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 2018

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

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

General comments

The EU can in general support this revised chapter. However, this chapter needs to be carefully revised, as some parts seem to be in a premature stage. Indeed, it remains unclear if some proposed new methods are sufficiently validated and applicable in routine laboratories. Specific comments are provided below.

Specific comments

LINES 9-10: In relation to the Coproantigen tests, we have a reflection for the experts to consider whether there are sufficiently validated copro-antigen tests of comparable quality everywhere.

LINE 10: For the statement that Coproantigen and coproDNA assays are "safe, fast and accurate" we believe it is necessary to provide appropriate references. The sensitivity of the coproDNA detection assay is very prone to inhibitors, which may be varying among individuals. Moreover, there are differences between different extraction methods, therefore it may not be possible to define this protocol as 'accurate'.

LINE 19: *E. granulosus sensu strict*o and not *sensu strictu.*

LINES 35-41: Very few studies are published using coproantigen detection as PCR methods are generally preferred. The move to PCR methods should be encouraged given the reliability of both methods putting more emphasis on molecular methods when compared to ELISA.

LINES 37-38: We suggest the inclusion of the limitations of the methods.

LINES 37-40: These sentences require appropriate references to support them.

LINE 52: Here and for the rest of the text, please use the nomenclature *E. oligarthr*a as referred by Romig *et al*. 2015.

LINE 65: There is no current evidence of the zoonotic potential of *E. felidis* and *E. shiquicus*. Both species are part of the five.

LINE 69: Given the zoonotic risk, we suggests adding the following sentence at the end of the paragraph (in line with other recently revised Manual chapters):
"Clinical specimens and eggs of *Echinococcus* spp. should be handled with appropriate biosafety and containment procedures as determined by biorisk analysis (see Chapter 1.1.4 *Biosafety and biosecurity: Standard for managing biological risk in the veterinary laboratory and animal facilities*)."

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TABLE 2 page 3: *E. equinus* (not *E. echinus*) in the cell in the last row and last column in others intermediate host from Southern Europe.

LINE 106: We suggest quoting up-to-date references, for example the work of Jenny Knapp and colleagues.

LINE 115: "Prevalence of *E. multilocularis* varied from 0 to >10%". To note that in high endemic area it exceeds 50%. We suggest a modification of this text to reflect prevalence in high endemic areas.

LINE 118: The inclusion of Myodes may need to be reviewed as Woolsey *et al*. 2016 showed that is a rather inefficient intermediate host.

TABLE 3: Agent identification; antigen detection: confirmation of clinical cases: only in combination with other methods, because of low specificity, therefore we suggest ++. Moreover, if the method is applied in the final host, there is usually no clinical disease. Prevalence of infection and surveillance: we suggest only ++ due to limitations in specificity. The same holds true for the following row "PCR".

Sections for adult worms in carnivorous definitive hosts more and more PCR appears to be used for surveillance but also in EU countries which needs to prove that are free to demonstrate population freedom for infection. We suggest adding '+' for this two categories in this first row.

LINE 183: We suggest the inclusion of a reference for the decontamination of infective (egg/ adult material) with heating at "70°C for 12 hours". Is the method applicable to fluids and to dry surfaces? If clear prove cannot be provided showing that this method is reliable, we suggest that it is deleted because of the potentially dangerous exposure of people to possibly infectious eggs.

LINE 184: We suggest the inclusion of a reference for the disinfection with "10% bleach" and the inclusion of the duration of the treatment. We also wish to highlight that the usual concentration of sodium hypochlorite is around 3.75%. Why 10% is proposed here?

LINE 239: It is not necessary to start DNA extraction from ethanol-fixed material as it can be done from fresh or thawed material not previously fixed. We suggest remove reference to ethanol-fixed.

LINES 251-252: Necropsies are useful in domestic dogs but also in cats for *E. multilocularis*. We suggest changing 'domestic dogs' with 'domestic carnivores'.

LINES 258-271: This paragraph refers to the examination of the living parasite without decontamination that is usually not preformed. We wish to note that it may not be necessary to provide a full detailed paragraph for this technique.

LINES 278-280: In most published studies, PCR methods were characterised using fecal samples from animals tested by either SCT or IST. The results showed no significant differences between PCR based methods and IST/SCT. Moreover Wahlström *et al*. 2016 applied latent class analysis to estimate the diagnostic sensitivity of SCT and one PCR-based method for detection of *E. multilocularis* infection in foxes. There was no significant differences between SCT and PCR concerning sensitivity. The text may need to be revised accordingly.

LINES 295-303: 1.3.3 Intestinal scraping technique. We suggest that this technique is not deleted as it is widely used. Its sensitivity is in the same range as the SCT if a sensitive protocol is followed (Tackmann *et al*., 2006). The method can be carried out if no PCR equipment is available.

LINE 295: It may be useful to at least name other different techniques available with references. Mainly the SSCT technique (Umhang *et al*. 2011) that could be added as similar to SCT but with the advantage of being less time consuming.

LINE 351: These tests are hardly commercially available (and if they are available, it is not always clear how they were validated) and are difficult to be established as inhouse tests in a laboratory. The description is not sufficient to establish inhouse tests of reliable and comparable quality if run in different laboratories.

LINE 466: Up to 0.5 g of the sample can be processed using commercial DNA extraction kits designed for faecal samples, but not 1-2 g. We suggest that this is corrected.

LINE 451: Abbasi *et al*. 2003 is incorrectly quoted: Abbasi *et al*. 2003 used the QIAmp DNA Stool Mini Kit (Qiagen, Hilden, Germany), which allows to process 0.2-0.5 g fecal sample, but not 1-2 g. We suggest that this reference is revised.

LINES 469-484: Literature referring to the validation of these techniques is missing. We suggest that this method is only included in the manual once it is fully validated.

LINES 474-476: It should also be mentioned that each commercial DNA extraction/PCR protocol needs to be validated before use. There is a high variability among the Testkits for DNA extraction in removing inhibitors. Also the PCR kits must be validated with respect to their performance with CoproDNA.

LINES 478-484: These lines only concern *E. granulosus* while the monitoring of *E. multilocularis* in Europe is very developed. Moreover, mainly for *E. multilocularis* the most commonly used approach in surveillance is the analysis of fox intestines and never coproELISA. Increasingly, the coproPCR approach (mainly real-time PCR) is replacing these intestinal analyses. We suggest incorporating this point here and more broadly in the document (as already mentioned for table 3).

LINES 479-480: It should also be mentioned that each commercial DNA extraction/PCR protocol needs to be validated before use. There is a high variability among the Testkits for DNA extraction in removing inhibitors. Also the PCR kits must be validated with respect to their performance with CoproDNA.

LINES 482-484: Validated DNA extraction/PCR protocols may be less expensive than the suggested combination as the copro-antigen ELISA is hardly available as a commercial kit and must therefore be first established in the lab. It is therefore difficult to recommend this test for large-scale epidemiological studies. Please consider deleting or at least modifying these sentences.

TABLE 4: for the reference Boufana *et al*. 2013, *E. canadensis* must be replaced by *E. shiquicus*.

LINE 550: We suggest replacing "molecules" with "vaccines", as that term seems more appropriate in this context.

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2.1.13 New World screwworm (*Cochliomyia hominivorax*) and Old World screwworm (*Chrysomya bezziana*)

General comments

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

Specific comments

LINE 160 (Table 1): We suggest adding "analysis" after "Mitochondrial DNA" in the first column.

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2.5.1 African horse sickness (infection with African horse sickness virus)

General comments

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

Specific comments

LINE 47: We suggest adding that AHSV can be inactivated at 72^{o}C for 120 min. followed by three blind passages in Vero Cell line.

LINE 308: In the section 'Interpretation of the results' we suggest avoiding the classification of results into positive and inconclusive based on a strict Ct-value cut-off, as Ct-values might be influenced by the used chemistry and/or PCR-machines and might thus differ between laboratories.

LINES 319-322: This sentence should be deleted, as it is just a repetition of the sentence before.

LINE 324: see comment above on line 308.

LINES 349-358: This paragraph has some editorial errors after using several fonts and font sizes in the same sentence.

LINE 522: This sentence should read "…with 70% ethanol and stained with 1% basic fuchsin ~~fuschsin~~".

LINE 570: We suggest adding the following after "[...] respectively.": "Serotypes 5 and 9 are not included in vaccine formulations".

2.5.3 Dourine

General comments

The EU can in general support this revised chapter. However, this chapter needs to be carefully revised. Indeed, some parts (e.g. Sections 1.1.1.2; 2.1.4; 2.3.) seem to be in a pre-final stage. The EU is ready to offer support the OIE in this regard.
Specific comments are provided below.

Specific comments

LINES 4 and 38: We suggest switching 'chronic and acute' as follows 'Dourine is an acute or chronic contagious disease of breeding equids that is transmitted directly from animal to animal during coitus.'

LINE 11: We suggest re-phrasing as follows 'Although dourine is often fatal, equids may act as latent carriers and may spontaneously recover.'

LINE 13: The positioning of the sentence 'Subclinical infections can occur' in the text seems awkward. A new location should be found for it, perhaps as an addendum to the following sentence 'Subclinical infections can occur, particularly in donkeys and mules which are more resistant to infection than horses and may remain as unapparent carriers.'

LINE 18: We suggest re-wording the following two sentences 'Clinical signs are characterised by periodic exacerbation and relapse, with infection typically being fatal. Recovery is rare. Moderate fever, local oedema of the genitalia and mammary glands, cutaneous eruptions, incoordination, facial and lip paralysis, paraplegia, ocular lesions, anaemia, and emaciation may all be observed.'

LINE 22: We suggest re-phrasing as follows, changing the tense of the latter part of the sentence and associating *T. evansi* infection with surra for readers who may not be well informed as regards trypanosomes 'Oedematous cutaneous plaques can be 5–8 cm in diameter and 1 cm thick. Although considered pathognomonic for infection with *T. equiperdum*, they are also occasionally reported in equids infected with *T. evansi* (colloquially known as surra).

LINE 26 and as follows down the document: We suggest abbreviating the complement fixation test as the CFT, rather than the CF test.

LINE 27: We suggest that antibodies do not need to be pre-fixed with 'humoral.'

LINE 31: We suggest providing the following abbreviation at this point 'Enzyme-linked immunosorbent assays (ELISAs) are also used.'

LINE 41: We suggest slightly changing the following sentence to the pleural and include a comma 'So far, no human cases have been reported.'

LINE 43: ‘Dourine is the only trypanosomosis that is not transmitted by an invertebrate vector.’ This sentence requires a reference.
LINE 47: We suggest that this sentence is too long and contains too few linking words. Perhaps it could be divided into two sentences as follows ‘Infection is transmitted during coitus due to the presence of the parasite in the seminal fluid and mucous exudate of the penis and sheath of the infected male and in the vaginal mucus of the infected female. Transmission occurs more commonly from stallion to mare rather than from mare to stallion.’

LINE 51: We would suggest re-phrasing as follows ‘Following tissue invasion, oedematous patches appear in the genital tract.’

LINE 51: A slight change would be beneficial ‘Parasites may subsequently pass into the blood, where they are carried to other parts of the body.’

LINE 53: We suggest re-wording this entire paragraph as below. The last sentence regarding the donkeys requires a reference.
‘The incubation period, severity and duration of the disease vary considerably. In South Africa, the disease is usually mild and chronic, capable of persisting for 6-24 months (Henning, 1955). In other areas such as North Africa and South America, the disease tends to be more acute and often lasts no more than 1–2 months. Although dourine is considered a fatal disease with an average mortality of 50% (especially in stallions), spontaneous recovery may occur. Subclinical infections are recognised, particularly in donkeys and mules which are more resistant to infection compared to horses. Subclinically infected equids remain a transmission risk; both semen and vaginal secretions from subclinically infected donkeys may contain infective trypanosomes.’
Mention of the Italian outbreak is out of keeping with the theme of this particular paragraph. If included, we suggest that it should be in the first paragraph of the Introduction, alongside a brief description of the current worldwide distribution of the agent.

LINE 61: We suggest re-phrasing ‘As trypanosomes are not continually present in the genital tract of infected animals, transmission does not necessarily take place at every mating.’

LINE 63: Re-phrasing of this sentence may be beneficial, for example to the following: ‘Transmission of infection can occur via contact with mucosal surfaces, such as may occur from mare to foal.’ If included, the latter point requires a reference.

LINE 66: We suggest re-phrasing the sentence to ‘Experimental challenge studies can only be conducted in a limited range of small mammal models, including rabbits, rats and mice.’

LINE 71: Re-structuring of this sentence may be beneficial ‘Dourine is marked by stages of exacerbation, remission and relapse; these vary in duration and may occur once or several times before death or recovery.’

LINE 75: We suggest rewording the two sentences: 'A pathognomonic sign is the appearance of raised oedematous skin plaques, which may be up to 5–8 cm in diameter and 1 cm thick. The lesions usually appear over the ribs but may occur anywhere on the body and typically persist for between 3 and 7 days. However, they are not a constant feature.'

LINE 78: A slight change is recommended **'**During each remission, an increasing extent of permanently thickened and indurated tissue can be seen.'

LINE 84: A slight phrase reversal is recommended **'**In heavy breed stallions, the oedema may extend over the whole floor of the abdomen.'

LINES 75 and 85: These two paragraphs should be amalgamated as they discuss the same topic.

LINE 85: We suggest re-phrasing as follows 'Pyrexia is intermittent. Nervous signs may include incoordination, which primarily affects the hind limbs, lips, nostrils, ears and throat. Nervous signs may also include facial paralysis, which is usually unilateral.'

LINE 106: This sentence is very long- we would suggest re-phrasing as follows:
'This is rarely possible because: (a) although the clinical signs and gross lesions in the developed disease may be pathognomonic, they cannot always be identified with certainty, e.g. in early stages or latent cases; (b) the trypanosomes are only sparsely present and are extremely difficult to find, even in oedematous areas; (c) the trypanosomes are only fleetingly present in the blood, and in small numbers that defy detection.'

LINE 108 and elsewhere: Latent infection versus subclinical infection should be defined and appropriately referenced if both terms are being used in the text.

LINE 108: We suggest that the differential diagnoses should be included in the Introduction, not in this paragraph.

LINE 116: We suggest re-phrasing as follows 'Recently, new putative *T. equiperdum* strains have been isolated in Ethiopia (Dodola), Italy (ICT 2011) and Venezuela (TeAp-N/D1). However, these isolates require further characterisation (Hagos *et al*., 2010; Perrone *et al*. 2009; Pascucci *et al.,* 2013).

LINE 120: 'Recently, other approaches have been studied and reported on (Claes *et al*., 2003).' If this sentence is to be included and referenced, the other approaches taken by this study should be listed.

LINE 121: We suggest re-phrasing to 'Trypanosomes are only present in low numbers in infected animals and are typically found in lymph and oedematous fluids from the external genitalia, vaginal mucus (Parkin, 1948) and exudates from skin plaques and mammary glands (Pascucci *et al*., 2013; Scacchia *et al*., 2011).'

LINE 133: The sentence refers to ‘nagana and surra.’ These are colloquial terms and there has been no prior discussion as to their causative agents. Their full identification should be provided for readers who are not familiar with trypanosomes. *T. evansi* has been briefly mentioned in the above text, but not in connection to surra; both subgenuses are mentioned later in the paragraph, but it is not clarified which causes what.

LINE 144: A slight correction as follows ‘More relevant for distinction is the presence of maxi-circles in *T. equiperdum* and their absence in *T. evansi,* which provides a possible differentiation between these two parasites (Li *et al*., 2007).’

LINE 145: We suggest re-phrasing as follows ‘Although some publications suggest that the absence of the VSG RoTat 1.2 could be a molecular marker to differentiate *T. equiperdum* from *T. evansi* (Claes *et al*., 2003; 2004), it is absent from some *T. evansi* strains (Ngaira *et al.,* 2005).’

LINE 158: We suggest re-phrasing as follows **‘**Following identification of a seropositive result and after a clinical examination, serological tests should be repeated twice at 15–20 day intervals and an accurate epidemiological investigation should be undertaken.’

LINES 158-162: The text proposed in this new point 1.3. "*Confirmation of dourine cases*" resembles a case definition that would usually be included in the *Terrestrial Code*. As the revision of the Code chapter on dourine is currently included in the work programme of the Code Commission, we invite the OIE to closely coordinate the ongoing revision of both the Manual and Code chapter on dourine between the BSC and the Code Commission on this point in order to avoid any possible overlaps or contradictions.

LINE 164: We think it is unnecessary to prefix ‘antibodies’ with ‘humoral.’

LINES 182, 316 and 326: There are excellent alternatives to “veronal buffer” available, which need to be mentioned, as barbital is a controlled narcotic substance in many countries.

LINE 187: We suggest removing the start of the sentence and re-phrase as follows ‘*T. equiperdum* seems to be differentiated from *T. evansi* type A by absence of the VSG RoTat 1.2 gene (Claes *et al*., 2003, 2004). Therefore, *T. equiperdum* OVI and BoTat 1.1 strains could be suitable as antigen sources.’

LINES 238-246: References like “SOPs: Ty02a and Ty02b, Institute of Molecular Pathogenesis, Friedrich-Loeffler-Institute, Jena, Germany” may not be too helpful. We propose that it would be a more sustainable solution to incorporate the necessary information from these external documents directly into the OIE document. Colleagues at the Friedrich-Loeffler-Institute have kindly offered to help the OIE in this regard if sufficient notice is provided.

LINE 240: The sentence "For details, please refer to the SOPs." is a bit unusual for the Terrestrial Manual. Indeed, it is not clear where these SOPs

have been published (peer reviewed paper, or on the website of a reference laboratory). Therefore, either a reference or link should be added, or the sentence should be deleted.

LINES 245-246: Referring to “sample ID numbers 13Ty299, 13Ty300 & 13Ty301” may not be helpful. We suggest to removing the sample ID numbers and replacing this sentence by “Culture adapted trypanosomes can be stored at -80°C and in liquid nitrogen.”

LINES 404-405: The ‘Bundesinstitut für Gesundheitlichen Verbraucherschutz und Veterinärmedizin’ no longer exists and the respective departments became part of the Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health in 2004. We suggest replacing the designation in order to facilitate contact.

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2.5.5 Equine encephalomyelitis (Eastern, Western and Venezuelan)

General comments

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

Specific comments

LINES 9 and 57: Horses are not dead-end hosts for VEEV epizootic strains. When the document talks about WEE and VEEV in the same sentence stating "humans and horses are incidental dead-end hosts" is incorrect. We suggest that the sentence reads "Horses and humans are incidental end hosts for WEE. In the case of VEEV, horses are not dead-end hosts".

LINE 15: "viruses" should be added after WEE

LINES 47 and 394: It is not clear what is meant by "the only acceptable vaccines"; perhaps this should be replaced with "the only recommended vaccines".

LINE 51: We suggest replacing the word "never" with "not" (style).

LINE 94: "virus" should be added after VEE ("enzootic VEE virus strains").

LINE 109: We suggest replacing "or non-infectious agents producing similar signs produce similar clinical signs" with "or non-infectious agents producing similar signs can produce similar clinical signs " for better readability.

LINE 126: We suggest replacing "Severe infection…" with "Severe clinical disease…".

LINE 150: We suggest to "reverse-transcription" as that is the correct term for the acronym RT-PCR (used e.g. on **LINE 251**, and also the other Manual chapters). (This change should consistently be made throughout the chapter, also e.g. in **LINES 209, 215**, etc.).

LINE 209: "Viral Isolates" should be "Viral isolates".

LINE 234: "°" is missing in annealing temperature. Must be "46°C".

LINE 247: "viral RNA" should be added after WEE.

LINE 360: In "The light absorbance of the test serum is measured at 405 nm" "of" should be deleted.

LINE 433: A "." should be added after extraneous agents.

LINE 443: "In an emergency epizootic situations" should read "In an emergency epizootic situation".

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LINE 451: “viruses” should be added at the end of the sentence.

LINE 498 *et seq*.: The batch potency is mentioned for WEE and EEE virus only. VEE is missing.

2.5.6 Equine infectious anaemia

General comments

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

Specific comments

LINES 7 and 36: We suggest including SIV in the list of lentiviruses.

LINE 8: This line suggests that all of (1) clinical signs, (2) pathological lesions, (3) serology and (4) molecular methods are required to make a diagnosis. However, the clinical signs and pathological lesions are non-specific and a diagnosis could not be made on them in isolation. Perhaps a better way to convey this would be to say 'Although EIA may be suspected on the basis of clinical signs and pathological lesions, confirmation of infection requires further serological and molecular based testing.'

LINE 11: We suggest the re-wording of this line as follows 'As EIA infection generates a persistent antibody response, all equids older than 12 months which test seropositive are identified as virus carriers.'

LINE 12: We suggest the re-wording of this line as follows 'In young equids less than 12 months of age, positive serological reactions may be due to maternally derived antibodies; therefore, identification of EIA infection status may have to rely solely on molecular techniques.'

LINE 13: We suggest re-wording to **'**As virus reservoirs, infected animals are a transmission risk to other equids.'

LINE 14: We suggest re-wording to 'The virus is primarily blood-borne; biting flies are mechanical vectors for the virus in nature and infection may also be spread via iatrogenic routes'.

LINE 15: It is unnecessary to state 'from a horse.' We suggest re-wording to 'Virus isolation may be performed by either inoculating suspect blood into a susceptible horse or into leukocyte cultures prepared from susceptible horses.'

LINE 16: As it is a summary, we suggest just saying 'positive serological reactions.' If the ELISA is specified, we would also include the AGID. We suggest re-wording as 'Recognition of infection in experimentally challenged horses may be made on the basis of clinical signs, haematological changes, positive serological reactions and/or detection of the virus by molecular techniques.'

LINES 18 and 19: "~~an immunodiffusion test or~~" should not be deleted.

LINE 20: We suggest re-wording to 'Successful virus isolation in horse leukocyte cultures is confirmed by the detection of specific EIA antigens, immunofluorescence assays, polymerase chain reaction based techniques or through the experimental challenge of culture fluids into susceptible horses.'

LINE 24: We suggest re-wording to 'Agar gel immunodiffusion (AGID) tests and enzyme linked immunosorbent assays (ELISAs) [only spelt out if it is the first time it is appearing in the text] are simple, reliable serological tests for the demonstration of EIAV infection.'

LINE 25: As the AGID is the first test stated in the previous sentence, it should really be the first test mentioned in this one too. We suggest re-wording to 'The AGID should be used to confirm ELISA positive results.'

LINE 28: It should be emphasised that a multitude of licensed and validated commercial tests are available that ensure high and stable quality.

LINE 29: Take the commas out of the following sentence 'An attenuated live vaccine was developed in the early 1970s and used extensively in China (People's Rep. of) between 1975 and 1990.'

LINE 33: We suggest replacing the last sentence: So far, no efficacious vaccines are available that confer protection against heterologous virus strains and simultaneously allow discrimination of vaccinated from infected animals.

LINE 34: It is important to note that in 30-70% of the cases the infections remains in its whole course clinically unapparent.

LINE 35: We suggest slightly re-wording the following 'The infection was formerly known as swamp fever and is limited to equids.'

LINE 47: 'Nucleic acid sequence comparisons have demonstrated a marked relatedness among these viruses.' This sentence requires a reference.

LINE 48: We suggest combining these two sentences **'**Once a horse is infected with EIAV, its blood remains infectious for the remainder of its life and it can potentially transmit the infection to other horses (Cheevers & McGuire, 1985).'

LINE 49: Replace "viraemic" by "virus".

LINE 50: insert: …transfer of blood "or contaminated secretion" of an infected horse…

LINE 51: We would give the Linnaean taxonomical classification for each family of flies that may be involved in transmission and only provide their colloquial name in brackets i.e. Tabanidae (commonly known as horseflies).

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LINE 52: We suggest specifying blood products and other blood contaminated equipment on this list 'Transmission can also occur by the iatrogenic transfer of blood through the use of contaminated blood products, needles, syringes, IV administration sets or other equipment.'

LINE 55: AGID and ELISA should only be fully spelt out if it is their first time appearing in the text. We would suggest re-wording this sentence to something along the lines of 'However, a recent study in mules indicated that infected animals with indeterminate AGID results and positive ELISA results can have viral loads at the same level as animals with stronger antibody responses and are therefore equally as likely to be a potential source of transmission (Scicluna *et al*., 2013).'

The last sentence describes a very special and rare case; However it should be clearly stated that the infection can in general be transferred by clinically inapparent equids to contacts and also to foals (Issel CJ, Adams WV, Meek L, *et al*. Transmission of equine infectious anemia virus from horses without clinical signs of disease. J Am Vet Med Assoc 1982;180:272–275).

LINE 70: insert "…noted with ELISAs "in rare cases".
ELISAs are since years validated by purpose, validated by licensing procedures and compared in national/ international proficiency tests (Nardini R, Autorino GL1 Issel CJ, Cook RF, Ricci I, Frontoso R, Rosone F, Scicluna MT. Evaluation of six serological ELISA kits available in Italy as screening tests for equine infectious anaemia surveillance. BMC Vet Res. 2017 Apr 14;13(1):105. doi: 10.1186/s12917-017-1007-6.+ proficiency test of the EU Reference Laboratory 2018).

Table 1: We suggest changes to the ELISA row as follows:

Method	Population…	Individual animal freedom…	Contribute to…	Confirmation of….	Prevalence of…	Immune status..
ELISA	**+++**	**+++**	**+++**	**++**	**+++**	n/a

LINE 71: We suggest re-wording to 'The AGID test is specific, thus has the advantage of distinguishing between EIA and non-EIA antigen–antibody reactions.'

LINE 72: We suggest re-wording to 'Discrepancies between either test methods or tests with questionable results can be further evaluated by via immunoblot analysis (Issel *et al*., 1999; 2013; Rusvai *et al*., 2009).'

LINE 101: A slight correction is required (EIAV not EAV) 'It has proven to be a sensitive technique to detect field strains of EIAV in white blood cells of EIA infected horses.'

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LINE 125: We suggest re-wording to 'The AGID test detects the production of precipitating antibodies produced as a result of EIA infection.' We support removing 'rapidly.'

LINE 126: This sentence could be re-phrased and a slight correction is required 'Specific reactions are indicated by the formation of precipitation lines between the EIA antigen and the test serum; their identity is confirmed by comparison with the reaction between the antigen and the positive standard serum.'

LINE 128: replace "alternately" by "alternatively".

LINE 167: H3BO3 should read H_3BO_3.

LINE 183: insert as the last sentence: 'Unspecific precipitin lines may occur that are not in identity with the standard lines and therefore cross the control lines'.

LINE 186: insert after "…be bled again after 3-4 weeks": "In rare cases antibodies are not detectable before 90 days after infection".

LINE 189: We suggest slightly altering this sentence to 'If the mare passes EIA antibodies to the foal through colostrum, then a period of 6 months or longer after birth must be allowed for these maternally derived antibodies to wane.'

LINES 193-196: We suggest re-wording to 'It should be noted that maternally derived antibodies can often be detected for up to 12 months of age, therefore alternative diagnostic methods should be considered such as PCR based techniques.'

LINE 197, 2.2. Enzyme-linked immunosorbent assay: information concerning USDA licensed tests is not useful without including a reference or link to a source where comprehensive information about the tests can be found (may be in the USDA homepage?); Further recommendations and standardisation should be provided by OIE and EU reference laboratories.

LINE 205: 'A positive test result by ELISA should be retested using the AGID test to confirm the diagnosis because false-positive results have often been noted with the ELISA.' This sentence requires a reference.

LINE 206: Replace "often" by "in rare cases".

LINE 209: We suggest that the sentence "This standard should not be used as the reference for minimum detection limits for the reaction" should be replaced by "This standard should not be used as the reference for minimum detection limits for the ELISA reaction".

LINE 217: add at the end of the sentence '…and because the live vaccine was able to spread in the equine population'.

LINE 220: A slight correction is required: ‘The use of a live EIAV vaccine needs to be very cautiously and carefully evaluated.’

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2.5.7 Equine influenza (infection with equine influenza virus)

General comments

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

Specific comments

LINE 80: We suggest adding “Equine influenza viruses have the potential to evolve, as found in an outbreak from vaccinated and unvaccinated horses. The virus was identified as part of Florida Clade 1. However, several genetic variants were identified during the outbreak suggesting that the virus evolved”. This is the reference for the above sentence: Patricia Filippsen Favaro, Wilson Roberto Fernandes, Dilmara Rischark, Paulo Eduardo Brandao, Sheila Oliveira de Souza Silva, Leonardo Jose Richtzenhain. Brazilian Journal of Microbiology Volume 49, Issue 2, April–June 2018, Pages 336-346.

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2.7.10 Peste des petits ruminants (infection with peste des petits ruminants virus)

General comments

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

Specific comments

LINE 26: We suggest deleting "(ELISA)", as no acronym is used in this paragraph for the other tests and the correct acronym ("IC-ELISA") is indicated **in LINE 112.**

LINE 50: We suggest replacing "Over the past 20 years" with "Since the late 1990s", as this will be more readable.

LINE 91 (Table 1): We do not support the "+" for the penside test in the column "individual animal freedom from infection prior to movement". Indeed, as the reliability of the test is not described in the chapter (see EU comment below), it should not be used for that purpose. For the same reason, "+++" should be changed to "++" in the columns "Contribute to eradication policies" and "Confirmation of clinical cases".

LINE 97: We suggest replacing "reverse-transcriptase" with "reverse-transcription" as that is the correct term for the acronym RT-PCR (see also EU comment on Chapter 2.5.5.). This change should be made throughout the chapter (e.g. in **LINE 153**).

LINE 177: We suggest replacing "procedure" with "instructions" for consistency.

LINES 177-179: We recommend that tissues should be included here, if the procedure/extraction kit used has been thoroughly validated for that purpose. For tissues, blood, white cells or swabs, RNA extraction based on magnetic beads or spin columns are also suitable. The resulting RNA is stored at –70°C (or –20°C if –70°C not available) until required.

LINES 273-282: Information should be included on the reliability of this pen-side test, such as diagnostic sensitivity and specificity. Indeed, it is unclear from the text whether this text was validated, and in what situations it can or cannot be used.

LINES 636-637: The last sentence does not read well and we suggest that it is rephrased (e.g. "None of these vaccines has yet been validated for field use.").

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

2.8.1 African swine fever (infection with African swine fever virus)

General comments

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

We wish to emphasise that there is a lack of scientific evidence for the importance of the role played by carrier pigs in the epidemiology of the disease. See specific comments below.

Serology is an important part of ASF diagnosis. However, it should be kept in mind that circulation of highly virulent strains will lead to a very slow increase in seroprevalence (e.g. in wild boar). It takes time before prevalence rates are high enough to allow reliable surveillance. See specific comments below.

Specific comments

LINE 26 *et seq*.: In case of an outbreak, antibody detection will help in any case (providing an idea on disease dynamics).

LINES 35-42: There is far too much detail here (regarding countries and year of ASF incursion) that is neither relevant for the purposes of the Manual nor correct – indeed, not in all countries mentioned in **LINES 39 and 40** have both hosts (domestic and wild pigs) been affected. Furthermore, the global ASF situation evolves rapidly, and since Manual chapters are updated only every 4 to 6 years, this information will quickly become outdated and erroneous. Therefore, we suggest keeping the text to a minimum and instead refer to the information publicly available in WAHIS that is kept up to date and now even provides weekly overview reports of the global ASF situation.

LINE 47: Please, consider a revision of the word “infectious” proteins. ASFV proteins are not infectious.

LINE 56: Of “the” VP72 gene.

LINE 69 *et seq*.: “apparently healthy virus carriers” are not a syndrome. In our opinion, “subclinical” would reflect the mild or almost non-existent clinical signs in some pigs.

LINE 71: Check usage of mortality rates/ case fatality rates.

LINE 76: Consider deleting “in nature”. The data is from experimental infections.

LINE 78: See above (Line 71). Check usage of mortality rates / case fatality rates.

LINE 85: There is lack of scientific evidence that long-term shedding occurs along with a certain detectability of the pathogen. We recommend including

that genome detection in some lymphnodes does not necessarily mean shedding of virus. The behavior of “carriers” is being discussed and there is no agreement among the scientific community that the long-term carrier pigs constitute the biggest problem in controlling the disease.

LINES 95 and 96: Check use of articles (delete “the” PCR and by “the” direct…) (linguistic).

LINE 108: There is some duplication in this paragraph. Consider deleting the second sentence.

LINE 125 - Table 1: Is there an overestimation of serology? It is beneficial beyond doubt, but at least for surveillance in wild boar, low sero-prevalences after years of virus circulation hamper the implementation. Also for eradication policies, the use of serology depends on the situation.
We suggest checking if there are many isolates non-haemadsorbing?

LINE 135: We suggest including bone marrow as a sample.

LINES 282-284: We wish to recommend that for PCR from wild boar samples, blood swabs should also be considered. For reference see Petrov *et al*., Vet. Mic. 2014.

LINE 294: We suggest a change of this sentence from “(e.g. if organ homogenate looks muddy and genomic DNA content is suspected to be huge).” to “(e.g. if organ homogenate looks turbid and/or an excessive genomic DNA content can interfere with DNA replication).”

LINES 302-303: We wish to recommend the inclusion of endogenous or exogenous extraction controls, to control for successful extraction and/or absence of inhibitors in every sample. For reference, see Haines *et al*., 2013, or Toussaint *et al*., J Virol Methods 2007.

LINE 453: It could be considered to exchange the quencher TAMRA by a BHQ-quencher.

LINES 552-553: We suggest deleting the last sentence of this paragraph (starting with "Further advice ...") as this is an unnecessary repetition of what is included at the end of the chapter (**LINE 989**).

LINE 557: We suggest inserting "commercial" before "vaccine is available", as there are experimental vaccines.

LINE 560 *et seq*.: The two sentences do not match and cannot be linked with “Moreover”. The strains circulating in Eastern and now also Central Europe and Asia, are highly virulent. This would mean that serology is difficult anyway.

LINE 572: Insert the nature of the test (lateral flow assay), consider including alternative sample matrices.
Furthermore, We suggest deleting "is" before "commercially available" (linguistic).

LINE 783: We suggest inserting "presumed" before "free from ASF", for reasons of clarity (sincere there are sera positive in the ELISA, and this is a confirmatory test, the outcome may well be that the areas indeed are not free).

LINE 855: We suggest inserting "commercially available" before "vaccine for ASF" (same rationale as above).

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

2.8.3 Classical swine fever (infection with classical swine fever virus)

General comments

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

Specific comments

LINES 106-110: We recommend that Porcine Reproductive and Respiratory Syndrome (PRRS) is included as it might also be a differential diagnosis to CSF.

LINES 275-284: We recommend that methods that allow simultaneous detection and differentiation of CSFV and ASFV are mentioned here, such as Haines *et al*., Plos One 2013.

LINE 649: We suggest replacing "ideal" with "optimal", for consistency with the wording used in **LINE 627**.

LINE 650: It will be useful to specify if "absence of transmission" includes vertical transmission, as this is indeed crucial for the epidemiology of pestiviruses.

LINE 662: We suggest rewording as follows: "Therefore, there is still room ..." (linguistic).

LINES 663-664: We suggest deleting the sentence starting with "Other approaches ...", as this information on techniques that are not yet avalable let alone validated is not relevant in an OIE standard.

LINE 670: WE suggest replacing "otherwise" with "previously", for reasons of clarity and consistency with wording used in the OIE Code.

LINE 676: We suggest deleting this sentence, as it is not relevant for this Manual chapter.

2.9.7 Mange

General comments

The EU supports this revised chapter and has no specific comments.

Specific comments

None.

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

3.1 Laboratory methodologies for bacterial antimicrobial susceptibility testing

General comments

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

Specific comments

LINE 35: We suggest the following addition as follows: "and ~~even~~ commensal organisms such as resistance to carbapenemases, colistin, linezolid, macrolids etc…"

LINE 45: We suggest the following addition as follows: "cost, reproducibility, accuracy, accessibility, and individual preference."

LINE 48: We suggest the following change: "day-to-day laboratory use routine".

LINES 49-51: We suggest that this sentence: "The reference susceptibility testing method is now generally considered to be broth dilution determination of minimum inhibitory concentration (MIC) and a reference method is available (ISO [International Organization for Standardization], 2006)" is changed to "Currently, the reference ~~susceptibility testing~~ AST method is the ~~now generally considered to be~~ broth micro-dilution method that determines~~ation of~~ the minimum inhibitory concentration (MIC) and ~~a reference method is available~~ described by the ISO [International Organization for Standardization], 2006)".

LINES 57-58: We suggest that this sentence: "As the science of AST has progressed, a greater understanding of the multiple factors that could affect the overall outcome of susceptibility testing has become clearer." Is changed to "By time ~~As the science of AST has progressed~~, a ~~greater~~ better understanding of the multiple factors that could affect the ~~overall~~ AST results ~~outcome of susceptibility testing~~ has become clearer."

LINE 57: We suggest adding this reference "Integrated surveillance of antimicrobial resistance in foodborne bacteria. Application of a One Health approach." Authors: WHO. Publication details: Number of pages: 76; Publication date: 2017; Languages: English; ISBN: 978 92 4 151241 1.

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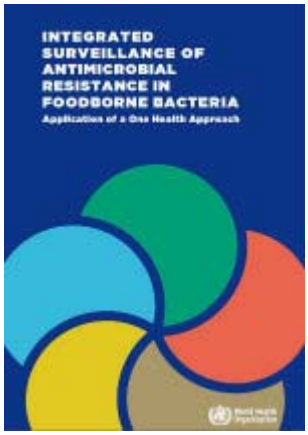

LINES 61-62: We suggest that this sentence: "In order to achieve standardisation of AST methods and comparability of AST results, the following requirements apply:" is changed to "The following requirements should be applied ~~in order to achieve~~ standardisation of AST methods and comparability of AST results~~, the following requirements apply~~:".

LINES 63-64: We suggest that this sentence: " i) the use of standardised AST methods and the harmonisation of AST test parameters (including choice of antimicrobial agents and subsequent interpretive criteria) are essential," is changed to "i) the use of standardised AST methods including ~~and~~ the harmonisation of AST test parameters such as media, inoculum, incubation time, quality controls, as well as the ~~(including~~ choice of antimicrobial agents and subsequent interpretive criteria~~)~~ are essential.

LINE 67: We suggest rephrasing this sentence to follow well-established guidelines such as those from CLSI and EUCAST which are based on ISO.

LINE 68: This sentence may be confusing as susceptibility data is not collected but generated and reported. The reference to ‘quantitatively’ data does it refer to MIC data?, if so we would suggest to also include this in the text as many might not know that quantitative refers to MIC.

LINE 69: We suggest using the term “reference laboratories”.

LINES 84-86: We wish to make you aware that WHO has already created a list and that at least the same classes should be tested: "Critically important antimicrobials for human medicine", 5th revision. Authors: WHO.

LINES 89-91: We suggest that the representative classed used for monitoring should be used under the ‘One Health’ context e.g. CIP and not ENRO etc.

LINES 95-96: We propose changing this sentence from: "the number of antimicrobials to be tested should be limited in order to ensure the relevance and practicality of AST." to "the number of antimicrobials to be tested should **comply to the guideline used such as CLSI, EUCAST and ISO and at least contain class representatives** ~~be limited in order~~ to ensure the relevance and practicality of AST."

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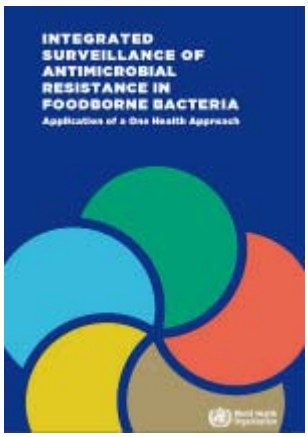
LINES 98-99: We wish to inform about the following document that contains a list of unusual phenotypic resistance, which ought to be carefully monitored and re-tested: "Integrated surveillance of antimicrobial resistance in foodborne bacteria. Application of a One Health approach." Authors: WHO. Publication details: Number of pages: 76; Publication date: 2017; Languages: English; ISBN: 978 92 4 151241 1.



LINES 109-111: We suggest the modification of this sentence from "i) once the bacterium has been isolated in pure culture, the optimum concentration of the ~~inocula~~ inoculum used in the test must be determined using a standardised method to obtain accurate and repeatable susceptibility results. Bacteria or other organisms used in AST testing should be from a fresh culture", to "i) once the bacterium has been isolated in pure culture, the ~~optimum concentration~~ density of the ~~inocula~~ inoculum used in the test must be ~~determined~~ adjusted using a standardised method such as a nephelometer or spectrophotometer to ensure a well-defined number of colony forming units (CFU) to obtain accurate and repeatable susceptibility results. Bacteria or other organisms used in AST testing should be from a fresh 24h culture".

LINES 112-113: We suggest changing this sentence from "ii) the composition and preparation of the agar and broth media used (e.g. pH, cations, thymidine or thymine, use of supplemented media)." to "ii) the composition and preparation of the agar and broth media used should apply to CLSI, EUCAST or ISO and take into account ~~(~~e.g. pH, cations, thymidine or thymine, use of supplemented media~~)~~".

LINE 114: We suggest changing "employed procedures" to "used procedures".

LINE 115: We suggest changing this sentence from "iii) the content of antimicrobial in the carrier (antibiotics used in microtitre plates, disk, strip, tablet)," to "iii) the content, range/ interval and concentration of the antimicrobials used (microtitre plates, disk, strip, tablet) should comply to CLSI, EUCAST and ISO standards and relevant to the species tested. ~~in the carrier (antibiotics used in microtitre plates, disk, strip, tablet),~~".

LINE 119: With our previous comment, this bullet vii) can now be deleted as its content has been merged with iii) above.

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LINE 121: We propose the following addition as follows "epidemiological cut-off values (ECOFFs))."

LINE 125: We propose changing this sentence from "The selection of an AST methodology may be based on the following factors:" to "The selection of an AST methodology may be ~~based on~~ influenced by the following factors:"

LINES 126-134: We propose that in this list of factors, a new factor "accessibility" is added to the list.

LINE 137: four methods are listed below.

LINE 138: The reference should be updated – there is a newer edition available.

LINES 139-142: We suggest rephrasing the methods to explain better the different methods: only two methods exists – diffusion (qualitative) and dilution (quantitative). Diffusion methods include disk diffusion and gradient strip tests and dilution methods, agar and broth where broth micro-dilution is the golden standard.

LINE 142: The 'gradient strip method' if included, should be given its own bullet point further down the text like the other methods.

LINES 144-148: Only paper disks are included in the CLSI method. We propose changing the following sentence from: "Disk diffusion refers to the diffusion of an antimicrobial agent incorporated at of a specified concentration into from disks or tablets or strips, into a solid culture medium that has been seeded with the selected inoculum isolated in a pure culture (see section 3). Disk diffusion is based on the determination of an inhibition zone around the disk, the diameter of which is proportional to the bacterial susceptibility to the antimicrobial present in the disk." to "Disk diffusion refers to the diffusion of an antimicrobial agent ~~incorporated~~ present in disks ~~or tablets~~ ~~at of a~~ at a specified concentration ~~into from disks or tablets or strips.~~, The disks ~~/ tablets~~ are applied to the surface of ~~into~~ a solid culture medium, normally Muller-Hinton agar according to CLSI, EUCAST and ISO that has been ~~seeded~~ inoculated with ~~the selected inoculum isolated in~~ a pure culture (see section 3). Disk diffusion result**t** is based on the measurement of the zone diameter of the ~~determination of an~~ inhibition zone ~~around the disk, the diameter of which is proportional to the bacterial susceptibility to the antimicrobial present in the disk~~."

LINE 149: We suggest the deletion of the word "seeded".

LINES 157-159: We propose changing this sentence from "Those antimicrobial agents that are very large molecules diffuse poorly in agar making disk diffusion methods unreliable for these compounds. For this reason disk diffusion methods are not recommended for the susceptibility testing of colistin (Matuschek *et al*., 2018)." to "Those antimicrobial agents that are very large molecules diffuse poorly in agar making disk diffusion methods unreliable and should not be used ~~for these compounds~~. For this

reason disk diffusion methods are for example not recommended for the susceptibility testing of colistin/ polymyxin (Matuschek *et al*., 2018)."

LINE 159: In this web link the EUCAST warnings concerning antimicrobial susceptibility testing products or procedures can be found: http://www.eucast.org/ast_of_bacteria/warnings/.

LINES 163-164: We propose changing this sentence from "Disk diffusion is straightforward to perform, reproducible, and does not require expensive equipment." to "Disk diffusion is ~~straightforward~~ easy to perform, reproducible if standardised, and does not require expensive equipment."

LINES 173-174: It may be worth pointing out that with automated zone-reading devices it is harder to detect contaminations.

LINE 175: We propose to change this sentence "The disks should be distributed evenly on the test plate so that the zones of inhibition…" to "The disks should be distributed evenly on the ~~test plate~~ agar surface allowing ~~so that~~ the zones of inhibition…".

LINE 180: suggest to include a new section here: There is a wide variation in disk quality from different manufacturers (Åhman, 2018). The disks should be purchased from a reliable source, accompanied by a certificate of analysis and each new batch should be tested with appropriate QC strains. Appropriate storing and handling of disks (CLSI, M02 Ed13, 2018) is essential to ensure that the antibiotic content in the disk is retained.

The references are:
https://www.sciencedirect.com/science/article/abs/pii/S1198743X18304567
and CLSI. Performance Standards for Antimicrobial Disk Susceptibility Test. 13th ed. CLSI standard M02, 2018.

LINE 182: We propose adding this text as follows "inoculum according to CLSI; EUCAST and ISO."

LINE 183: We propose change "lighter" to "sparse".

LINES 183-184: The reference used is a national standard not widely used. We propose to use CLSI as reference.

LINES 186-190: We propose to change the following sentence from "The aim of the broth and agar dilution methods is to determine the lowest concentration of the assayed antimicrobial that inhibits the visible growth of the bacterium being tested in either broth or on agar (MIC, usually expressed in µg/ml or mg/litre). The range of concentrations tested in broth and agar dilution methods generally but not necessarily includes 1 mg/litre and with doubling dilutions either side of that value as considered appropriate" to "The aim of the broth and agar dilution methods is to determine the lowest concentration of the ~~assayed~~ antimicrobial that inhibits the visible growth of the bacterium being tested in either broth or the surface of the ~~on~~ agar (MIC, usually expressed in µg/ml or mg/litre). The range of concentrations tested in broth and agar dilution methods generally includes ~~1 mg/litre with doubling~~

two-fold dilutions e.g. 1 mg/L - 2 mg/L etc. ~~either side of that value as considered appropriate~~."

LINES 190-193: Suggest the formulation: However, the MIC does not represent an absolute a value lying exactly at a concentration which was tested. The 'true' MIC is a point between the lowest test concentration that inhibits the growth of the bacterium and the next lower test concentration. Therefore, MIC determinations performed using a dilution series are considered to have an inherent variation of ±1 one dilution.

LINE 196: We propose adding text as follows: "resistant **/** wildtype, non-wild type)".

LINES 198-199: We propose to change the following sentence from: "Antimicrobial susceptibility dilution methods appear to be more reproducible ~~and quantitative~~ than agar disk diffusion and broth microdilution is now considered the reference test" to "Antimicrobial susceptibility dilution methods ~~appear to be~~ are more reproducible and quantitative than agar disk diffusion ~~and~~ **which is why** broth micro-dilution is ~~now considered~~ the current reference test method".

LINES 200-201: This argument is difficult to understand. Suggest to delete it.

LINES 203-205: We recommend checking this statement with experts, as we believe that this is not recommended as its very laborious and difficult to ensure purity and this is why panels are commercially available. If a lab attempts to produce agar or broth micro-dilution plates, they should follow the CLSI manual. It is more than just making two-fold dilutions – there is a very complex dilution system to follow. For reference SOPs can be found here: http://antimicrobialresistance.dk/protocols.aspx and https://www.eurl-ar.eu/protocols.aspx.

LINES 208-209: Suggest: "...a suspension of a bacterium of a predetermined optimal concentration."

LINE 214: lyophilised or dried prediluted…

LINES 223-224: This statement needs a reference. The labour cost for the QC programme needed when using disk diffusion is high and depending on how many antibiotics that are tested the total cost may be equal for both methods.

LINES 208-224: We recommend including the advantages of broth dilution methods, which are numerous.

LINES 218-219: In relation to the sentence "will facilitate the comparability of results among laboratories" it may be useful mentioning that standard EU panels have been developed and used in the EU AMR monitoring of food and animals and based on the EU regulation / decision.

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LINES 223-224: The use of antimicrobial plates and associated equipment is a decision for each individual lab as they have the advantage that can be inoculated by multi-channel pipettes.

LINES 228-229: The two lines have been deleted but should probably be retained. The statement is probably true and should be kept. Many consider agar dilution the Gold Standard especially when it comes to fastidious bacteria and development/evaluation of new protocols.

LINE 239: It may be worth taking into account that producing agar plates, which are not commercially available is not easy and a very laborious task.

LINE 241: We believe this sentence is incorrect as plates can be used up to 3 weeks or more depending on the results of the QC strains.

LINE 243: The purity of the inoculum should always be verified through streaking a sample of each inoculum on a suitable nonselective agar plate and incubate overnight. Thus the purity of the inoculum should not be in doubt. Suggest to delete this point.

LINE 245: The CLSI reference should be update to latest version.

LINE 246: The bullet points and numbering should be harmonised with the points mentioned above.

LINE 250: suggest to rephrase to "...compared with results of other methods…" the references do not only deal with agar dilution.

LINES 259-263: This has been updated and to avoid having to change it everything it is update we propose to leave this out or to ensure that it refers to the newest versions and content of the CLSI 2017 versions.

LINE 264: We now have CLSI 2017 versions.

LINES 275-305: It is probably best to have this section revised and updated by somebody that knows more about WGS and the application of bioinformatics tools.

LINE 342: Non wild-types are described as microbiologically resistant. We believe that it is more accurate to describe non- wild type isolates as isolates that have MICs above the ECOFF, rather than the microbiological cut-off.

LINES 360-370: We recommend that latest reference and guidelines are used. For example, new CLSI guidelines are issued annually with updates. We will not recommend beginning the list with national guidelines e.g. BSAC as this will not ensure harmonisation but make it all fragmented. It should be emphasised that CLSI, EUCAST are those to follow.

LINES 264-265: it should be noted that this test could also be carried out using dilution methods.

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LINES 300-302: suggest to rephrase this sentence to "However, despite the new influx of genotypic tests, documented and agreed upon phenotypic AST methods will still be required to detect unknown and emerging resistance mechanisms among bacterial pathogens".

LINE 324: "…enable a bacterial isolate to be….".

LINES 326 and 334: We suggest updating the reference to the latest edition.

LINE 371: We suggest deleting the text in brackets (formerly NCCLS).

LINES 372, 380, 387, and 390: We suggest updating CLSI references to latest editions.

LINE 402: We proposed the following changes to the table:

Susceptibility testing method	**International standard available**	**Published methods available**	**Use in national surveillance programmes**
Broth (micro) dilution MIC determination	Yes (ISO 20776-1) CLSI, EUCAST	Yes CLSI, EUCAST	Yes, broth microdilution MIC determination is preferred
Agar dilution MIC determination	No CLSI, EUCAST	Yes CLSI, EUCAST	Not widely used
Disc diffusion test	No, CLSI, EUCAST	Yes CLSI, EUCAST A number of different methods are available. These are not in general equivalent.	**May be used,** but broth microdilution MIC determination is preferred

Furthermore, under Agar dilution MIC determination: it is stated that it is a Former reference method (last column). However, according to CLSI broth dilution and agar dilution are both still reference methods.

The Breakpoint method is described here, but has not been described in the entire document.

Under Disc diffusion test: EUCAST should be spelled with capital letters (6th column).

LINE 500: We suggest rephrasing to "…will still be required to detect new and emerging resistance….".

LINES 513-527: These five CLSI documents should be updated to latest editions.