European Union Reference Laboratory for Fish Diseases
National Veterinary Institute, Technical University of Denmark, Copenhagen

DIAGNOSTIC MANUALS FOR LISTED NON-EXOTIC FISH DISEASES: STATUS AND IMPLEMENTATION

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Commission Decision for surveillance and diagnostic methods:

EUROPEAN COMMISSION

Brussels, XXX
SANCO/6084/2009
(POOL/G2/2009/6084/6084-EN.doc)
[…](2015) XXX draft

COMMISSION IMPLEMENTING DECISION

of XXX

laying down rules for the application of Directive 2006/88/EC as regards requirements for surveillance and diagnostic methods

(Text with EEA relevance)

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The Decision lays down rules for:

- (a) **the surveillance, buffer zones, sampling and diagnostic methods** to be used by Member States in connection with the disease status of the Member States or zones or compartments thereof for the non-exotic aquatic animal diseases listed in Part II to Annex IV to Decision 2006/88/EC (the 'listed diseases');
- (b) **the diagnostic methods** to be used for laboratory examinations in the case of the suspicion or confirmation of the presence of listed diseases; and
- (c) **the minimum control measures to be applied** in the event of the suspicion or confirmation of a listed disease in a Member State, zone or compartment not declared free of that listed disease.

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Article 3
Minimum requirements for eradication and surveillance programmes

- Member states shall ensure that the rules on surveillance and eradication programmes, buffer zones, sampling and diagnostic methods set out in Annex I and the specific methods and detailed procedures set out in Annex II are complied with, **when disease-free status is to be granted, withdrawn or restored** for a Member State or for a zone or compartment thereof for one or more of the listed diseases.

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Article 4
Minimum requirements for diagnostic methods and specific procedures

- Member States shall ensure that the **control methods** set out in Annex I and **the specific diagnostic methods and detailed procedures** set out in Annex II are complied with, when laboratory examinations are carried out in order to confirm or rule out the present of a listed disease.

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Article 5
Minimum control measures for the containment of and minimum requirements for the lifting of containment measures in Member States, zones or compartments not declared free of listed diseases

- Member States shall ensure that the minimum control measures and minimum requirements for **the lifting of containment measures** set out in Annex I are complied with, when carrying out control measures and the lifting of containment measures for one or more of the listed diseases in a Member State or in a zone or compartment thereof not declared free of those listed diseases.

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Two Annexes

EUROPEAN COMMISSION

Brussels, XXX
SANCO/6084/2009 ANNEX
(POOL/G2/2009/6084/6084-EN
ANNEX.doc)
[...](2015) XXX draft

ANNEXES 1 to 2

ANNEXES

to the

COMMISSION IMPLEMENTING DECISION

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Annex I: Surveillance and control methods

ANNEX I

SURVEILLANCE AND CONTROL METHODS

I. Introduction

This Annex sets out:

(a) requirements for eradication and surveillance programmes, as provided for in Article 44 of Directive 2006/88/EC, and the sampling and diagnostic methods to be used to declare disease-free status for Member States or zones or compartments thereof as provided for in Chapter VII of that Directive;

(b) sampling and diagnostic methods to be used for laboratory examinations in the case of the suspicion of and to confirm the presence of the non-exotic diseases listed in Part II of Annex IV to Directive 2006/88/EC (the 'listed diseases') as provided for in Articles 28(a) and 57(b) of that Directive;

(c) the containment measures to be taken in the case of confirmation of the presence of a listed disease, as provided for in Article 39 of Directive 2006/88/EC, and the measures to be taken in order to obtain Category III health status for a Member State, zone or compartment that previously had Category V health status.

The requirements set out in this Annex cover the following listed diseases:

1.	Viral haemorrhagic septicaemia (VHS)	Part 1
2.	Infectious hematopoietic necrosis (IHN)	Part 1
3.	Koi herpes virus (KHV) disease	Part 2
4.	Infectious salmon anaemia (ISA)	Part 3
5.	Infections with *Marteilia refringens*	Part 4
6.	Infections with *Bonamia ostreae*	Part 5
7.	White spot disease (WSD)	Part 6

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Annex II: Diagnostic methods

ANNEX II

DIAGNOSTIC METHODS AND DETAILED PROCEDURES

I. Introduction

This Annex sets out the detailed procedures for the diagnostic methods to be used for the laboratory examination in the eradication and surveillance programmes set out in Annex I to this Decision, and in order to confirm or rule out the suspected presence of the following non-exotic diseases listed in Part II to Annex IV to Directive 2006/88/EC ('the listed diseases') in accordance with Article 57 (b) to that Directive:

1.	Viral haemorrhagic septicaemia (VHS)	Part 1
2.	Infectious hematopoietic necrosis (IHN)	Part 1
3.	Koi herpes virus (KHV) disease	Part 2
4.	Infectious salmon anaemia (ISA)	Part 3
5.	Infections with *Marteilia refringens*	Part 4
6.	Infections with *Bonamia ostreae*	Part 5
7.	White spot disease (WSD)	Part 6

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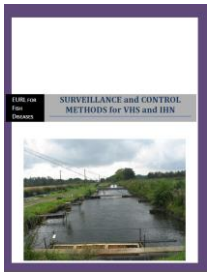
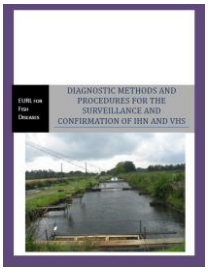
Two parts for each listed disease on www.eurl-fish.eu/ Diagnostic Manuals. VHS & IHN in one





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VHS & IHN: What´s new? I. surveillance, eradication and maintenance

- Replace the Commission Decision 2001/183/EC
- Category I zones / compartments >< approved free zones / farms in non-approved zones
- General requirements for health inspections and sampling for VHS and IHN
- Specific requirements to obtain disease-free health status (category I):
- Surveillance programmes: Model A (2-y) and B (4-y) kept
- Eradication programmes: protection zone and a surveillance zone
- Synchronised fallowing (3 wk – 6 wk in all)
- Requirements for change from Cat.V to Cat.III
- Requirements for the maintenance of disease-free health status

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VHS & IHN: What´s new? II. Diagnostic and sampling methods

- Organs to be sampled: same & 10 fish pools
- Diagnostic methods: Virus isolation in cell culture ***OR RT-qPCR !***
- Sample size reduced for the 2-year program from 150 to 75 (design prevalence 5%!)
- 4-year program no changes.
- Maintenance of status: Risk based in high medium low.

Risk level	Number of health inspections	Number of fish in the sample[3]
High	2 every year	30
Medium	1 every year	30
Low	1 every 2 years	30
Maximum number of fish per pool: 10		

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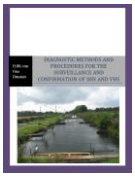
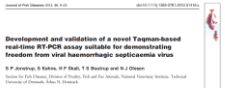
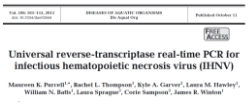
VHS & IHN: What´s new?
Detailed diagnostic methods Part I



- 3 parts:
- **for the surveillance of VHS and IHN**
- **for the confirmation of or to rule out the suspicion of VHS and IHN in suspected outbreaks**
- **for titration to verify the susceptibility of the cell cultures to infection**
- **Detailed procedures for sampling and diagnostic method for RT-PCR and RT-qPCR (RT-qPCR for VHSV: Jonstrup et al. 2013 for IHNV: Purcell et**

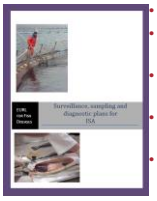


Development and validation of a novel Taqman-based real-time RT-PCR assay suitable for demonstrating freedom from viral haemorrhagic septicaemia virus



Universal reverse-transcriptase real-time PCR for infectious hematopoietic necrosis virus (IHNV)

ISA: What´s new?
I. surveillance, eradication and maintenance



- Replace the Commission Decision 2003/466/EC
- To contain infections with **HPR deleted ISAV**
- Very similar to VHS/IHN decision.
- Developed in close collaboration with OIE ref lab for ISA (VI, Norway)
- General requirements for health inspections and sampling for ISA
- Specific requirements to obtain disease-free health status (category I): only 2-year model
- 5-fish pools 2x75 fish/year

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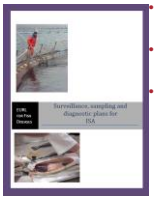
ISA: What´s new?
II. surveillance, eradication and maintenance



- Eradication programmes: protection zone and a surveillance zone
- Synchronised fallowing (3 months – 6 wk synchronised)
- Risk based requirements for the maintenance of disease-free health status.
- NB: Not for salmonids in fresh water without Atlantic salmon

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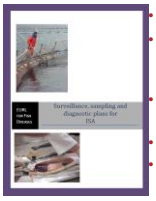
ISA: What´s new?
Detailed diagnostic methods Part I



- 2 parts in Diagnostic procedures:
- **Sampling for the surveillance and control of ISA**
- **Procedures for the surveillance and for the confirmation of the presence of or to rule out the suspicion of ISA**
- Mid-kidney only! + eventually kidney, heart, spleen, liver or pyloric caeca
- RT-qPCR for surveillance
- For diagnostics: RT-PCR, sequencing, cell cultivation, IFAT, Histology, IHC

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KHVD: What´s new?
I. surveillance, eradication and maintenance

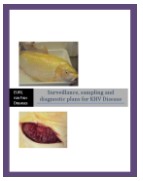
- All new!! Based on 2 d expert group meeting in Feb. 2014, at EURL in Cph.
- Follow the VHS/IHN template.
- General requirements for health inspections and sampling for KHV. Temperature requirement: >15°C
- If possible keep carp for 24-72 hours at 15°C-26°C
- Specific requirements to obtain disease-free health status (category I):
- Surveillance programmes: Model A (2-y) and B (4-y)

		Number of clinical inspections per year (two years)	Number of laboratory examinations per year (two years)	Number of fish in the sample
Farms/sampling sites	First two years of the surveillance period	2	2	75[1]
Maximum number of fish per pool: 2				

[1] Samples must be taken from the number of fish that will ensure the detection of KHV with a 95% confidence if the design prevalence is 5%.

		Number of clinical inspections per year	Number of laboratory examinations per year	Number of fish in the sample
Farms/sampling sites	First two years of the surveillance period	1	1	30
Farms/sampling sites	Second two years of the surveillance period	2	2	30
Maximum number of fish per pool: 2				

KHVD:
II. surveillance, eradication and maintenance



- Eradication programmes: protection zone and a surveillance zone
- Synchronised fallowing (3 wk – 6 wk in all)
- Restocking with Cat I fish or (until 2020) with Cat II
- Requirements for change from Cat.V to Cat.III.
- Requirements for the maintenance of disease-free health status

Risk level	Number of health inspections	Number of fish in the sample
High	**2 every year**	**30**
Medium	**1 every year**	**30**
Low	**1 every 2 years**	**30**
Maximum number of fish per pool: 2		

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KHVD:
Detailed diagnostic methods

- Sampling: Gill and kidney – in addition spleen, encephalon, intestine in separated sample
- 5 fish pool in acute cases allowed, else 2-fish pools
- Non-lethal samples (blood, gill swabs or biopsy, mucus scrape) possible for valuable fish.
- PCR: Bercovier et al. 2005
- qPCR Gilad et al. 2004 *DAO* **60**
- For surveillance only real time PCR.

Final remarks

- The Diagnostic manuals passed the PAFF Committee in April and are now in the process of adoption.
- Has to be translated into 23 languages!
- Planned final adoption 15 September 2015
- The Decision is based on numerous discussions , ammendments and changes over a long period. Many compromises had to be taken. It is impossible to make everybody happy- but we are as close as possible.
- Please read it! Make your competend authorities and your directors aware of the rules.
- These are not guidelines but mandatory procedures to follow

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Thank you for your attention!

EAFP Pre-conference workshop on diagnostic test validation

Held in conjunction with the EAFP meeting, Canary Islands, Spain on September 6th

If you need guidance on the best strategies for use of experimental and/or field studies to obtain estimates of diagnostic sensitivity and specificity, and tips for analyzing these data this workshop is for you. There will be approximately 4 hours of lecture/discussion and the remaining 3.5 hours will be problem-based with use of Medcalc (and TAGS) software to analyze data assuming a perfect (imperfect) reference standard. Examples will be based on OIE-listed diseases of finfish, mollusks and crustaceans and participants are encouraged to bring their own data or provide relevant examples for discussion.

The workshop is limited to a maximum of 25 participants, see below for course outline.

The fee for the workshop will depend on room charges and costs for lunch, and number of attendees and has not been finally determined at the time of this posting. Estimated costs for the day are expected to be no more than 50 euros for students and 75 euros for professionals.

Please e-mail the lead instructor, Ian Gardner, as soon as possible, if you are interested in attending so you don't miss out. Further course updates, registration information and will be sent to those that have expressed interest by June 1, 2015:

Ian Gardner, Canada Excellence Research Chair (Aquatic Epidemiology)
Atlantic Veterinary College, Prince Edward Island
E-mail: *igardner@upei.ca*

Canada Excellence Research Chairs
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Course Outline

Evaluation of the accuracy of diagnostic tests for aquatic animal diseases: tips, tricks and traps Instructor: Ian Gardner, Canada Excellence Research Chair (Aquatic Epidemiology), UPEI

8:00 - 8:30.	Introduction: OIE context of fitness for purpose; analytical and other criteria; what's new in test evaluation (lecture)
8:30 – 10:00.	Test evaluation with a perfect reference standard (field data) – estimation of 95% confidence intervals, receiver-operating characteristic analysis for comparing continuous tests (lecture)
10:00 -10.30	Analysis of experimental challenge studies for diagnostic sensitivity – some tips (lecture and discussion)
10:30-10:45	Coffee break
10:45-12:15	Discussion of participant data sets and hands-on analysis (1.5 hr of discussion/computer lab using Medcalc)
12:15-13:00	Lunch
13:00- 14:00	Test evaluation without a perfect reference standard (field data) – when is it most necessary and why?; basic principles underlying method, practical example using web-based software (TAGS), example reanalyzed from a Bayesian perspective using prior information (lecture)
14:00 – 16:00	Discussion of participant data sets and hands-on analysis ctd. (2 hr of discussion/computer lab using Medcalc/TAGS) including a 15 min coffee break at about 15:00
16:00 – 16:30	Design and reporting considerations for diagnostic test accuracy studies (30 min of lecture and discussion)
16:30 -17:00	Questions and answers; wrap-up (30 min of discussion)

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