



World Organisation  
for Animal Health



# Launch of the Regional Aquatic Animal Health Laboratory Network for Africa (RAAHLN-AF)

5 – 7 December 2023 Pretoria, South Africa







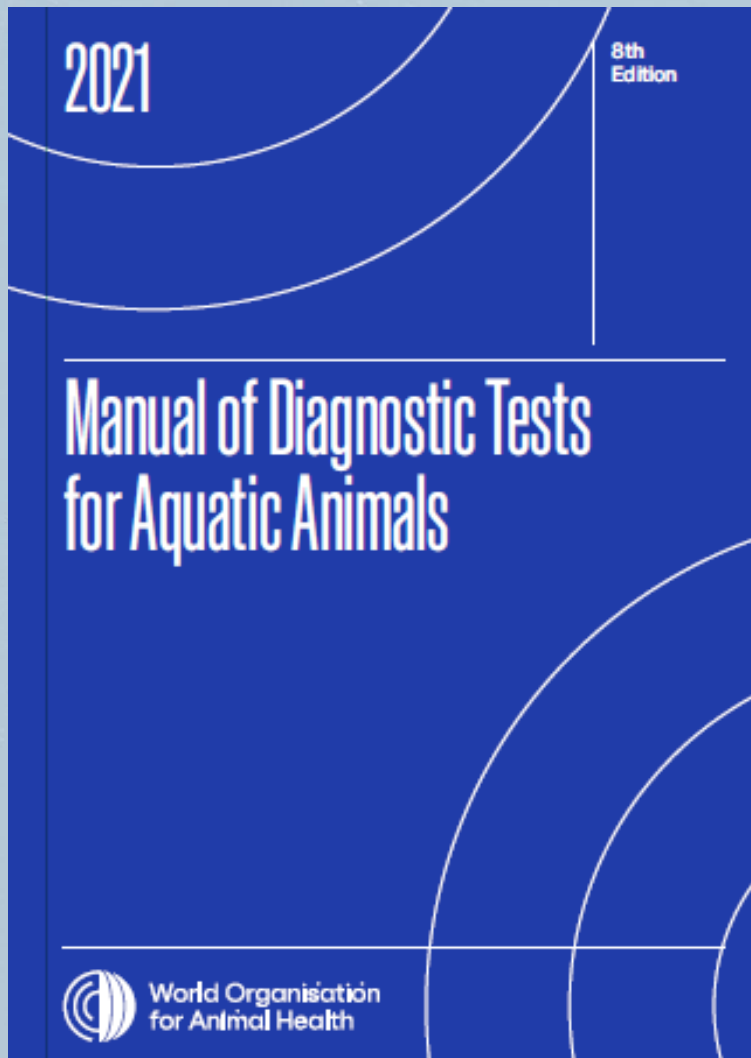
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# Provisions of the WOAHA Aquatic Animal Health Manual

5 – 7 December 2023 Pretoria, South Africa



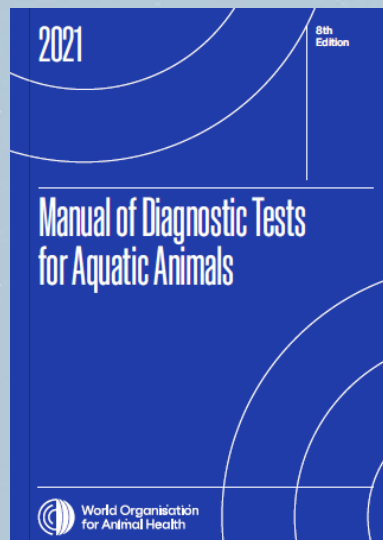


## WOAH Aquatic Manual

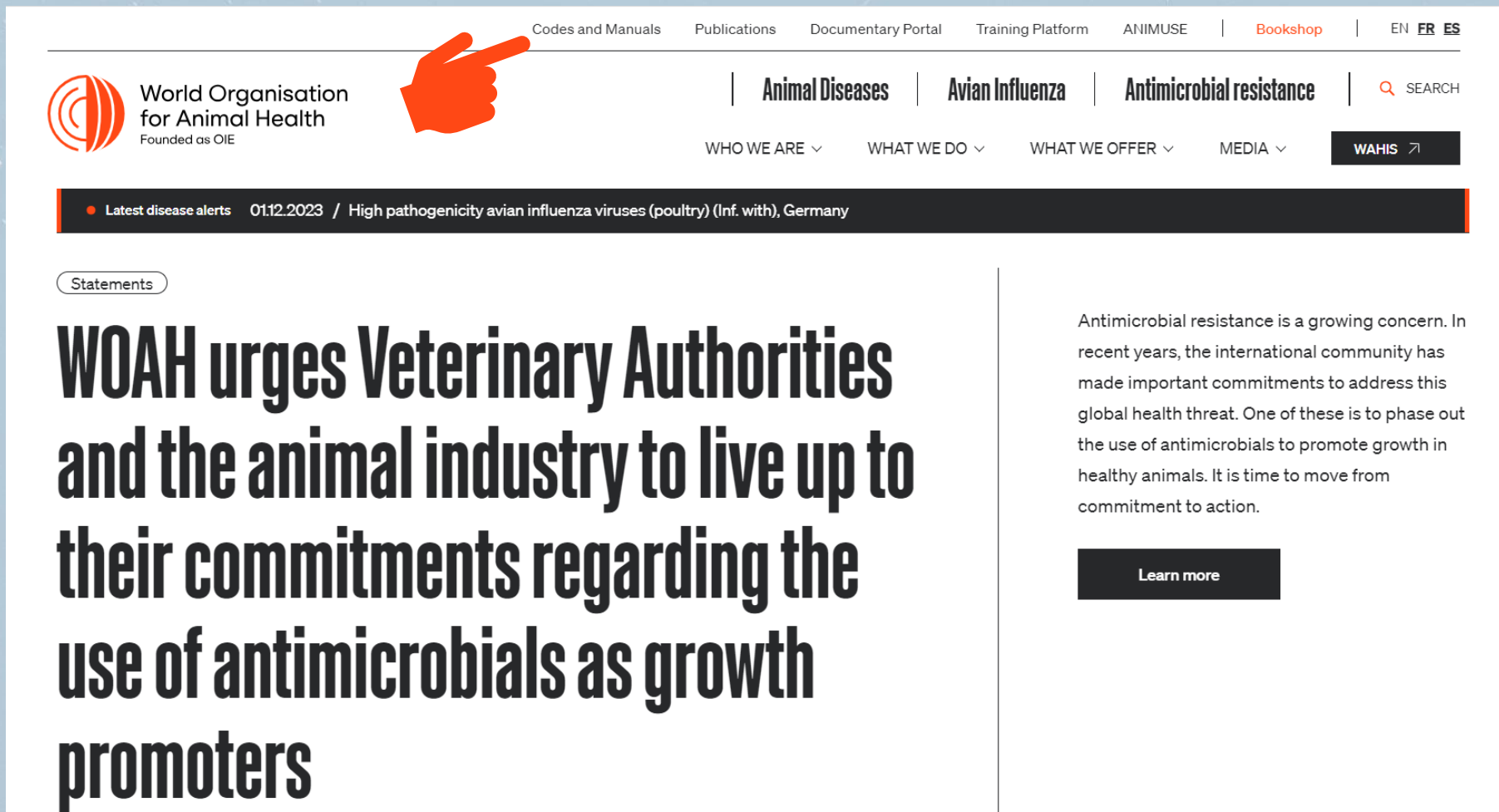
1. Internationally agreed recommendations to support effective laboratory testing capacity.
2. Provides standards and specific methods for disease diagnosis.
3. Presents a network of WOAH reference laboratories and collaboration centers.




# The Manual of Diagnostic Tests for Aquatic Animals



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• Latest disease alerts 01.12.2023 / High pathogenicity avian influenza viruses (poultry) (Inf. with), Germany

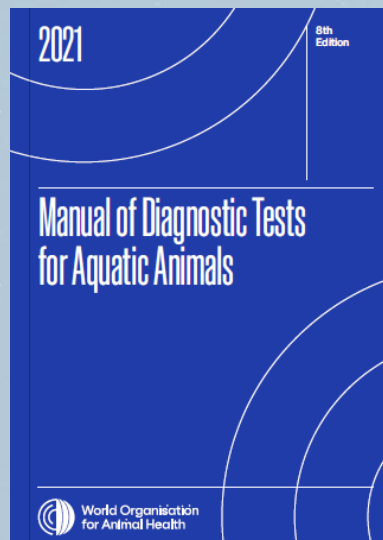
Statements

## WOAH urges Veterinary Authorities and the animal industry to live up to their commitments regarding the use of antimicrobials as growth promoters

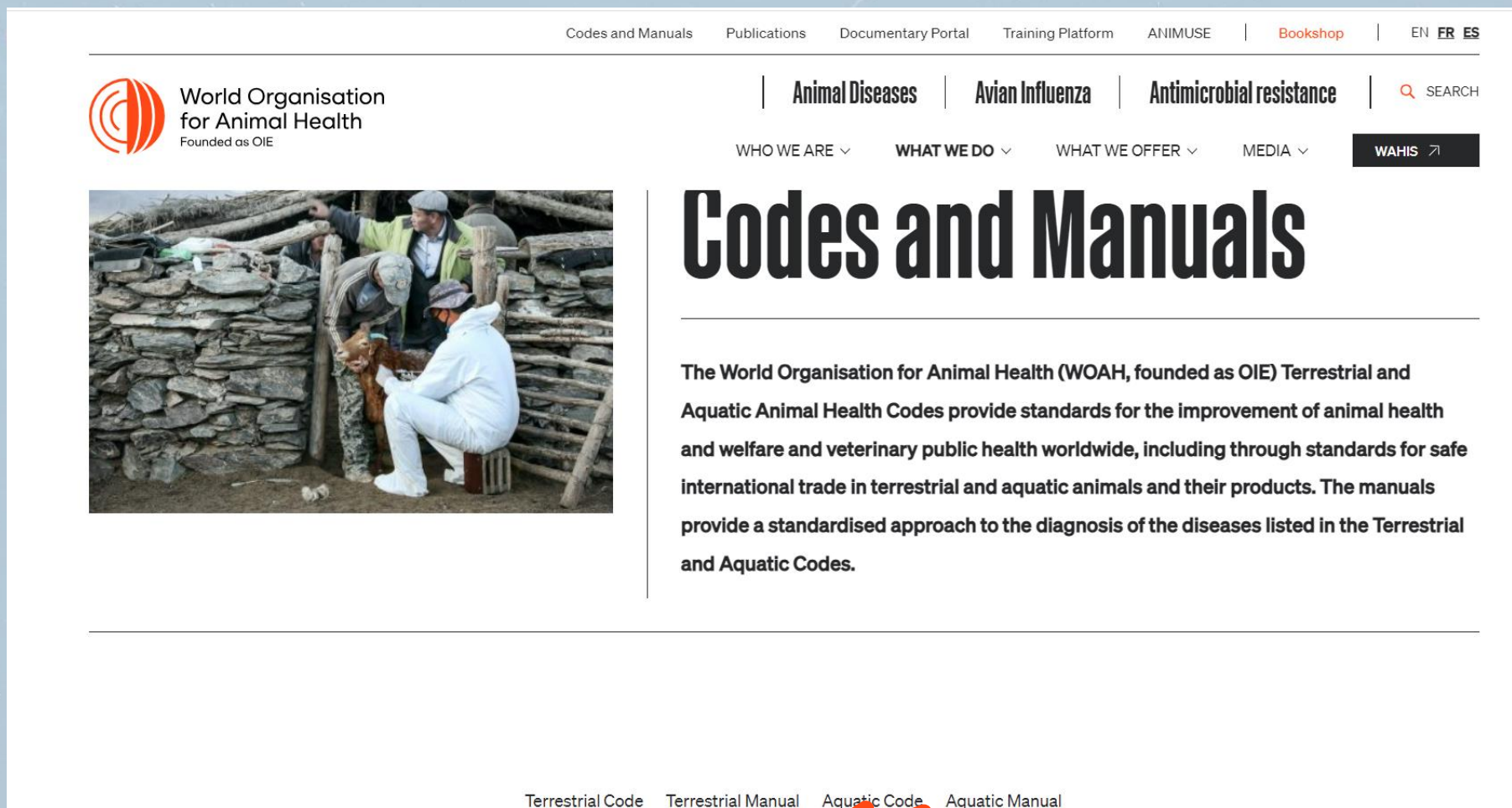
Antimicrobial resistance is a growing concern. In recent years, the international community has made important commitments to address this global health threat. One of these is to phase out the use of antimicrobials to promote growth in healthy animals. It is time to move from commitment to action.

[Learn more](#)


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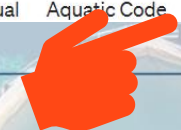
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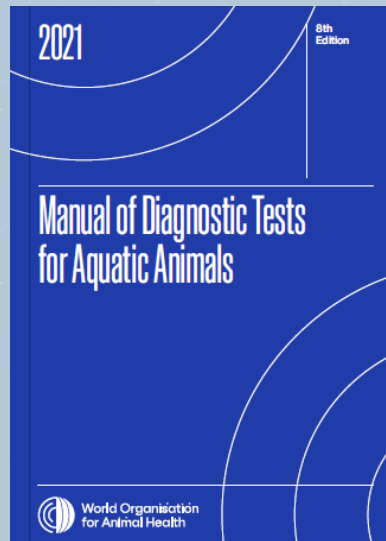
# Codes and Manuals

The World Organisation for Animal Health (WOAH, founded as OIE) Terrestrial and Aquatic Animal Health Codes provide standards for the improvement of animal health and welfare and veterinary public health worldwide, including through standards for safe international trade in terrestrial and aquatic animals and their products. The manuals provide a standardised approach to the diagnosis of the diseases listed in the Terrestrial and Aquatic Codes.

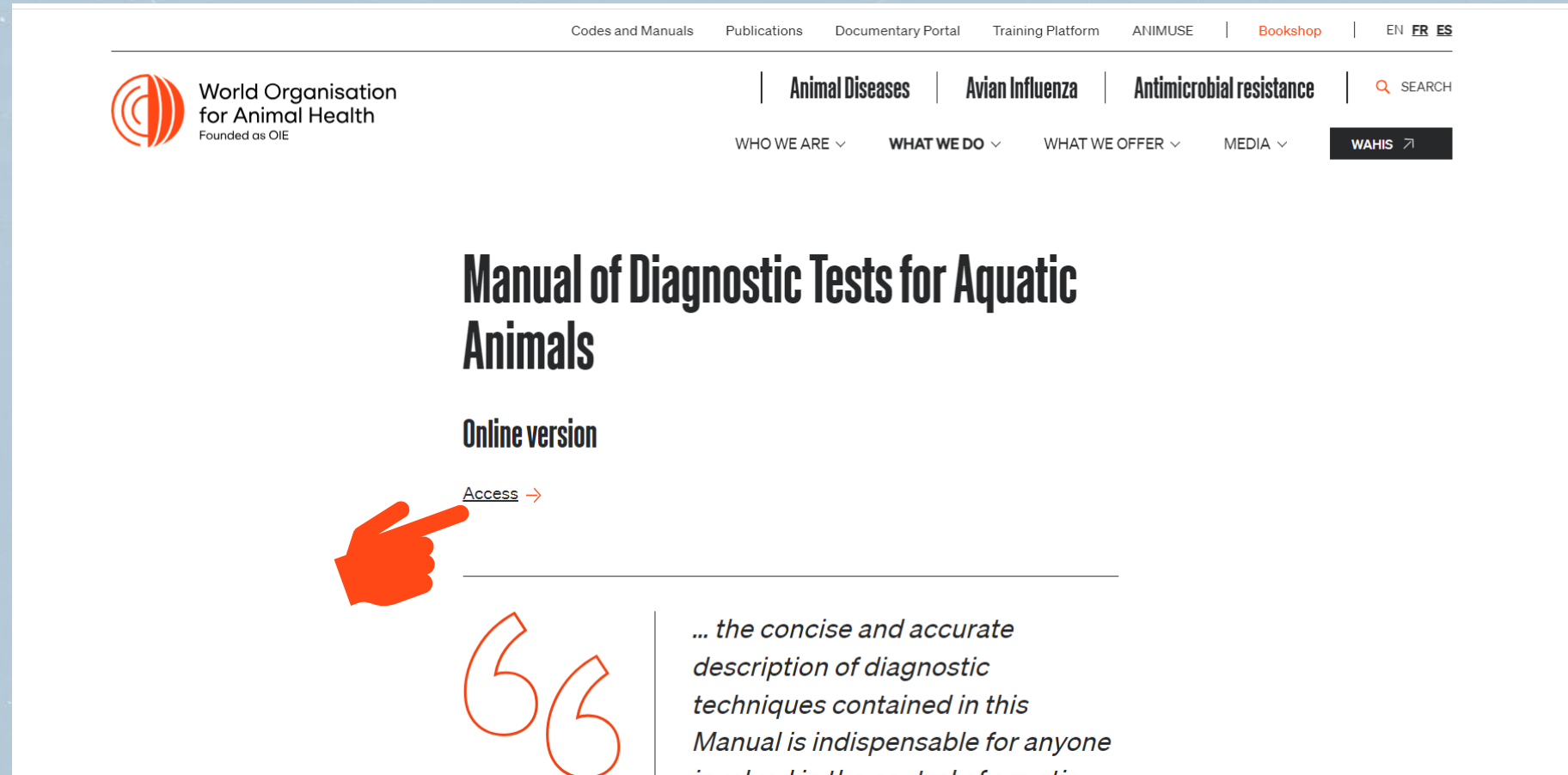
[Terrestrial Code](#) | [Terrestrial Manual](#) | [Aquatic Code](#) | [Aquatic Manual](#)




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## Manual of Diagnostic Tests for Aquatic Animals

Online version

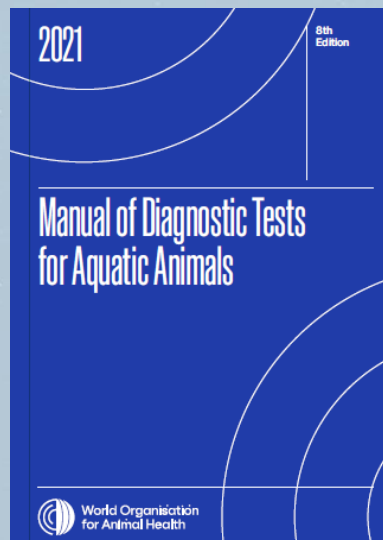
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... the concise and accurate  
description of diagnostic  
techniques contained in this  
Manual is indispensable for anyone  
involved in the control of aquatic




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*Manual of Diagnostic Tests for Aquatic Animals, tenth edition 2023*

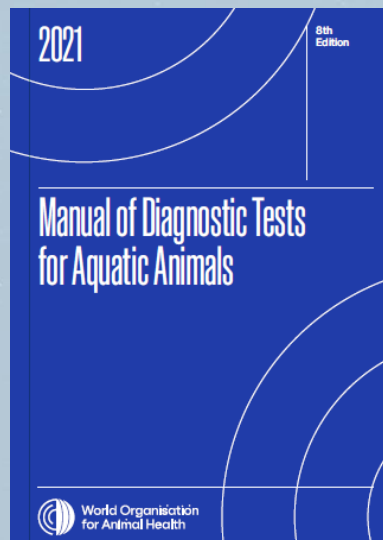
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*Contents*

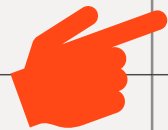
	Foreword	
	Introduction	
	Contributors	

# The Manual of Diagnostic Tests for Aquatic Animals

- General provisions (e.g. quality management for labs, principles and methods of validation)

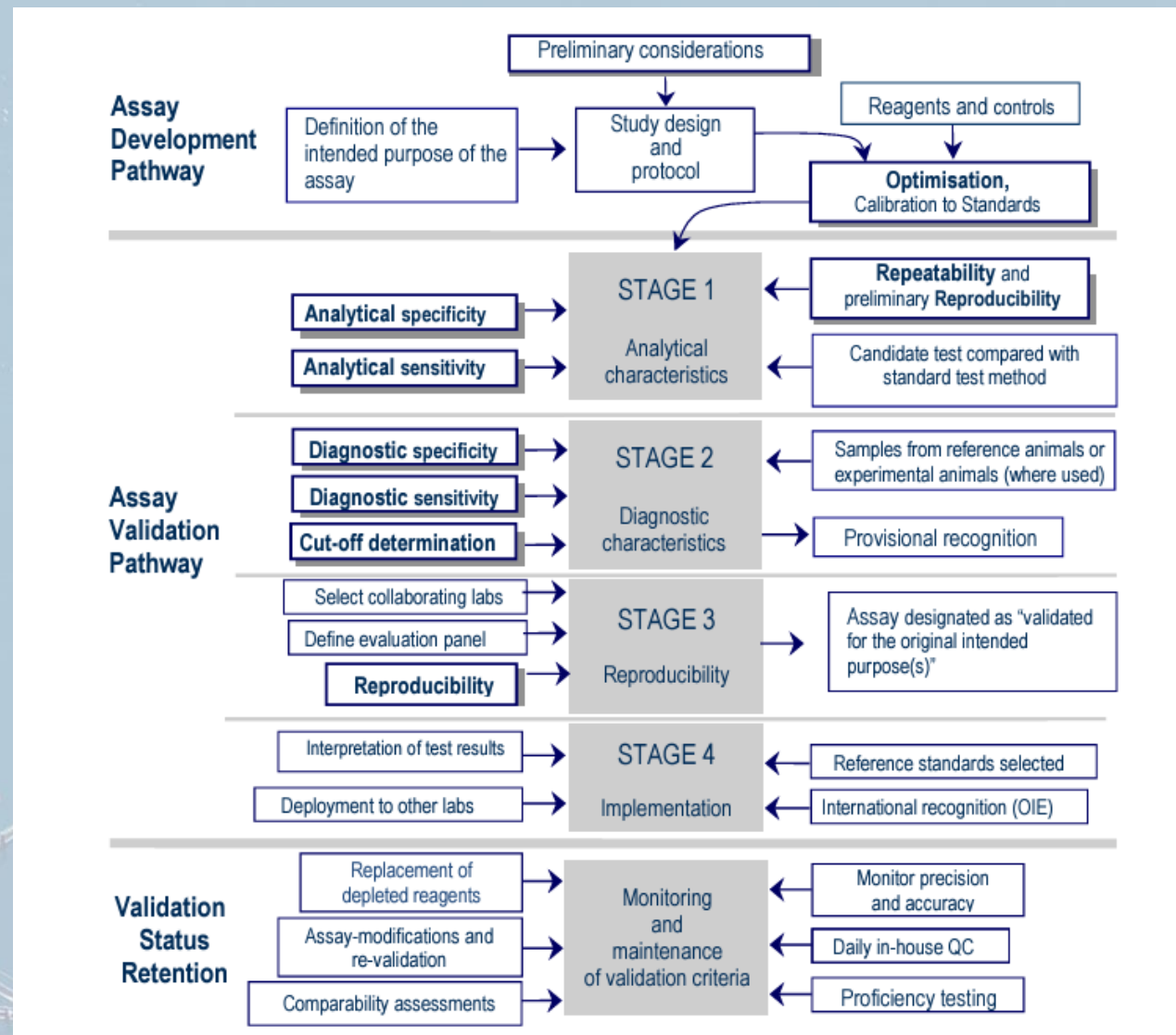
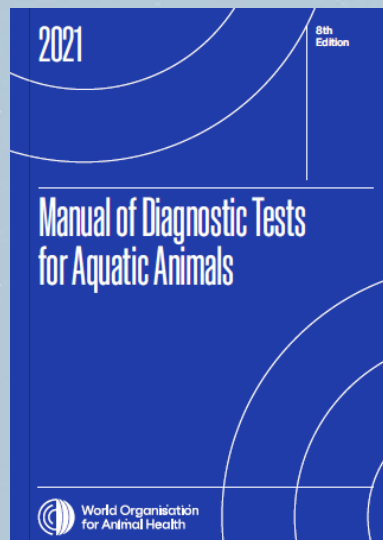


Part 1	General provisions
Section 1.1	Introductory chapters
Chapter 1.1.1.	Quality management in veterinary testing laboratories (version adopted in May 2017)
Chapter 1.1.2.	Principles and methods of validation of diagnostic assays for infectious diseases (version adopted in May 2013)



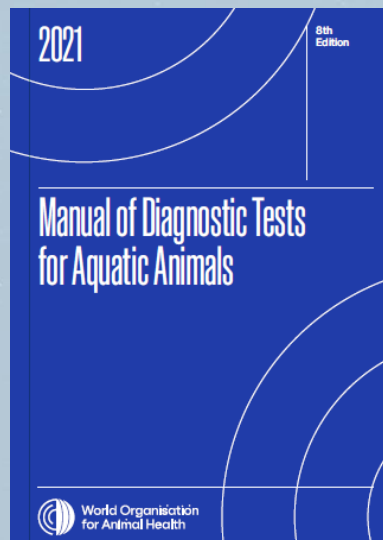


# The Manual of Diagnostic Tests for Aquatic Animals



# The Manual of Diagnostic Tests for Aquatic Animals

Disease-specific provisions (e.g. disease information, host factors, epidemiology, specimen collection, diagnostic methods, recs for purpose of use, case definitions)



<b>Part 2</b>	<b>Recommendations applicable to specific diseases</b>
<b>Section 2.1.</b>	<b>Diseases of amphibians</b>
<b>Section 2.2.</b>	<b>Diseases of crustaceans</b>
<b>Section 2.3.</b>	<b>Diseases of fish</b>
<b>Section 2.4</b>	<b>Diseases of molluscs</b>



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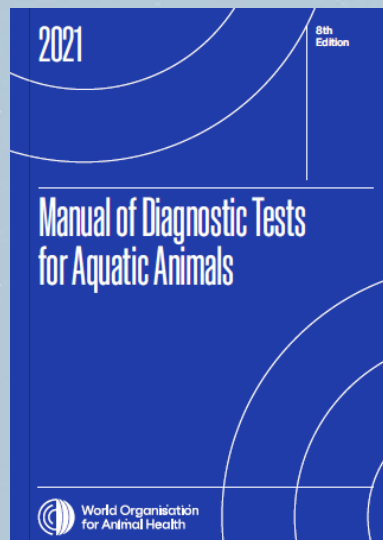


Table 4.1. WOAHA recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals

Method	A. Surveillance of apparently healthy animals				B. Presumptive diagnosis of clinically affected animals				C. Confirmatory diagnosis <sup>1</sup> of a suspect result from surveillance or presumptive diagnosis			
	Early life stages <sup>2</sup>	Juveniles <sup>2</sup>	Adults	LV	Early life stages <sup>2</sup>	Juveniles <sup>2</sup>	Adults	LV	Early life stages <sup>2</sup>	Juveniles <sup>2</sup>	Adults	LV
Wet mounts												
Histopathology												
Cytopathology												
Cell culture		++	++	1		++	++	1		++	++	1
Real-time PCR												
Conventional PCR		++	++	1		++	++	1		++	++	1
Amplicon sequencing										+++	+++	1
<i>In-situ</i> hybridisation												
Immunohistochemistry						++	++	1				
Bioassay												
LAMP												
Ab-ELISA												
Ag-ELISA						++	++	1				
IFAT						++	++	1				

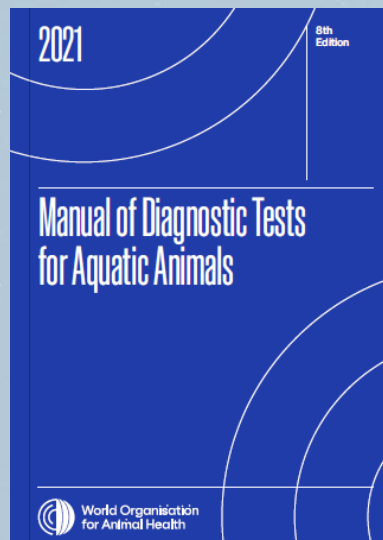
LV = level of validation, refers to the stage of validation in the WOAHA Pathway (chapter 1.1.2); PCR = polymerase chain reaction; LAMP = loop-mediated isothermal amplification; Ab- or Ag-ELISA = antibody or antigen enzyme-linked immunosorbent assay.

<sup>1</sup>For confirmatory diagnoses, methods need to be carried out in combination (see Section 6).

<sup>2</sup>Early and juvenile life stages have been defined in Section 2.2.3.

Shading indicates the test is inappropriate or should not be used for this purpose.

# The Manual of Diagnostic Tests for Aquatic Animals



## 6.1. Apparently healthy animals or animals of unknown health status <sup>1</sup>

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 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:

- i) A positive result from a real-time PCR assay
- ii) A positive result from a conventional nested PCR assay.

### 6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with KHV is considered to be confirmed if at least one of the following criteria is met:

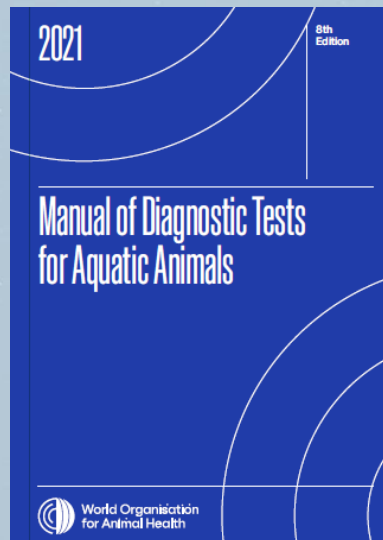
- i) Detection of KHV in tissue samples by real-time PCR followed by conventional PCR and sequencing of the amplicon
- ii) Detection of KHV in tissue samples by real time PCR followed by conventional nested PCR and sequencing of the amplicon

## 6.2. Clinically affected animals

No clinical signs are pathognomonic for infection with KHV however, they may narrow the range of possible diagnoses.



# The Manual of Diagnostic Tests for Aquatic Animals



## 6.2.1. Definition of suspect case in clinically affected animals

The presence of infection shall be suspected if at least one of the following criteria is met:

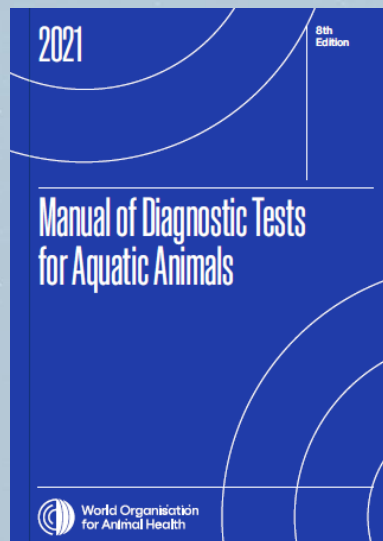
- i) Gross pathology or clinical signs associated with infection with KHV as described in this chapter, with or without elevated mortality
- ii) Histopathological changes consistent with infection with KHV as described in this chapter
- iii) KHV typical CPE in cell culture
- iv) A positive result by a real-time PCR
- v) A positive result by a conventional (single round or nested) PCR
- vi) A positive result by LAMP assay
- vii) A positive result by IFAT

## 6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection shall be confirmed if at least one of the following criteria is met:

- i) KHV isolation in cell culture followed by virus identification by conventional PCR or conventional nested PCR and sequencing of the amplicon
- ii) Detection of KHV in tissue samples by real-time PCR followed by conventional PCR or conventional nested PCR and sequencing of the amplicon
- iii) A positive result by LAMP assay followed by conventional PCR or conventional nested PCR and sequencing of the amplicon
- iv) A positive result by IFAT followed by conventional PCR or conventional nested PCR and sequencing of the amplicon

# The Manual of Diagnostic Tests for Aquatic Animals



## 6.3.1. For presumptive diagnosis of clinically affected animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (n)	DSp (n)	Reference test	Citation
Real-time PCR (Durand & Lightner, 2002)	Diagnosis	Clinically diseased shrimp from farms	Gill, pleopod	<i>Penaeus monodon</i>	100% (n=71)	100% (n=71)	Real-time PCR	Moody et al., 2022
Real-time PCR (Sritunyalucksana et al., 2006)	Diagnosis	Clinically diseased shrimp from farms	Gill, pleopod	<i>Penaeus monodon</i>	100% (n=71)	100% (n=71)	Real-time PCR	Moody et al., 2022

DSe = diagnostic sensitivity, DSp = diagnostic specificity, n = number of samples used in the study, PCR = polymerase chain reaction.

\*The nested PCR (Lo et al., 1996a) is linked to false positives for WSSV when they are used to test species of *Cherax quadricarinatus* (Claydon et al., 2004).

## 6.3.2. For surveillance of apparently healthy animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (n)	DSp (n)	Reference test	Citation
Real-time PCR (Durand & Lightner, 2002)	Surveillance in apparently healthy animals	Wild populations of crustaceans	Gill, pleopod	<i>Penaeus merguensis</i> , <i>P. esculentus</i> , <i>P. plebejus</i> , <i>Metapenaeus endeavouri</i> , <i>M. bennettiae</i>	76.8% (n=1591)	99.7% (n=1591)	Bayesian latent class analysis	Moody et al., 2022
Real-time PCR (Sritunyalucksana et al., 2006)	Surveillance in apparently healthy animals	Wild populations of crustaceans	Gill, pleopod	<i>Penaeus merguensis</i> , <i>P. esculentus</i> , <i>P. plebejus</i> , <i>Metapenaeus endeavouri</i> , <i>M. bennettiae</i>	82.9% (n=1591)	99.7% (n=1591)	Bayesian latent class analysis	Moody et al., 2022



Thank you

