EUROPEAN UNION

Brussels,
SANCO/G2/SC/ lo D(2011) D/ 1094634

Subject: **OIE Manual of Standards for Diagnostic Tests for aquatic animals**

Dear Director General,

Please find here attached the comments of the EU on the draft modified chapters of the OIE Manual of Standards for Diagnostic Tests for aquatic animals, sent for comment on 9 August 2011.

I trust you will find this useful and I thank you for your continued cooperation.

Yours sincerely,

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Bernard Van Goethem
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Annexes: 1

Copy: All Directors / Chief Veterinary Officers of the EU 27 and Croatia, Iceland, Liechtenstein, Norway, Switzerland and Turkey.

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ANNEX

EU COMMENTS

ON THE PROPOSED CHANGES TO OIE MANUAL OF DIAGNOSTIC TESTS FOR AQUATIC ANIMALS

Presented for comments August 2011 with deadline 1 October 2011

Chapters

00. SUMMARY AND GENERAL COMMENTS

The EU notes that of the draft OIE Aquatic Manual chapters that were sent for comment in August 2011, there are five disease specific chapters proposed for diseases that have previously been de-listed by the OIE and for which there where no chapters in the 2009 printed edition of the OIE Aquatic Manual. The EU would in principle support the inclusion of chapters in the Aquatic Manual on other diseases than those that are included in the OIE list of diseases. However, it is essential that also those chapters are kept up-to-date. Furthermore, the resources spent on chapters concerning non-listed diseases should be seen in the light of the OIE work plan.

The EU would be interested in the rationale behind reintroducing chapters on these specific diseases. Indeed, there are other recently de-listed diseases of importance, such as BKD, for which chapters have not been proposed for reintroduction in the Aquatic Manual. It would be preferable to develop criteria for the inclusion of disease specific chapters in the Aquatic Manual for diseases that are not listed in the OIE Aquatic Animal Health Code.

The EU has a number of comments and suggestions for amendments to the draft Aquatic Manual chapters submitted for comments and would therefore very much welcome a second round of consultation of OIE Members prior to the presentation of those revised draft chapters for adoption at the next OIE General Session.

Part 2. Recommendations applicable to specific diseases
General Introduction

General comments

The EU supports the proposed changes.

Specific comments

None

Diseases of crustaceans: listed
2.2.0. General Information

General comments

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

Specific comments

LINES 79, 107 and 109: The EU would propose the following to be added at the end of the line: "except in the case of necrotising hepatopancreatis".

LINES 259 – 261: The EU would propose the following amendments, taking into account that for two of the currently listed diseases, TEM can be used as a confirmatory diagnosis.

"Electron microscopy (EM – transmission or scanning) is a valuable research tool for the study of disease in crustaceans. However, However, EM methods are only available as confirmatory methods for some diseases ~~not routinely used for diagnosis of the diseases listed by the OIE, and the application of EM is limited to specific purposes~~ (see disease-specific chapters in this *Aquatic Manual*)."

LINE 277: The EU would propose to add the following at the end of the sentence: "with a syringe that is half full with fixative/ethanol/RNALater® to prevent immediate lysis."

Diseases of crustaceans: listed
2.2.1. Crayfish plague (*Aphanomyces astaci*)

General comments

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

Specific comments

LINE 30: The EU would propose to replace the word "less" by the word "below", for linguistic reasons.

LINES 79 – 80: The EU would propose to replace the words "blood vessels" by the word "haemal sinuses".

LINE 323: The EU would propose to replace the word "remaining" by the word "being".

LINE 536: The EU would propose the following sentence to be added after the full stop:

"Endogenous control can be used to assess whether degradation may have occurred."

LINES 710 – 712 and 756 – 757: These references are not mentioned in the text and should therefore be removed.

Diseases of crustaceans: listed
2.2.2. Infectious hypodermal and haematopoetic necrosis

General comments

The EU in general supports the proposed changes but has a comment.

Specific comment

LINE 590: In table 5.1 the EU would request the OIE to reconsider whether it is appropriate to designate an "a" to histopathology as a confirmatory matter, taking into account the lack of well described examples of pathology associated with DNA viruses in decapod crustaceans.

Diseases of crustaceans: listed
2.2.3. Infectious myonecrosis

General comments

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

Specific comments

LINES 32 – 34: The EU would propose that lines 32-34 are replaced by the following:

"IMN disease is not the same disease as white tail disease of Penaeid shrimp and white tail disease of *Macrobrachium rosenbergii*. These 2 diseases exhibit gross and histological signs that mimic IMN, but which is caused by 2 different types of virus: a nodavirus named *Paneaus vannamei* novavirus – PvNV (Tang *et al*.,2007) and a nodavirus named *Macrobrachium rosenbergii* nodavirus – *Mr*NV (See 2.2.07)."

LINE 34: The word "novavirus" should be replaced by "nodavirus".

LINE 315: In table 5.1, the EU questions the designation "a" for histopathology as a confirmatory diagnosis. Given the wide range of idiopathic conditions that also cause abdominal muscle whitening in long-bodied crustaceans, and the mimic condition caused by PvNV or MrNV/XSV, the destination "c" or even "d" appears to be more appropriate.

LINE 315: In table 5.1, the EU questions the designation "a" for histopathology as a confirmatory diagnosis. Given the wide range of idiopathic conditions that also cause abdominal muscle whitening in long-bodied crustaceans, and the mimic condition caused by PvNV or MrNV/XSV, the destination "c" or even "d" appears to be more appropriate.

Diseases of crustaceans: listed
2.2.4. Necrotising hepatopancreatitis

General comments

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

Specific comments

LINE 5: The EU would propose adding a reference to the following article, which should subsequently be added in the reference section:

"LIGHTNER, D.V., REDMAN, R.M. & BONAMI, J.R. (1992). Morphological evidence for a single bacterial etiology in Texas Necrotizing Hepatopancreatitis in *Penaeus vannamei* (Crustacea: Decapoda). *Dis.Aquat.Org.*, ***13***, 235239."

LINE 32: In the view of the EU the statement that NHPB is highly sensitive to freezing appears to be in contradiction with the first part of the paragraph.

LINE 162: The word "Heces" should be replaced by "Faeces".

LINE 385: In table 5.1, the EU questions the designation "a" for Histopathology as a confirmatory diagnosis for NHP. Since necrotic HP tubules are a relatively common occurrence in crustacean hosts sampled from the wild, it is perhaps not suitable that NHP associated with the causal agent descried in this chapter, to use histopathology as a confirmatory tool. It may be more suitable as presumptive diagnostic tool, with a specific molecular test (ISH/PCR) to confirm disease.

Furthermore, the EU would propose to add "RT-PCR, QPCR" after "DNA probes" in the Method column of the table.

Diseases of crustaceans: listed
2.2.5. Taura syndrome

General comments

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

Specific comments

LINE 59: The EU would propose adding a reference to the following article at the end of the line, as it gives the most recent summary of susceptibility to Taura syndrome. Subsequently, the following reference should be added in the reference section:

"Stentiford GD, Bonami JR & Alday-Sanz V (2009). A critical review of susceptibility of crustaceans to Taura syndrome, Yellowhead disease and White Spot Disease and implications of inclusion of these diseases in European legislation. *Aquaculture*, **291**, 1–17)."

LINE 344: The EU suggests deleting the word "recent", as 2003, the year the study was published, would not seem recent in 2012, the earliest possible year of adoption of that amendment.

LINE 349: "An" should be replaced by "A".

LINE 267: The following text should be added in the beginning of the line to highlight that the ensuing text may not be relevant for all species:

"The following text describes penaeid shrimp pathology and may not be relevant for other host species."

Diseases of crustaceans: listed
2.2.6. White spot disease

General comments

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

Specific comments

LINE 33: The EU would propose adding a reference to the following article at the end of the line, as it gives the most recent summary of susceptibility to WSD. Subsequently, the following reference should be added in the reference section:

"Stentiford GD, Bonami JR & Alday-Sanz V (2009). A critical review of susceptibility of crustaceans to Taura syndrome, Yellowhead disease and White Spot Disease and implications of inclusion of these diseases in European legislation. *Aquaculture*, **291**, 1–17."

LINE 76: The EU proposes adding adding a reference to the following article after the word "Mediterranean". Subsequently, the following reference should be added in the reference section:

"Stentiford and Lightner (2011). Cases of White Spot Disease (WSD) in European shrimp farms. *Aquaculture*, **319**, 302–306."

LINE 197: The EU would invite the OIE to consider the inclusion of a Taqman real time PCR method in this chapter, as described in the following article:

Durand, S. V. & Lightner, D. V. (2002). Quantitative real time PCR for the measurement of white spot syndrome virus in shrimp. *Journal of Fish Diseases*, **25**, 381–389.

LINES 389: "Chilled" should be replaced by "chill".

LINE 422, in table 5.1: The EU questions the appropriateness of giving the designation "a" to Histopathology as confirmatory diagnosis, given that there are relatively few published examples of DNA viruses infecting the epithelial tissues of crustaceans and further, given the current status of Wssv as the single member of the family *Nimaviridae*. At present, we know relatively little about mimic infections in decapods but it is likely that there are some.

Diseases of crustaceans: listed
2.2.7. White tail disease

General comments

The EU supports the proposed changes but has some comments.

Specific comments

LINE 15: Please remove one "0" from "20005". Idem **LINE 17**.

LINE 16: The EU would propose to add the word “sequenced” between “first” and “satellite”, for reasons of precision.

LINE 161: The EU suggests deleting the citation "(Ravi *et al*., 2010)" as it is redundant. Indeed, that citation appears in line 167, after the text relating to it.

Diseases of crustaceans: listed
2.2.8. Yellow head disease

General comments

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

Specific comments

LINE 42: The EU would request the references to be added concerning the susceptibility of Antarctic krill (Euphasia superba) to YHD. To the knowledge of the EU, Antarctic krill is not known to be susceptible to YHD.

LINE 54: The EU would propose to add a reference to the following article at the end of the line, as it gives the most recent summary of susceptibility to YHD. Subsequently, the following reference should be added in the reference section:

"Stentiford GD, Bonami JR & Alday-Sanz V (2009). A critical review of susceptibility of crustaceans to Taura syndrome, Yellowhead disease and White Spot Disease and implications of inclusion of these diseases in European legislation. *Aquaculture*, **291**, (2009) 1–17.".

LINE 269: Please replace "4.2.7." by "4.2.6.".

Diseases of crustaceans: delisted
2.2.9. Spherical baculovirosis (*Penaeus monodon*-type baculovirus)

General comments

The EU supports the proposed changes.

Specific comments

None

Diseases of crustaceans: delisted
2.2.10. Tetrahedral baculovirosis (*Baculovirus penaei*)

General comments

The EU supports the proposed changes.

Specific comments

None

Diseases of fish: listed
2.3.0. General information

General comments

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

Provided VER and OMV are included in the *Aquatic Manual*, these diseases should be referred to as appropriate in this chapter, as suggested in the specific comments below.

Specific comments

LINES 33: The EU would suggest adding a new point 1.2.5 with the following wording:
"1.2.5 For Viral encephalopathy and retinopathy (VER)

For 2-4 cm: take the whole head
Fish 4 cm to adults: take the encephalon and when possible eyes and spinal cord."

LINES 107 – 117: The cell line SSN-1 should be added.

LINE 144: References to VER and OMV should be added.

LINE 153: Temperatures should be indicated for VER and OMV.

LINE 304: The EU propose that the words "or below" be added after "-20°C" (2 x in that line), as also lower temperatures would be appropriate.

Diseases of fish: listed
2.3.1. Epizootic haematopoetic necrosis

General comments

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

The EU would invite the OIE to consider including the following reference: Ariel E. & Bang Jensen B. (2009). Challenge studies of European stock of redfin perch, *Perca fluviatilis* L. and rainbow trout, *Oncorhyncus mykiss* (Wallbaum) with epizootic haematopoietic necrosis virus (EHNV). *Journal of Fish Diseases* 32 (12), 1017-1025.

Specific comments

LINE 77: The EU would propose to revise the text on Redfin perch. Infection trials in Europe did not find EHNV to be extremely pathogen to perch.

LINES 409 – 410: The EU would not agree with the following statement: "ELISA is useful for both diagnosis and certification."

ELISA for detection of virus directly in tissue material is only useful for diagnosis of clinical cases as the sensitivity is too low compared to cell culture techniques. It is therefore less suitable for surveillance in clinical healthy fish for demonstrating freedom of disease.

LINE 772: In the view of the EU the designations in table 5.1 would benefit from a reassessment, in particular as regards ELISA, see above comment. In the opinion of the EU PCR should be regarded as a useful method for surveillance, and thus be given the designation "a" or "b" rather than "d".

LINE 777 onwards: Following a reassessment of table 5.1, chapter 6 should also be revised.

LINE 784: The EU would invite the OIE to reassess the statement "For practical purposes, EHNV can only be detected in fish that are clinically affected of that have died with the infection." In experimental trials it has been possible to re-isolate the virus from clinically healthy fish.

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Diseases of fish: listed
2.3.2. Epizootic ulcerative syndrome

General comments

The EU in general supports the proposed changes but has several comments.

Specific Comments

LINES 53 – 54: The EU would propose deleting the following sentence: "Some fish, such as common carp (*Cyprinus capio*), Nile tilapia (*Oreochromis niloticus*) and milk fish (*Chanos chanos*), are considered to be resistant to EUS (Lilley *et al.*, 1998)."

In the opinion of the EU that reference does not provide strong evidence for resistance of the species referred to. There is evidence that both carp and tilapia are susceptible to infection by injection (Wada *et al*. 1996, Khan *et al*. 1998). Although this evidence might not be sufficient to demonstrate that these species are susceptible, it is in the view of the EU insufficient evidence to refer to these species as "resistant" to EUS.

LINE 55: In table 2.1, the EU would invite the OIE to revise the list of susceptible species to EUS, taking into account the new guidance when this becomes available, as it appears that species for which there is evidence suggesting susceptibility is not included in the list. The EFSA Scientific Opinion of the Panel on AHAW on a request from the European Commission on aquatic animal species susceptible to diseases listed in the Council Directive 2006/88/EC (The EFSA Journal (2008), 1-144) may provide useful information in this context.

Goldfish (*Carassius auratus*) is one of the species that should be assessed and for which scientific literature suggests its susceptibility, such as (Miyazaki & Egusa 1972, 1973) in Japanese, cited by Hatai in (Baldock *et al*. 2005), (Hatai 1980) in Japanese, cited by Hatai in (Baldock *et al*. 2005), (Hatai *et al*. 1994), Bondad-Reantaso *et al*. (1999) and (Phadee *et al*. 2004).

It would be useful if the fact that differences in susceptibility between species exist was highlighted.

LINES 64 – 65: The EU would suggest that the second half of the following sentence is deleted (See justification of amendments to lines 53-54):

"Experimental infections demonstrated that goldfish are susceptible (Hatai *et al.*, 1977; 1994)~~, but common carp (Wada *et al.*, 1996) and Nile tilapia (Khan et al., 1998) are resistant."~~

LINE 73: The word "skeleton" should be replaced by "skeletal".

LINES 76 – 78: The EU would propose that the text of Section 2.2.5 is replaced by the following:

"Generally, most infected fish die during an outbreak but mild or moderate EUS–infected fish could recover, and therefore potentially become carriers of *A. invadans*. Whilst there are no data to indicate that fish can be lifelong carriers of *A. invadans*, the seasonality of EUS could be explained by sub clinically affected carriers and / or survival of the pathogen in the environment for long periods. Currently, there is little data to support or refute either theory."

The reasons for this proposal, is firstly, that the statements made in this section are not backed up by evidence. Secondly, there are circumstantial evidence that may support the presence of carriers, i.e. the seasonality of EUS actually suggests that there may indeed be not clinically affected carriers.

LINES 92 – 95: The EU would propose that the text of Section 2.3.2 is replaced by the following text which is more nuanced and elaborated than the current text:

"The prevalence of EUS in the wild and in aquaculture farms may be high in endemic areas when high levels of mortality are observed, but can vary substantially. There is very limited data on prevalence of disease or infection at other times. Uncontrolled water exchange in fish farms in endemic areas will result in EUS outbreaks in most of the farms that culture susceptible fish species."

LINES 109 – 112: The EU would propose the following addition to this section:

"When EUS spreads into a fish culture pond, such as a snakehead fish pond, high morbidity (>50%) and high mortality (>50%) might be observed in those years that have a long cold season, with water temperatures between 18 and 22°C. However, mortality and morbidity may vary greatly. Some infected fish may recover when the cold period is over."

LINES 114, 116 and 410: The EU propose the following amendments to this sentence, for reasons of linguistic clarity:

"EUS occurs mostly ~~during periods of low~~ at water temperatures of ~~or~~ 18–22°C and after periods of heavy rainfall (Bondad-Reantaso *et al.*, 1992)."

LINE 116: The EU would request that the words "low temperatures" be replaced by a concrete temperature range, as the current formulation is ambiguous.

LINES 149 – 151: The EU is of the opinion that this section should either be deleted or reworded, with appropriate references included, see comment on lines 53-54 above.

LINES 185 – 188: The EU would propose deleting these sentences. Firstly, because it is a repetition of what is described later in this section. Secondly, the EU would not agree with histopathology as a main confirmatory method, please see comments on Table 5.1.

LINE 271: The EU would request the OIE for clarification on which dose which should be used for Streptomycin. In line 271 it is stated 100 µg/ml^{-1}, whereas in the EUS Technical handbook, referred to in line 469, it is stated 10 mg/ml.

LINE 403: In table 5.1, the EU would propose the following amendments in table:

Table 5.1. *Methods for targeted surveillance and diagnosis*

Method	**Targeted surveillance**			**Presumptive diagnosis**	**Confirmatory diagnosis**
	Fish fry	**Juveniles**	**Adults**		
Gross signs	b ~~c~~	b ~~c~~	b ~~c~~	c ~~b~~	d ~~c~~
Direct LM; observation of the oomycete hyphae in tissues, fresh squash	d	d	d	c ~~b~~	c ~~b~~
FISH; observation of the oomycete hyphae in tissues	d	d	d	a	a
Histopathology	c	c	c	b	c ~~a~~
Isolation of *A. invadans* and confirmatory identification by bioassay or PCR	d	d	d	a	a
PCR of tissue extracts	d	d	d	a	a
Sequence analysis	d	d	d	d	a
Transmission EM of tissues	d	d	d	d	d

LM = light microscopy; FISH = fluorescent peptide nucleic acid *in-situ* hybridisation;

In the view of the EU, gross signs cannot be considered to have good diagnostic sensitivity.

Identification of hyphae is not a sufficiently diagnostic tool to be rated "a" for presumptive or confirmation. *A. invadans* cannot be distinguished from other infections.

In the opinion of the EU histopathology is not a sensitive method to diagnose EUS as similar changes may be seen with other infections. Molecular techniques would be required to confirm a diagnosis.

Absence of mycotic granulomas should not be considered as evidence that the investigated condition is not EUS. Not all fish species shown to be susceptible develop the mycotic granulomas: *A. invadans* was isolated from Bluegills (*Lepomis macrochirus*), largemouth bass (*Micropterus salmoides*) and American shad (*Alosa sapidissim*), but granulomas were not observed despite fungal hyphae in histological sections of dermis and skeletal muscle (Sosa *et al*. 2007b). Similar observations were made in Channel catfish (*Ictalurus punctatus)*, black bullhead (*Ameiurus melas*) (Hawke *et al*. 2003) and European catfish (*Silurus glanis*) (Oidtmann *et al*. 2008). Instead of epitheloid granulomas, other inflammatory responses included multinucleated

giant cells. Furthermore, highly susceptible life stages of highly susceptible species (e.g. fingerlings of *Puntius sp*. (barb), Indian major or minor carp) may die before a pronounced immune response can develop (Chinabut and Roberts, 1999). Several other factors may decrease the likelihood that mycotic granulomas are observed, including where (i.e. how far from the skin lesion) the tissue samples are taken from and the degree of necrosis in the lesion.

Therefore, histopathology could lead both to false negatives and false positives.

LINE 410: The EU would propose replacing "22°C or below" by "18-25°C", in the interest of coherence with line 422 and clarity.

LINE 423: The EU would propose the following addition between "OR" and "the presence of branching [...]": "the presence of mycotic granulomas in muscle tissue OR".

LINES 427 – 429: The EU would propose that this paragraph is amended to read:

"A confirmed case of EUS is defined as a suspect case ~~that has produced typical mycotic granulomas in affected tissues or organs OR~~ that has been identified as positive by the PCR or FISH detection techniques OR that *Aphanomyces invadans* has been isolated and confirmed by either bioassay, PCR, or sequence analysis."

In the opinion of the EU a case of EUS cannot be confirmed by means of mycotic lesions, as also other diseases may cause this. Furthermore, the EU would request the OIE to specify which bioassay should be used.

References for the comments to the chapter on EUS:

Baldock FC, Blazer V, Callinan RB, Hatai K, Karunasagar I, Mohan CV, Bondad-Reantaso MG (2005) Outcomes of a Short Expert Consultation on Epizootic Ulcerative Syndrome (EUS): Re-examination of Causal Factors, Case Definition and Nomenclature. In: Walker PJ, Lester RG, Bondad-Reantaso MG (eds) Diseases in Asian Aquaculture V. Fish Health Section, Asian Fisheries Society, Manila, Philippines

Bondad-Reantaso M, Kishio H, Osamu K, Shinpei W, Kunika W, Dizon M (1999) Aphanomyces from EUS-infected fish from the Philippines and Bangladesh and MG-infected fish from Japan: 2. Pathogenicity studies. In: Fourth Symposium on Diseases in Asian Aquaculture: Aquatic Animal Health for Sustainability November 22-26, 1999, Cebu International Convention Center, Waterfront Cebu City Hotel, Cebu City, Philippines Book of abstracts

EFSA (2008) Scientific Opinion of the Panel on AHAW on a request from the European Commission on aquatic animal species susceptible to diseases listed in the Council Directive 2006/88/EC. *The EFSA Journal (2008), 1-144.*

Hatai K (1980) Studies on pathogenic agents of saprolegniasis in fresh water fishes. In: Special Report of the Nagasaki Prefecture Institute of Fisheries 8

Hatai K, Nakamura K, Rha SA, Yuasa K, Wada S (1994) Aphanomyces infection in dwarf gourami (Colisa lalia). Fish Pathol 29:95-99

Khan MH, Marshall L, Thompson KD, Lilley JH (1998) Susceptibility of five fish species (Nile tilapia, rosy barb, rainbow trout, stickleback and roach) to intramuscular injection with the oomycete fish pathogen, Aphanomyces invadans. Bulletin of the European Association of Fish Pathologists Weymouth 18:192-197

Miyazaki T, Egusa S (1972) Studies on mycotic granulomatosis in freshwater fish I. Mycotic granulomatosis in goldfish. . Fish Pathol 7:15-25

Miyazaki T, Egusa S (1973) Studies on mycotic granulomatosis in freshwater fish II. Mycotic granulomatosis prevailed in goldfish. Fish Pathol 7:125-133

Phadee P, Kurata O, Hatai K, Hirono I, Aoki T (2004) Detection and Identification of Fish-Pathogenic Aphanomyces piscicida Using Polymerase Chain Reaction (PCR) with Species-Specific Primers. J Aquat Anim Health 16:220-230

Wada S, Rha S, Kondoh T, Suda H, Hatai K, Ishii H (1996) Histopathological comparison between ayu and carp artificially infected with Aphanomyces piscicida. Fish Pathol 31:71-80

Diseases of fish: listed
2.3.3. Gyrodactylosis (*Gyrodactylus salaris*)

General comments

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

Specific comments

LINE 6: The EU would propose adding the words "and other salmonids" after "(*salmo salar*)", as Atlantic salmon is not the only salmonid to be susceptible to GS.

LINE 19 – 21: The EU would suggest that this paragraph is deleted, as it is referring to a debate without any conclusion and is completely without references.

LINE 23 – 24: Reference is missing for statements on survival of detached parasites.

LINE 42: The EU would invite the OIE to consider whether Eels should be regarded as susceptible to GS, taking into account the new guidance when this becomes available. Eels have also been shown to carry *G. salaris* for periods of 8 days under experimental conditions (Bakke, Jansen, Hansen, 1991, Can J Zoo, v69, 733-737).

LINE 98 onwards: The EU would invite the OIE to consider whether it would be appropriate to include in this section more details on the use of Rotenone and Aluminium sulphate to control GS.

LINES 100 and 109: The EU would propose to replace "not feasible" by "not available", as it could in theory be feasible, but none are in fact available.

LINE 105: The OIE would request the OIE to clarify, if feasible, what is meant by "slightly acidic solutions".

LINES 132 – 145: The EU would request the OIE to reconsider the wording of this section. It would be easier to state that fish/fins have to be fully submerged in ethanol and moving freely in the container. The EU would also suggest recommending 96-100% ethanol as it will always become diluted which may lead to difficulties with DNA extraction.

LINE 155: The EU would propose the following addition in the end of line 155: "DNA extraction from formaldehyde preserved material is very difficult."

LINE 166: The EU would propose the following addition in the end of line 166: "Formaldehyde preserved material is not suitable either.", as it is almost impossible to both ID species using morphology and obtain any decent sequence.

LINE 170: The EU would request the OIE to consider whether it is possible to provide a more precise description replacing the words "a few tens", and also specify whether this statement is applicable to all live stages of fish, including fry.

LINE 254: The EU would propose adding the following in the end of line 254: "It is recommended to digest parasites on a microslide covered with a cover slip before microscopic examination."

LINE 282: The EU would suggest that a recommended protocol for DNA extraction or at least some references for different protocols, e.g. proteinase K-based lysis buffer (Matejusova *et al*. 2001) is added.

LINES 286 – 291: The EU would request the OIE to consider whether it is appropriate to mention Real time PCR in this section. A real-time (TaqMan) multiplex PCR targeting ITS rDNA was recently developed for the simultaneous identification of *G. salaris*, *Gyrodactylus derjavinoides* and *Gyrodactylus truttae* (Collins *et al*., 2010). The *G. salaris* assay within the multiplex can be used as a singleplex for detection of *G. salaris* only. As with conventional PCR amplification and sequencing of ITS rDNA, the real-time assay does not differentiate between *G. salaris* and *G. thymalli* and subsequent mitochondrial cytochrome oxidase I (COI) gene analysis must be performed on positive samples. The assay is used in some labs for routine screening but data on its performance is still relatively limited.

LINES 290 – 291: The EU would propose to replace "(Matejusová *et al*., 2001; Ziętara & Lumme, 2003)" by the following text:

"(Cunningham 1997; Ziętara & Lumme, 2003). If partially degraded material is analysed, the ITS1 and ITS2 spacers can be amplified in two separate reactions using primer sets and PCR conditions described in Matejusova *et al*. (2001)."

Consequently, the following reference should be added in the reference section:

[ADD REFERNCE TO CUNNINGHAM 1997]

Diseases of fish: listed
2.3.4. Infectious haematopoetic necrosis

General comments

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

Specific comments

LINE 52: The EU would invite the OIE to consider whether European eel (*Anguilla anguilla*) should be regarded as susceptible to IHN, taking into account the new guidance when this becomes available (Bergmann *et al*. 2003).

LINE 151: The EU would request the OIE to add references to this statement.

LINES 279 – 280: The EU would propose to make the following amendment in the text in the parenthesis: "(either by stomacher, blender, ~~or~~ mortar and pestle with sterile sand or any other suitable and validated homogenizer)".

LINES 281 – 286: The EU would propose to move the following text to section 3.4., as it fits better under that heading:

"If a sample consisted of whole fish less than 4 cm long, these should be minced with sterile scissors or a scalpel, after removal of the body behind the gut opening. If a sample consisted of whole fish with a body length between 4 cm and 6 cm, the viscera, including kidney, should be collected. If a sample consisted of whole fish more than 6 cm long, tissue specimens should be collected as described above. The tissue specimens should be minced with sterile scissors or a scalpel, homogenised and suspended in transport medium."

Diseases of fish: listed
2.3.5. Infectious salmon anaemia

General comments

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

The EU supports the idea of limiting the case definition of the disease to infection with virulent ISA as foreseen in the proposed amendments to the scope (lines 5-10). However, the EU would emphasise the importance of ensuring consistency with the OIE Manual and Code as regards the case definition.

Specific comments

LINES 534 – 543: The EU would propose the following amendments in this paragraph, for reasons of clarification:

~~None of the methods described above have been evaluated for the purpose of declaration of freedom of virus or disease, as data on prevalence and distribution of ISAV in subclinically infected fish population are lacking.~~ Regular health inspections combined with investigation for ISA when increased mortality is associated with one of the given clinical signs and/or pathological changes consistent with ISA is ~~may be~~ an efficient way of obtaining data on the occurrence ~~prevalence~~ of ISA in farmed populations. ~~Alternatively,~~ ~~i~~ In addition to regular health inspections, testing for ISAV, preferentially by PCR-based methodology, at certain intervals may be carried out, ~~in addition to regular health inspections,~~ However, the significance of positive findings of ISAV (HPR-deleted or HPR0) by PCR alone for the risk of developing ISA disease is not clear, and therefore any positive findings would have to be carefully interpreted. ~~The presence of ISAV HPR0 in sea reared Atlantic salmon without clinical signs (see Section 2.1.1) may complicate the management of infected farms.~~

Diseases of fish: listed
2.3.6. Koi herpesvirus disease

General comments

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

The EU would encourage the OIE to include a description of RT-PCR in chapter 4 on diagnostic methods as this method is both more sensitive and specific than conventional PCR.

Specific comments

LINE 37: The EU would propose to replace the word "strain" by "isolate" as there is no proper definition of KHV strains. Even the isolates KHV-U and KHV-I are different in some genomic parts.

LINE 76: The EU would invite the OIE to revise the list of susceptible species to EUS, taking into account the new guidance when this becomes available, including assessing whether Sturgeon is susceptible.

The EFSA Scientific Opinion of the Panel on AHAW on a request from the European Commission on aquatic animal species susceptible to diseases listed in the Council Directive 2006/88/EC (The EFSA Journal (2008), 1-144) may provide useful information in this context.

LINE 77: The EU would propose to replace the phrase " common carp (*Cyprinus carpio carpio*), koi carp (*Cyprinus carpio koi*) and ghost carp (*Cyprinus carpio goi*), and hybrids of these varieties." by common carp and koi carp (*Cyprinus carpio*)" as they are all the same species.

LINES 195 – 196: The sentence "In Europe this includes Austria, Belgium, Czech Republic, Denmark, France, Italy, Luxembourg, The Netherlands, Poland, Switzerland and the United Kingdom (Bergmann *et al.,* 2006; Haenen *et al.,* 2004; Novotny *et al.,* 2010)." should be replaced by "In Europe KHV has been detected in many countries in all parts of the continent (Bergmann *et al.,* 2006; Haenen *et al.,* 2004; Novotny *et al.,* 2010)".

LINE 198: A reference appears to be missing after "Malaysia".

LINE 365: The EU would request the OIE to consider the inclusion of IFAT and ISH with separated fish leukocytes. (Bergmann *et al*. 2009 and Bergmann *et al*. 2010).

LINE 375: The EU would propose that it in the paragraph on ELISA is mentioned that those assays will need at least 10^5 virus particles to show positive signals. Since this is only likely to happen during outbreaks of KHVD, ELISA is not an appropriate method for KHV surveillance in healthy populations.
LINE 692-693: The EU would request the OIE for clarification on the definition of a confirmed case in point 7.2 (i), in particular whether a) and b) are

alternative or cumulative requirements, taking into account point iv) of the definition of a suspect case, which defines a single positive result as a suspect case.

Diseases of fish: listed
2.3.7. Red sea bream iridoviral disease

General comments

The EU supports the proposed changes.

Specific comments

None

Diseases of fish: listed
2.3.8. Spring viraemia of carp

General comments

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

Specific comments

LINES 75: The EU proposes deleting the words "identified in", for linguistic reasons.

LINES 111 and 140: The EU would encourage the OIE to include the specific references as has been done elsewhere in the chapter.

Diseases of fish: listed
2.3.9. Viral haemorrhagic septicaemia

General comments

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

The EU would recommend that it is considered whether a description of QPCR is included in the Manual.

Specific comments

LINES 24 – 26: The genome should be arranged in its natural order, namely: N-P-M-G-NV-L.

LINE 43: The EU would propose to move the heading 2.1.1 above line 2.1, in order to follow the structure of the other chapters and to facilitate precise referencing.

LINE 52: The reference is incomplete and should read: "Ito *et al*., 2010".

LINE 95: The EU would propose to replace the reference to "Smail, 1999" with "Smail and Snow, 2011", which is a more recent article.

LINE 112: The EU would propose to add the word "to" after "rainbow trout", for linguistic reasons.

LINE 115: The word "interier" should be replaced by "interior".

LINE 116: In the title of table 2.1. the word "identified" should be added after "Fish species", for linguistic reasons.

LINE 122: The EU would propose to amend the title of Table 2.2 to read "Fish species from which VHSV has been isolated but with insufficient data to list them as susceptible to VHSV according to the criteria listed by the European Food Safety Authority report on susceptible species (EFSA, 2008)"

LINE 116, In table 2.2, the boxes containing "Siluriformes (catfish)" and "Ictaluridae (North American freshwater catfish)" should be merged with the boxes below.

LINE 126: The EU would propose to replace the following words "VHS might causes" with "VHSV may cause", for linguistic reasons.

LINE 145: A full stop is missing after "North America".

LINE 157: A full stop is missing after "temperature".

LINE 187: The EU would propose to move the heading 2.3.1 above line 152, in order to follow the structure of the other chapters and to facilitate precise referencing.

LINE 221: "36%" should be replaced by "36‰".

LINE 246: "2002b" should be replaced by "2002a,b", as both articles are relevant.

LINE 381: The EU propose to add ", validated homogenizer" after "blender", as this would also be an appropriate method to homogenise.

LINE 542 and onwards: "PBST" should be replaced by "PBS-T", as both of these are in use.

LINE 730: The EU suggests that "PCR" be replaced by RT-PCR, as the latter is the preferred method.

LINE 741: The EU would propose to replace "Anonymous" with "EFSA", as this is the way EFSA usually is quoted.

LINE 744: The original paper is written in German. The EU would therefore propose to include the original German title in the reference. The title is "Vergleichende Untersuchungen über die Stabilität von vier fischpathogenen Viren (VHSV,PFR, SVCV, IPNV)"

LINE 911: In the opinion of the EU, this reference is not correct. The reference should be:

"Skall H.F., Olesen N.J. & Mellergaard S. (2005). Viral haemorrhagic septicaemia virus in marine fish and its implications for fish farming – a review. *J. Fish Dis.*, **28**, 509–529."

Diseases of fish: delisted
2.3.10. *Oncorhynchus masau* virus disease

General comments

The EU supports the proposed changes.

Specific comments

None

Diseases of fish: delisted
2.3.11. Viral encephalopathy and retinopathy

General comments

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

Specific comments

LINE 63: The EU would invite the OIE to consider whether the below species should be included in the list of susceptible species in table 2.2. There are for all the below species publications suggesting their sustainability.

Order	**Family**	**Common name**	**Latin name**
Clupeiformes	Clupeidae	Big-eye sardine	*Etrumeus teres*
Gadiformes	Gadidae	Pacific cod	*Gadus macrocephalus*
Pleuronectiformes	Pleuronectidae	Winter flounder	*Pleuronectes americanus*
Pleuronectiformes	Soleidae	Senegalese sole	*Solea senegalensis*
Scorpaeniformes	Sebastidae	Rockfish	*Sebasticus marmoratus*
Perciformes	Carangidae	Chub mackerel	*Scomber japonicus*
Perciformes	Serranidae	Longtooth grouper	*Epinephelus bruneus*
Perciformes	Serranidae	Humpback grouper	*Cromileptes altivelis*
Perciformes	Serranidae	Brown-spotted grouper	*Epinephelus chlorostigma*
Perciformes	Malacanthidae	Japanese tilefish	*Branchiostegus japonicus*
Perciformes	Mullidae	Red mullet	*Mullus barbatus*
Perciformes	Sciaenidae	White weak fish	*Atractoscion nobilis*
Perciformes	Sciaenidae	Meagre	*Argyrosomus regius*

Perciformes	Apogonidae	Narrow stripe Cardinal fish Cardinal fish	*Apogon exostigma*
Perciformes	Anarhichadidae	Wolf fish	*Anarchichas minor*
Perciformes	Oplegnathidae	Rock bream	*Oplegnathus fasciatus*
Perciformes	Eleotridae	Sleepy cod	*Oxyeleotris lineolatus*
Perciformes	Cichlidae	Tilapia	*Oreochromis niloticus*
Perciformes	Pomacanthidae	Threespot dascyllus	*Dascyllus trimaculatus*
Perciformes	Pomacanthidae	Lemonpeel angelfish	*Centropyge flavissimus*
Perciformes	Acanthuridae	Convict surgeonfish	*Acanthurus triostegus*
Perciformes	Acanthuridae	Lined surgeonfish	*Acanthurus lineatus*
Perciformes	Acanthuridae	Scopas tang	*Zebrasoma scopas*

LINE 44: The word "dead" should be replaced by "death".

LINE 134: In the opinion of the EU, Tunisia should be added to the list of countries that have reported the detection of VER.

Diseases of molluscs: listed
2.4.0. General information

General comments

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

As specific chapters on infections with abalone herpes-like virus, *Xenohaliotis californiensis* and ostreid herpes virus 1 are presented in this version of the manual, the EU suggests including some references on these infections in the "Key references for further reading" section (see proposed references below).

The following references are proposed to be added to the "Key references for further reading" section:

- Infection with abalone herpes-like virus:

CHANG P.H., KUO S.T., LAI S.H., YANG H.S., TING Y.Y., HSU C.L. & CHEN H.C. (2005). Herpes-like virus infection causing mortality of cultured abalone *Halitotis diversicolor supertexta* in Taiwan. *Dis. Aquat. Org.*, **65**, 23–27.

CORBEIL S., COLLING A., WILLIAMS L.M., WONG F.Y.K., SAVIN K., WARNER S., MURDOCH B., COGAN N.O.I., SAWBRIDGE T.I., FEGAN M., MOHAMMAD I., SUNARTO A., HANDLINGER J., PYECROFT S., DOUGLAS M., CHANG P.H. & CRANE M.ST.J. (2010). Development and validation of a TaqMan® PCR assay for the Australian abalone herpes-like virus. *Dis. Aquat. Org.*, **92**, 1–10.

HOOPER C., HARDY-SMITH P. & HANDLINGER J. (2007). Ganglioneuritis causing high mortalities in farmed Australian abalone (*Haliotis laevigata* and *Haliotis rubra*). *Aus. Vet. J.*, **85**, 188–193.

SAVIN K.W., COCKS B.G., WONG F., SAWBRIDGE T., COGAN N., SAVAGE D. & WARNER S. (2010). A neurotropic herpesvirus infecting the gastropod, abalone, shares ancestry with oyster herpesvirus and a herpesvirus associated with the amphioxus genome. *Virol. J.*, **7**, 308. http://www.virologyj.com/content/7/1/308.

TAN J., LANCASTER M., HYATT A., VAN DRIEL R., WONG F. & WARNER S. (2008). Purification of a herpes-like virus from abalone (*Haliotis* spp.) with ganglioneuritis and detection by transmission electron microscopy. *J. Virol. Methods*, **149**, 338–341.

- Infection with Xenohaliotis californiensis:

ANDREE K.B., FRIEDMAN C.S., MOORE J.D. & HEDRICK R.P. (2000). A polymerase chain reaction for detection of genomic DNA of a Rickettsiales-like prokaryote associated with Withering Syndrome in Black Abalone (*Haliotis cracherodii*). *J. Shellfish Res.*, **19**, 213–218.

BALSEIRO P., ARANGUREN R., GESTAL C., NOVOA B. & FIGUERAS A. (2006). *Candidatus* Xenohaliotis californiensis and *Haplosporidium montforti* associated with mortalities of abalone *Haliotis tuberculata* cultured in Europe. *Aquaculture*, **258**, 63–72.

FRIEDMAN C.S., ANDREE K.B., BEAUCHAMP K.A., MOORE J.D., ROBBINS T.T., SHIELDS J.D. & HEDRICK R.P. (2000). "*Candidatus* Xenohaliotis californiensis" a newly described pathogen of abalone, *Haliotis* spp., along the west coast of North America. *Int. J. Syst. Evol. Microbiol.*, **50**, 847–855.

FRIEDMAN C.S., BIGGS W., SHIELDS J.D. & HEDRICK R.P. (2002). Transmission of withering syndrome in black abalone, *Haliotis cracherodii* Leach. *J. Shellfish Res.*, **21** (2), 817–824.

- Infection with ostreid herpes virus 1:

DAVISON A.J., TRUS B.L., CHENG N., STEVEN A.C., WATSON M.S., CUNNINGHAM C., LE DEUFF R.M. & RENAULT T. (2005). A novel class of herpesvirus with bivalve hosts. *J. Gen. Virol.*, **86**, 41–53.

LE DEUFF R.-M. & RENAULT T. (1999). Purification and partial genome characterization of a herpes-like virus infecting the Japanese oyster, *Crassostrea gigas*. *J. Gen. Virol.*, **80**, 1317–1322.

PÉPIN J. F., RIOU A. & RENAULT T. (2008). Rapid and sensitive detection of ostreid herpesvirus 1 in oyster samples by real-time PCR. *J. Virol. Methods*, **149**, 269–276.

RENAULT T., LE DEUFF R.-M., LIPART C. & DELSERT C. (2000). Development of a PCR procedure for the detection of a herpes-like virus infecting oysters in France. *J. Virol. Methods*, **88**, 41–50.

RENAULT T., LE DEUFF R.-M., COCHENNEC N. & MAFFART P. (1994). Herpesviruses associated with mortalities among Pacific oyster, *Crassostrea gigas*, in France – comparative study. *Revue de Médecine Vétérinaire*, **145**, 735–742.

SEGARRA A., PEPIN J.F., ARZUL I., MORGA B., FAURY N. & RENAULT T. (2010). Detection and description of a particular *Ostreid herpesvirus 1* genotype

Specific comments

LINE 15: The EU is of the opinion that it is not very clear what exactly is meant by the new added sentence ("To obtain sections of most organs, take a shallow and a deep histology section from each block"). This should be clarified or could be replaced by the following:

"Sections are prepared in order to include most organs.".

LINE 26: The EU is of the opinion that it is not very clear what exactly is meant by the following words: "removal of several pedal sections". This should be clarified.

LINE 56: Please replace the word "laps" by the word "palps".

LINES 139 and 215: Please replace the word "molluscan" by the word "mollusc".

LINE 194: In the view of the EU the description of the composition of the 1GF4 fixative is not correct. The EU would propose that it reads as follows:

"*Stock 1G4F solution (may be held at 4° C for up to 3 months):
120 ml 37-40% buffered formalin solution**
20 ml 50% glutaraldehyde
360 ml tap water

**Buffered formalin solution:
1 litre 37-40% formaldehyde
15 gm disodium phosphate (Na2HPO4)
0.06 gm sodium hydroxide (NaOH)
0.03 gm phenol red (pH indicator)

Working solution (should be prepared immediately prior to use):
500 ml filtered ambient seawater
500 ml Stock 1G4F solution*"

LINE 206: Please add the words "but tissue optimum thickness is 2-3 mm" after the words "electron microscopy".

LINES 230-231: The EU would propose the following amendments in section 2.4.3:
"After the blocks have been cooled on a cold plate, which allows the paraffin to solidify, histological sections of about ~~2-3~~2–5 µm are cut using a microtome. The sections are recovered on histological slides, drained and dried ~~overnight at 60°C~~ for up to 1 hour 40-42°C or overnight at room temperature. Drying the samples ~~at this temperature~~ allows the excess moisture to be eliminated and thus the sections adhere to the slides."

LINE 237: In order not to be too prescriptive, in the parenthesis, please insert the words "for example" before "95%, […]".

LINE 261: To the EUs knowledge fixation of the samples is carried out directly in 3% glutaraldehyde for 1 to 4 hour. The sentence “Fixation for longer periods leads to membranous artifacts” is therefore not correct since over fixation does not exist with glutaraldehyde fixative, and should thus be deleted.

LINE 363: The EU suggests amending the sentence as follows:

"[…] which are then dried overnight at room temperature or ~~baked overnight~~ in an oven at 40°C.".

LINE 369: In order not to be too prescriptive, please insert the words "10 to" before the words "30 minutes".

LINES 277 and 279: Please replace the word "Gluteraldehyde" by the word "glutaraldehyde".

Diseases of molluscs: listed
2.4.1. Infection with abalone herpes-like virus

General comments

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

In particular, it is noted that the molecular techniques in section 4.3.1.2.3. are very well presented, however no information is given in relation to sensitivity, specificity and validation of any of the recommended techniques. The EU recommends adding such information.

Specific comments

LINE 15: The EU is of the opinion that it is not very clear what exactly is meant by the new added sentence ("To obtain sections of most organs, take a shallow and a deep histology section from each block"). This should be clarified.

LINE 130: The EU suggests adding the following sentence:

"If no diagnostic method is available for juvenile abalone, targeted surveillance should be performed on adult abalone."

LINES: 140 – 141: The EU is of the opinion that an explanation is needed for the proposed new sentence on the pooling of samples ("Abalone tissue samples collected for AbHV surveillance using qPCR should not be pooled"). Indeed, the pooling of samples from several animals allows analysing of more individuals at a lower price.

LINES 272 – 286: The EU is of the opinion that it is generally problematic to fix a threshold to determine if a sample is positive or not since these values can vary from one apparatus to another and from one laboratory to another. Therefore, there should be some flexibility for these values to be validated in each diagnostic laboratory. This could be attained by adding the following after the word "criteria" in Line 273: " as guideline when validating the technique in house". Furthermore, the EU would be grateful for an explanation as to why these changes have been proposed.

LINE 396: In table 5.1, columns larvae and PLs, there should preferably be one technique in category "a", e.g. PCR.

Diseases of molluscs: listed
2.4.2. Infection with *Bonamia exitiosa*

General comments

The EU in general supports the proposed changes but has one comment.

Specific comments

LINES 27 and 82: The EU suggests replacing the reference to "Pichot *et al*., 1979" by the more recent publication by "Narcisi *et al*., 2010".

Consequently, in the reference section, the following reference should be added:

"Narcisi V., Arzul I., Cargini D., Mosca F., Calzetta A., Traversa D., Robert M., Joly J. P., Chollet B., Renault T. & Tiscar P. G. (2010). Detection of Bonamia ostreae and Bonamia exitiosa (Haplosporidia) in Ostrea edulis from the Adriatic Sea (Italy). *Diseases of Aquatic Organisms*. **89**,79-85."

Diseases of molluscs: listed
2.4.3. Infection with *Bonamia ostreae*

General comments

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

Gaping molluscs may be moribund animals but they might also be dead. As a general comment for molluscs, the recommendation for sampling gaping animals can lead to material that is useless for histology.

Specific comments

LINES 62 – 64: There seems to be a mistake in the sentence, which should be reworded as follows:

"Polymerase chain reaction (PCR) positive signal observed in *Crassostrea gigas* suggests that this species may act as a carrier or reservoir of *B. ostreae* (Lynch *et al*., 2010).".

LINE 70: Please replace the word "palleal" by the word "pallial".

LINE 305: It is unclear what is meant by "the first conventional assay". It would be more appropriate to use the names of the authors of the related reference.

Idem in **LINE 311** ("the first conventional PCR assay ").

Idem in **LINE 316 (**"second TaqMan PCR assay").

Diseases of molluscs: listed
2.4.4. Infection with *Marteilia refringens*

General comments

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

Specific comments

LINE 86: The high prevalence estimates are only applicable to infection in *O. edulis*. This should be clarified by adding the words "in *O. edulis*" after the words "up to 98%".

LINES 131 – 132: It is unclear what is meant by "or when temperature is at a maximum for mussels". If this implies that there is a different threshold temperature for infection in mussels, that temperature should be stated. Otherwise, that part of the sentence should be replaced by the following:

"[…] or when temperature reaches the yearly maximum (Carrasco *et al*., 2007).".

LINE 350: The EU suggests adding the words "or equivalent" after the words "in xylene".

Diseases of molluscs: listed
2.4.5. Infection with *Perkinsus marinus*

General comments

The EU in general supports the proposed changes but has one comment.

Specific comments

LINE 409: The EU is of the opinion that in table 5.1, column seed, there should preferably be one technique in category "a", e.g. PCR.

Diseases of molluscs: listed
2.4.6. Infection with *Perkinsus olseni*

General comments

The EU in general supports the proposed changes but has one comment.

Specific comments

LINE 405: The EU is of the opinion that in table 5.1, column seed, there should preferably be one technique in category "a", e.g. PCR.

Diseases of molluscs: listed
2.4.7. Infection with *Xenohaliotis californiensis*

General comments

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

Specific comments

LINE 319: The EU suggests deleting the following part of sentence "[…] and is not commercially available" as this information is not relevant here.

Idem in **LINE 328:** ("[…] and is not commercially available").

Idem in **LINE 372:** ("It is not commercially available").

LINE 374: The EU suggests adding a reference to the DNA sequence at GenBank. Please add the words "(GenBank Accession AF133090)" after the words "target bacterium".

Diseases of molluscs: delisted
2.4.8. Infection with *Mikrocytos mackini*

General comments

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

Specific comments

LINE 15: Please replace "2001" by "2011".

LINE 119: The meaning of this sentence is unclear. It should be clarified whether only tissue fragments from the same animal may be pooled.

LINES 233 – 234: The EU proposes deleting the sentences "*Mikrocytos mackini* is an intracellular parasite. Because oyster cell lines are not available, it is impossible to ascertain if *M. mackini* can be maintened with oyster cells in vitro." Indeed, information could be obtained using primary cell cultures. It is possible to maintain oyster cells alive (not in routine). Thus, mentioning "Not applicable" would be enough.

Diseases of molluscs: not listed
2.4.9. Infection with ostreid herpesvirus-1

General comments

The EU in general supports the proposed changes and has several comments.

Specific comments

LINES 5 – 6: For reasons of clarity, the EU suggests amending the sentence as follows:

"For the purpose of this chapter, infection with ostreid herpesviruse 1 (OsHV-1) is considered to be a viral infection due to OsHV-1 affecting mainly the Pacific cupped oysters, *Crassostrea gigas*."

LINES 17 – 19: The EU suggests deleting the following sentence:

"OsHV-1 overall genome structure is similar to that of certain mammalian herpes viruses (e.g. herpes simplex virus and human cytomegalovirus)."

LINE 31: Please replace "in 2008" by "since 2008" and delete "and subsequently in 009 and 2010".

LINE 32: As mortalities have been associated with other variants, the EU suggests these be mentioned with relative mortality rates.

LINE 62: The EU suggests that it is indicated which species should preferentially be sampled.

Furthermore, the EU suggests adding the following information after the words "naturally infected":

"Young stages including larvae, spat and juveniles seem to be more susceptible to the infection. The virus is more easy to detect in moribund animals than in healthy ones."

LINES 63 – 66: The EU is of the opinion that this paragraph is more related to the section on transmission and therefore suggests moving this paragraph to section 2.3.1 "Transmission mechanisms".

LINES 67 – 69: Similarly, this paragraph should be moved to section 2.2.7 "Known or suspected wild aquatic animal carriers".

LINE 73: It is suggested to add two references to this section, so as to read as follows:

"(Lipart & Renault, 2002; Renault *et al*.; 1995; Schikorsky *et al*., 2011a; Arzul *et al*., 2002)"

LINE 119: The EU suggests that the outbreaks in France in 2008-2010 be referred to the new variant, by adding the following sentence:

"The increased mortalities were associated with the emergence OsHV-1 µvar."

LINE 122: Please add Morocco to the list of countries.

LINE 129: The EU suggests amending the first part of the sentence as follows:

"Mortalities associated with the detection of OsHV-1 are more frequent during summer, which might suggest […]"

LINE 177: The following recommendations should be added to section 2.4.8. "General husbandry practices":

"Moribund and dead oysters should be destroyed whenever feasible. Equipment used in an infected zone should not be sent and used in a non-affected zone. without adequate cleaning and disinfection"

LINE 186: The EU suggests adding the following sentence:

"However, the effect of pooling samples on PCR/ Real Time PCR sensitivity has not been evaluated.".

LINE 191: Please add the words "and digestive gland tissues" after the words "Gonad tissues".

LINES 228 – 234: The EU is of the opinion that this paragraph would be better placed in section 4.3 relating to "Agent detection and identification methods", rather than in section 4.2 “Clinical methods”. Indeed, that paragraph describes viral particles in transmission electron microscopy and not ultra structural changes and should therefore be moved to section 4.3.1.1.

LINE 280 onwards: Section 4.3.1.2.3 on molecular techniques is in the view of the EU incomplete. It only contains a brief introductory test, followed by a longer section on SyberGreen. The EU would suggest that the text is revised, including the following aspects:

1. Samples to be taken: The current text could be retained.

2. Extraction: The current text could be retained, but it should be indicated that any appropriate DNA tissue kit may be used, eg QIAgen.

3. Introduction of PCR methods: The text should include a description of all primer sets used to date (with references) and how they have been used (i.e. in conventional or real time - Sybr®Green, TaqMan – assays). To include a table, similar to the one used in the chapter on Abalone herpes virus, may be beneficial.

4. Choice of PCR method: A text should be drafted providing advice on which PCR method to use, in which the following should be taken into consideration: Single round conventional assays are appropriate to confirm suspected clinical cases of OsHV1 during an outbreak. However, their lack of sensitivity makes them less suited suitable for use in targeted surveillance (ie detection

of virus in clinically normal oysters). The use of C9/C10 or DPFor /DPRev primers in a real time Sybr®Green is recommended as an assay with proven high sensitivity and suitable for use in targeted surveillance. The DPFor /DPRev primer pair might be useful because it targets the DNA polymerase catalytic subunit which is supposed to present less polymorphism than other part of the viral genome especially the C region. Other assays such as the real time TaqMan assay (Martenot *et al*. 2010) and two round amplification of a conventional PCR (nested assay) may also achieve a similar level of sensitivity and be suitable for use in targeted surveillance.

5. Sybr®Green PCR assays: The current text could be retained.

6. Other PCR methods: Seperate sections should be devoted to other PCR methods, such as conventional, nested and TaqMan.

7. Identification of OsHV1 µvar : A description of the appropriate methods to use for the identification of OsHV-1µvar should be included. In the view of the EU, the use of conventional PCR using C2C6 or the CFCR primer sets and sequencing analysis is needed to identify the new variant.

LINES 292 – 298: This paragraph should be replaced by the following: "*Technical procedures*: DNA extraction is accomplished by spin-column methodology using commercially available kits (e.g. QIAgen – Qiamp tissue mini kit) according to the manufacturer's protocol".

LINES 329 – 330: This sentence is very prescriptive. The EU suggests replacing it by the following:

"Real Time PCR analysis should preferably be performed in triplicate.".

LINE 391: Please replace the word "was" by the word "is".

LINES 392 – 399: This paragraph should be replaced by the following:

“The detection steps are performed according to the manufacturer’s instructions.”

LINE 437: In table 5.1, column 1, please insert the words "PCR and" before the words "Real time PCR".

LINE 441: In the view of the EU it should in section 6 be made clear that both Taqman and Sybr Green assays are suitable.

LINES 444 – 450: The EU would propose the following wording for Chapters 7.1 and 7.2.:

"7.1 Definition of a suspect case

A suspected case of infection with OsHV-1 is a positive result by any one of the following methods: histology, PCR or real time PCR.

7.2 Definition of a confirmed case

i) In the case of mortality of susceptible species in a country, zone or compartment in which OsHV-1 was previously detected, a confirmed case of infection with OsHV-1 is a positive result by any one of the following methods: PCR, Real time PCR or ISH.

ii) In other cases, a confirmed case is a positive result of PCR confirmed by
a) sequencing of the PCR product or
b) ISH".