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 2020[1]

[1] The draft chapters can be downloaded from the following address:
http://web.oie.int/downld/Terr_Manual/MAILING_OCT_2020.zip

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

1.1.1. Management of veterinary diagnostic laboratories

General comment:

The EU can in general support this revised chapter.

During the COVID-19 pandemic, many veterinary laboratories have tested human samples to increase national testing capacities during times of acute crisis in a true One Health approach. The OIE has also provided guidance on Veterinary Laboratory Support to the Public Health Response for COVID-19. This should be mentioned in the chapter.

Specific comments are provided below.

Specific comments:

LINE 44: We wonder what is meant by “external organisations”. The sentence previously refers to public sector laboratories, private sector laboratories and university laboratories – however it’s not clear what “external organisations” are.

LINES 46-49: By starting this paragraph with “The governance of public sector veterinary laboratories […]” it suggests that the chapter refers only to public sector laboratories. Assuming that this Chapter refers to all rather than all veterinary diagnostic laboratories, I suggest that the paragraph makes this explicit, as follows:

“This chapter sets out the general principles of ~~veterinary laboratory~~ governance and management for all types of veterinary laboratories that should ensure that Veterinary Services have access to reliable, trustworthy laboratory services.”

LINES 70-73: The recommendations sound reasonable, but for official national reference laboratories business plans etc. are not always useful since there is an official task and the structure and the task are laid down in legal instruments. Therefore, this paragraph should be redrafted to reflect the situation in many countries with national reference laboratories provided by the government. In those laboratories also budget plans are connected to the official task and the government structure.

LINE 126: Section 6.1. should include training of new personnel. Training is key to avoid accidents. Therefore, clear training concepts have to be implemented here as a special point.

LINE 134: Please insert “experimental” or “laboratory” before “animals”.

LINES 160-190: The title of Section 6.2. should be changed to “biosecurity and biosafety”, as both are dealt with. Reference is made to the definitions contained in the glossary of the Terrestrial Manual.

LINES 273-274: This is relevant not only for maintaining trade and renewal of disease freedom, but also for gaining access to trade and for attaining disease freedom status in the first place. This should be reflected in the text.

LINES 275-277: We wonder whether it is appropriate to refer to the Aquatic Manual as well in this context, as this is a chapter in the Terrestrial Manual and the Aquatic Manual may contain its own guidelines. (This comment is relevant also for the reference to the Aquatic Code in **LINE 194**.)

LINE 308: Section 3. asks for several very specific conditions (list of cost items etc.) which are handled in very different ways in many countries due to the national regulations e.g. for government institutions (e.g. using global budget or full-cost regimes). Here a more general description allowing national variation in a broad range should be implement to reflect the situation in many laboratories.

3.1.3. Bluetongue (infection with bluetongue virus)

General comment:

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

Specific comments:

LINES 17-20; 68-69: This statement is very general and certainly is not valid across the board in all countries and all situations. Furthermore, such a statement on the possible economic impact of a disease and trade restrictions seems to go beyond the remit of the OIE Manual and seems especially inappropriate in the introduction section. We therefore suggest deleting the entire sentence (starting with "Outbreaks of BT disease ..."). For the same reasons, the words "and impact" should be deleted from the title of this section (**LINE 66**).

LINE 64: Please add ", BTV-1 and BTV-4" after "BTV8".

LINE 74: Please replace "importance" with "significance".

LINE 77: The EU suggests adding a sentence here, as follows:

"In the EU, based on a scientific assessment of EFSA, of the 27 BTV serotypes, the strain group BTV (1–24) has been listed for Union intervention under the EU Animal Health Law (Regulation (EU) No 2016/429), while the strain group BTV-25–30 has not."

Reference: EFSA scientific opinion "Assessment of listing and categorisation of animal diseases within the framework of the Animal Health Law (Regulation (EU) No 2016/429): bluetongue" (2017 European Food Safety Authority. EFSA) https://www.efsa.europa.eu/en/efsajournal/pub/4957

LINES 124-126: For the reasons stated above, we suggest deleting this sentence as well (starting with "The ability to ...").

Table 1: As regards the detection of the immune response, the EU considers that choosing serotype specific virus neutralisation test as a recommended method for the purpose "Individual animal freedom from infection prior to movement" is not appropriate because it should be done against the 27 serotypes, which is really cumbersome. Instead, C-ELISA should be recommended for this purpose, as it is a very rapid and simple method producing at least the same sensitivity.

LINES 355-356: It is unclear what is meant by "normal serum wells" in this context. We would suggest replacing it with a reference to negative control or negative serum or similar.

LINE 386: The statement "officially recognised web sites" is problematic in an OIE standard. Indeed, it is unclear what exactly is meant (officially recognised by whom?).

LINE 669: It is unclear what is meant by “problem samples”.

LINE 827: The words “authorisation/registration/licensing” in the title should be replaced by “regulatory approval”, as this is the wording that was used in other recently adopted Manual chapters.

3.1.6. Echinococcosis (infection with *Echinococcus granulosus* and with *E. multilocularis*)

General comment:

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

Specific comments:

LINES 51 and 410: Please replace the words “is registered” for “has gained regulatory approval”, as the term “registration” is used only in some OIE member countries whereas the term “regulatory approval” works globally and has been used in other recently adopted Manual chapters.

Table 2: Concerning detection of the agent in definitive hosts for the purpose of population freedom from infection, the PCR is not recommended (-). Nevertheless, this tool has proved to be useful notably for detection of *E. multilocularis* in a few EU Member States (Sweden, Croatia and France). The national surveillance of non-investigated areas in France will also be realized using this approach of coproqPCR. We would therefore recommend this approach as it quite fast, potentially non-invasive (faeces collected from the environment) and very specific and sensitive. So (-) may be substituted by (++) (or even (+++)).

LINE 163: Reference to the detection of adult worms in faeces needs to be deleted as adult worms are present only in intestines and even after deworming (except purgation) the worms are not present in the faeces since they are lysed before (contrary to larger worms such as *Taenia* spp. or nematodes). The detection in faeces is possible only via coproDNA (tissue or eggs) or coproantigen.

LINE 201: It seems that there is a confusion since it is the germinative layer (not laminated layer) which is inside the cyst cavity and can be easily removed.

LINE 208: From ethanol-fixed but also frozen material.

LINES 346-347: Again faecal samples can be frozen to perform further PCR diagnostic.

LINES 361-367: We disagree with this part as indicated above since large scale surveillance (several hundreds of faeces samples) can currently be tested by copro(q)PCR as is already done in some countries. Furthermore as only dogs is mentioned it seems that it concern only *E. granulosus* so adding foxes for *E. multilocularis* would be useful. It is not clear whether in Europe any surveillance program is based on coproELISA in screening but rather by direct detection of *E. multilocularis* coproDNA by copro(q)PCR.

TABLE 3: In the entry for “Cox1, NAD, rrnS”, *E. multilocularis* should be included in the species column.

3.1.12. Leptospirosis

General comment:

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

Specific comments:

LINE 498: The words "authorisation/registration/licensing" in the title should be replaced by "regulatory approval", as this is the wording that was used in other recently adopted Manual chapters. Furthermore, the term "registration" should be replaced with "approval" in other parts of the text (**LINE 500**).

3.1.23. Vesicular stomatitis

General comment:

The EU can in general support this revised chapter.

In general, we question whether it is useful to retain the acronym "VSV", as it it not clear what this refers to. Indeed, there is no single "vesicular stomatitis virus". The ICTV-official species names are Indiana vesiculovirus, Alagoas vesiculovirus, Cocal vesiculovirus and New Jersey vesiculovirus. It is confusing to use acronyms of different styles (e.g., "VSIV" alongside "NJ" or "VSV NJ"). Therefore, we suggest deciding on one style and use it consistently throughout the chapter. Also, please delete all references to "serotype" in the chapter as these are obsolete.

Specific comments are provided below.

Specific comments:

LINE 114: It may be useful clearly indicate that probang samples should only be collected from ruminants, not from pigs and certainly not from horses. Also, saliva samples or swabs (taken by vigorously rubbing over lesions) are more useful than probang samples are when no epithelium can be recovered. It is notoriously difficult to take a good probang sample, and the requisite skill set is becoming increasingly rare among contemporary field veterinarians (as are the tools themselves). To make an accurate diagnosis of an acute clinical case with lesions, a bad probang is much less useful than a good swab.

LINE 118: "Mycostatin" is a trade name, which should be avoided. The proper name of the antifungal compound is "nystatin". Also, there is more than one polymyxin; please specifiy whether what is meant is polymyxin B?

LINE 182: Should be "2000 × g". Why is "g" in bold?

LINES 165; 185: "Vero" or "VERO"?

LINE 277: "Veronal" is a trade name for barbital. Not only should trade names be avoided in the Manual; barbiturates are controlled substance in many countries and should be replaced by other suitable alternatives, such as specific Complement Fixation Test Buffers that do not contain barbiturates and are commercially available.

LINE 309: The statement "PCR techniques have not been routinely used for screening diagnostic cases for viruses causing VS" is very much obsolete and should be deleted. PCR techniques are in fact the method of choice for differential diagnosis of vesicular disease and have been for decades, which is even states elsewhere in the text.

LINE 347: It may be worth pointing out here that the Indiana P179F/P793R and New Jersey P102F/P831R primers from Table 3 are also very useful for sequencing.

LINES 423-425: The wording here is not very clear. In the current version of the chapter, it is recommended to carry out the neutralization test with 1000 TCID50 of virus per well (see line 409). Please note that this is a virus dose, not a concentration. It does not matter whether the amount of liquid in a well is 50 µl or 100 µl, you always add 1000 TCID50 of virus. The proposed new text says to "add 50 µl per well of (…) stock at a dilution (…) containing 200 TCID50 per 50 µl". So, the virus dose added to the well is 200 TCID50. Then, in parentheses, it says "final dilution – mixture virus/serum = 100 TCID50/100µl" (line 425). Please note that the instructions do not specify the volume of the serum dilution to use, but common practice is to use the same volume of (diluted) serum and (diluted) virus. Therefore, if there were 50 µl serum in the well, and we added 50 µl of virus dilution (containing 200 TCID50, see above), we now have 100 µl of liquid in the well that contains 200 TCID50 of virus, not 100 TCID50 as stated in line 425 and not 1000 TCID50 as in the current specification of the test. Is it intended to change the virus dose used for the VNT or is it simply phrased poorly? If such a change is indeed intended, why? It actually is quite nice to have the VS VNT at 1000 TCID50 instead of 100 TCID50, because it reduces false positive results.

LINE 601: The word "authorisation" in the title should be replaced by "regulatory approval", as this is the wording that was used in other recently adopted Manual chapters. Furthermore, the term "authorisation/licensing" should be replaced with "approval" in other parts of the text **(LINES 688, 693**).

3.2.7. Varroosis of honey bees (infestation of honey bees with *Varroa* spp.)

General comment:

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

Specific comments:

LINE 31: Phoresis or phoresy is a non-permanent, commensalistic interaction in which one organism attaches itself to another solely for the purpose of travel. Since the Varroa mite is feeding on the adult bee it cannot be defined as 'phoretic '. We suggest consistently using the term "dispersal phase" as suggested in Traynor *et al*., 2020.

LINES 63-68: While the EU in general supports these statements, we feel this academic issue could lead to ambiguity and thus confusion as to the definition of the disease for the purposes of the Manual (we note that the definition is very clear in the corresponding chapter of the OIE Terrestrial Code). Indeed, if left as is, the reader could get the impression that there currently is no longer or not yet a clear definition of varroosis, which actually concerns more the scientific community than the veterinary services and diagnostic laboratories that need to deal with the disease. We therefore suggest adding wording to restore clarity, without anticipating the global consensus among scientists as to a possible future definition. We feel these changes would also help explain why all agent detection methods in this chapter are directed at *Varroa* mites, and not bee viruses, nor the assessment of clinical signs. The EU therefore suggests the following amendments to the text:

"Traditionally this disease has been defined as the infestation of honey bees with *Varroa spp*. However, due to the growing knowledge on viruses vectored by *V. destructor* and their confirmed important role in *Varroa*-induced colony collapse, this definition is no longer representative of the full process of the disease in *A. mellifera* (Genersch *et al*., 2010; Rosenkranz *et al*., 2010). An accurate and globally agreed definition of varroosis in *A. mellifera* is in progress among the scientific community, with efforts put into clarifying the precise role of *V. destructor* itself and the role of the different viruses carried by the mite, and thereby contextualising their relative importance in the complex of signs observed. Until then, for the purposes of this Manual chapter, varroosis is linked to the detection of *Varroa spp*., regardless of the occurrence of clinical signs (see Section B. Diagnostic Techniques)."

LINE 71: There is a more recent and appropriate reference for discussing the viruses that are or are not transmitted by *Varroa*:

Yañez O, Piot N, Dalmon A, de Miranda JR, Chantawannakul P, Panziera D, Amiri E, Smagghe G, Schroeder DC, Chejanovsky N (2020) Bee viruses: Routes of infection in Hymenoptera. Frontiers in Microbiology 11: e943. DOI: 10.3389/fmicb.2020.00943.

3.3.4. Avian influenza (including infection with high pathogenicity avian influenza viruses)

General comment:

The EU thanks the OIE for having taken into account some of the comments we have provided previously. However, we cannot support this revised chapter as presented. Specific comments are provided below.

Specific comments:

LINE 70: Please replace "H5Nx" with "HPAI", as indeed this not only concerns H5 subtypes.

LINES 73-74; 119: Contrary to Manual conventions agreed in recent years further to the adoption of Chapter 1.1.4. in 2015, references are included here to specific laboratory biosafety containment levels. In line with other recently adopted disease specific Manual chapters, we would suggest deleting lines 73-74 and the parenthesis in line 119.

LINE 108: "Section B2" should read "Section B1.1.1.". Indeed, B2 deals with serological tests and is not relevant.

LINES 132-133: Please replace "insensitive" with "intensive", and "isolated" with "isolates" (editorial / typographic).

LINES 133-178: The corresponding paragraphs in chapter 3.3.14. "Newcastle disease" should be strictly identical with these, since sample collection, pre-analytical treatment, egg inoculation and incubation, allantoic fluids collection are entirely the same in case of clinical suspicion. Distinction between avian influenza virus infection and Newcastle disease virus infection is not possible based on clinical and epidemiological investigations alone; field samples and laboratory procedures need therefore to be the same. You will find below a face-to-face comparison of all instances where there is a difference between chapters and where harmonization should be the case. *(Please note that all highlighted phrases should be compared between chapters and harmonised. Phrases in green may be allowed to stand as they are, but the aim should be to have a unique text, allowing maybe only phrases highlighted in grey, which may be virus specific, to remain.)*

Chapter 3.3.4. avian influenza	*Chapter 3.3.14. Newcastle disease*
Samples taken from dead birds should include intestinal contents (faeces) or cloacal swabs and oropharyngeal swabs. Samples from trachea, lungs, air sacs, intestine, spleen, kidney, brain, liver and heart should also be collected and processed either separately or as a pool. When pooling samples the brain should be collected and processed first (to avoid cross contamination with other tissue types) and kept separate as presence of virus in the brain may be an	*When investigations of ND are the result of severe disease and high mortality in poultry flocks, it is usual to attempt virus isolation from recently dead birds or moribund birds that have been killed humanely. Samples from dead birds should consist of oropharyngeal or tracheal swabs, as well as samples collected from lung, kidneys, intestine (including contents), caecal tonsils, spleen, brain, liver and heart tissues. These may be collected separately or as a pool, although brain and*

indicator of HPAI. Further pools should be made consistent with known virus tropisms between HPAI and LPAI, i.e. grouped at the level of respiratory, systemic and gastrointestinal. *Samples from live birds should include both oropharyngeal and cloacal swabs. To avoid harming them, swabbing of small delicate birds should be done with the use of especially small swabs that are usually commercially available and intended for use in human paediatrics. Where these are not available, the collection of fresh faeces may serve as an alternative (caution that some influenza A viruses in birds can have a strong respiratory tropism). Similar swab samples can be pooled from the same anatomical site (i.e. cloacal swabs with cloacal swabs, oropharyngeal swabs with oropharyngeal swabs), and most commonly pooling of 5 or occasionally 11 samples, but specific swab types should be used (Spackman et al., 2013).* *The samples should be placed in isotonic phosphate-buffered saline (PBS), pH 7.0–7.4 with antibiotics or a solution containing protein and antibiotics. The antibiotics can be varied according to local conditions, but could be, for example, penicillin (2000 units/ml), streptomycin (2 mg/ml), gentamycin (50 µg/ml) and mycostatin (1000 units/ml) for tissues and oropharyngeal swabs, but at five-fold higher concentrations for faeces and cloacal swabs. It is important to re-adjust the pH of the solution to pH 7.0–7.4 following the addition of the antibiotics. It is recommended that a solution for transport of the swabs should contain protein to stabilise the virus (e.g. brain–heart infusion, up to 5% [v/v] cattle serum, 0.5% [w/v] bovine albumen or similar commercially available transport media). Faeces and finely minced tissues should be prepared as 10–20% (w/v) suspensions in the antibiotic solution. Suspensions should be processed as soon as possible after incubation for 1–2 hours at room temperature. When immediate processing is impractical, samples may be stored at 4°C for up to 4 days. For prolonged storage, diagnostic samples and isolates should be kept at –80°C but for transport on dry ice (≤-50C) is widely used. Repeated freezing and thawing should be avoided.* *The preferred method of growing influenza A viruses is by the inoculation of specific pathogen free (SPF) embryonated chicken eggs, or specific antibody negative (SAN) eggs. The supernatant fluids of faeces, swabs or tissue suspensions obtained through clarification by centrifugation at 1000 g are inoculated into the amniotic (for primary isolation) and allantoic sacs of three to five embryonated SPF or SAN chicken eggs of 9–11 days' incubation. The eggs are incubated at 37°C (range 35–39°C) for 2–7 days. Eggs containing dead or dying embryos as they arise, and all eggs remaining at the end of the incubation period, should*	*intestinal samples are usually processed separately from other samples. The brain should be collected and processed first (to avoid cross contamination with other tissue types and kept separate since presence of virus in the brain may be an indicator of NDV.* *Samples from live birds should include both tracheal or oropharyngeal and cloacal swabs, the latter should be visibly coated with faecal material. Swabbing may harm small, delicate birds, but the collection of fresh faeces may serve as an adequate alternative. Further the type of swabs used may affect test sensitivity or validity with thin wire or plastic shafted swabs preferred. Similar swab samples can be pooled from the same anatomical site (i.e. cloacal swabs with cloacal swabs, oropharyngeal swabs with oropharyngeal swabs), and most commonly pooling of 5 or occasionally 11 samples.* *The samples should be placed in isotonic phosphate buffered saline (PBS), pH 7.0–7.4, containing antibiotics. Protein-based media, e.g. brain–heart infusion (BHI) or tris-buffered tryptose broth (TBTB), have also been used and may give added stability to the virus, especially during shipping. The antibiotics can be varied according to local conditions, but could be, for example, penicillin (2000 units/ml); streptomycin (2 mg/ml); gentamycin (50 µg/ml); and mycostatin (1000 units/ml) for tissues and tracheal swabs, but at five-fold higher concentrations for faeces and cloacal swabs. It is important to readjust the concentrated stock solution to pH 7.0–7.4 before adding it to the sample. If control of Chlamydophila is desired, 0.05–0.1 mg/ml oxytetracycline should be included. Faeces and finely homogenised tissues should be prepared as 10–20% (w/v) suspensions in the antibiotic solution. Suspensions should be processed as soon as possible after incubation for 1–2 hours at room temperature. When immediate processing is impracticable, samples may be stored at 4°C for up to 4 days. For prolonged storage, diagnostic samples and isolates should be kept at –80°C but for transport on dry ice (≤–50°C) is widely used. Repeated freezing and thawing should be avoided.* *1.2 Virus isolation* *The preferred method of growing AOAV-1 viruses is by the inoculation of specific pathogen free (SPF) embryonated chicken eggs, or specific antibody negative (SAN) eggs. The supernatant fluids of faeces or tissue suspensions and swabs, obtained through clarification by centrifugation at 1000 g for about 10 minutes at a temperature not exceeding 25°C, are inoculated in 0.2 ml volumes into the allantoic cavity of each of three to five embryonated SPF or SAN chickens' eggs of 9–11 days incubation. After inoculation, these are incubated at 35–37°C for 2–7 days. To accelerate the final isolation, it is possible to carry out up to two passages at a 1- to 3-day intervals, obtaining results comparable to two passages at 2–7-day intervals (Alexander & Senne, 2008a). Eggs containing dead or dying embryos as they arise, and all eggs remaining at the end of the*

first be chilled to 4°C for 4 hours or overnight, and the amnio-allantoic fluids should then be recovered and tested with a screening test (such as haemagglutination [HA] test), influenza A type-specific test (such as agar gel immunodiffusion test [AGID] or solid-phase antigen-capture enzyme-linked immunosorbent assays [ELISA]) or influenza A subtype-specific test (such as haemagglutination inhibition [HI] and neuraminidase [N] inhibition [NI] tests) or a molecular test to detect influenza A specific nucleic acid signatures (such as real-time reverse transcription polymerase chain reaction [RT-PCR]) as described later (see Section B.3.2). Detection of HA activity, in bacteria-free amnio-allantoic fluids verified by microbiological assay, indicates a high probability of the presence of an influenza A virus or of an avian orthoavulavirus (formerly avian paramyxovirus). Fluids that give a negative reaction should be passaged into at least one further batch of eggs	*incubation period, should first be chilled to 4°C for a minimum of 2 hours or overnight (and checked for embryo death before proceeding) and the allantoic fluids tested for haemagglutination (HA) activity. Fluids that give a negative reaction should be passed into at least one further batch of eggs. Routine checks for contamination should be conducted by streaking samples in Luria Broth agar plates and reading these at 24 and 48 hours of incubation against a light source. For larger numbers of sample initial culture could be in tryptose phosphate broth. Contaminated samples can be treated by incubation with increased antibiotic concentrations for 2–4 hours (gentamicin, penicillin g, and amphotericin b solutions at final concentrations to a maximum of 1 mg/ml, 10,000 U/ml, and 20 µg/ml, respectively). Samples heavily contaminated by bacteria that cannot be removed by centrifugation or controlled by antibiotics can be filtrated through 0.45 and 0.2 micron sterile filters. Filtration should be used only when other methods fail because aggregation may significantly reduce virus titre.*

LINE 175: “Section B.3.2.” should read “Section B.1.2.2.”. Section B.3.2. does not exist in the revised text.

LINE 180: “Section B.3.1.” should read “Section B.2.2.”. Section B.3.1. does not exist in the revised text.

LINES 193-194: We note that **LINE 544** also mentions BPL for inactivation of the influenza A antigen for AGID; this should also be added here “following by treatment with 0.1% formalin or 1% betapropiolactone”.

LINE 198: We note that the term “licencing” is used here and throughout the text, when referring to regulatory approval of vaccines. As the terms used in member countries differ widely (e.g. licensing, registration, authorisation), the EU suggests using wording that is accepted worldwide and is also used in other Manual chapters adopted recently, such as “approval” or “regulatory approval”.

LINE 224: Please delete “of virus” (editorial).

LINE 276: The EU is of the opinion that the term “similar” is too vague. As the Terrestrial Manual is intended for worldwide use and must also be clearly interpreted by less experienced and skilled laboratory workers, it is better to be as clear as possible. To avoid any misinterpretation, we suggest it could be specified as follows:

“Multi–basic cleavage sites containing several basic amino acids in the critical position of the cleavage site of the hemagglutinin molecule (HA0) currently remains the identifying sign of HPAI strains; therefore, the isolate being tested to confirm this characteristic will be considered to be HPAI even if the sequence is unusual and different from the one observed so far.”.

Furthermore, it should be clearly understood that any new isolate should also be tested in vivo by IVPI.

LINES 281-292: The EU does not support keeping this part of the text in the Manual chapter. As explained in our previous comments (https://ec.europa.eu/food/sites/food/files/safety/docs/ia_standards_oie_eu_position_terrestrial_manual_202002.pdf), the EU is of the opinion that the Manual should as a general rule not contain any information on notification obligations of member countries. Indeed, this is the remit of the Code and subject to regular changes. Having information in this regard in the Manual that is possibly diverging from the Code would be highly confusing, could lead to international trade problems, and is thus inappropriate. We therefore urge the OIE to delete this information from this Manual chapter and instead insert a reference to the relevant Code chapter.

LINE 304: Please reword to "to exclude the presence of any HPAI virus." (editorial).

LINES 319-320: "[(A/chicken/Pennsylvania/1/83 (H5N2) and A/goose/Guangdong/2/96 (H5N1), A/turkey/England/87-92BFC/91 (H5N1) or A/chicken/Texas/298313/04 (H5N2)]" should read "[~~(~~A/chicken/Pennsylvania/1/83 (H5N2) and A/goose/Guangdong/2/96 (H5N1), A/turkey/England/87-92BFC/91 (H5N1) or A/chicken/Texas/298313/04 (H5N2)]". Editorial: deletion of an opening bracket.

LINES 340-341: Please reword as follows: "routinely applied as a first choice for the diagnosis of Influenza A" (editorial).

LINES 381-382: "(at least of H5, H7 and H9 but increasingly bespoke assays for other subtypes)" should read "(at least for H5, H7 and H9 subtypes, and more recently developed assays are also available for other subtypes)".

LINES 392-393: Please reword as follows (editorial): "[...] to t hose of virus isolation when used on tracheal and oropharyngeal swabs of chickens and turkeys [...]".

LINE 399: The EU suggests deleting the words "or 'TaqMan®'" as direct references to commercial products or brand names should be avoided in the Manual unless strictly necessary.

LINES 411-412: Please reword as follows (editorial): "[...] detection of the M (or NP) gene fulfilling the criteria for a suitable screening test. For subtype [...]".

LINE 434: Please insert the word "the" before "neuraminidase subtype" (editorial).

LINE 445: Please insert the word "assays" after "validated" (editorial).

LINE 489: "Section 1.1.1." should read "Section B.1.1.1.".

LINES 550-551: The distance among the wells is very important. We suggest inserting the words "at a distance of about 3 mm from each other," after "5 mm in diameter". This follows the procedure described in:

Terregino C. & Capua I. (2009). Conventional diagnosis of avian influenza. In: Avian Influenza and Newcastle Disease, Capua I., ed. AD Springer Milan, Milan, Italy.

LINE 553: Please replace "50 µl" with "25-30 µl". Indeed, there are too many volumes of 50 µl; note that it is not possible to put in the wells this amount of reagents with a gel thickness of 2-3 ml as indicated here.

LINES 568-570: The paragraph on HA/HI test must be harmonised with the revised text proposed for ND: U-shaped plates are now mentioned for ND. The sentence should therefore read "The following recommended examples apply in the use of V-bottomed (U- bottomed can be used but care in reading is required as the clarity is less defined) microwell plastic plates in which the final volume for both types of test is 0.075 ml."

Actually, in our experience with U-shaped plates compared to V-shaped plates, it is the quality and the nature of the plastic itself that probably makes the difference in terms of clarity of the test and not the shape of the well: we routinely use U-plates and this is explicitly indicated in the corresponding French AFNOR standardized methods NF U47-011 / NF U47-036-1 / NF U47-036-2.

LINES 623-624: This should read "Nonspecific inhibition of agglutination can be caused by steric inhibition when the tested serum contains antibodies against the same N subtype as the H antigen used in the HI test."

LINES 628-630: this should read "~~To prevent steric nonspecific inhibition the H antigen used to test unknown serum must be of a different N subtype than the unknown sera, or~~ Alternatively, the H antigen used can be recombinant or purified H protein that lacks N protein." When testing unknown sera, the solution set down in the first part of the sentence does not apply, since the nature of the N-elicited antibodies is precisely unknown.

LINES 634-639: We suggest turning this paragraph into a new Section 2.4. entitled "Neuraminidase inhibition tests". Indeed, although this description is purposely kept short, it should maybe appear as a separate section, as the title of Section 2.3. refers to HA and HI tests only.

LINES 775-779: This should read "For inactivated vaccines against HPAI or LPAI virus ~~challenge~~, potency tests may rely on the measurement of immune response or challenge and assessment of morbidity, mortality (HPAI only) and quantitative reduction in challenge virus replication in respiratory (oropharyngeal or tracheal) and intestinal (cloaca) tracts."

LINE 834: This should read "However, recombinant live vaccines have limitations in that they may have reduced replication and […]".

LINES 897-898: “The H5 RNA particle vaccine has been demonstrated to be an effective booster vaccine to replace rg inactivated H5Nx vaccine (Bertran et al., 2017).” should be placed between LINES 892 and 893, since the displaced lines deal with the same vaccine as LINES 889-892.

LINES 894-896: This should read “Since this category of vaccine only contain the specific influenza A haemagglutinin protein, they are easily amenable to serological DIVA testing using assays designed for identifying antibodies to the nucleoprotein/matrix protein.”

LINES 943-945: This should read “In 1995, Mexico implemented influenza A vaccine use for poultry as one tool in the HPAI control strategy, with eradication of HPAI strain by June 1995, but as H5N2 LPAI viruses continued to circulate, ~~and~~ H5N2 vaccination was maintained (Villarreal, 2007).”

LINES 1202-1204: This should read “The spread of high pathogenicity H5Nx avian influenza throughout Asia, Africa and Europe has led to an increase in the number of laboratories performing diagnostics for this pathogen. High pathogenicity avian influenza (HPAI) viruses, in general, are a serious threat to birds and mortality is often 100% in susceptible chickens.”

3.3.14. Newcastle disease (infection with Newcastle disease virus)

General comment:

The EU thanks the OIE for having taken into account comments we have provided previously. However, we cannot support this revised chapter as presented. Specific comments are provided below.

Specific comments:

LINE 8: We suggest inserting the words "At present" before "there are 21 serotypes" as more could well be described in the near future.

LINE 13: Please insert the words "and enteric" after "severe respiratory" as these strains may induce signs other than respiratory problems such as enteric disease.

LINE 101: The statement "NDV is a human pathogen" is very strong, as strictly speaking NDV is certainly not an out-and-out human pathogen. We would suggest rewording as follows:

"AOAV-1 may infect humans normally causing ~~is a human pathogen and the most common sign of infection in humans is~~ conjunctivitis that develops [...]".

LINE 108: We are aware of only one single patient, therefore would prefer keeping "patient" instead of "patients".

LINES 128-129: Please replace "insensitive" with "intensive", and "isolated" with "isolates" (editorial / typographic).

LINES 334-353: With reference to the specific EU comment on Chapter 3.3.4. avian influenza, the EU does not support keeping this part of the text. Instead of paraphrasing the Terrestrial Code, there should be a simple reference to it. Indeed, the Code may be revised independently from the Manual, resulting in divergence of text and thus confusion, possibly leading to international trade problems. Therefore, as stated previously and as a general rule, no case or disease definitions should be contained in the Manual, if such definitions are already contained in the Code. For the same reason, the words "and therefore NDV" in **LINE 37** should be deleted.

LINE 992: Please replace "Once registered and licensed" with "Once regulatory approval has been gained", as this wording is accepted worldwide and is used in other Manual chapters adopted recently. Similarly, the term "licensed" should be avoided in other parts of the text, and preferably be replaced by "approved".

3.4.4. Bovine genital campylobacteriosis

General comment:

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

Specific comments:

LINES 33-35: The EU supports the proposed revision of Section C. on vaccines of this chapter and feels the information on vaccines in the summary needs to be clearer to avoid any ambiguity. We thus suggest the following changes to the text:

"While t~~T~~here are commercial vaccines available, and autologous vaccines based on isolated strains from farms ~~may~~ can be used. ~~. T~~there is a lack of scientific evidence overall of the effectiveness of vaccination."

LINES 370-371: As PCR seems unsuitable overall, and it is being deleted from Table 1, this sentence and the mentioning of several PCR assays published in the scientific literature in Section 1.9. could be misunderstood. There should therefore be a clear statement added here that the OIE does not at present recommend use of PCR assays as a laboratory method to detect the agent.

LINE 418: It is stated that the sensitivity of this assay cannot be estimated. This seems to indicate the test was not properly validated according to OIE standards. We would recommend mentioning this explicitly in the text.

3.5.3. Infection with *Trypanosoma equiperdum* (dourine in horses)

General comment:

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

Specific comments:

LINE 122: A comma is missing after “clinical signs” (editorial).

LINE 153: Please insert the words “morphologically” after “to distinguish”.

LINE 161: Please consider rewording the sentence as follows: “For typical strains, the parasites length ranges from 15.6 to 31.3 µm.”

LINE 229: Please replace “ess.” with “essential”.

Reference in generals: Please check, there are a number of species names not written in italic style (for example in line 515).

LINES 513-516: We note this is a case definition of Dourine, which should preferably be included in the corresponding Code chapter. However since the current Code chapter does not contain a case definition, and the ongoing revision of that Code chapter will take some more time, we support the text of the Manual chapter as presented. We are confident that there is ongoing close coordination between the BSC and the Code Commission on this point that will avoid any inconsistencies or overlaps.

LINES 518-520: We note that the new text proposed is not related to vaccines but rather concerns disease control, which strictly speaking is outside the remit of the Manual. However since this is very limited and general text we are ready to accept it as proposed.

LINE 530: The species name should be italicised (in this and several other references).

LINES 531-533: The German Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV) was dissolved in 2002 and its task have since been assumed by other German federal institutions. Our attempts to get access to these Working Protocols failed. Thus, this reference needs to be removed and replaced if possible.

3.5.5. Equine encephalomyelitis (Eastern, Western and Venezuelan)

General comment:

The EU can in general support this revised chapter.

We note that the complement fixation test is shortened to "CF test". However in the Dourine and Glanders chapter it is shortened to "CFT". For consistency, we suggest using "CFT" in this chapter as well.

Specific comments are provided below.

Specific comments:

LINES 8-10: The information on Old World alphaviruses seems irrelevant for the summary and should rather be moved to section A. Introduction (for example after Line 84).

LINE 537: The words "authorisation/registration/licensing" in the title should be replaced by "regulatory approval", as this is the wording that was used in other recently adopted Manual chapters. Furthermore, the term "registration" should be replaced with "approval" in other parts of the text (**LINE 539**).

LINE 46: The use of newly hatched chicken for virus isolation is deleted in the summary, however is mentioned as method for this purpose in **LINES 233-235**. This should preferably be harmonised.

LINE 294: Table 3 should be supplemented with primer/probe for VEEV from Vina-Rodriguez *et al*., 2016 to allow researchers to verify the RT-PCR with field strains as mentioned in the text, as follows:

Virus	Reagent designation	Genome position	Sequence (5'–3')	Amplicon length
EEE	EEE 9391, primer F	9391–9411	ACA-CCG-CAC-CCT-GAT-TTT-ACA	69
	EEE 9459c, primer R	9459–9439	CTT-CCA-AGT-GAC-CTG-GTC-GTC	
	EEE 9414 Probe	9414–9434	TGC-ACC-CGG-ACC-ATC-CGA-CCT	
WEE	WEE 10,248 primer F	10,248–10,267	CTG-AAA-GTC-GGC-CTG-CGT-AT	67
	WEE 10,314c primer R	10,314–10,295	CGC-CAT-TGA-CGA-ACG-TAT-CC	
	WEE 10,271 Probe	10,271–10,293	ATA-CGG-CAA-TAC-CAC-CGC-GCA-CC	
VEE	AlphaVIR966 primer F	151-178	TCC-ATG-CTA-ATG-CYA-GAG-CGT-TTT-CGC-A	98
	AlphaVIR966 primer R	248-225	TGG-CGC-ACT-TCC-AAT-GTC-HAG-GAT	
	INEID-VEEV Probe	193-218	TGA-TCG-ARA-CGG-AGG-TRG-AMC-CAT-CC	

3.6.1. Myxomatosis

General comment:

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

Specific comments:

LINE 63: MYXV infections were confirmed in brown hares in France and Ireland during the 1950s (Lucas *et al*.,1953; Collins, 1955) and, more recently, in Great Britain (Barlow *et al*., 2014).

Reference: Barlow, A.; Lawrence, K.; Everest, D.; Dastjerdi, A.; Finnegan, C.; Steinbach, F. Confirmation of myxomatosis in a European brown hare in Great Britain. Vet. Rec. 2014, 175, 75–76.

In several places in the text, reference is made to a new strain of MYXV, however we note inconsistencies in the wording used to describe this new strain, which may cause confusion as it is not clear whether what is being referred to is in fact the same thing. We would thus suggest a consistent wording (see **LINES 64-66** "*However, a new cross-species jump of a genetic variant of MYXV has recently been observed in both Great Britain and Spain, where European brown hares (Lepus europaeus) and Iberian hares (L. granatensis) respectively were found dead with lesions consistent with those observed in myxomatosis*"; **LINE 103** "*The new MYXV isolate from hares in Spain (MYXV Toledo) is a recombinant strain*..."; **LINE 269** "*This method is also able to detect a recombinant MYXV found in the Iberian Hares*"; **LINE 514** "*"new" Hare strain*").

LINES 64-66: Too much interpretation – sentence in original paper of (Garcia-Bocanegra et al., 2019): "*The absence of myxomatosis cases in sympatric wild rabbits suggests differences in the susceptibility to the MYXV strain implicated in the outbreak between these lagomorph species.*"

LINES 93-94: It is not clear what is meant with "virulence" – is it really the virulence of the virus or is it the expression of clinical symptoms; original wording in the paper of Marlier *et al*., 2000: "*No relationship could be established between the presence of specific antibodies to MV and the observed pulmonary lesions or the results of the bacteriological examinations. A significant trend was established between the severity of the lesions and the results of the bacteriological examinations.*"

LINE 510: The words "authorisation/registration/licensing" in the title should be replaced by "regulatory approval", as this is the wording that was used in other recently adopted Manual chapters. Furthermore, the term "registration" should be replaced with "approval" in other parts of the text (**LINE 512**).

3.8.6. Porcine reproductive and respiratory syndrome

General comment:

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

Specific comments:

LINE 33: Please replace “types” by “species”.

LINE 104: The word “insect” is repeated twice (editorial).

LINE 212: Please delete the word "set" (editorial).

LINE 226: Please delete the word "east". PRRSV-1 west strains are also highly variable and in our opinion also difficult to be detected with published methods.

LINE 227: Real-Time RT-PCR is not prone to post-PCR contamination, this is the case only for conventional RT-PCR and nested RT-PCR.

LINE 232: In addition, nucleic acid test (NAT) techniques also have the advantage that they allow rapid differentiation between the two subtypes.

LINE 315: Please specify that this refers to PRRSV-2 isolates.

LINES 313-324: ORF5 sequencing is now currently used in the field to discriminate between wild type and vaccine PRRSV-1 strains. This should be mentioned more clearly in **Section B 1.3.** Ideally a short protocol for ORF5 sequencing including primer sequences should be added.

LINE 324: Full genome sequencing should also be mentioned as a valuable tool to understand the epidemiological and viral evolution in many field cases.

LINE 571: Please replace “both Types 1 and 2” by “both PRRSV-1 and 2”.

LINE 573: We suggest specifying that commercial ELISAs are mainly using recombinant N protein as antigens.

LINE 596: 2 freeze-thaw cycles are sufficient.

LINE 597: Please specify that the antigens are previously inactivated using b-propiolactone treatment.

LINES 611-613; 617: Please specify that serum, controls and antibodies are diluted in saturation buffer (PBS-2% milk-10% FBS).

LINES 633 and 638: We note that the words “are licensed” is used, when referring to the regulatory approval of vaccines. As the terms used in member countries differ widely (e.g. licensing, registration, authorisation), the EU suggests using wording that

is accepted worldwide and is also used in other Manual chapters adopted recently, **LINE 640:** Please specify that some vaccines are now authorised for a use in piglets younger than 3 weeks of age: 2 weeks or even as young as 1 day of age.

such as “approval” or “regulatory approval”. We therefore suggest replacing “are licensed” with “have gained regulatory approval”.

LINES 652-656, Section C 2.1.1.: As full genome sequencing is now available in many laboratories, it should be recommended to determine the full genome sequence of the PRRS MSV. This reference sequence could then be used to control the genetic stability of the PRRS MLV during the production process or during the serial in vivo passages.

LINES 657-664, Section C 2.1.2.: In complement to culture, PCR (which is much more sensitive than culture) might also be used to detect extraneous virus that could be present in the PRRS MSV.

LINES 744-759, Section C 2.3.2.: As MLV vaccines are frequently used in piglets bearing PRRSV specific maternally derived antibody (MDA), the interference of MDA with the efficacy of MLV vaccine need to be evaluated.

3.9.6. *Listeria monocytogenes*

General comment:

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

Specific comments:

LINE 43: Please rephrase to "[...] or molecular serotyping using polymerase chain reaction grouping [...]"

LINE 78: Please add hedgehog to Table 1. Reference:

Listeria Monocytogenes infection of free living western European hedgehogs (*Erinaceus Europaeus*): J Zoo Wildl Med. 2019 Mar 1;50(1):183-189.

LINE 154: We suggest referring also to HACCP in food processing and avoidance of cross contamination from the environment.

LINE 259-261: The sentence lacks clarity and should be deleted or reworded.

LINE 270: also refer to ISO 11290-1 Detection of *L. monocytogenes* and *L. spp* for products intended for human consumption and for feeding animals and environmental samples in area of food production and food handling.

LINES 291-292: Why give typical colony morphology for Oxford and not Palcam, suggest remove for Oxford.

LINE 446: This is not required in the ISO standards, it is optional.

LINE 616: We suggest adding another target gene PrfA to distinguish *L. monocytogenes* from *L. spp*.

3.9.11. Zoonoses transmissible from non-human primates

General comment:

The EU supports the revised chapter.

Specific comments:

None