The new Aquatic Manual template for disease-specific chapters and what it means for Reference Laboratory experts

OIE regional expert consultation meeting on aquatic animal disease diagnosis and control
15-16 November 2018, Bangkok, Thailand

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# OIE ad hoc Group on the *Aquatic Manual*

Established in early 2016 and met in April, 2016 and January, 2017 (also by email)

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</tbody>
</table>
OIE ad hoc Group on the *Aquatic Manual*

**Terms of Reference**

1. Review the structure of Section 4 *Diagnostic methods* and propose an amended structure to include information on the validation status for each diagnostic test described. The structure of this information should be consistent with the *Aquatic Manual* Chapter 1.1.2. *Principles and methods of validation of diagnostic assays for infectious diseases.*

2. Work with Reference Laboratory experts as necessary to update information on assay validation in Section 4 *Diagnostic methods* of each disease-specific chapter.

3. Review Section 5 *Rating of tests against purpose of use.* Consider whether the current categorisation of tests (a to d) is appropriate and propose an alternative if necessary. Provide technical guidance that could be used by chapter authors on how tests should be rated for purposes of use: for example as a) surveillance to demonstrate freedom b) surveillance to determine occurrence or distribution c) presumptive diagnosis and d) confirmatory diagnosis.

4. Work with Reference Laboratory experts as necessary to update information in Section 5 *Rating of tests against purpose of use* of each disease-specific chapter.

5. Revise Section 7 *Corroborative diagnostic criteria.* The OIE *Aquatic Animal Health Code* defines a case definition as a set of criteria used to distinguish a case animal or an epidemiological unit from a non-case; and a case as an individual aquatic animal infected by a pathogenic agent, with or without clinical signs. A consistent structure for case definitions should be proposed that provides for clear definitions for a suspect case and a confirmed case. Technical guidance that can be used by chapter authors should be developed and include the purpose of case definitions (e.g. for surveillance) and factors that should be taken into account when defining criteria for case definitions.

6. Work with Reference Laboratory experts as necessary to update information in Section 7 *Corroborative diagnostic criteria* of each disease-specific chapter.
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General observations

1. Variability in how the same sections are structured across different Chapters.
2. Variability in the corroborative diagnostic criteria section.
3. Old protocols that should be updated (e.g. newer extractions systems and master mixes).
4. Information included that really is not really relevant for detection and identification

→ The old template was modified from the viewpoint of a user who wanted to (for the purposes of notification to the OIE):
   - Detect the specific pathogen in clinically-affected animals (suspect case)
   - Confirm the detection in clinically-affected animals (confirmed case)
   - Detect the specific pathogen in apparently healthy animals (suspect case)
   - Confirm the detection in apparently healthy animals (suspect case)

   *In either instance, if the criteria for a confirmed case can not be met, the case stays as a suspect case and OIE notification is not required (additional sampling and testing can be undertaken).*
OIE ad hoc Group on the *Aquatic Manual*

There is a process for migration of OIE *Aquatic Manual* Chapters to the new template (which will be explained later).

**Changes to:**
- The order of some of the Sections (re-arrangement)
- Section 2.2.1 and Section 2.2.2
- Modification of Table 5.1 to become Table 4.1
- Changes to Section 7 which is now Section 6
- Addition of Appendix V

....which I will now explain and feel free to ask questions (and there will be time for a general discussion at the end).
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**Major outcomes:**

1. Re-arranged a number of sections

2. Two sections to be completed by the relevant *ad hoc* Group on susceptible species
   - **Section 2.2.1** Susceptible host species
   - **Section 2.2.2** Species with incomplete evidence for susceptibility

   **Terms of Reference**
   1. Consider evidence required to satisfy the criteria in Chapter 1.5.
   2. Review relevant literature documenting susceptibility of species for OIE listed fish diseases.
   3. Propose susceptible species for OIE listed diseases for fish based on Article 1.5.7.
   4. Propose susceptible species for OIE listed diseases for fish based on Article 1.5.8.
   5. Test the proposed new Aquatic Code Article 1.5.9. that is the listing of susceptible species at a taxonomic ranking of genus or higher [insert pathogen name].

3. Modified **Section 2.4.** ”Biosecurity and disease control strategies”
   - Only includes items that would interfere with detection (e.g. recent vaccination with a heat-killed vaccine may give PCR positive test results).

4. Created **Table 4.1.** “OIE recommended diagnostic methods and their level of validation for surveillance of healthy animals and investigation of clinically affected animals”

..........
### Table 4.1. “OIE recommended diagnostic methods and their level of validation for surveillance of healthy animals and investigation of clinically affected animals”

<table>
<thead>
<tr>
<th>Method</th>
<th>A. Surveillance of apparently healthy animals</th>
<th>B. Presumptive diagnosis of clinically affected animals</th>
<th>C. Confirmatory diagnosis(^1) of a suspect result from surveillance or presumptive diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early life stages(^2)</td>
<td>Juveniles(^2)</td>
<td>Adults</td>
</tr>
<tr>
<td>Wet mounts</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cytopathology(^3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histopathology(^3)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cell or artificial media culture</td>
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<tr>
<td>Real-time PCR</td>
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<tr>
<td>Conventional PCR</td>
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<tr>
<td>Amplicon sequencing(^4)</td>
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<tr>
<td>In-situ hybridisation</td>
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<tr>
<td>Bioassay</td>
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<tr>
<td>LAMP</td>
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<td></td>
<td></td>
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<tr>
<td>Ab ELISA</td>
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<td></td>
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<tr>
<td>Ag ELISA</td>
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<tr>
<td>Other antigen detection methods(^5)</td>
<td></td>
<td></td>
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<tr>
<td>Other serological method(^6)</td>
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</tbody>
</table>

LV = level of validation, refers to the stage of validation in the OIE Pathway (chapter 1.1.2); PCR = polymerase chain reaction. [give definitions of abbreviations as appropriate; nPCR = nested PCR, etc. NB “RT-PCR” is reserved for reverse transcriptase-polymerase chain reaction methods. “real-time PCR” should always be stated in full and refers to probe-based and SYBR green assays]

\(^1\)For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). \(^2\)Early and juvenile life stages have been defined in Section 2.2.3. \(^3\)Cytopathology and histopathology can be validated if the results from different operators has been statistically compared. \(^4\)Sequencing of the PCR product. \(^5\)Specify the test used. Shading indicates the test is inappropriate or should not be used for this purpose.
OIE ad hoc Group on the Aquatic Manual

Major outcomes:

5. Provided standardised options for **Section 6.** Corroborative diagnostic criteria

6.1. Apparently healthy animals or animals of unknown health status
   6.1.1. Definition of suspect case in apparently healthy animals
   6.1.2. Definition of confirmed case in apparently healthy animals

6.2. Clinically affected animals
   6.2.1. Definition of suspect case in clinically affected animals
   6.2.2. Definition of confirmed case in clinically affected animals

6.3. Diagnostic sensitivity and specificity for diagnostic tests
   [Include table from appendix V]
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**Section 6.3. Diagnostic sensitivity and specificity for diagnostic tests (AbHV example)**

6.3.1. **For presumptive diagnosis of clinically affected animals**

DSe: = diagnostic sensitivity, DSp = diagnostic specificity, qPCR: = real-time polymerase chain reaction.

<table>
<thead>
<tr>
<th>Test type</th>
<th>Test purpose</th>
<th>Source populations</th>
<th>Tissue or sample types</th>
<th>Species</th>
<th>DSe (n)</th>
<th>DSp (n)</th>
<th>Reference test</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Real Time PCR</td>
<td>Diagnosis</td>
<td>Clinically diseased abalone from farms and processing plants</td>
<td>Pleuropedal ganglion or pedal nerve cords</td>
<td>Need to check the species to include here</td>
<td>100 (30)</td>
<td>100 (30)</td>
<td>Histopathology</td>
<td>Need to check the reference to insert here</td>
</tr>
</tbody>
</table>

6.3.2. **For surveillance of apparently healthy animals**

DSe: = diagnostic sensitivity, DSp = diagnostic specificity, qPCR: = real-time polymerase chain reaction.

<table>
<thead>
<tr>
<th>Test type</th>
<th>Test purpose</th>
<th>Source populations</th>
<th>Tissue or sample types</th>
<th>Species</th>
<th>DSe (n)</th>
<th>DSp (n)</th>
<th>Reference test</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Real Time PCR</td>
<td>Surveillance</td>
<td>Naturally AbHV infected and AbHV free</td>
<td>Pleuropedal ganglion or pedal nerve cords</td>
<td>Wild blacklip, and farmed hybrid abalone</td>
<td>96.7 (29)</td>
<td>99.7 (1644)</td>
<td>Histopathology</td>
<td>Corbeil <em>et al.,</em> 2010</td>
</tr>
</tbody>
</table>
Section 6. Corroborative diagnostic criteria

6.1. Apparently healthy animals or animals of unknown health status

Apparently healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link(s) to an infected population. Geographic proximity to, or movement of animals or animal products or equipment, etc., from a known infected population equate to an epidemiological link. Alternatively, healthy populations are sampled in surveys to demonstrate disease freedom.

6.1.1. Definition of suspect case in apparently healthy animals

[The information in 6.1.1 and 6.1.2 is pathogen specific. Any positive test result from assays listed in Table 4.1 with a designation of ++ or +++ would usually be considered a suspect positive test result. It should be noted whether a recommended test can be used for all or only some susceptible species. Authors should advise caution if tests are used on species for which validation studies have not been completed.]

The presence of infection shall be suspected if: a positive result has been obtained on at least one animal from at least one of the following diagnostic tests:

[Authors: delete criteria that are non-relevant to the pathogen in question]

i) Positive result by a recommended molecular or antigen or antibody detection test

ii) Cyto- or histopathological changes consistent with the presence of the pathogen or the disease

iii) Visual observation (direct or by microscopy) of the pathogen

iv) Cytopathic effect in cell culture (viruses)

v) Culture and isolation (e.g. bacterial colonies indicative of the pathogen)

vi) Bioassay

6.1.2. Definition of confirmed case in apparently healthy animals

[In principle, at least two independent tests, one from column Test A and one from column Test B in Table A, are required for a confirmed case. One of the tests may have been undertaken as part of a screening programme and resulted in a suspect case. Ideally both tests are run on the same samples with positive test results for both.]

The presence of infection shall be confirmed if: positive results have been obtained on at least one animal from two test used in the following combination:

[number i) to n) combinations of tests]

Reference Laboratories should be contacted for specimen referral when testing laboratories cannot undertake any of the recommended test methods and testing is being undertaken that will result in notification to the OIE.
### Section 6. Corroborative diagnostic criteria

#### Appendix 2. Combination of tests with positive results required for a confirmed case – in apparently healthy or clinically affected animals (for use by experts – not for publication in the Manual)

1. A and B do not indicate order in which tests are conducted except where indicated; 2. In-situ hybridisation or immunocytochemistry should follow histopathological examination.

[If one epidemiological unit, e.g. a fish, meets the definition of confirmed case then all the animals in that population are considered to have the same status.]

<table>
<thead>
<tr>
<th>Test A (presumptive)</th>
<th>Test B (confirmatory)</th>
</tr>
</thead>
</table>
| 1  | Cytopathology  
|    | or Histopathology  
|    | And Conventional PCR test and amplicon sequencing  |
| 2  | Any PCR test, including LAMP  
|    | And Conventional PCR targeting non-overlapping region of the genome and amplicon sequencing  |
| 3  | Histopathology  
|    | And In-situ hybridisation  
|    | or Immunohistochemistry  |
| 4  | Pathogen (virus, bacterium, oomycete, etc.) isolation  
|    | or Bioassay  
|    | And Conventional PCR and amplicon sequencing  
|    | or In-situ hybridisation  
|    | or Immunocytochemistry (with specific antibody)  |
| 5  | Antigen detection  
|    | And Conventional PCR test and amplicon sequencing  
|    | or Pathogen isolation and confirmation (see row 4)  |
| 6  | Visual observation of the pathogen  
|    | And Conventional PCR and amplicon sequencing  |
OIE ad hoc Group on the *Aquatic Manual*

**Process for migration OIE *Aquatic Manual* Chapters to the new template**

1. AAHSC decide which Chapters are going to be updated.
2. Member of the *ad hoc* Group on the Aquatic Manual assigned to the Chapter and contacts the Reference Laboratory experts.
3. AHG Member transfers the text across (to create a clean copy), including existing tests into Table 4.1 and the text into Section 6. Corroborative diagnostic criteria and sends to the Reference Laboratory experts.
4. AHG Member works with Reference Laboratory experts as necessary to update information in Table 4.1 and Section 6.
5. Reference Laboratory experts can make changes to the Chapter in Word using Track Changes. Opportunity to update the Chapter with new information in any Section, including diagnostic tests (opportunity to update older molecular methods to newer molecular methods [NM and EP need to update advice to authors about this]).
6. Any changes need to refer to peer-reviewed literature (must be published).
7. Reference Laboratory experts send the changes back to the AHG Member who checks everything is fine (sends any changes back to the Reference Laboratory experts) then submitted to the OIE AAHSC.

- AHG Member is there to advise on the process and requirements for Table 4.1 and Section 6.
- If there are any disagreements these are resolved by the AAHSC
- The process is currently being undertaken with the RSIV and SVCV Chapters.
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**Experience doing it previously**

- Chapter 2.3.8 Red sea bream iridoviral disease
- Dr Yasuhiko Kowato is the Reference Laboratory expert
- Good opportunity as Dr Kowato is at AFDL for 12 months

- AHG Members need to provide a clear description of what needs to be done
- Need good communication about what can be changed and what cannot be changed
- Good opportunity to update the Chapter as well
- Migrated to new template, discussed changes to Table 4.1 and Section 6
- Dr Kowato is updating the Chapter to include new assays and information about assay specificity
- Currently undertaking work to provide estimates of DSe and DSp, particularly for surveillance testing in apparently healthy animals (will be added when published).

- Ongoing process with ongoing communication
- Much easier being in the same room, looking at the same computer
OIE ad hoc Group on the *Aquatic Manual*

- Feedback already provided will be used to update the advice to authors
- Feedback form this meeting will be used to update the advice to authors
- AAHSC will determine the priority for Chapters to be updated
- Possibility to update Chapter 2.X.0 General Information
  - General advice (e.g. fixation for histology and TEM, sample preservation for molecular testing). Chapter 2.X.0 Section then referred to in the pathogen-specific Chapter unless there are “special” factors to be considered.
- If anything is unclear, ask questions, that way the process can be made simpler for everyone