EU COMMENTS

ON THE PROPOSED CHANGES TO OIE MANUAL OF DIAGNOSTIC TESTS AND VACCINES FOR TERRESTRIAL ANIMALS

PRESENTED FOR COMMENTS IN MARCH 2011

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CHAPTER 1.1.3: Quality management in veterinary testing laboratories

General comments

The EU can support the proposed changes only if its comments pertaining to accreditation are taken into account.

Indeed, the EU is of the opinion that accreditation is not a prerequisite for good laboratory quality management, and that other, less cost-intensive tools such as the OIE Quality Standard and Guidelines for Veterinary Laboratories of 2008 can be used by laboratories to enhance the quality of their work.

Furthermore, even though the modified chapter appears to maintain the two options for quality management (accreditation and the OIE standard), some parts of the text seem to imply that accreditation is mandatory. Indeed, the sections on proficiency testing and strategic planning are addressed solely to accredited laboratories.

Finally, the overall objective of the chapter should be clarified. Indeed, it remains unclear if the chapter is meant as a guidance document for laboratories considering accreditation, detailing certain aspects of the ISO/IEC 17025 standard in relation to veterinary laboratories and the demands of the accreditation bodies. Should this be the case, it should be clarified that the interpretations of the ISO/IEC standard and the accreditation procedure merely represent the views of the chapters authors and were not endorsed by the accreditation bodies. On the other hand, should the chapter be prescriptive for veterinary laboratories, it should be clarified to which laboratories it applies, as parts of the chapter are addressed only to laboratories considering accreditation or that were already accredited.

In order not to jeopardise veterinary laboratories, especially in developing countries, by adopting too demanding standards, and in order to clarify the objectives of the chapter, the EU strongly suggests these aspects be reviewed before the modified chapter can be considered for adoption.

Specific comments

LINES 14-18: The EU is of the opinion that there is no "need" for accreditation in laboratory quality management, as there are other acceptable quality management systems in place, e.g. the OIE quality standard of 2008. Thus, the EU proposes the sentence to read as follows:

"The need for mutual recognition of test results for international trade and the acceptance of international standards such as the ISO/IEC[1] ~~International Standard~~ 17025 standard for laboratory accreditation *(*ISO/IEC, 2005 ~~*8*~~*)*

[1] International Organization for Standardization/International Electrochemical Commission.

requires good ~~also affect the need and requirements for~~ laboratory quality management programmes."

Furthermore, in **LINE 41**, the sentence "The OIE standard on this subject is a useful guideline" should not be deleted.

LINE 35: Please insert the words ", strain/genotype characterisation" after the words "turnaround time".

LINE 37: Please insert the words "(e.g. reference laboratory)" after the words "other laboratories".

LINE 77: Please insert the words "(e.g. with the participation in proficiency tests on a regular basis)" after the words "methods used".

LINE 89: Please insert the "ISO/IEC" prior to "17025".

LINES 144-145: The proposed new sentences ("The fact that a test is recommended does not necessarily mean that a laboratory is competent to perform it. Accreditation to ISO/IEC 17025 provides this evidence") should be deleted. Indeed, whereas the first sentence is self-evident, the second implies that only accredited laboratories are reliable to perform recommended tests.

LINE 257: Please insert the words ", virus isolation" after the word "immunofluorescence".

LINE 366: Please insert the words "; organizing in house or external meetings about diagnostics and quality assurance/management" after the word "conferences".

LINE 369: Please insert the words "; writing and reviewing publications about diagnostic methods" after the word "publications".

LINE 369: Please insert the words "Training programs including" prior to the word "visits to other laboratories".

CHAPTER 1.1.6: Laboratory methodologies for bacterial antimicrobial susceptibility testing

General comments

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

Specific comments

LINE 188: The EU suggests inserting the word "lyophilised" before the words "prediluted antibiotics".

LINE 209: Please replace "36" by "60".

LINE 213: Please add the following after the words "within a week":

"(or less depending on the antimicrobials tested)".

LINE 281: Please replace "mg/ml" by "µg/ml".

LINE 322: Please replace the words "CLSI ~~/NCCLS~~" by the words "CLSI (formerly NCCLS)".

LINE 341: Please replace the word "reference" by the word "referenced".

CHAPTER 2.1.2: Aujeszky's disease

General comments

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

Specific comments

LINE 35: The word "in" should be inserted before the words "a variety of mammals".

LINES 56-57: The EU suggests amending the sentence to take into account recent developments, to read as follows:

"Aujeszky's disease is endemic in many parts of the world, but several countries have successfully completed eradication programmes, e.g. the United States of America (USA), Canada, New Zealand and many Member States of the European Union (EU)."

LINES 106-109: The detailed protocols of GB and GE real-time PCRs should be included in the text.

LINES 154-345: The EU would like encourage the publication of the SOPs of the tests prescribed for international trade on the website of the OIE reference laboratory.

LINES 201, 253, 268 and 271: For reasons of clarity, the word "sample" should be inserted after the word "serum".

LINES 384-387: The text of this paragraph is repeated in **LINES 402-405**. The EU therefore suggests deleting the text from lines 384-387 and amending the text in **LINE 402** to read as follows:

"Both the MSV and the MCS must be shown to be free from mycoplasma, […]".

CHAPTER 2.1.16: Trichinellosis

General comments

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

Specific comments

LINE 5: The EU suggests replacing the words "survive less than two months" by "develop in".

LINE 9: Please insert the word "species" after the word "Trichinella".

LINE 16: The EU suggests deleting the words "and abdominal muscles", as abdominal muscles are no predilection sites.

LINE 25: The words "and game" should be replaced by ", game and indicator wildlife species such as foxes".

LINE 45: Please insert the words "by ELISA" after the words "have been detected".

LINE 70-71: The EU suggests replacing the words "in pigs, the tongue usually contains the highest concentration of larvae, followed by the diaphragm" by the following:

"in pigs, the diaphragm pillar~~tongue~~ usually contains the highest concentration of larvae, followed by the tongue~~diaphragm~~".

LINE 80: Please delete the repeated word "be" prior to the words "also be detected".

LINE 91: A comma should be added after the word "experimentally".

LINE 107: Please replace the words "*trichinellosis* in horses" by the words "*Trichinella* infection of horses".

LINES 108-109: Please replace the words "infected meat" by the words "meat harbouring *Trichinella* larvae".

LINE 116: Please replace the words "Zoonotic risk" by the words "Laboratory infection".

LINES 126-127: The EU suggests adding references to the relevant standards in the EU, Canada and the USA mentioned here. As the EU standard (i.e. Regulation (EC) No 2075/2005) is already in the list of references, please add the words "(European Commission, 2005)" after the words "standards in the EU" in **LINE 126**, and also references to the standards of Canada and the USA in **LINE 127**.

LINES 127-128: The EU suggests deleting the sentence “However, a number of other official methods not currently used routinely are not recommended because of their lack of efficiency or reliability”, as it is contradictory.

LINES 244-245: For reasons of clarity, the EU suggests replacing the words "(*Equivalent method A, EC No. 2075/2005* ~~*Method 4: 84/319/EEC*~~)" by the following:

"(Equivalent method A, Regulation (EC) No 2075/2005, European Commission, 2005 ~~*Method 4: 84/319/EEC)"*~~

LINE 247: The EU suggests adding a further method considered as equivalent in EU legislation as new section B.1.b)iii):

"iii) Automatic digestion method for pooled samples of up to 35 g (Trichomatic 35): this method involves an automated digestion chamber and a membrane filter for the recovery and examination of larvae (*Equivalent method C, Regulation (EC) No 2075/2005, European Commission, 2009).*"

Consequently, **LINES 270-272** should be deleted.

LINE 268: The EU suggests replacing "EU" by "the European Union", as the acronym is not used elsewhere in the text.

LINE 294: Please replace the words "for the detection of trichinellosis" by the words "for the detection of *Trichinella* infection".

LINE 327: The EU suggests replacing the words "by serial passage in mice or rats" by the words "by serial passage in mice ~~or~~, rats or guinea pigs", as guinea pigs are perfect hosts with high larval burdens and a longer life span.

LINES 362-363: The EU suggests deleting the words "produced by Kirkegaard and Perry Laboratories, Gaithersburg, Maryland, USA", as preference to certain commercial providers should generally be avoided.

LINE 386: The EU suggests displaying the reference as follows:

"EUROPEAN COMMISSION (2005). Commission Regulation (EC) No 2075/2005 of 5 December 2005 laying down specific rules on official controls for Trichinella in meat: *Official Journal of the European Union* L **338**, 60–82 (Regulation as last amended by Commission Regulation (EC) No 1245/2007: *Official Journal of the European Union* L **281**, 19-20)."

CHAPTER 2.3.4: Avian influenza

General comments

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

Specific comments

LINE 28: The EU suggests inserting the words "naturally occurring" before the words "virulent strains".

LINE 81: The EU suggests inserting the words "naturally infected" before the words "chickens"

LINE 93: The EU suggests inserting the following sentence after the word "pathognomonic":

"In certain host species such as Pekin ducks some HPAI viruses do not necessarily provoke significant clinical disease."

LINE 96: please insert the words "and/or adverse environmental conditions" after the words "exacerbating infections".

LINE 140: Please insert the words "recovered and" before the words "tested with".

LINE 145: Please insert the words "(sterility assay on blood agar required)" after the words "in bacteria-free amnio-allantoic fluids".

LINE 181: Please insert the words "or recombinant" after the word "purified".

LINE 212: Please insert the words "naturally occurring" prior to the words "HPAI viruses to date".

LINE 282: The EU suggests inserting the following sentence after the words "(Imai *et al*., 2006)":

", but may be sensitive to viral mutations affecting the target regions (Postel *et al*., 2010)".

Accordingly, the following should be added to the reference list:

"POSTEL A., LETZEL T., FRISCHMANN S., GRUND C., BEER M. & HARDER T. (2010). Evaluation of two commercial loop-mediated isothermal amplification assays for detection of avian influenza H5 and H7 hemagglutinin genes. *J. Vet. Diagn. Invest*., **22**, 61-66."

LINES 494-495: The EU suggests inserting the following words after the words "(Smith *et al*., 2006; Swayne & Kapczynski, 2008b)":

"and Egypt (Grund *et al.*, 2011)".

Accordingly, the following should be added to the reference list:

"GRUND C., ABDELWHAB E.S., ARAFA A.S., ZILLER M., HASSAN M.K., ALY M.M., HAFEZ H.M., HARDER T.C. & BEER M. (2011). Highly pathogenic avian influenza virus H5N1 from Egypt escapes vaccine-induced immunity but confers clinical protection against a heterologous clade 2.2.1 Egyptian isolate. *Vaccine*. [Epub ahead of print] PubMed PMID: 21244859."

LINE 533: The EU suggests inserting the words "(Rudolf *et al.*, 2010)" after the words "when given in high challenge doses".

Accordingly, the following should be added to the reference list:

"RUDOLF M., PÖPPEL M., FRÖHLICH A., BREITHAUPT A., TEIFKE J., BLOHM U., METTENLEITER T., BEER M. & HARDER T. (2010). Longitudinal 2 years field study of conventional vaccination against highly pathogenic avian influenza H5N1 in layer hens. *Vaccine*, **28**, 6832-6840."

LINE 484: Please replace the words "live virus" by the words "replication-competent virus".

LINE 595: Please add the sentence "Viral infectivity must not remain." after the words "[…] or SAN embryos.".

LINE 606: Taking into consideration the European Pharmacopoeia, the EU suggests inserting a specific value here, i.e. to add the following sentence after the words "significant HI titre in SPF or SAN birds":

"The vaccine complies with the requirements if three weeks after the vaccination the mean HI titres of birds vaccinated intramuscularly with 1/50 of the dose is equal to or higher than 1:16 (4.0 log2) against the homologous antigen".

LINE 817: The EU suggests adding the following sentence at the end of the paragraph:

"Rapid turn-over rates of some poultry populations and slaughter at young age (broilers) may severely hamper efforts to induce resilient population immunity."

CHAPTER 2.3.7: Duck virus enteritis

General comments

The EU can support in general the proposed changes but has some specific comments that should be taken into acount.

Moreover, the method described in this chapter for the extraction of viral DNA (section B.1.e, lines 123-150) seems to be outdated and should be replaced with standardized, kit-based DNA extraction protocols which do not require harmful phenol/chloroform extraction.

Specific comments

LINE 5: The EU suggests replacing the words "a herpesvirus" by the words "an *alpha-herpesvirus* (*anatid herpesvirus-1*).

LINE 11: Please insert the word "primary" prior to the words "cell cultures".

LINE 27: Please add the following sentence after the words "order *Anseriformes* ":

"The disease is a potential threat to commercially reared, domestic and wild waterfowl."

LINES 27-28: Please replace "a herpesvirus" by "*anatid herpesvirus-1*"

LINE 33: The words "in laying flocks" should be added after "in egg production".

Furthermore, in **LINE 33**, the EU suggests adding the following sentence before the words "In chronically infected […]":

"In domestic ducks the incubation period ranges from 3-7 days. Mortality usually occurs 1-5 days after the onset of clinical signs and is often more severe in susceptible adult breeder ducks."

LINE 34: Please add the words "latently infected" prior to the word "carriers".

LINE 37: After the word "species,", please insert the words "immune status,".

LINE 38: Please insert the word "with" before the words "the virulence of the virus".

Furthermore, in **LINE 38**, please insert the following after the words "the virulence of the virus":

"Similarly, as infection progresses within a flock, more clinical signs are typically observed."

LINES 38-39: The EU suggests amending the sentence as follows:

"In breeder ducks the range of signs include ‘sudden deaths’, photophobia associated with partially closed, ~~eye watering and~~ pasted eye-lids ~~associated with photophobia~~, polydyipsia, loss of appetite, ataxia~~, watery diarrhoea~~ and nasal discharge."

Furthermore, in **LINE 40**, after the words "ruffled feathers" please insert ", watery diarrhoea"

LINE 43: After the words "loss of weight", please insert "conjunctivitis and serous ocular discharge,"

LINES 44-52: The EU suggests amending the paragraph as follows:

"At necropsy, ~~there is little evidence of emaciation in~~ adult ducks that have died are typically in good body condition. In mature males, prolapse of the penis may occur. In mature females, haemorrhages may be observed in ovarian follicles. The gross lesions are characterised by vascular damage, with tissue haemorrhages, ~~and~~ free blood in the body cavities and intestinal lumen and a range of lesions affecting the digestive tract mucosa. These latter lesions progress with the course of the disease and include initial mucosal haemorrhages and eruptions and intense annular congestion, leading to pseudo-membranous or diphtheritic mucosal~~, eruptions, or annular haemorrhages and diphtheroid lesions of the mucosal surfaces of the digestive tract,~~ lesions. Necrotic degenerative changes are evident in~~of~~ the lymphoid and~~organs and retrograde changes of the~~ parenchymatous organs. In the liver this manifests as irregularly distributed pinpoint haemorrhages and white foci giving a speckled appearance. In ducklings lesions of the lymphoid tissues tend to be more prominent than visceral haemorrhages. Collectively, these lesions are pathognomonic for DVE. The pathology and histopathology of DVE in white Pekin ducks has been reviewed (Sandhu & Metwally, 2008 ~~23~~). Microscopic lesions are characterised by vascular damage and its consequences in visceral organs. Eosinophilic intranuclear inclusions and cytoplasmic inclusions in epithelial cells of the digestive tract are typically present. Birds that recover from natural infection are suggested to be immune to re-infection, but latency (in the trigeminal ganglion) and reactivation of virus is recognised."

LINES 59-63: As many diagnostic laboratories do not have access to SPF duck eggs and consequently primary duck cell cultures, it would be advisable to indicate the source of a commercially available continuous cell line that is susceptible to DVE infection, if available.

LINE 88: For animal welfare reasons, the following sentence should be added to this paragraph on *in vivo* virus isolation:

"However, for reasons of animal welfare, the alternative *in vitro* diagnostic methods described in this section should preferably be used for virus isolation, if available."

LINE 113: The protocol of the quantitative real-time PCR assay should also be described, as real time methods may offer increased sensitivity and would avoid time-consuming gel electrophoresis.

LINE 118: The EU suggests adding the following sentence after the words "[…] other protocols exist":

"Recently, a LAMP-based nucleic acid amplification method for the detection of DVE DNA has been published (Ji *et al*., 2009)."

Accordingly, the following should be added to the reference list:

"Ji J., Du L.Q., Xie Q.M., Cao Y.C., Zuo K.J., Xue C.Y., Ma J.Y., Chen F. & Bee Y.Z. (2009). Rapid diagnosis of duck plagues virus infection by loop-mediated isothermal amplification. *Res. Vet. Sci*., **87**, 53-58."

LINE 186: After the words "for 20 minutes", please add the words "(alternative, safe products are available to visualise PCR products, e.g. RedSafe™ or GelRed™)".

LINE 205: After the sentence ending with "[…], or in cell cultures", the EU suggests adding the following sentence:

"For laboratories lacking duck embryos, serological diagnosis is possible by virus neutralisation, using a chicken embryo fibroblast adapted DVE strain and primary chicken embryo fibroblasts (CEF)."

LINES 231-234: The EU suggests amending the paragraph as follows:

"A live attenuated virus vaccine can be used to control DVE in birds over 2 weeks of age (Richter & Horzinek, 1993 ~~22~~). The live vaccine virus is not thought to spread by contact from vaccinated to unvaccinated ducklings. Fattening or breeding ducks may be vaccinated subcutaneously or intramuscularly to produce an active immunity. Maternally-derived immunity in ducklings is reported to decline rapidly and progeny of breeders vaccinated with a live attenuated vaccine are fully susceptible.~~The vaccine virus is not thought to spread by contact from vaccinated to unvaccinated ducks, as the unvaccinated birds remain susceptible to infection.~~"

LINE 236: Please put the words "Mondal *et al.*, 2010" in brackets.

CHAPTER 2.3.14: Newcastle disease

General comments

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

Specific comments

LINE 48: The EU suggests inserting the words "and phylogenetic analysis" after the words "serological testing".

Furthermore, in line **LINE 48**, the word "serotypes" should be replaced by "subtypes".

LINE 62: The word "enteric" should be deleted after the word "Asymptomatic".

LINE 134: Please insert the word ", NDV" after the word "viruses".

LINE 205: Please replace the word "virulence" by the words "clinical signs".

LINE 248: The protocol of the general APMV-1 real-time PCR assay (Kim *et al*., 2008) should be described. Alternatively, it could be published on the website of the OIE reference laboratory, along with the protocols of other PCR methods described in this chapter.

LINES 402-405: For reasons of clarity, the EU suggests replacing the sentence starting with "The European Union […]" by the following:

"The European Union ~~in 1993~~ stated in their Commission Decision 93/152/EEC (European Commission, 1993) that for routine ND vaccination programmes the viruses used as live NDV vaccines are to be tested under specific conditions and have an ICPI of less than 0.4 or 0.5, depending on the dose of vaccine given".

Accordingly, the following should be added to the reference list:

"EUROPEAN COMMISSION (1993). Commission Decision of 8 February 1993 laying down the criteria for vaccines to be used against Newcastle disease in the context of routine vaccination programmes (93/152/EEC): *Official Journal of the European Communities* L **59**, 35 (Decision as amended by Decision 2010/633/EC: *Official Journal of the European Union* L **279**, 33)."

LINE 487: Please replace the word "morality" by the word "mortality".

LINES 487-490: The last sentence of this paragraph should be deleted, as it seems inappropriate to introduce experimental vaccines in this section dealing with conventional vaccines.

LINES 510-514: The following sentence should be deleted: "This risk is reflected in Commission Decision 93/152/EEC, which restricts the use of viruses used for inactivated vaccine in member states of the European Union from 1 January 1995 to those for which the master seed has been tested and

shown to have an ICPI of <0.7 if no fewer than 108 EID50 are administered to each bird".

Indeed, Commission Decision 93/152/EEC was recently amended and the ICPI requirement for inactivated vaccines was deleted. However, the requirements in relation to ICPI limits for live vaccines were maintained (see comment as to line 402 above).

LINES 677-686: Some text seems to be missing in this paragraph, as it starts with the efficacy test for inactivated vaccines in the USA and finishes with a potency test for live vaccines that is different from the one used in the USA. It is unclear from the text which potency test and country other than the USA is being referred to in the last part of that paragraph. Furthermore, it is unclear which potency test does not need to be repeated on each vaccine batch. Indeed, from what is said in lines 607 – 618, in the USA each serial batch of inactivated vaccines needs to be tested for potency, whereas the European Pharmacopoeia does not require repeating of the potency test on each batch.

LINE 725: Please replace the word "Mebatsop" by the word "Mebatsion".

CHAPTER 2.4.1: Bovine anaplasmosis

General comments

The EU can support the proposed changes.

Specific comments

None

CHAPTER 2.4.11: Enzootic bovine leukosis

General comments

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

Specific comments

LINE 72: The EU suggests inserting references as follows: following "consumption of milk from infected cows", please add "(Perzova *et al*., 2000; Burmeister *et al*., 2007)".

In the references section, please add the following 2 entries:

"Burmeister T., Schwartz S., Hummel M., Hoelzer D. & Thiel E. (2007). No genetic evidence for involvement of Deltaretroviruses in adult patients with precursor and mature T-cell neoplasms. *Retrovirology*, **4**, 11."

"Perzova R.N., Loghran T.P., Dube S., Ferrer J., Esteban E. & Poiesz B.J. (2000). Lack of BLV and PTLV DNA sequences in the majority of patients with large granular lymphocyte leukaemia. *Br. J. Haematol*.,**109**, 64-70."

LINE 127: The EU suggests replacing the words "during the analysis" by the words "throughout the protocol", for reasons of clarity.

Furthermore, in **LINE 128**, the word "analysis" should be replaced by "protocol".

Finally, in LINE 129, the words "[…] assay, negative controls (water blanks), etc." should be replaced by the words "[…] assay~~,~~ and negative controls (e.g. water blanks)~~, etc~~."

LINES 162-213 (Nested PCR): The EU would suggest that the previous method is re-published as opposed to this new proposed method: firstly due to the presence of the mimic there is no need to obtain BLV control material, which is often difficult to obtain. Secondly, the new suggested method uses 1 microgram of DNA, double the quantity required for the previous method. Finally, the current method has the combination of house-keeping genes and the mimic as controls which would be useful for laboratories which are not familiar with molecular techniques.

Consequently, in **LINE 122**, the words "nested and real-time" prior to the words "PCR assay described […]" should be deleted.

LINE 217: The EU suggests adding the following after "ethidiumbromide":

"(alternative, safe products are available to visualise PCR products, e.g. RedSafe™ or GelRed™)".

LINES 230-289 (Real-time PCR procedure): The EU would advise against including the real-time protocol as the method is not published yet nor has it

been validated via ring trials between Reference Laboratories. The data generated from the protocol has not been distributed to the other Reference Laboratories or discussed at the annual Reference Laboratories meeting. In addition there currently isn't an EBL expert at the Institute that designed the assay in case any third party laboratories require help or advice in set-up or trouble shooting.

LINE 302: For reasons of consistency with other chapters, the words "Commission of the European Comunities, 2009" should be replaced by "European Comission, 2009".

Hence, the corresponding reference in **LINES 559-561** should be replaced by the following:

"EUROPEAN COMMISSION (2009). Commission Decision of 15 December 2009 amending Annex D to Council Directive 64/432/EEC as regards the diagnostic tests for enzootic bovine leucosis (2009/976/EU): *Official Journal of the European Union* L **336**, 36-41.

LINE 308: The reference to the above Commission Decision should not be repeated here, as that Decision recognising the new standard serum for EBL diagnostics was issued based on and explicitly making reference to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Sixth Edition, 2008. Thus the words "(Commission of the European Communities, 2009)" should be deleted.

CHAPTER 2.4.12: Haemorrhagic septicaemia

General comments

The EU can support the proposed changes.

Specific comments

None

CHAPTER 2.4.17: Trichomonosis

General comments

The EU can support the proposed changes and has some editorial comments.

Specific comments

LINE 18 and 187: The word "fetuses" should be replaced by "foetuses".

LINES 83, 87, 184 and 274: The word "fetus" should be replaced by "foetus".

LINE 135: The word "recently" should be deleted, as the method in question was published in 1999.

LINES 185, 238 and 276: The word "fetal" should be replaced by "foetal".

LINES 331-333: For reasons consistency, the EU recommends to display the reference as follows:

EUROPEAN UNION (1988). Council Directive of 14 June 1988 laying down the animal health requirements applicable to intra-community trade in and imports of deep-frozen semen of domestic animals of the bovine species (88/407/EEC): *Official Journal of the European Communities* L **194**, 10–23 (Directive as last amended by Directive 2008/73/EC: *Official Journal of the European Union* L **219**, 40–54).

CHAPTER 2.5.7: Equine influenza

General comments

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

Specific comments

LINE 8: The EU suggests inserting the words "highly pathogenic" prior to the words "avian H5N1".

LINES 39 and 113: Please insert the words "antigen capture" before the words "enzyme-linked".

LINE 98: Please replace the words "by personnel" by the words "and indirectly by contaminated personnel".

LINE 107: Please insert the word "silently" prior to the word "contribute".

LINE 110: Please insert the word "acute" prior to the word "equine".

LINE 111: Please replace the words "illness, or on the demonstration of a serological response to infection" by the following:

"illness~~, or on~~. Alternatively, the demonstration of a serological response to infection in paired serum samples is attempted".

LINE 120: Please insert the words "and class II safety cabinets" after the words "level 2".

LINE 130: Please replace the sentence "Only one sample should be processed at a time" by the following:

"Sample processing follows GLP guidelines avoiding any cross contamination risks."

LINE 143: Please insert the word "also" prior to the word "occurred".

LINE 154: Please replace "to the exclusion of ~~in preference to~~ virus isolation" by the words "for exclusion diagnosis".

LINE 205: The EU suggests replacing the subtitle by the following:

"Serological haemagglutinin subtyping".

LINE 219: The EU suggests adding the following sentence at the end of the paragraph:

"Also, modes of immunization (e.g., number of revaccinations etc.) have an influence on serum specificity/cross reactivity. Sera obtained three weeks after a single antigen application are considered to be most discriminative."

LINE 223: The EU suggests replacing the subtitle by the following:

"Serological neuraminidase subtyping".

LINE 284: Please replace "(12)" by the words "(JOHN & FULGINITI, 1966)".

Furthermore, in **LINE 284**, the EU suggests adding the following sentence at the end of the paragraph:

"Safety precautions while handling ether must be strictly observed and work is confined to a fume hood."

LINE 406: Please insert the word "glycoprotein" prior to the word "subunits".

LINE 442: Please insert the word "global" prior to the words "Equine Influenza".

LINE 533: Please delete the repeated word "that".

LINE 574: The EU suggests the first sentence to read as follows:

"For inactivated or subunit vaccines absence of viral infectivity should be proven by passages in 10 fertilised hen eggs."

CHAPTER 2.8.1: African swine fever

General comments

The EU can support the proposed changes.

Specific comments

None