OFFICE INTERNATIONAL DES EPIZOOTIES

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DIAGNOSTIC MANUAL FOR AQUATIC ANIMAL DISEASES

Third edition, 2000

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FOREWORD

The Office International des Epizooties (OIE) is an intergovernmental organisation that was established in 1924 in order to promote world animal health. Its main activities are as follows:

- 1 To collect and disseminate to its Member Countries, information (including emergency information) on the occurrence, course and treatment of animal diseases.
- 2 To provide guidelines and standards for health regulations applicable to international trade in animals.
- 3 To promote and co-ordinate research on the pathology, diagnosis, treatment and prevention of animal diseases when international collaboration in such research is desirable.

Aquatic animals are included in the concept of 'animals' above. Diagnostic proceSdures for some aquatic animal diseases used to be included in the OIE International Animal Health Code (1986 edition), but it became clear that separate publications specific to aquatic animal health were needed. The reasons are that the conditions, problems and requirements in this field are different to those encountered in other animals, and that international trade in aquatic animals and their products is intensifying and increasing in importance.

The purpose of this Diagnostic Manual for Aquatic Animal Diseases (referred to hereafter as the Manual) is to provide a uniform approach to the diagnosis of the diseases listed in the OIE International Aquatic Animal Health Code (referred to hereafter as the Code), so that the requirements for health certification in connection with trade in aquatic animals and aquatic animal products, can be met.

Although many publications exist on the diagnosis and control of aquatic animal diseases, the Manual is a key document describing the methods that can be applied to the OIE notifiable and other significant diseases in aquatic animal health laboratories all over the world, thus increasing efficiency and promoting improvements in aquatic animal health world-wide.

The task of compiling the Manual was assigned to the OIE Fish Diseases Commission, and all the chapters were circulated to OIE Member Countries for comments and revision. The Manual will be continually revised and updated as new information on aquatic animal diseases in general, and new emerging diseases in particular, becomes available. It is intended to publish a new edition approximately every three years; intermittent changes will be available on the OIE Web site.

Dr Jean Blancou Director General, OIE

Prof. Tore Håstein
President, Fish Diseases Commission

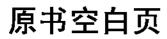
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ist of OIE Referen	ce Laboratories and Collaborating Centre for diseases of fish, molluscs and crustaceans 275

PART 1

GENERAL PROVISIONS



INTRODUCTION

The clinical signs in fish with the diseases listed in the OIE International Aquatic Animal Health Code (referred to hereafter as the Code) are not pathognomenic. Moreover, these infections may occur as subclinical infections of asymptomatic pathogen carriers.

The only dependable approach for diagnosis of fish diseases therefore lies in the specific identification of the pathognus using laboratory methods. These methods, which are suitable for the diagnosis of isolated cases of disease as part of national aquatic animal health surveillance/control programmes, form the main contents of this the *Diagnostic Manual of Aquatic Animal Diseases* (referred to hereafter as the *Manual*).

Such health surveillance programmes aim to determine, from the results provided by standardised laboratory procedures performed with samples collected according to defined rules, the health status of aquatic animal stocks from a particular production site and even a geographical zone or entire country. The satisfactory implementation of such aquatic animal health surveillance/control programmes, requires the existence of both adequate legislation and resources in each country interested in aquatic animal health.

The diagnostic methods presented in this *Manual* are all direct diagnostic methods. Due to the insufficient development of serological methodology, the detection of antibodies to pathogens in fish has not thus far been accepted as a routine diagnostic method for assessing the health status of fish populations. However, the validation of some serological techniques for diagnosis of certain infections could arise in the near future, rendering the use of serology more widely acceptable for diagnostic purposes. At present, the only diagnostic methods that are accepted in those countries where aquatic animal health control programmes are implemented, are based either on isolation of the pathogen followed by its specific identification, or on the demonstration of pathogen-specific antigens using an immunological detection method. General information on diagnostic techniques for fish diseases is given in Chapter I.1.

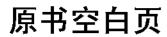
Molluscan and crustacean diseases differ in some ways from fish diseases. For example, diagnostic methods must be direct because these animals do not produce antibodies to pathogens. General information on diagnostic techniques for mollusc diseases is given in Chapter I.2. and for crustacean diseases in Chapter 1.3.

As detailed in Parts 2, 3 and 4 of the *Code*, the list of notifiable diseases of aquatic animals includes only major diseases of proven actional limited geographical range. The OIE Fish Diseases Commission, therefore, recommended the creation of a list entitled 'Other significant diseases'. The diseases on this list include:

- those that are serious, but thet have a broad geographical distribution;
- those causing significant mortality, that are transmissible and of limited geographical range, but for which the aetio-logical agent has not yet been identified, or for which standard diagnostic methods are not yet available;
- those with the potential for causing large losses, but which are too new for the geographical range to be defined or for
 the essential epizoological elements to be understood.

It is expected that the diseases on this list will either be elevated to notifiable status or dropped from the hat as new information is obtained.

The Manual includes descriptions of diagnostic methods for these 'Other significant diseases' as well as for the notifiable diseases.



ABBREVIATIONS

Ab	antibody	FCS	fetal calf serum
AEC	aminoethyl carbazole	FEV	fish encephalitis virus
Ag	antigen	FTTC	fluorescein isothiocyanate
AO	acridine orange	GAV	gill-associated virus
BCIP	5-bromo-4-chloro-3-indoyl phosphate	GP	glucose peptone (broth)
BF-2	bluegill fry (cell line)	GPY	glucose peptone yeast (broth)
BKD	bacterial kidney disease	H&E	hematoxylin and eosin
BMN(V)	baculoviral midgut gland necrosis virus	HBSS	Hank's basal salt solution
BP	Baculovirus penaei	HEPES	N-2-hydroxyethyl-piperazine-N-2-ethanesul-
BSA	bovine serum albumin		fonie acid
BSS	balanced salt solution	HP	hepatopancreas
CCO	channel catfish ovary (cell line)	HRPO	horseradish peroxidase
CCV(D)	channel catfish virus (disease)	IF	immunofluorescence
CFA	complete Freund's adjuvant	IFAT	indirect fluorescent antibody test
CHSE-214	4	lg	immunoglobulin
CIA	Cowdry type A inclusion bodies	IHHNV	infectious hypodermal and haematopoietic
CMS	cardiomyopathic syndrome		necrosis virus
CPE	cytopathic effect	IHN(V)	infectious haematopoietic necrosis (virus)
CSHV	coho salmon herpesvirus	IPN(V)	infectious pancreatic necrosis (virus)
CSTV	coho salmon tumour virus	ISA	infectious salmon anaemia
DEPC	diethyl pyrocarbonate	KDM-2	kidney disease medium
DIG	digoxigenin	LOS	lymphoid organ spheroids
DNA	deoxyribonucleic acid	LOV	lymphoid organ virus
dNFP	deoxynucleotide triphosphate	LPS	lipopolysaccharide
DTT	dithiotreital	MAb	monoclonal antibody
ECV	European catfish virus	MBV	Penaeus monodon-type baculovirus
EDTA	ethylene diamine tetra-acetic acid	MCMS	mid-crop mortality syndrome
EHN(V)	epizootic haematopoietic necrosis (virus)	MEM	minimal essential medium
ELISA	enzyme-linked immunosorbent assay	m.o.i.	multiplicity of infection
EPC	Epithelioma papulosum cyprini (cell line)	M-MLV	marine leukaemia viros
ERA	EUS-related Aphanomyces	MSX	multinucleate sphere X
ESC	enteric septicaemia of catfish	NAb	neutralising antibody
ESV	European sheatfish virus	NBT	nitrohlue tetrazolium
EUS	epizootic ulcerative syndrome	OCT	embedding medium for frozen tissue specimens
FAT	fluorescent antibody test	OKV	Oncorhynchus kisutch virus
FBS	fetal bovine serum	OMV(D)	Oncorhynchus masou virus (disease)

OPD	o-phenylenediamine	SM(V)	spawner-isolated mortality (virus)
PAGE	polyacrylamide gel electrophoresis	SPF	specific pathogen free
PBS	phosphate buffered saline	SSC	standard saline citrate
PBST	phosphate buffered saline containing Tween	SSO	seaside organism
PCR	polymerase chain reaction	SSS	sonicated salmon sperm
PFU	plaque forming units	SVC(V)	spring viraemia of carp (virus)
PIB	polyhedral inclusion body	TEM	transmission electron microscopy
POB	polyhedral occlusion body	TMB	tetramethylbenzidine
RDS	runt deformity syndrome	Tris	tris (hydroxymethyl) aminomethane
RHV	rainbow trout herpesvirus	TS(V)	Taura syndrome (virus)
RKV	rainbow trout kidney virus	VER	viral encephalopathy and retinopathy
RNA	ribonueleic acid	VHS(V)	viral haemorrhagic septicemia (virus)
RSD	red spot disease	VN	virus neutralisation
RSIV(D)	red sea hream iridoviral (disease)	VNN(V)	viral nervous necrosis (virus)
RTG-2	rainbow trout gonad (cell line)	WSBV	white spot disease baculovirus
RT-PCR	reverse-transcription polymerase chain reac-	WSD	white spot disease
	tion	WSIV(D)	White sturgeon iridoviral (disease)
RVC	ribonucleoside vanadyl complex	WSSV	white spot syndrome virus
SBL	seabass larva (cell line)	WSV	white spot virus
SDS	sodium dodecyl sulfate	YHD	yellowhead disease
SHK-1	salmon head kidney (cell line)	YHV	yellowhead virus
SJNNV	striped jack nervous necrosis virus	YTV	yamame tumour virus
SKDM	selective kidney disease medium		

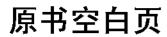
DEFINITIONS

The International Aquatic Animal Health Code (companion volume to this Manual) contains a list of definitions that may be consulted for the meaning of terms used in this Manual. Some terms that are not used in the Code but that appear in the Manual, are defined below:

Fry newly hatched fish larvae.

Sensitivity the proportion of true positive tests given in a diagnostic test, i.e. the number of true positive results divided by the number of true positive and false negative results.

Specificity the probability that absence of infection will be correctly identified by a diagnostic test, i.e. the number of true negative results divided by the number of true negative and false positive results.



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QUALITY MANAGEMENT IN VETERINARY DIAGNOSTIC LABORATORIES

SUMMARY

In any testing work, the final expression of quality is a reliable result. This is the product of thought, planning, care, knowledge, skill, experience, and environment. Good laboratory practice, quality control, and quality assurance are inter-related and comprise one complex subject of increasing importance in the conduct of biological assays. In recent years this subject, including process control of laboratory work, has been increasingly implemented at the international level. The advent of widely recognised international standards, such as the International Standard ISO/IEC 17025 (1), and the increased application in laboratories of total quality management and the ISO/IEC 9000 series (2), have made it imperative that laboratories have a formal, visible, and appropriate quality system.

KEY ELEMENTS OF QUALITY IN TESTING

The activities and elements necessary to achieve quality in testing may be described in the following six categories.

1. THE LABORATORY ENVIRONMENT

Before developing any test or conducting a test, a laboratory must ensure it has appropriate facilities and resources. These are outlined in the two categories that follow.

a) Laboratory, building and grounds

The laboratory must have:

- i) Adequate funding for operation;
- ii) Safety and security;
- iii) Adequate space;
- iv) Adequate lighting;
- Appropriate and dependable environmental control, e.g. edequate and/or appropriately controlled ventilition, vibration levels, dust, sound, temperature, humidity, electromagnetic interference, and electrical power supply;
- vi) Necessary and properly housed and maintained equipment, instruments, and materials, including both internal and applicable external equipment. Equipment must be traceable, calibrated, edequate for the job, capable of achieving the accuracy required, and otherwise fit for the intended purpose;
- vii) Good bousekeeping;
- viii) Appropriate storage and archiving;
- ix) Access to necessary literature references.

b) Personnel