

DTU






DIAGNOSTIC METHODS FOR THE SURVEILLANCE AND CONFIRMATION OF KHVD

31st May 2021 – Shared session AW for national reference laboratories for fish and crustacean diseases

CHAPTER 2.3.7.

INFECTION WITH KOI HERPESVIRUS

<https://www.eurl-fish-crustacean.eu/fish/diagnostic-manuals/khv>



EURL FOR FISH DISEASES

DIAGNOSTIC METHODS AND PROCEDURES FOR THE SURVEILLANCE AND CONFIRMATION OF KHV DISEASE

KHVD LIST E (2018-1882)

Gill necrosis
Increase mortality
endophtalmia



Passive surveillance from MS- although some MS initiated targeted surveillance and quest to eradication (hungary Italy etc)



Pics from CEFAS
Gov.uk



KHVD LIST E (2018-1882)

Susceptible species

Common carp and koi carp (*Cyprinus carpio*)

Vector species

Goldfish (*Carassius auratus*), grass carp (*Ctenopharyngodon idella*)

Currently we refer to OIE manual

https://www.oie.int/en/what-we-do/standards/codes-and-manuals/aquatic-manual-online-access/?id=169&L=1&htmfile=chapitre_koi_herpesvirus.htm

CHAPTER 2.3.7.

INFECTION WITH KOI HERPESVIRUS

- We will revise what was provided by the EURL in 2014 – and included in 2015-1554 and upload a new manual for
- **DIAGNOSTIC METHODS AND PROCEDURES FOR THE SURVEILLANCE AND CONFIRMATION OF KHV DISEASE**



EURL
FOR FISH
DISEASES

DIAGNOSTIC METHODS AND
PROCEDURES FOR THE
SURVEILLANCE AND
CONFIRMATION OF KHV
DISEASE



For Surveillance - Detailed diagnostic methods and procedures for the surveillance of KHVD

- To conduct surveillance:
- For the detection of KHV, **gill and kidney shall be collected**; **tissue material of up to two fish may be pooled**. Furthermore, non-lethal samples such as blood, gill swabs, gill biopsy, mucus scrape may be used in certain cases (namely very valuable fish may be used in the case of the suspicion of the presence of KHV).
- FOR SURVEILLANCE For the surveillance of KHV, a qPCR **shall** be used
- qPCR for KHV detection The qPCR described (gilad et al., 2004) as follows shall be used:
 - Forward primer (KHV-86f): 5'- GACGCCGGAGACCTTGTG -3';
 - Reverse primer (KHV-163r): 5'- CGGGTTCTTATTTTTGTCCTTGTT -3';
 - and probe (KHV-109p): 5'-FAM- CTCCTCTGCTCGGCGAGCACG -3'.

For Surveillance - Detailed diagnostic methods and procedures for the surveillance of KHVD

- For the surveillance of KHV, **a qPCR shall be used**.

If positive samples appear in an area not previously confirmed positive, the test results shall be confirmed either:

(a) by sequencing of a PCR or nested PCR product from the samples. The obtained clean consensus sequence shall match (by at least 98 %) with these reference sequences.

(b) or alternatively, samples may be sent to a national reference laboratory for confirmation.

Table 2.1

Primers and conditions for the nested PCR assay targeting all cyprinid herpesviruses (CyHV-1, CyHV-2 and CyHV-3)

Primer name	Sequence	Cycling conditions	Product size
CyHVpol-forward	5'-CCAGCAACATGTGCGACGG-3'	First round PCR 1 cycle: 95 °C 2 minutes 40 cycles: 95 °C for 30 seconds 55 °C for 30 seconds 72 °C for 45 seconds 1 cycle: 72 °C for 10 minutes	362 bp
CyHVpol-reverse	5'-CCGTARTGAGAGTTGGCGCA-3'		
Primer name	Sequence	Cycling conditions	Product size
CyHVpol-internal forward	5'-CGACGGVGGYATCAGCCC-3'	Second round PCR 1 cycle: 95 °C 2 minutes, 40 cycles: 95 °C for 30 seconds 55 °C for 30 seconds 72 °C for 45 seconds 1 cycle: 72 °C for 10 minutes	339 bp
CyHVpol-internal reverse	5'-GAGTTGGCGCAYACYTTCATC-3'		

For Diagnostics - Detailed diagnostic methods and procedures for the confirmation of the presence of or to rule out the suspicion of KHVD

- For the detection of KHV, gill and kidney shall be collected; in addition spleen, encephalon and intestine may be included in an additional separated sample. **In acute cases, tissue material of up to five fish may be pooled.** Furthermore, non-lethal samples such as blood, gill swabs, gill biopsy, mucus scrape may be used in certain cases (namely very valuable fish may be used in the case of the suspicion of the presence of KHV).
- Agent detection and identification by Polymerase chain reaction (PCR) based methods
- Same qPCR for surveillance (gilad et al.,2004)

OR

- The assay described in this point targeting the Thymine kinase (TK) gene of KHV shall be used. Bercovier 2005
 - Forward primer (KHV-TKf): 5'-GGGTTACCTGTAC GAG-3';
 - Reverse primer (KHV-TKr): 5'-CACCCAGTAGATTA TGC-3'.



New challenges with KHV diagnostics and surveillance

It has been reported that variants of KHV may escape the methods recommended for diagnostics and surveillance.

Engelsma et al.,2013 DAO

Stone et al.,2019 AW for NRL for fish diseases

Presence of at least 3 isolates which are not recognised by Gilad and Bercovier assay

Further discussion and validation needed to select the diagnostic methods.

Stay tuned 😊

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Detection of novel strains of cyprinid herpesvirus closely related to koi herpesvirus

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