

Robust and rapid real-time multiplex RT-PCR identification of two notifiable aquatic viruses by virotype® IHN/VHSV RT-PCR Kit

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Introduction

INDICAL BIOSCIENCE (INDICAL) has developed a real-time RT-PCR method, enabling rapid simultaneous identification and differentiation of *Infectious Hematopoietic Necrosis Virus* (IHNV) and *Viral Hemorrhagic Septicemia Virus* (VHSV) RNA in fish tissue. IHNV and VHSV are economically significant pathogens in aquaculture, responsible for high mortality events and trade restrictions. Rapid and reliable detection of these viruses is essential for disease surveillance, outbreak response, and certification programs.

This study evaluated the analytical and diagnostic performance of the kit with field samples from various geographic regions. The kit targets highly conserved regions of the IHNV and VHSV genomes and includes an endogenous internal control to monitor RNA extraction efficiency and PCR inhibition. RNA was extracted using the IndiMag® Pathogen Kit and tested according to the manufacturer's instructions.

Materials and Methods

Sample material

- In-vitro RNA of IHNV and VHSV (INDICAL) as well as field samples provided by Friedrich-Loeffler Institut (FLI) and from veterinary state laboratories, Germany.

virotype IHNV/ VHSV RT-PCR Kit

- The virotype IHNV/VHSV RT-PCR Kit is intended for the simultaneous detection and differentiation of RNA from *Infectious Hematopoietic Necrosis Virus* (IHNV) and *Viral Hemorrhagic Septicemia Virus* (VHSV) from tissue samples from salmonids and cell culture supernatant.



virotype IHNV/VHSV RT-PCR Protocol

Table 1. virotype IHNV/VHSV RT-PCR protocol for simultaneous identification of IHNV and VHSV RNA and endogenous Internal Control in ~64 min run-time on the BioRad® CFX96™ cyclers.

Pathogen/Internal Control	Reporter	Temperature	Time	Number of Cycles
IHNV	Cy ⁵	50°C	10 min	1
VHSV	FAM™	95°C	2 min	1
Endogenous Internal Control	JOE/ VIC/ HEX™	95°C	5 s	40
Passive reference	ROX™	60°C	30 s	

Results: High Analytical Sensitivity

The virotype IHNV/VHSV RT-PCR Kit enables the identification of as few as 2 in vitro RNA copies/well of IHNV and VHSV (Figure 1) using individual titration series (in triplicate) of IHNV and VHSV in vitro RNA on the BioRad CFX 96.

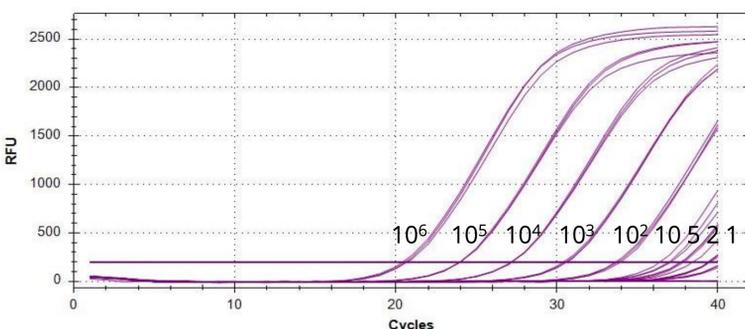


Figure 1a. Titration series of in vitro IHNV RNA in triplicate using the virotype IHNV/VHSV RT-PCR protocol.

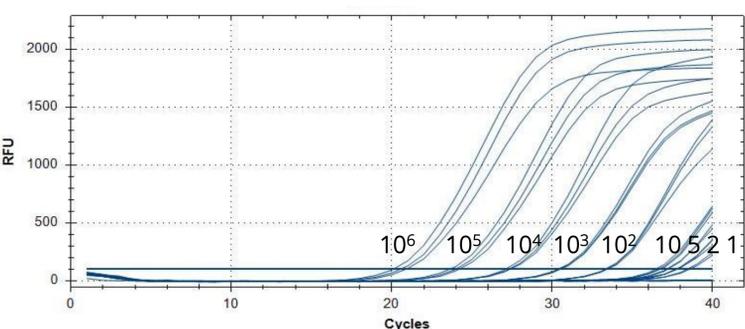


Figure 1b. Titration series of in vitro VHSV RNA in triplicate using the virotype IHNV/VHSV RT-PCR protocol.

Results: Analytical Specificity

To ensure reliable identification of IHNV and VHSV lineages, RNA samples from known genotypes were kindly provided by the FLI and tested with the virotype IHNV/ VHSV RT-PCR Kit on the BioRad CFX 96 instrument (Table 2). The kit correctly detected each IHNV and VHSV genotype in concordance with the reference assay.

Table 2. RNA samples tested using virotype IHNV/ VHSV RT-PCR Kit on the BioRad CFX 96 instrument.

Sample	Reference Assay*		virotype IHNV/VHSV RT-PCR Kit	
	C _T (IHNV)	C _T (VHSV)	C _T (IHNV)	C _T (VHSV)
Infectious Hematopoietic Necrosis Virus (IHNV)				
IHNV/K203 (gt E)	20.15	-	19.82	-
220-90 (gt M)	28.65	-	28.81	-
93-057 (gt U)	20.27	-	20.17	-
Viral Hemorrhagic Septicemia Virus (VHSV)				
VHSV/FI13 (gt Ia)	-	18.00	-	16.80
99-292 (gt IVa)	-	23.78	-	24.05
U13653-1 (gt IVb)	-	22.67	-	22.30
CA-NS04-1 (gt IVc)	-	21.73	-	22.23

- = no C_T, gt = genotype

* In-house RT-PCR of the reference laboratories

Results: 100% Diagnostic Exclusivity

RNA samples of IPNV (genotypes I, II, IV, and V), ISAV (genotypes I and II), and SAV (genotypes I and II) were kindly provided by the FLI and tested with the virotype IHNV/VHSV RT-PCR Kit on the BioRad CFX 96 instrument. The RNA samples were negative with the virotype IHNV/VHSV RT-PCR Kit, confirming the high specificity of the kit.

Results: High diagnostic sensitivity and specificity

To confirm diagnostic sensitivity and specificity, RNA extracts from 367 fish field samples were tested with the virotype IHNV/ VHSV RT-PCR Kit and the reference method. The virotype IHNV/ VHSV RT-PCR Kit had 99.4% diagnostic sensitivity and 100% diagnostic specificity for IHNV and VHSV. The virotype IHNV/ VHSV RT-PCR Kit demonstrated slightly lower CT values compared to the reference methods.

Table 3. Diagnostic sensitivity, specificity and efficiency for IHNV and VHSV of the virotype IHNV/VHSV RT-PCR Kit.

virotype IHNV/VHSV RT-PCR Kit (IHNV; Cy5)	comparative data				
	Total	367	Reference-positive	167	Reference-negative
positive	166	true positive	166	false-positive	0
negative	201	false-negative	1	true-negative	200
Diagnostic sensitivity:			99.4 %		
Diagnostic specificity:			100 %		
Diagnostic efficiency:			99.7 %		

virotype IHNV/VHSV RT-PCR Kit (VHSV; FAM)	comparative data				
	Total	367	Reference-positive	154	Reference-negative
positive	153	true positive	153	false-positive	0
negative	214	false-negative	1	true-negative	213
Diagnostic sensitivity:			99.4 %		
Diagnostic specificity:			100 %		
Diagnostic efficiency:			99.7 %		

Results: High repeatability of the virotype IHNV/ VHSV RT-PCR Kit

The intra-assay and inter-assay variability was tested with six RNA samples and seven replicates of the samples using the virotype IHNV/VHSV RT-PCR Kit on the BioRad CFX 96 instrument (Figure 2). The intra-assay coefficient of variation (CV) was 0.68% and 0.75% for IHNV and VHSV, respectively. The inter-assay coefficient of variation (CV) was 0.88% and 0.50% for IHNV and VHSV, respectively confirming the high repeatability of this kit.

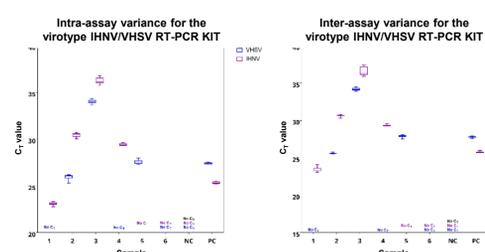


Figure 2. Testing intra- and inter-assay variance of 6 samples and the NC and PC of the virotype IHNV/ VHSV RT-PCR Kits on the BioRad CFX 96 instrument. No C_T indicates the RNA was negative.

Conclusion

The virotype IHNV/VHSV RT-PCR Kit provides a robust, rapid, and sensitive tool for the identification of two notifiable aquatic viruses. Its compatibility with automated extraction platforms and multiplex PCR format supports high-throughput workflows in diagnostic laboratories. The virotype IHNV/VHSV RT-PCR Kit is a valuable addition to surveillance and outbreak response efforts, facilitating early detection and minimizing the spread of disease in susceptible fish populations.

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