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NRLs for fish diseases in Europe
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UPDATED LABORATORY DIAGNOSTIC PROCEDURES FOR DETECTING IHNV RNA BY RT-qPCR

Dear colleagues,

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niven

In relation to the recent outbreak of IHN in Denmark occurred on May 18th 2021 https://www.foedevarestyrelsen.dk/english/Animal/AnimalHealth/Animal%20diseases/IHN/Pages/default.aspx, it is necessary to inform that we have observed unsatisfactory performances of the RT-qPCR protocols currently described in the diagnostic manual on the EURL website https://www.eurl-fish-crustacean.eu/fish/diagnostic-manuals/ihn. Both recommended methods, the validated RT-qPCR described in the publication by Purcell et al., 2013 (two-steps method) and Cuenca et al., 2020 (one-step method), have shown low sensitivity in detecting the IHNV variant present in Denmark in May 2021

The low sensitivity of these methods is very likely caused by a mismatch in the region where the probe IHNV N 818MGB (Purcell et al. 2013) hybridize with the N-gene of IHNV. As a consequence of this, the diagnostic procedures for detection of IHNV have been modified by using the IHNV specific probe (IHNV-qP826) described in Hoferer et al., 2019. According to a validation carried on by the authors of this publication, the use of this modified probe (IHNV-qP826) together with the original Purcell et al. 2013 primers will detect all known European IHNV isolates.

It may be noticed that the use of the IHNV-qP826 probe described by Hoferer et al. 2019 has not been validated for isolates outside Europe, and indeed we have preliminary indications that the method may perform poorly when detecting some isolates from North America and/or Asia.
Therefore, until further validation of the method is conducted, the EURL recommends that if one laboratory is relying only on RT-qPCR assays to conduct surveillance to demonstrate freedom of infection with IHNV, samples shall be tested in parallel with both RT-qPCR protocols (Hoferer et al.,2019 and Purcell et al.,2013; OR Hoferer et al.,2019 and Cuenca et al.,2020). The same apply if confirmatory diagnostic testing of clinical IHNV suspected case is conducted only by RTq-PCR methods.

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If the aim of the testing is to detect or rule out presence of the European strains of IHNV the Hoferer et al protocol will be sufficient to use. An *interim* modified protocol in the IHNV diagnostic manual will be uploaded on the webpage of the EURL for fish and crustacean diseases.

Alternatively the use of cell culture and subsequent virus identification by immunochemical methods or end point RT-PCR, as described in the diagnostic manual, remains the gold standard and does not appear to be affected by the genetic variance of the IHNV isolates.

Best regards,

The EURL Team

References

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- Cuenca, A., Vendramin, N., & Olesen, N. J. (2020). Analytical validation of one-step realtime RT-PCR for detection of infectious hematopoietic necrosis virus (IHNV). Bulletin of The European Association of Fish Pathologists, 40(6), 261-272.

- Hoferer M, Akimkin V, Skrypski J,Schütze H, Sting R. Improvement of a diagnostic procedure in surveillance of the listed fish diseases IHN and VHS. J Fish Dis. 2019; 42:559–572. https://doi.org/10.1111/jfd.12968

ANNEX : Primer sets and Probe to be used for RT-qPCR detection of IHNV RNA

Name	**Sequence 5'→3'**	**Nt position**	**References**
IHNV-qP826	FAM-AGC GGG ACA GGR ATG ACA ATG GTG BHQ1	826–849	Hoferer et al.,2018
IHNV N 818MGB	FAM-TGA GAC TGA GCG GGA CA-NFQ/MGB	818–834	Purcell et al. (2013)
IHNV-F796	AGA GCC AAG GCA CTG TGC G	796–814	Purcell et al. (2013)
IHNV-R875	TTC TTT GCG GCT TGG TTG A	875–857	Purcell et al. (2013)