OIE Sub-Regional Training Course on Diseases of Fish
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The Aquatic Manual

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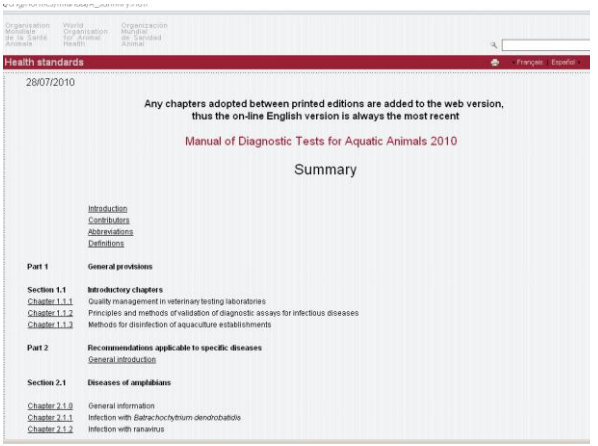
Manual of Diagnostic Tests for Aquatic Animals ('Aquatic Manual')

'Aquatic Manual'

- Sixth edition of the *Manual of Diagnostic Tests for Aquatic Animals (2009)*

Available on-line www.oie.int



Purpose of Aquatic Manual

- Provide internationally agreed standardised approach to the diagnosis of OIE-listed diseases (*Aquatic Code*)
- Facilitate international trade in aquatic animals and their products by:
  - ensuring harmonisation of diagnostic testing
  - avoiding differences in interpretation of results
  - ensuring quality of diagnostic tests

Recognised as the international standard by the WTO

Purpose of Aquatic Manual

- A key and unique document describing diagnostic methods that can be applied to the OIE-listed diseases in aquatic animal health laboratories all over the world ➡ improved aquatic animal health worldwide;
- Describes diagnostic laboratory methods which are suitable for the detection of disease as part of a national aquatic animal health surveillance/control programme, or as part of a programme to underpin claims of freedom from a specific disease;
- To assist with the development of surveillance methodologies for OIE-listed diseases;
- Surveillance programmes aim to determine, from the results provided by standardised lab. methods performed with samples collected according to define rules (*Aquatic Code*), the health status for a country, zone or compartment for a specified disease.

Who uses the Aquatic Manual?

- Laboratories carrying out veterinary diagnostic tests and surveillance;
- Competent Authorities in Member Countries.

CONTENTS

Part 1 General Provisions

Section 1.1 Introductory Chapters

Ch 1.1.1 Quality management in veterinary testing laboratories

Ch 1.1.2 Principles and methods of validation of diagnostic assays for infectious diseases

Ch 1.1.3 Methods for disinfection of aquaculture establishments

CONTENTS

Part 2

- **Recommendations Applicable to Specific Diseases**

Diseases of Amphibians – in preparation
Diseases of Crustaceans
Diseases of Fish
Diseases of Molluscs



General information

Each Section starts with general information - amphibians (pending), crustaceans, fish, molluscs

A. Sampling
1. Assessing the health status of the epidemiological unit
2. General processing of samples

B. Material and biological products required for the isolation and identification of pathogens

CHAPTER X.X.X.
DISEASE X

1. Scope

"For the purpose of this chapter, DISEASE NAME is considered to be INFECTION WITH PATHOGEN NAME."

2. Disease information

2.1. Agent factors
- 2.1.1. Aetiological agent, agent strains
- 2.1.2. Survival outside the host
- 2.1.3. Stability of the agent (effective inactivation methods)
- 2.1.4. Life cycle (if applicable)

Disease information (cont…)

2.2. Host factors
- 2.2.1. Susceptible host species
- 2.2.2. Susceptible stages of the host
- 2.2.3. Species or sub-population predilection (probability of detection)
- 2.2.4. Target organs and infected tissue
- 2.2.5. Persistent infection with lifelong carriers
- 2.2.6. Vectors
- 2.2.7. Known or suspected wild aquatic animal carriers

2.3. Disease pattern
- 2.3.1. Transmission mechanisms
- 2.3.2. Prevalence (in wild and farmed populations for the detection method used, under different conditions)
- 2.3.3. Geographical distribution
- 2.3.4. Mortality and morbidity
- 2.3.5. Environmental factors (e.g. temperature, salinity, season, etc.)

Disease information (cont…)

2.4. Control and prevention
- 2.4.1. Vaccination
- 2.4.2. Chemotherapy
- 2.4.3. Immunostimulation
- 2.4.4. Resistance breeding
- 2.4.5. Restocking with resistant species
- 2.4.6. Blocking agents
- 2.4.7. Disinfection of eggs and larvae
- 2.4.8. General husbandry practices

3. Sampling

- 3.1. Selection of individual specimens
- 3.2. Preservation of samples for submission
- 3.3. Pooling of samples
- 3.4. Best organs or tissues
- 3.5. Samples/tissues that are not suitable (i.e. not possible to detect)

4. Diagnostic methods

4.1. Field diagnostic methods
(observation of the animal and its environment)
- 4.1.1. Clinical signs
- 4.1.2. Behavioural changes

4.2. Clinical methods
(effects of the pathological agent on the host, rather than on agent detection)
- 4.2.1. Gross pathology
- 4.2.2. Clinical chemistry
- 4.2.3. Microscopic pathology
- 4.2.4. Wet mounts
- 4.2.5. Smears
- 4.2.6. Fixed sections
- 4.2.7. Electron microscopy/cytopathology

4.3. Agent detection and identification methods
(methods that detect, possibly isolate and amplify, and identify the agent)
- 4.3.1. Direct detection methods
  - 4.3.1.1. Microscopic methods
  - 4.3.1.2. Agent isolation and identification
- 4.3.2. Serological methods

5. Rating of tests against purpose of use

- This information is used to determine which test is appropriate for what purpose.
- E.g. a particular method may be highly suitable to diagnose clinical cases of disease in individual animals of a certain age group, but the same method may be unsuitable for assessing the infection status of large numbers of clinically healthy animals.
- Each *Manual* disease chapter includes a Table comparing different methods for targeted surveillance and diagnosis of Disease X.
- It is an assessment of the test's 'fitness for purpose'.

Method	Targeted surveillance				Presumptive diagnosis	Confirmatory diagnosis
	Larvae	PLs	Juveniles	Adults		
Gross signs	d	d	c	c	c	d
Bioassay	d	d	d	d	c	b
Direct LM	d	d	c	c	c	c
Histopathology	d	c	c	c	a	a
Transmission EM	d	d	d	d	d	a
Antibody-based assays	d	d	c	c	a	b
DNA Probes □ *in situ*	d	d	c	c	a	a
PCR	d	b	a	a	a	a
Sequence	d	d	d	d	d	a

a = the method is the **recommended method** for reasons of availability, utility, & diagnostic specificity & sensitivity;
b = the method is a **standard method** with good diagnostic sensitivity & specificity;
c = the method has **application in some situations**, but cost, accuracy, or other factors severely limits its application;
d = the method is presently **not recommended** for this purpose.

- The rating of the different diagnostic methods are somewhat subjective as suitability involves issues of reliability, sensitivity, specificity and utility.
- Although not all of the tests listed as **category a** (the recommended method) or **category b** (a standard method) have undergone formal standardisation and validation, their routine nature and the fact that they have been used widely without dubious results, makes them acceptable.

6. Test(s) recommended for targeted surveillance to declare freedom from Disease X.

- Describes methods, based on the information provided in point 1- 4, and assessed in 5, for targeted surveillance to declare freedom from disease as outlined in the *Aquatic Code.*

7. Corroborative diagnostic criteria

7.1. Definition of suspect case
7.2. Definition of confirmed case

- Defines what constitutes a suspect case of disease, and a confirmed case of disease
- For example, a certain level of mortality at the right time of the year, in susceptible animals, together with matching clinical signs, liver lesions and histopathology could be sufficient for suspicion of Disease X. Several combinations may be possible.
- A confirmed case could be defined where in addition to the above, the agent has been detected. However, detection of viable agents without any clinical signs could also constitute a confirmed case.
- This information is required:
  - for the purpose of disease investigations, especially in cases where 'free' status is threatened.
  - when surveillance of healthy populations yields controversial results, e.g. positive PCR signals in the absence of any other evidence of infection.

Aquatic Manual

Note:

- Chapter on *Aquatic animal health surveillance* (1.4.) is in the *Aquatic Code*.
- *Guide for Aquatic Animal Health Surveillance* (2009)
  - additional text on surveillance

Thank you