and has a few specific comments.

Specific comments

LINE 4: Please add "**Description of the disease:**" at the beginning of this paragraph (consistency with other Manual chapters).

LINE 66: Please replace the word "killed" by "inactivated", as this is the more appropriate term.

EU COMMENTS
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CHAPTER 2.7.13.: Scrapie

General comments

The EU can in general support this revised chapter. However, the statements in lines 40-42 on the definition of scrapie in the Terrestrial Code are unacceptable and should be deleted. Indeed, asserting the scope of a Terrestrial Code chapter in the Terrestrial Manual is not possible. However, clarifying the scope of this Manual chapter as regards classical and atypical scrapie in the first paragraph is important, and the same should be done in the BSE chapter (see also EU comment on Chapter 2.4.6. Furthermore, and for the same reasons, the reference to the definition of scrapie in the Terrestrial Code in LINES 98-98 should equally be deleted.

In general terms, since the BSE and Scrapie Manual chapters are updated in parallel, the OIE should consider more consistency between both chapters as regards the wording related to the prion hypothesis and in particular whether PrP^{Sc} are to be considered the "agent" or not. Indeed, there are slight differences between the chapters, with the scrapie chapter stating clearer that PrP^{Sc} are widely regarded as causative agent while the BSE chapter states that the nature of the agent is hypothetical.

Specific comments

LINE 38: As regards atypical scrapie, the EU proposes to replace "may not be contagious and may, in fact, be a spontaneous degenerative condition of older sheep" by "is likely to be a non-contagious and a spontaneous degenerative condition of older sheep".
Indeed, as indicated by EFSA (http://www.efsa.europa.eu/en/efsajournal/pub/4197 and http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/1945.pdf), the prevalence of atypical scrapie is quite homogeneous over stream, time and space, and there is no statistical difference of the observed atypical scrapie frequencies between the general population and the flocks where a positive case had been identified.

LINE 39: Please add the words "and rarely of goats" after "condition of older sheep".

LINE 48: Please introduce "in animals of some genotypes" (replacing "in some animals").

LINE 53: Please delete the word "which".

LINE 54: Please delete the word "rather".

LINE 61: It is presumed that by "between parturition and weening" it is meant that transmission occurs after parturition. Later in the chapter, in LINE 185,

the text still reads "in the period from parturition to weaning", which seems more relevant. Therefore, the current wording should be kept also in LINE 61.

LINES 75-77: It is suggested to re-order the wording for clarity, since it is important that immunodetection is also specifically linked to target areas. Proposed new sentence structure: "Diagnosis is confirmed by demonstration of vacuolation or the immunodetection of PrPSc in target areas within the brain."

LINE 100: After the sentence ending with "Europe, North America, Asia and Africa", the EU proposes to add the following sentence: "There is no evidence of a causal link between Classical or Atypical scrapie and human TSEs". Indeed, this is the main conclusion of the 2015 EFSA opinion on the zoonotic potential of scrapie prions[1] and it is important to recall it early in the text.

LINE 115: Spelling. "Transmissable" should be "transmissible".

LINE 118: Please insert "relatively" before "resistant".

LINES 176-177: Please slightly reword the sentence as follows: "[...] is unknown, and ~~to~~ objective~~ly~~ establishing of freedom from infection [...]".

LINE 181: Please insert "a" between "scrapie" and "significant".

LINE 183: Please insert "and atypical" after classical, then "have been described" instead of "has been described".

LINE 184: Please insert "and classical scrapie has been observed" before "in captive moufflon".

LINE 185: For reasons of consistency, the wording in LINE 185 should be aligned to the changes proposed in LINE 60, as follows: "~~The infection in sheep may be passed~~ Classical Scrapie may be transmitted from ewe [...]".

LINE 197: The "j" in Creutzfeldt-Jacob disease should be capitalised.

LINE 289: Please add the words "samples giving" between "which" and "positive".

LINE 295: The EU proposes to replace "Annex X of EC regulation 999/2001" by "Annex X of Regulation (EC) No 999/2001, as last amended".
Indeed, reference to Regulation (EC) No 999/2001 should be indicated this way.

LINES 314-319: The EU is of the opinion that the paragraph is a bit unclear as regards the population to be targeted and the tissues to be tested. In addition, it should be in line with the Terrestrial Code provisions, which do not require testing of animals younger than 18 months of age to determine the

[1] http://www.efsa.europa.eu/en/efsajournal/pub/4197.

freedom from scrapie at establishment level. We therefore suggest amending the paragraph to read as follows:
"Due to the complex epidemiology of scrapie, the part of the population that should be targeted for sampling as well as the tissues to be analysed differs with the different purposes of the testing. Surveillance for prevalence of the disease could limit tissue examination to the CNS of adult sheep and goats for the reasons given above. However, testing to estimate disease prevalence needs to take into account a number of factors, including the stratification of the sheep-farming industry, dose or level of infection within particular flocks, frequency of disease and relative involvement of the LRS in different genotypes, and the effect of genotype/agent strain combination on incubation period."

LINES 328-334: This is misleading, because it sounds like every TSE case in small ruminants in Europe undergoes this full procedure. The TSE discriminatory testing should be mentioned here. Only cases with an unclear result in this test will undergo the complete procedure.

LINE 367: Please delete "such as are" from the text in parenthesis as it makes the sentence read very awkwardly.

LINE 411: It is suggested to remove the comma after "sampled".

LINE 449: It is suggested to remove the comma after "cases".
Furthermore, it should either stay as "to confirm a diagnosis of clinical suspect classical scrapie" or be changed to "to indicate a diagnosis of classical scrapie". To say "to indicate a diagnosis of clinically suspect" doesn't make sense. Confirmatory testing will either confirm suspicion or indicate a diagnosis, but not indicate a suspicion, since these animals are already suspect.

LINES 470-472: The information in parenthesis does not seem to apply appropriately to the text preceding it. It should refer to the list of antibodies, not the list of reference laboratories. Given that a specific link to the relevant document containing the antibody information is given later in the paragraph, the whole section in parenthesis should be deleted.

LINE 497: Please replace the word "system" by "signal".

LINES 530-531: It is suggested to delete the following sentence: "The rapid tests rely on the optimisation of the reagents used for extraction and digestion and specific antibodies for detection, which negates the need for lengthy ultracentrifugation steps.". Indeed, the sentence gives the impression that the diagnostic sensitivities of rapid tests and protocols including ultracentrifugation are comparable, which is not the case.

LINE 564: After "strategies based on genetic selection for resistance to classical scrapie", the EU proposes to add "in sheep". This is to ensure consistency with the next sentence.

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LINES 572-573: The EU proposes replacing "homozygous for arginine at codon 171" by "i.e. animals of genotypes which encode alanine on both alleles at codon 136, arginine on both alleles at codon 154 and arginine on both alleles at codon 171 (so-called ARR/ARR animals)".
Indeed, this reference is more precise. According to the 2006 EFSA opinion on the breeding programme for TSE resistance in sheep[2], a breeding programme to select ARR/ARR sheep is an appropriate strategy to control and eliminate classical scrapie in sheep based on genetic selection for resistance.

LINES 575-576: The EU proposes deleting the sentence "This may hamper a strategic approach to eliminating scrapie infection from national sheep flocks or geographical regions by adopting a national genetic breeding programme", which is redundant with the previous sentence.

LINE 575: Propose moving paragraph break. The sentence currently opening the final paragraph ("This may hamper [...]" should be at the end of the penultimate paragraph. It makes more sense for the final paragraph to start at "Limited knowledge concerning the prevalence [...]".

LINES 580-581: The EU proposes to replace the sentence "Limited knowledge concerning the prevalence and epidemiology of atypical scrapie indicates that an alternative breeding strategy would be required for this form of the disease" by "A breeding programme selecting ARR/ARR sheep will however not ensure resistance to atypical scrapie".
Indeed, there is no reason to suggest that an alternative breeding strategy is needed to address atypical scrapie, since atypical scrapie is most probably not contagious, is sporadic and not zoonotic, as mentioned at the beginning of this chapter. It is therefore appropriate to focus breeding programmes on resistance to classical scrapie.

LINES 601-602, 654-655, 674-675, 691-693, 705-706: References should not be removed as these are mentioned in LINE 144.

[2] http://www.efsa.europa.eu/en/efsajournal/pub/382.

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CHAPTER 2.9.4.: Cryptosporidiosis

General comments

The EU can in general support this revised chapter and has some specific comments.

Specific comments

LINE 7: Please change to "27 valid *Cryptosporidium* species" (see: Ryan U, Hijjawi N. New developments in Cryptosporidium research. Int J Parasitol. 2015, 45(6):367-73).

LINE 23: Enzyme immunoassay shows a very low specificity; the use of this test should therefore not be encouraged due to the risk of false positive results; furthermore, this test does not allow detection of all Cryptosporidium species.

LINE 29: Please insert the word "subtyping" before "schemes".

LINE 62, TABLE 1: Please replace "Homoeo-thermic" by "Homeothermic".

LINE 62, TABLE 1: Please add "goat" as major host of *C. xiaoi* (see: Rieux A, Paraud C, Pors I, Chartier C. Molecular characterization of Cryptosporidium spp. in pre-weaned kids in a dairy goat farm in western France. Vet Parasitol. 2013;192(1-3):268-72).

LINE 64: The title of section 2 ("Description of the disease in livestock") should be changed to "Description of the disease in animals", as this section also describes cryptosporidiosis of companion animals and birds.

LINE 68: The sentence "Healthy and adult animals can also shed oocysts [...]" may be changed to underline that these animals do not shed C*. parvum* oocysts.

LINE 74: Please replace the word "unapparent" by "asymptomatic".

LINES 92-95: The text should be general, therefore please remove information on the country where parasites have been detected.

LINE 100-101: The text should be general and focused on cryptosporidiosis in cats, therefore please remove from the sentence the following information: "and it may be helpful to include cryptosporidiosis in the differential diagnosis of chronic feline diarrhoea".

LINE 112 -113: The sentence is awkwardly phrased, please change to: "Cryptosporidiosis in turkeys caused by *Cryptosporidium baileyi* is similar to that observed in chicken".

LINE 117 -118: The sentence is awkwardly phrased, please change to: "*Cryptosporidium meleagridis* infects turkeys, other poults and humans"

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LINES 119 -120: The sentence is awkwardly phrased, please change to: "*Cryptosporidium galli* produces a disease in adult hens and some wild and exotic birds. Unlike the life cycle stages of either *C. meleagridis or C. baileyi,* infection with *C. galli* is limited to the epithelial cells of the proventriculus".

LINE 129: It should be "morbidity" instead of "morbility".

LINE 170: In the title of Table 1, the name of the disease is misspelled (a "p" is missing).

LINE 170, TABLE 1: It is suggest to delete the two lines using antigen detection by CI and by ELISA, and the line concerning antibody detection by ELISA because antibodies can be detected after but not during infection; furthermore the sensitivity can be very low.

LINE 184: Please insert the word "by" before "a reference laboratory test".

LINE 190: The specificity of the serological tests is questionable because these tests have not been validated.

LINE 202: Instead of 2.5% $K_2Cr_2O_7$ it should be 5%.

LINE 210: Preservatives may interfere with PCR-based tests; faecal samples can be preserved in ethyl alcohol for subsequent PCR identification.

LINES 338, 374: It should be "eyepiece" instead of "eye piece".

LINES 395-401: These sentences confirm the very poor sensitivity and specificity of immunological tests; it is therefore suggested not to refer to these tests, at least in the summary.

LINE 402: The range of species detected by this test is unknown; further the test does not allow distinguishing *Cryptosporidium* species. This information should be clearly reported in the text.

LINE 432: Please replace the word "developed" by "detected".

LINES 452, 471: Please add PCR after dIFM, as follows: "[...] such as dIFM or PCR".

LINE 484: DNA extraction and PCR amplification from formalin fixed samples is notoriously difficult. To preserve faecal samples to be tested by PCR, the sample should be stored in 90% ethyl alcohol.

LINES 537-538: The EU does not agree to include a statement on control programmes in the section on requirements for vaccines. Indeed, this is outside of the scope of this section. The sentence should therefore be amended as follows: "There is no ~~control programme for cryptosporidiosis, neither is there a~~ rigorously tested and accepted vaccine available."

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CHAPTER 2.9.9.: Salmonellosis

General comments

The EU can in general support this revised chapter and has some specific comments.

Specific comments

LINE 15: Please insert the words "food borne" before the words "zoonotic disease".

LINE 58: The EU does not agree with the change from "may" to "will usually". Indeed, this is not valid as a general statement, as vaccines (live and inactivated) may vary in their influence on serology results.

LINES 329-331: Please amend the sentence as follows: "In 2007, a standard method for detection of *Salmonella* from primary animal production has been published (ISO 6579:2002/Amd. 1:2007, ISO, 2007)."

LINE 338: Please capitalise the word "Salmonellae".

LINE 348: Please amend the title of section 1.1.2. to "Selective enrichment media".

LINE 352: Please add the following after "Rappaport-Vassiliadis broth": "and Modified semi-solid Rappaport-Vassiliadis (MSRV) agar."

LINE 420: Please insert "chromogenic agar (e.g." before "Rambach agar)".

LINE 421: Please insert "one plate of chromogenic agar (e.g." before "Rambach agar)".

LINE 427: Please insert "biochemically, using composite media, such as TSI, LDC and urea, or commercial biochemical tests such as API ID 32 E. Confirm to serogroup level by" after "lysine desoxycholate agar)".

LINE 431-432: Please amend the sentence to read as follows: "Biochemically and serologically confirmed isolates can then be submitted for serotyping to a reference laboratory."

LINES 442-443: Please replace the sentence after "environmental samples" by the following:
"A miniaturised MPN method has been described in ISO/TS 6579-2 (ISO, 2012) and was based on the publication of Fravalo et al. (2003). Furthermore, quantitative real time [...]".

LINE 448: please replace the word "sera" by "antisera".

LINE 460: Please insert the following sentence after "[...] typing sera.":

"More details on serotyping of Salmonella is described in ISO/TR 6579-3:2014 and by Grimont and Weill (2007).".

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GUIDELINE 3.6.9.: Comparability of assays after minor changes in a validated test method

General comments

The EU can in general support this revised chapter, however has a few comments that should be taken into account before adoption.

The chapter is titled comparability of assays after minor changes. However, most of the examples used are major changes according to the definitions given. Indeed, the two Hendra assays are based on different genes, so if the proposal was to change from one to the other it would entail a full validation exercise.

It would be much clearer if the examples used were minor changes such as manual vs automation or different extraction methods.

The chapter contains a lot of descriptive statistics, but is light on what is actually being compared. For example the scatter plots in Figure 2 are likely comparing Ct values, but this is not specified. The raw values are not particularly relevant to comparability of the assays, because they are different assays based on different genes and to adjusted values to a control or cut-off would be more useful.

Furthermore, with regard to the use of CV% in these guidelines, from a statistical point of view it is important to transform the data to make them homoscedastic, which means that they are normally distributed on the whole output range. CV% has been introduced for observations in which the variation changes with the observation, so the SD increases. From a statistical point of view, it is a solution to solve the problem of poor homoscedasticity, but it is the wrong solution. Transformation (e.g. log transformation) is the best solution. Therefore, use of CV% should be discouraged.

Specific comments

LINES 28-31 and 45: There seems to be an inconsistency between the text of LINES 28-31 which says change of test specimen requires verification and the Table 1 at LINE 45 which describes change of specimen types as a major change requiring full validation. In the example used in LINE 28 (cloacal vs tracheal swabs), this is a major difference based on the biology of the pathogen and its distribution in the host, which would require a full validation; i.e. latter information in Table 1 is correct. This should be reflected consistently in LINES 28-31.

LINE 67: Please amend the sentence as follows:
"It is important to agree on acceptance criteria prior to the evaluation study to evaluate the outcome […]".

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LINE 75: Figure 1 step 3 - samples should be analysed "blind". Furthermore, in Figure 1 step 5, please insert "Document and retain" before "Final decision about comparability […]".

LINE 113: Pease add the following under table 3:
"All parametric statistical methods are based on normal distributions. In tests that are not normally distributed the Coefficient of Variation is sometimes used. However, transformation of data (e.g. logarithmic transformation) is preferred and then the Standard Deviation and other statistics can be used. The use of the Coefficient of Variation should be discouraged in test validation studies."

LINE 166: There is a need to consider the diluent, i.e. it is better to make dilutions in the sample matrix than in the buffer, as is described in the validation guidelines documents.

LINE 175: We would not advise making the decision that an assay was not comparable on a single run/plate as detailed here.

LINE 226: After the first paragraph, please add the following:
"It is important that the data generated and the decision making process regarding whether the proposed change is acceptable is clearly documented and retained to show an audit trail".