OFFICE INTERNATIONAL DES EPIZOOTIES

Organisation mondiale de la santé animale World organisation
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DIAGNOSTIC MANUAL FOR AQUATIC ANIMAL DISEASES

Third edition, 2000

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World organisation for animal health

DIAGNOSTIC MANUAL FOR AQUATIC ANIMAL DISEASES

THIRD EDITION, 2000

本手册系由OIE鱼病委员会根据成员国意见编辑并由OIE国际委员会批准

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12rue de Puouy, 75017 巴黎 法国

电话：(33-1)44151888

传真：(33-1)42670987

电报：EPIZOTI 642285F

E-mail: oie@oie.int

http: \ www.oie.int

This *Manual* has been edited by the OIE Fish Diseases Commission on the basis of comments received from Member Countries, and approved by the International Committee of the OIE

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OFFICE INTERNATIONAL DES EPIZOOTIES, 2000
12, rue de Prony, 75017 Paris, FRANCE
Telephone: 33-(0)1 44 15 18 88
Fax: 33-(0)1 42 67 09 87
Electronic mail: oie@oie.int
WWW:http://www.oie.int

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

2000

CONTENTS

PART 1

GENERAL PROVISIONS

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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 pathognomonic. Moreover, these infections may occur as subclinical infections nf asymptomatic pathogen carriers.

The only dependable approach for diagnosis of fish diseases therefore lies in the specific identification of the pathogens 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 *Maunal*).

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 validetion 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 thet 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 ioformation 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 oely major diseases of proven aetiology and 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, lut thet have a broad geographical distribution;
- those causing significant mortality, that are transmissible and of limited geographical range, but for which the aetiological 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.

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ABBREVIATIONS

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

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

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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AUTHORS AND PROFESSIONAL ADDRESS AT THE TIME OF WRITING

Dr D. Alderman, The Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Weymouth Laboratory, Barrack Road, The Nothe, Weymouth, Dorset DT4 8UB, United Kingdom

Dr I. Anderson, Oonoomba Veterinary Laboratory, Queensland Department of Primary Industries, P. O. Box 1085, Oonoomba QLD 4810, Australia

Dr T. Atle Mo, National Veterinary Institute, Ullevålsveien 68, P.O. Box 8156 Dep., 0033 Oslo, Norway

Dr F. Berthe, IFREMER, Laboratoire de pathologie des invertébrés, BP 133, 17390 La Tremblade, France

Dr S. Bower, Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, British Columbia V9R 5K6, Canada

Prof. Shiu-Nan Chen, National Taiwan University, Department of Zoology and Fishery Research, No. 1 Roosevelt Road, Section 4, Taipai, Taiwan 10764, Taipei China.

Dr B. Dannevig, National Veterinary Institute, Ullevå lsveien 68, P.O. Box 8156 Dep., 0033 Oslo, Norway

Dr I. East, Aquatic Animal Health Unit, Office of the Chief Veterinary Officer, Agriculture, Fisheries and Forestry-Australia, GPO Box 858, Canberra ACT 2601, Australia

Dr B. Edgerton, Animal Biosecurity Policy, Agriculture, Fisheries and Forestry-Australia, GPO Box 858, Canberra ACT 2601, Australia

Dr K. Falk, National Veterinary Institute, Ullevålsveien 68, P.O. Box 8156 Dep., 0033 Oslo, Norway

Dr T. W. Flegel, Department of Biotechnology, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand

Prof. J. Fryer, Oregon State University, Corvallis, Oregon 97331-3804, United States of America

Dr H. Grizel, IFREMER, 1 rue Jean Vilar, 34200 Sète, France

Dr L.A. Hanson, Fish Diagnostic Laboratory, College of Veterinary Medicine, Mississippi State University, Box 9825, Spring Street, Mississippi 39762, United States of America

Prof. T. Håstein, Dr B. Dannevig & Dr K. Falk, National Veterinary Institute, Ullevå lsveien 68, P.O. Box 8156 Dep., 0033 Oslo, Norway

Prof. R. Hedrick, Department of Medicine & Epidemiology, School of Vetcrinary Medicine, University of California, One Shields Ave, Davis, California 95616, United States of America

Prof. B.J. Hill, The Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Weymouth Laboratory,

Barrack Road, The Nothe, Weymouth, Dorset, DT4 8UB, United Kingdom

Dr P.M. Hine, National Institute of Water, and Atmospheric Research, P.O. Box 14-901, Kilbirnie, Wellington, New Zealand

Dr A. Hyatt, Australian Animal Health Laboratory, P.O. Bag 24, Geelong, Victoria 3220, Australia

Dr R. Jacobson, Diagnostic Laboratory, College of Veterinary Medicine, Cornell University, Ithaca, NY 14850 – 5786, United States of America

Dr N. Jörgen – Olesen, Danish Veterinary Laboratory, Hangovej 2, 8200 Aarhus N, Denmark

Dr P. de Kinkelin, Unité de Virologie et Immunologie moléculaires, Centre de Recherche de Jouy – en – Josas, Institut National de la Recherche Agronomique, 78350 Jouy – en – Josas Cedex, France

Prof. D.V. Lightner, Aquaculture Pathology Laboratory, Department of Veterinary Science, University of Arizona, Building 90, Room 202, Pharmacy/Microbiology, Tucson, AZ 85721, Tucson, AZ 85721, United States of America

Dr C. Michel, Unité de Virologie et Immunologie moléculaires, Centre de Recherche de Jouy – en – Josas, Institut National de la Recherche Agronomique, 78350 Jouy – eu – Josas Cedex, France

Dr B. Munday, University of Tasmania, School of Science and Technology, P.O. Box 1214, Launceston, Tasmania 7250, Australia

Dr T. Nakai, Faculty of Applied Biological Science, Hiroshima University, Higashi – Hiroshima 724, Japan

Dr K. Nakajima, National Research Institute of Agricolture, 422 – 1 Nakatsuhamaura, Nansei – cho, Watarai – gun, Mie 516 – 01, Japan

Dr L. Owens, Department of Microbiology and Immunology, James Cook University, Townsville, QLD 4811, Australia

Dr R. Subasinghe Food and Agriculture Organization of the United Nations, Fisheries Department, Room NF 514, Viale della Terme di Caracalla, 00100 Rome, Italy

Dr K. Tonguthai, Aquatic Animal Health Research Institute (AAHRI), Department of Fisheries, Kaesarts University Campus, Jatujak, Bangkok 10900, Thailand

Dr R. Whittington, Elizabeth Macarthur Agricoltural Institute, PMB 8, Camden NSW 2570, Australia

Dr A. Wiegers, USDA, APHIS, National Veterinary Services Laboratories, P.O. Box 844, Ames Iowa 50010, United States of America

Dr J. Winton, Western Fisheries Research Center, 6505 N.E. 65th St., Seattle, Washington 98115, United States of America

Dr M. Yoshimizu, Microbiology Department, Faculty of Fisheries, Hokkaido University, 3 – 1 – 1 Minato – cho, Hakodate, Hokkaido 041 – 0821, Japan

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 nt 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;
v) Appropriate and dependable environmental control, e.g. edequate and/or appropriately controlled ventihution, 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