APPLICATION NOTE

Rapid detection using the IndiMag® Pathogen Kit and the virotype® IHNV/VHSV RT-PCR Kit

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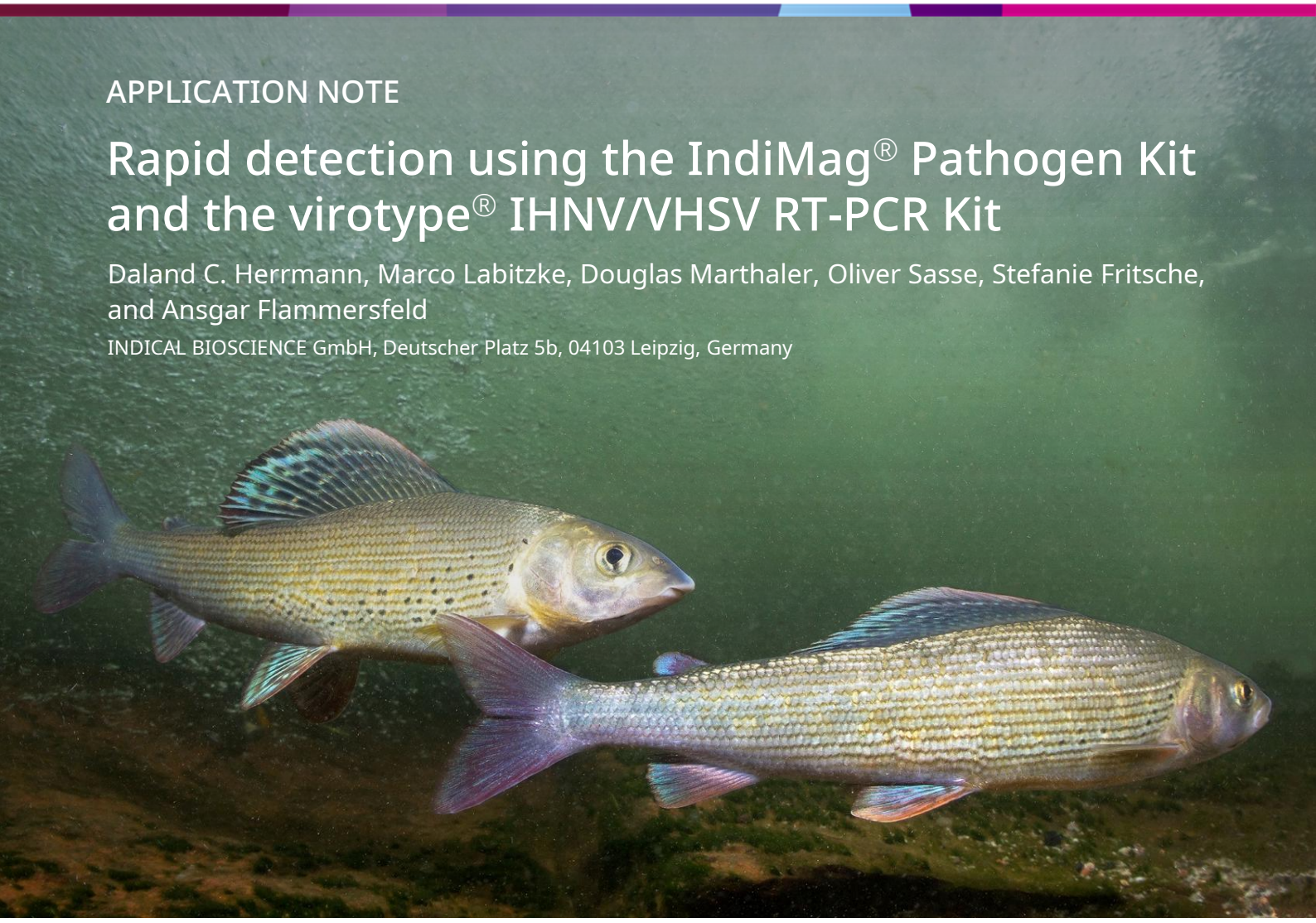

Introduction

Infectious Hematopoietic Necrosis Virus **(IHNV)** and ***Viral Hemorrhagic Septicemia Virus*** **(VHSV)** are highly contagious RNA viruses that spread systemically and cause hematopoietic necrosis and hemorrhagic lesions, leading to high mortality rates and economic losses in salmonid species, particularly affecting rainbow trout and other farmed fish [1].

Effective monitoring and rapid diagnosis are critical to prevent outbreaks and ensure biosecurity [2]. Real-time Reverse Transcription Polymerase Chain Reaction (real-time RT-PCR), provides a fast, sensitive, and highly specific method for detecting viral RNA in fish tissues and is key to controlling the spread of these viruses, maintaining healthy fish populations, and protecting the aquaculture industry [2, 3].

In this application note, INDICAL's **IndiMag Pathogen Kit** for DNA/RNA extraction, coupled with the **virotype IHNV/VHSV RT-PCR Kit** illustrate a reliable and efficient solution for the molecular detection of *Infectious Hematopoietic Necrosis Virus* and *Viral Hemorrhagic Septicemia Virus* to support disease surveillance and management in fish farming.

Keywords: *Biosecurity, pathogen detection, viral RNA, IHNV (Infectious Hematopoietic Necrosis Virus), VHSV (Viral Hemorrhagic Septicemia Virus), RNA extraction, real-time RT-PCR*

Materials and Methods

Sample preparation and RNA extraction

IHNV-positive (VHSV-negative) fish samples from a recent outbreak were kindly provided by a Veterinary State Laboratory in Germany. Organs (brain, kidney, liver, and spleen) were dissected, minced into small pieces and pooled into organ homogenates.

The organ homogenates were divided to enable a direct comparison between the **IndiMag Pathogen Kit** (INDICAL BIOSCIENCE) with **Pretreatment T1** and the **Reference Method** according to the official protocol published in the "Amtliche Methodensammlung" (Official collection of analytical methods) by the Friedrich-Loeffler-Institut (FLI) using silica spin columns for RNA extraction [4, 5, 6].

The protocol with the IndiMag Pathogen Kit with Pretreatment T1 was as follows:

1. 25 mg of the organ mix was transferred into 2 mL tubes containing 2.38 mm metal PowerBeads® (QIAGEN) and 300 µL of Phosphate-buffered saline (PBS) was added.
2. The samples were homogenized for 1 minute at 6.5 m/s using the FastPrep®-24 instrument (MP Biomedicals, LLC) and centrifuged for 2 minutes at 4,000 × g.
3. Then, 200 µL of the supernatant was used for RNA extraction with the IndiMag Pathogen Kit on the KingFisher™ Flex (Thermo Fisher Scientific Inc.).

The protocol using the Reference Method was as follows:

1. The organ mix was homogenized using a mortar and pestle, transferred into a 2 mL tube, and centrifuged for 15 minutes at 2,000 × g.
2. Then, 70 µL of the supernatant was mixed with 70 µL PBS and 560 µL Buffer AVL (QIAGEN), briefly vortexed, and incubated at room temperature for 15 minutes.
3. The RNA was extracted by silica column according to the manufacturer's protocol.

The IndiMag Pathogen Kit is available in two formats:

- Manual-fill where the user adds all reagents to the required plates.
- Prefilled format for the IndiMag 2, IndiMag 48s, and KingFisher™ systems, where reagents are pre-dispensed into plastics and only the sample and Lysis Buffer need to be added.

The IndiMag Pathogen Kit facilitates lysis, binding, and purification of total nucleic acids.

Detection and amplification

The extracted nucleic acids were analyzed with the **virotype IHNV/VHSV RT-PCR Kit** (INDICAL BIOSCIENCE), following the manufacturer's instructions [7].
This ready-to-use triplex RT-qPCR assay enables the simultaneous identification and differentiation of RNA from IHNV (Cy5) and VHSV (FAM) across all major European, North American, and Pacific genotypes, and includes an endogenous salmonid β-actin control (HEX) to support result interpretation.

Figure 1: virotype IHNV/VHSV RT-PCR Kit (Cat. no. VT290005)

The virotype IHNV/VHSV RT-PCR Kit is approved by the Friedrich-Loeffler-Institut (FLI-C 133), Germany's national reference laboratory for animal health and zoonoses.

In brief, the PCR reactions (25 µL total volume, including 5 µL RNA input and 20 µL of Master Mix) were performed on a Bio-Rad® CFX96™ PCR cycler (Bio-Rad, Hercules, CA, USA) [7].

The analytical sensitivity of the virotype IHNV/VHSV RT-PCR Kit was previously validated, with a limit of detection of 2 copies per reaction for IHNV and VHSV.

The correlation coefficient and PCR efficiency are 0.987 and 110.1 % for IHNV and 0.994 and 111.2 % for VHSV, respectively [8].

Representative application results

The virotype IHNV/VHSV RT-PCR Kit was employed to compare the extraction chemistries of the IndiMag Pathogen Kit with Pretreatment T1 and the Reference Method, using IHNV-positive (VHSV-negative) fish tissue homogenate samples.

In this dataset, the IndiMag Pathogen Kit and the Reference Method equivalently extracted genomic nucleic acids, with both methods illustrating strong and consistent amplification of the salmonid β-actin EC indicating efficient and reproducible RNA recovery. The IndiMag Pathogen Kit with Pretreatment T1 yielded a lower mean C_T of 2.27 for IHNV-RNA identification, suggesting improved extraction performance compared to the Reference Method (Table 1). The samples tested negative for VHSV with the virotype IHNV/VHSV RT-PCR Kit.

Table 1: Comparative C_T values of IHNV-RNA and the endogenous Internal Control (EC) extracted using the IndiMag Pathogen Kit with Pretreatment T1 vs. the Reference Method. C_T values are shown for IHNV-positive fish organ homogenate samples. All samples tested negative for VHSV (data not shown).

Material and sample number	Reference Method		IndiMag Pathogen Kit	
	IHNV C_T	EC C_T	IHNV C_T	EC C_T
Fish organ homogenate #1	33.80	19.17	33.73	19.05
Fish organ homogenate #2	24.32	16.73	21.10	18.16
Fish organ homogenate #3	18.00	16.68	15.32	18.33
Fish organ homogenate #4	20.09	17.70	18.87	17.26
Fish organ homogenate #5	22.40	16.94	19.97	17.86
Fish organ homogenate #6	38.42	16.25	32.80	17.07
Fish organ homogenate #7	19.16	18.92	14.92	17.63
Fish organ homogenate #8	16.47	15.30	15.51	16.37
Fish organ homogenate #9	18.65	15.07	14.70	16.29
Fish organ homogenate #10	17.47	16.06	16.15	15.93
Fish organ homogenate #11	16.87	15.87	15.40	15.81
Fish organ homogenate #12	26.71	16.06	25.58	16.65
Fish organ homogenate #13	27.62	16.53	26.53	16.26
Mean C_T	23.08	16.75	20.81	17.13

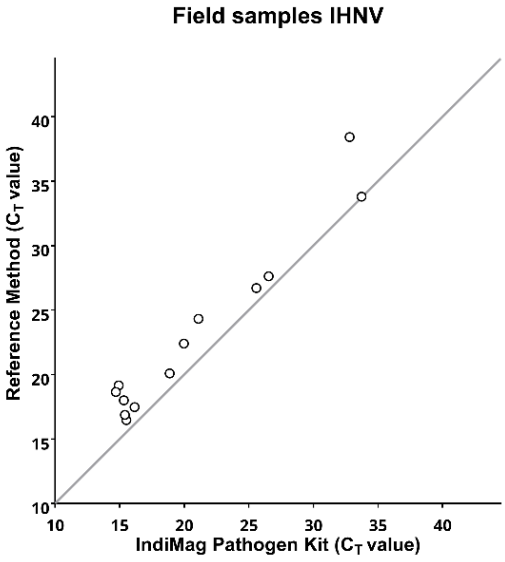

Figure 2: Graphical comparison of C_T values of the IHNV-positive fish organ homogenate samples 1-13 obtained using the IndiMag Pathogen Kit method with Pretreatment T1 and Reference Method as shown in Table 1.

Conclusion

INDICAL's IndiMag Pathogen Kit with Pretreatment T1 workflow delivers consistent, high-quality nucleic acid extraction from fish tissue samples, ensuring robust performance comparable to the Reference Method. Specifically designed for tissue matrices, it releases more viral material into the supernatant, providing a reliable alternative to the gold standard. In addition to the manual-fill format, the IndiMag Pathogen Kit is also available in prefilled versions for automated nucleic acid purification platforms such as the IndiMag 2, IndiMag 48s, KingFisher, and equivalent systems, further enabling efficient processing of large sample numbers with reduced hands-on time and increased laboratory capacity.

The virotype IHNV/VHSV RT-PCR Kit provides a highly sensitive and specific tool for the reliable detection of IHNV and VHSV in aquaculture samples. It covers all major genotypes (European, North American, and Pacific) and demonstrates no cross-reactivity with other aquatic viruses. The assay is officially approved by the FLI (FLI-C 133), Germany's Federal Research Institute for Animal Health [4, 7].

Comprehensive validation data [8] are available upon request to support regulatory or operational implementation.

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Access these and other related INDICAL protocols on the respective INDICAL BIOSCIENCE product pages. For a complete overview of INDICAL's solutions for nucleic acid extraction, detection, and instrumentation, please visit shop.indical.com or contact your local INDICAL representative.

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