World Organisation for Animal Health

Launch of the Regional AquaticAnimal Health Laboratory Network for Africa (RAAHLN-AF)

5 – 7 December 2023 Pretoria, South Africa

ARC • LNR

UNION AFRICAINE
BUREAU INTERAFRICAIN DES RESSOURCES ANIMALES

FishGov 2 Project

World Organisation for Animal Health

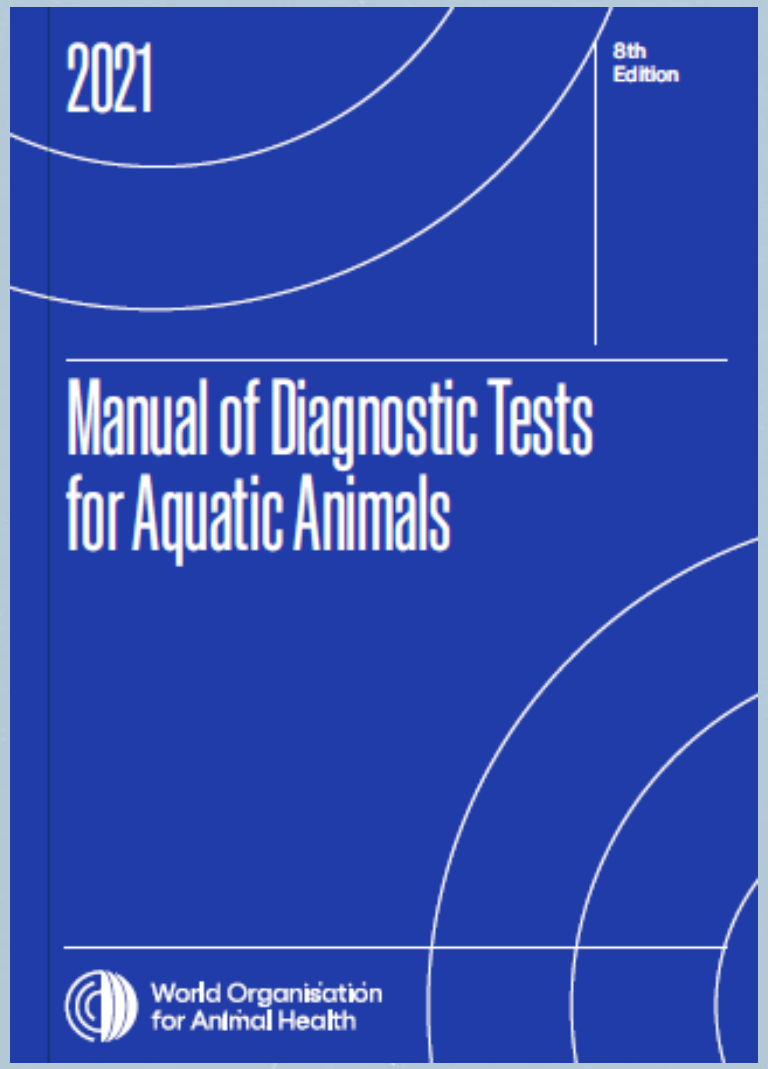
Provisions of the WOAH Aquatic Animal Health Manual

5 – 7 December 2023 Pretoria, South Africa

ARC • LNR

UNION AFRICAINE
BUREAU INTERAFRICAIN DES RESSOURCES ANIMALES

FishGov 2 Project



WOAH Aquatic Manual

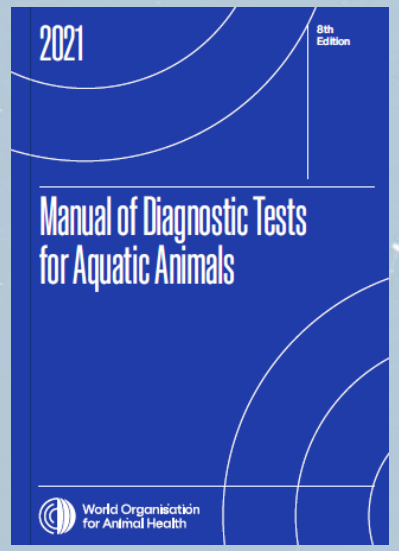
1. Internationally agreed recommendations to support effective laboratory testing capacity.

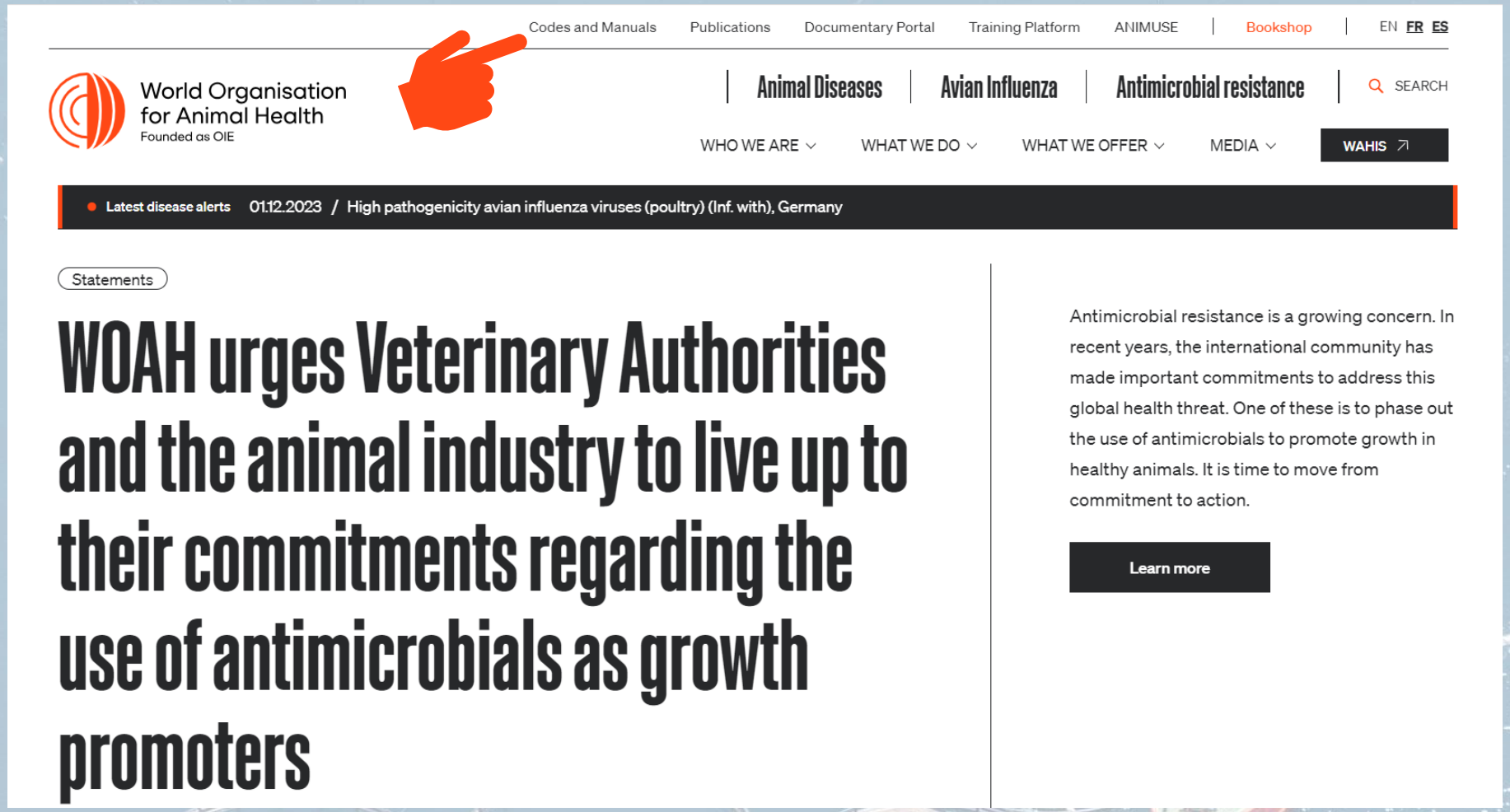
2. Provides standards and specific methods for disease diagnosis.

3. Presents a network of WOAH reference laboratories and collaboration centers.

The Manual of Diagnostic Tests for Aquatic Animals

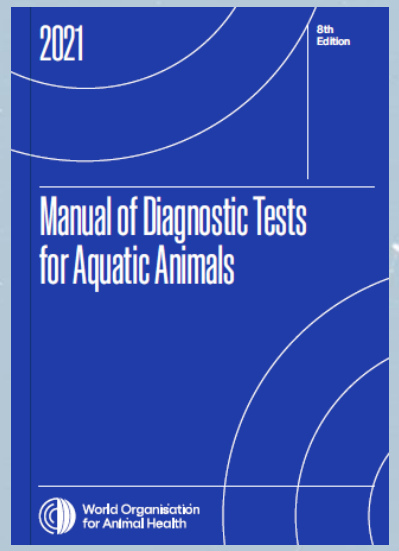
https://www.woah.org/en/home/

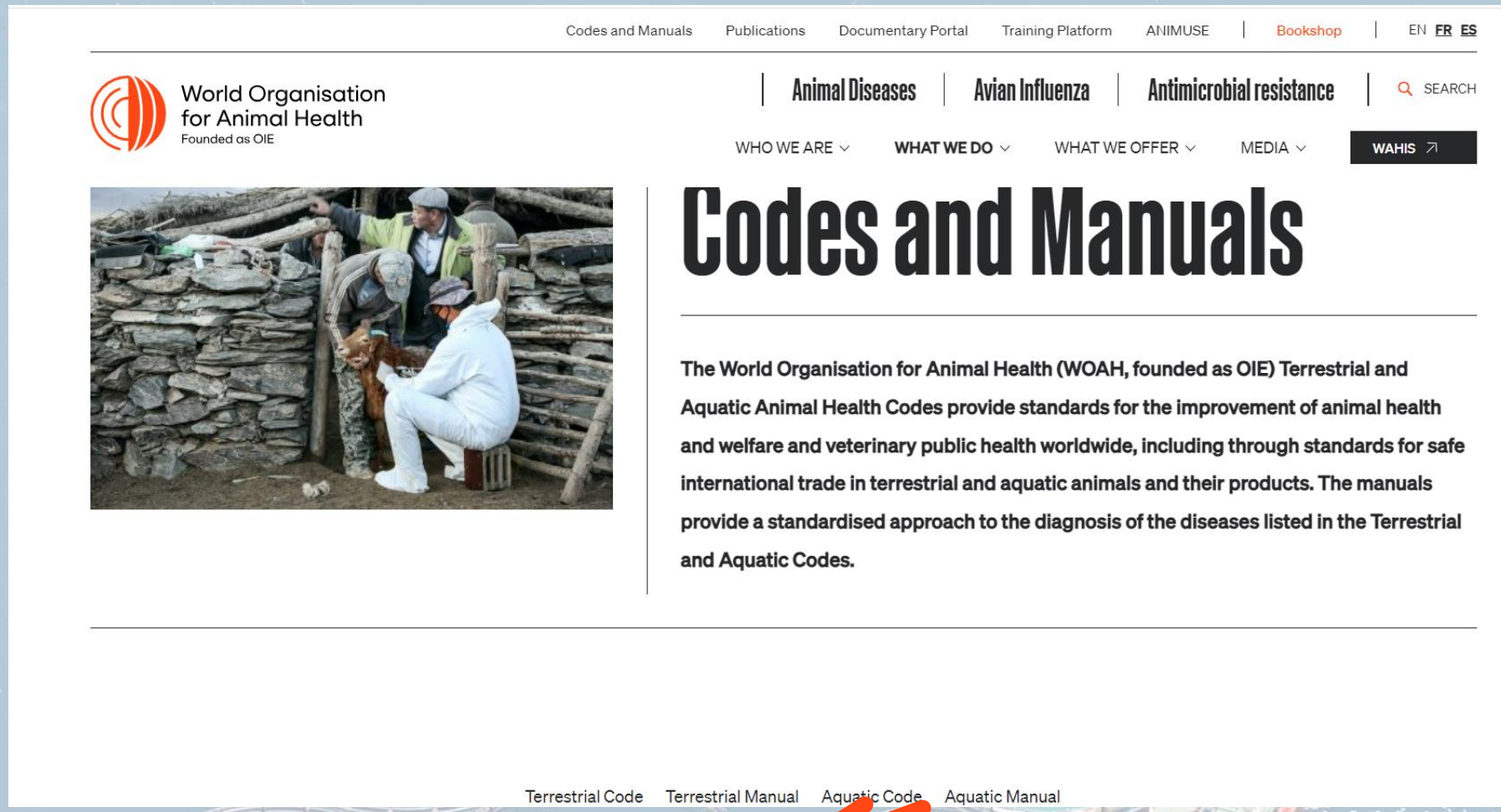




The Manual of Diagnostic Tests for Aquatic Animals

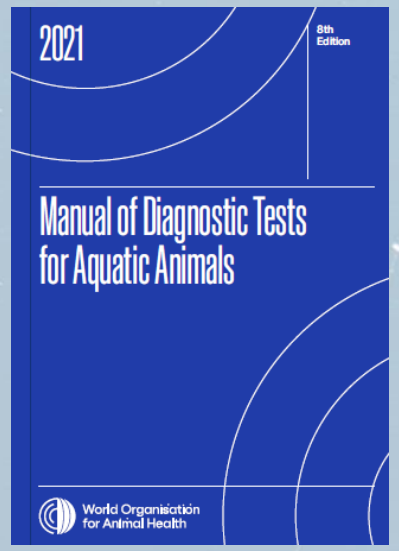
https://www.woah.org/en/home/

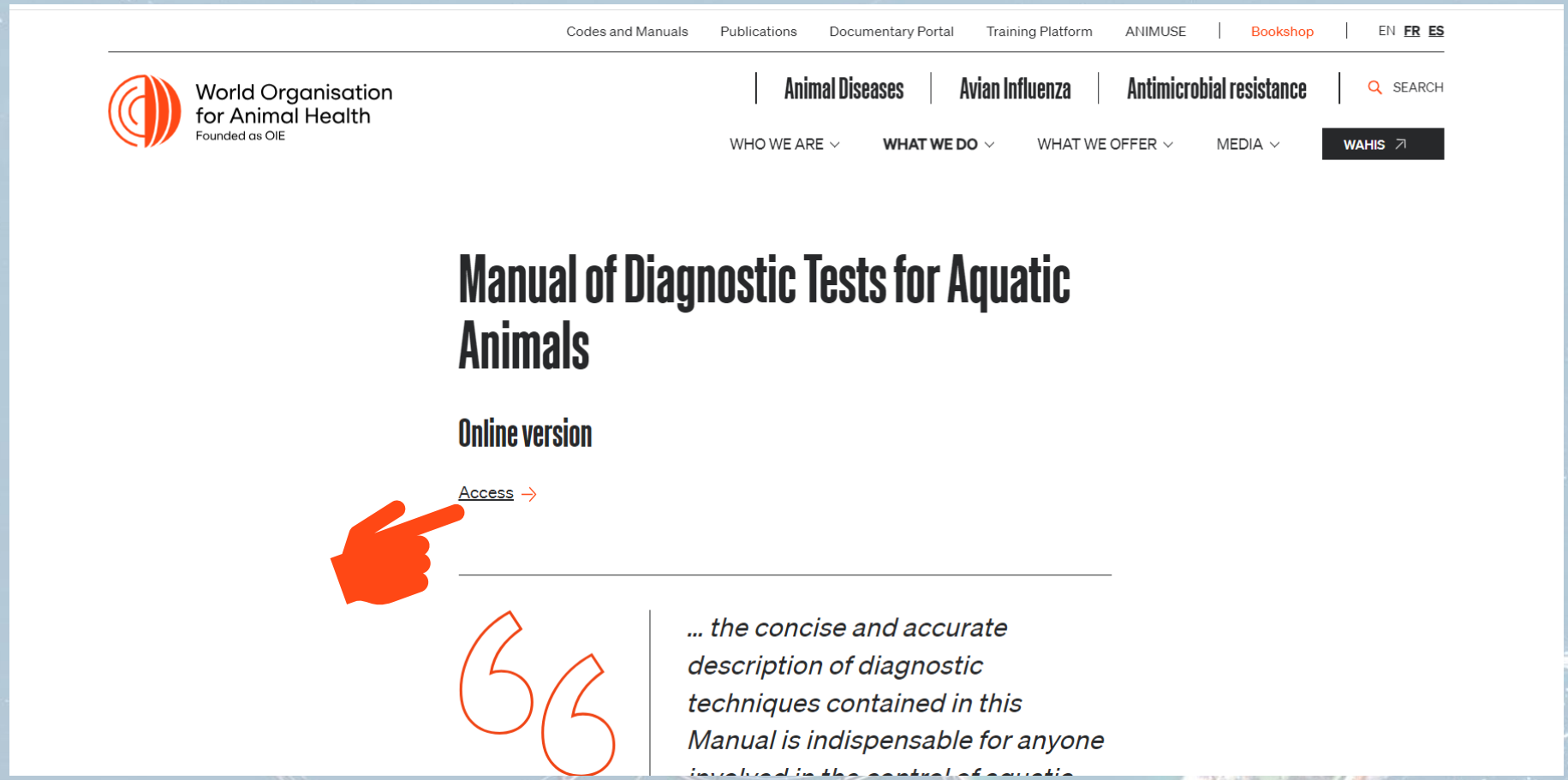




The Manual of Diagnostic Tests for Aquatic Animals

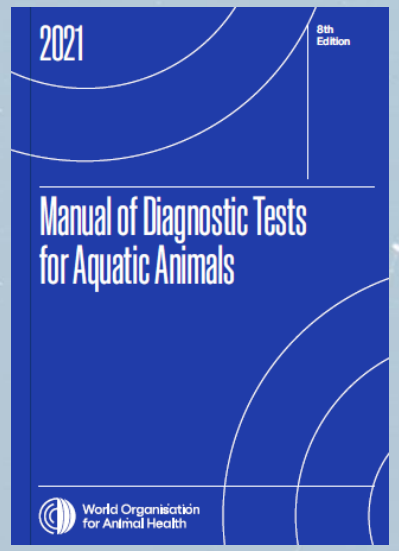
https://www.woah.org/en/home/

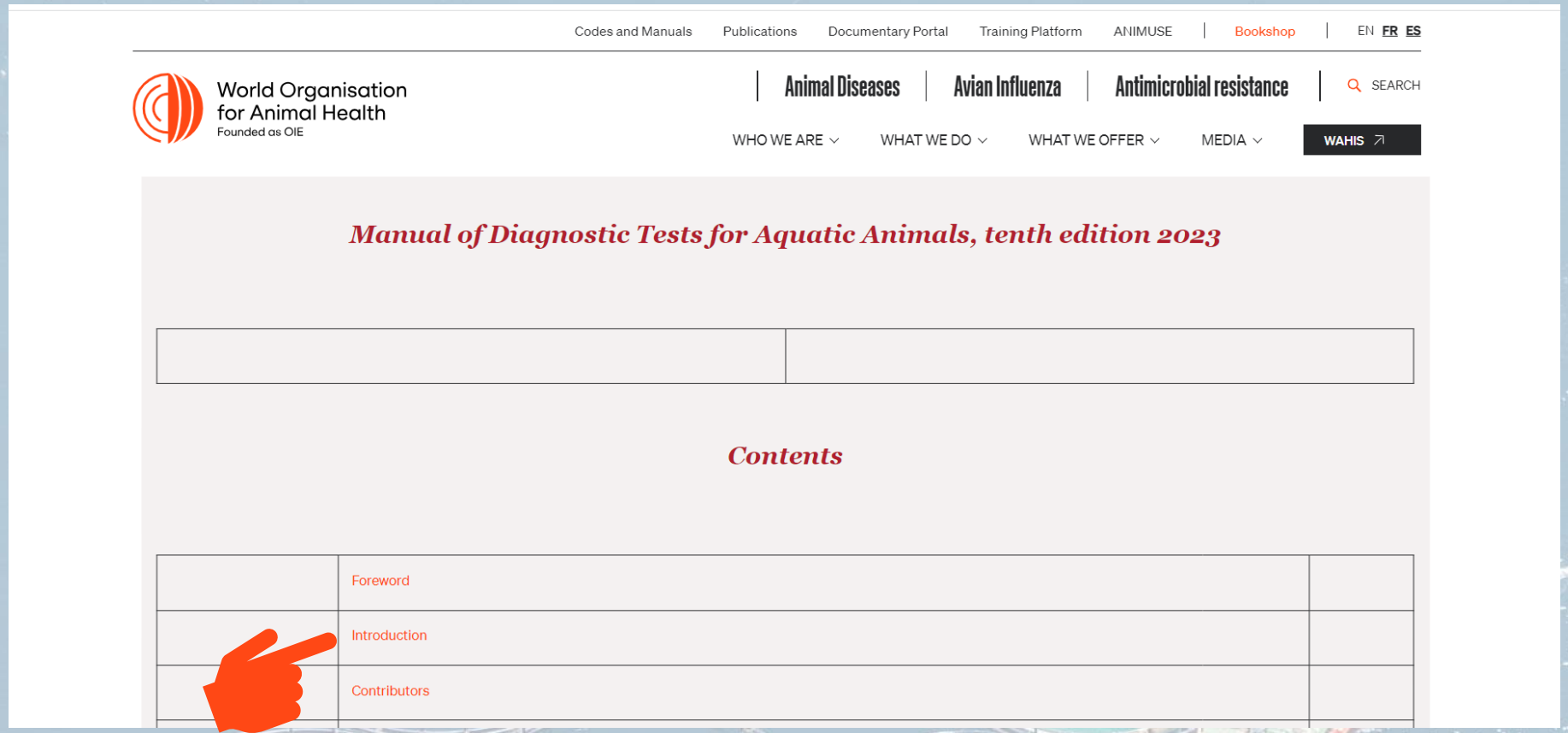




The Manual of Diagnostic Tests for Aquatic Animals

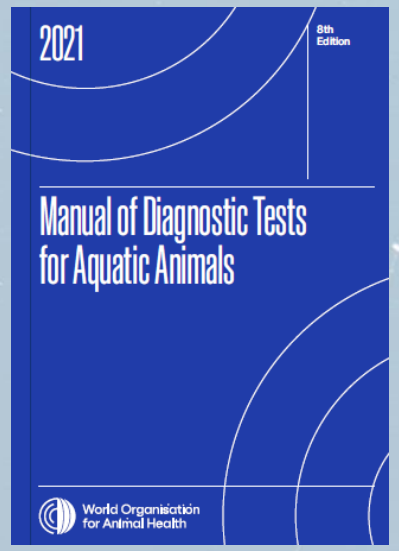
https://www.woah.org/en/home/





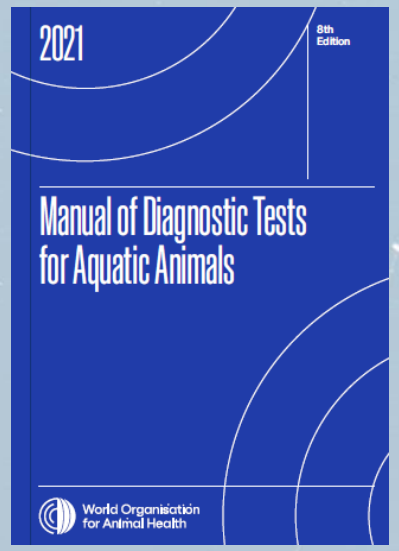
The Manual of Diagnostic Tests for Aquatic Animals

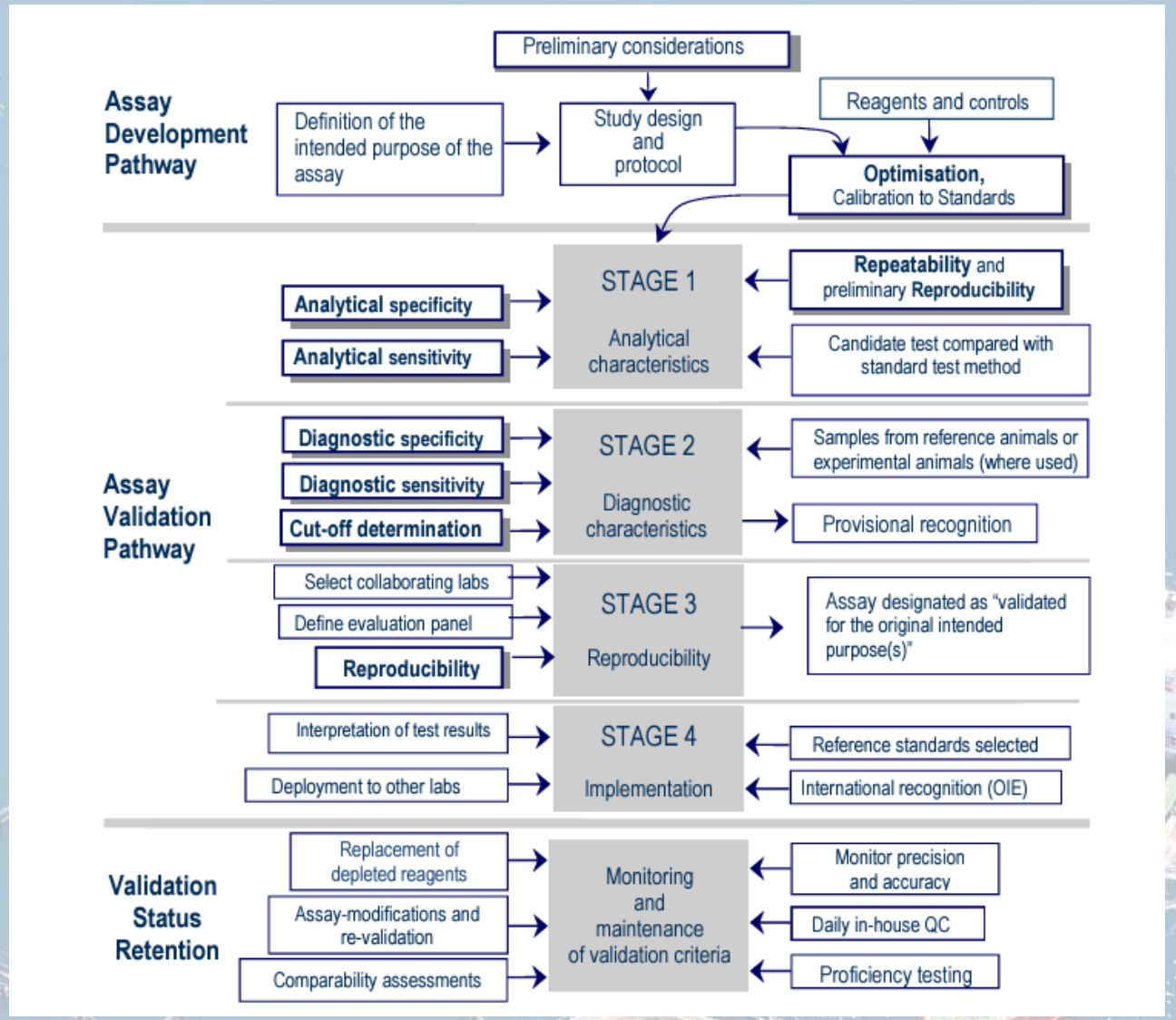
- General provisions (e.g. quality management for labs, principles and methods of validation)



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)

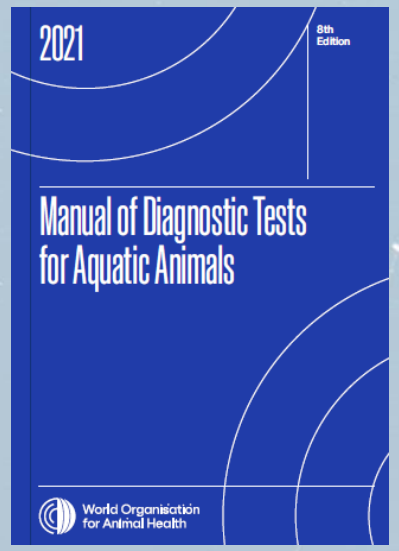
The Manual of Diagnostic Tests for Aquatic Animals





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)



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

The Manual of Diagnostic Tests for Aquatic Animals

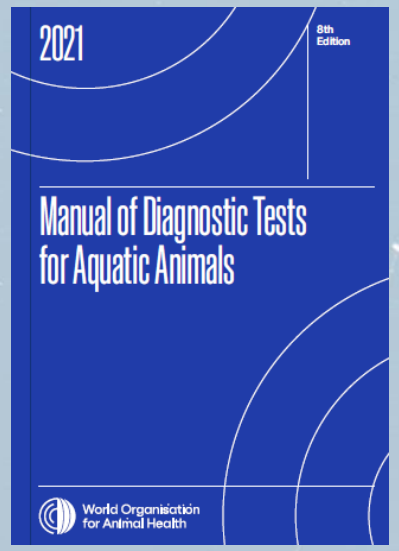


Table 4.1. WOAH recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals

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

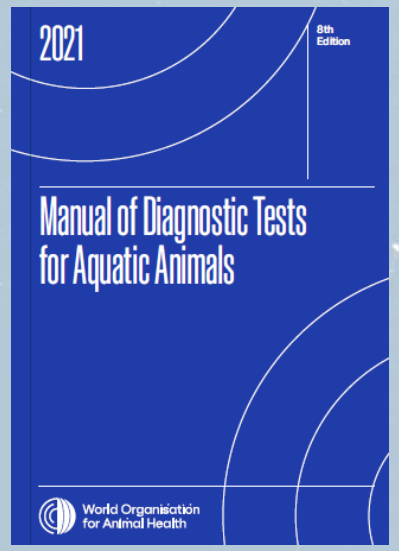
LV = level of validation, refers to the stage of validation in the WOAH Pathway (chapter 1.1.2); PCR = polymerase chain reaction; LAMP = loop-mediated isothermal amplification; Ab- or Ag-ELISA = antibody or antigen enzyme-linked immunosorbent assay.

[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.

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 [1]

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:

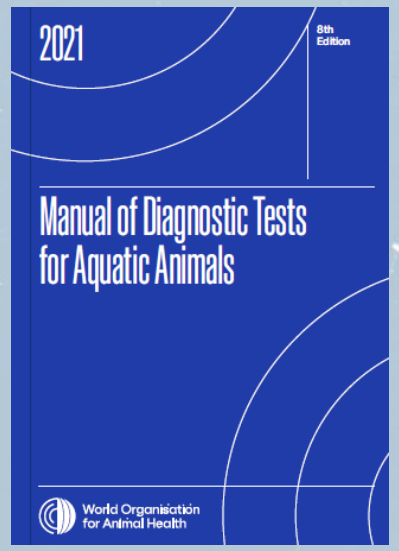
i) Detection of KHV in tissue samples by real-time PCR followed by conventional PCR and sequencing of the amplicon

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:

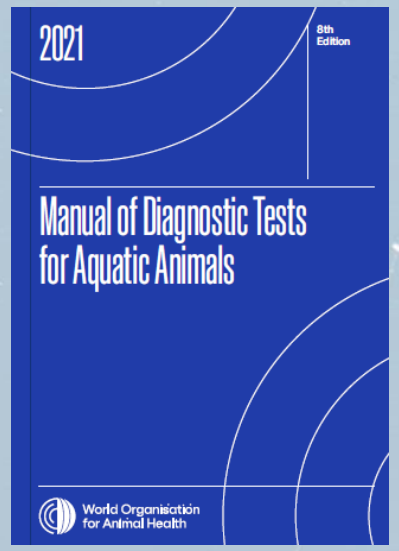
i) KHV isolation in cell culture followed by virus identification by conventional PCR or conventional nested PCR and sequencing of the amplicon

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	*Penaeus monodon*	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	*Penaeus monodon*	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	*Penaeus merguiensis, P. esculentus, P. plebejus, Metapenaeus endeavouri, M. bennettae*	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	*Penaeus merguiensis, P. esculentus, P. plebejus, Metapenaeus endeavouri, M. bennettae*	82.9% (*n*=1591)	99.7% (*n*=1591)	Bayesian latent class analysis	Moody *et al.*, 2022

Thank you