

## Commission Decision 2015-1554: The new diagnostic manual for NON-EXOTIC aquatic animal diseases

Niels Jørgen Olesen, Niccolò Vendramin,  
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for Fish Diseases Copenhagen, Denmark,

## COMMISSION IMPLEMENTING DECISION (EU) 2015/1554 of 11 September 2015 :

- laying down rules for the application of Directive 2006/88/EC as regards requirements for surveillance and diagnostic methods
- The Decision apply from 1 April 2016.



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### The Decision lays down rules for:

- (a) the surveillance, buffer zones, sampling and diagnostic methods for the **non-exotic aquatic animal diseases**
- (b) **the diagnostic methods** to be used for laboratory examinations in the case of the suspicion or confirmation
- (c) **the minimum control measures to be applied**

### Two Annexes

- **ANNEX I**  
**SURVEILLANCE AND CONTROL METHODS**
- **ANNEX II**  
**DETAILED DIAGNOSTIC METHODS AND PROCEDURES**

## Annex I: Surveillance and control methods

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ANNEX I  
SURVEILLANCE AND CONTROL METHODS

I. Introduction

This Annex sets out:

(a) requirements for evaluation and surveillance programmes, as provided for in Article 48 of Directive 2006/88/EC, and the sampling and diagnostic methods to be used in those programmes for Member States or zones or compartments thereof as provided for in Chapter VI of that Directive;

(b) sampling and diagnostic methods to be used for laboratory examination in the case of the suspicion of and to confirm the presence of the diseases listed in Part I of Annex IV to Directive 2006/88/EC (the listed diseases) as provided for in Article 51 and 52 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 53 of Directive 2006/88/EC, and the measures to be taken in order to attain the Category III health status for a Member State, zone or compartment that has 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 haematopoietic necrosis (IHN)	Part 1
3.	Kidney herpesvirus (KHV) disease	Part 2
4.	Infectious salmon anaemia (ISA)	Part 3
5.	Infection with <i>Aeromonas salmonicida</i>	Part 4
6.	Infection with <i>Aeromonas caviae</i>	Part 5
7.	White spot disease (WSD)	Part 6

## Annex II: Diagnostic methods

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ANNEX II  
DETAILED DIAGNOSTIC METHODS AND PROCEDURES

I. Introduction

This Annex sets out the detailed procedures for the diagnostic methods to be used for the laboratory examination in the evaluation 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 haematopoietic necrosis (IHN)	Part 1
3.	Kidney herpesvirus (KHV) disease	Part 2
4.	Infectious salmon anaemia (ISA)	Part 3
5.	Infection with <i>Aeromonas salmonicida</i>	Part 4
6.	Infection with <i>Aeromonas caviae</i>	Part 5
7.	White spot disease (WSD)	Part 6

## VHS & IHN: What's new?

### I. surveillance, eradication and maintenance



- Replace the [Commission Decision 2001/183/EC](#)
- Category I zones / compartments
- 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 following (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 <sup>3</sup>
High	2 every year	30
Medium	1 every year	30
Low	1 every 2 years	30

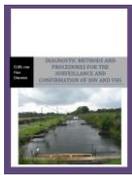
Maximum number of fish per pool: 10

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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



## ISA: What's new?

### I. surveillance, eradication and maintenance



- Replace the [Commission Decision 2003/466/EC](#)
- To contain infections with **HPR deleted ISAV**
- 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 following (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!!
- 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			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	2	2	75 <sup>1</sup>	Farms/sampling sites	First two years of the surveillance period	1	1	30
	Maximum number of fish per pool: 2				Farms/sampling sites	Second two years of the surveillance period	2	2	30
	<small><sup>1</sup> 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%.</small>					Maximum number of fish per pool: 2			

## KHVD:

### II. surveillance, eradication and maintenance

- Eradication programmes: protection zone and a surveillance zone
- Synchronised following (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 2015/1554 now finally approved and adopted!
- No possibility for making specific references in the legislative text- therefore consult the EURL webpage for further references and details.
- Please read them! Make your competent 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!