



Molecular Techniques in Diagnostic Pathology of Fish: A New Era in Aquatic Animal Health Management

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How to cite this article:

Bardola, M., Shahi, N., Kala, K., Singh, B. and Mallik, S. K. 2026. Molecular Techniques in Diagnostic Pathology of Fish: A New Era in Aquatic Animal Health Management. *Blue Spectrum*. 1(1): 87-92.

ABSTRACT

The intensification of aquaculture has increased the risk of infectious diseases, creating a need for rapid and reliable diagnostic tools. Molecular techniques have transformed fish disease diagnosis by enabling sensitive and specific pathogen detection, often before clinical signs appear. This article reviews major approaches, including PCR, qPCR, ddPCR, LAMP, RPA, RAA, ERA, CRISPR-based diagnostics, DNA barcoding, FISH and molecular biosensors, highlighting their applications, advantages and limitations in aquatic animal health management. Particular emphasis is placed on the integration of isothermal amplification with CRISPR and portable detection platforms for rapid, field-deployable diagnosis. Emerging technologies such as nanopore sequencing, eDNA, metagenomics, lab-on-a-chip and smartphone-assisted diagnostics offer further opportunities for real-time disease surveillance. Despite challenges related to cost, standardization and field validation, continued advances in portable and affordable molecular diagnostics are expected to strengthen biosecurity, disease management and sustainable aquaculture.

KEYWORDS

Fish pathology; Molecular diagnostics; Aquatic animal health; CRISPR-based diagnostics; Isothermal amplification.

Introduction

Aquaculture is one of the fastest growing food production sectors worldwide with an average annual growth rate of 5.1% (FAO, 2026). However, the rapid intensification of aquaculture has increased the occurrence of infectious diseases caused by bacteria, viruses, fungi and parasites, leading to significant economic losses and threatening sustainable production. To prevent these losses conventional diagnostic methods such as clinical examination, microscopy, histopathology and biochemical assays, remain important but are often labour intensive, time consuming and less sensitive for detecting pathogens during the early stages of infection (Jaies *et al.*, 2024). Recent advances in molecular biology have revolutionized fish diagnostic pathology by enabling rapid, highly sensitive and specific detection of pathogens.

This article highlights the major molecular diagnostic tools used in fish pathology, their applications, advantages, challenges and emerging innovations that are shaping the future of aquatic animal health management.

How Molecular Diagnostics Works?

Molecular diagnosis identifies disease-causing organisms by detecting their unique genetic material (DNA or RNA) in fish tissues, body fluids or environmental samples. The process begins with sample collection from infected fish by collecting swabs from anterior kidney, buccal area, eye, liver, spleen and lower intestine (Shahi *et al.*, 2018) and their culture or preservation in suitable media. Nucleic acids are then extracted and amplified using molecular techniques such as PCR, LAMP or RPA to increase the amount of target genetic material. The amplified products are subsequently detected through fluorescence, gel electrophoresis, lateral-flow strips, sequencing or biosensor based platforms to confirm the presence of specific pathogens.

Targeting Fish Pathogens with Specific Primers

For detecting a pathogen, specific primers are designed to target unique genetic regions. The choice of target gene depends on

the pathogen and the objective of the assay. For bacterial pathogens, conserved genes such as *16S rRNA*, *gyrB*, *groEL* and virulence-associated genes (e.g., *aerA* of *Aeromonas hydrophila*) are commonly targeted, while viral assays amplify specific genomic regions having high conserved score are targeted. For example, in Indian isolates of Tilapia Lake Virus (TiLV) segment 6 have a high conserve score of 97.58 (Thangadurai *et al.*, 2026) showing a high potential of target gene for developing a primer. Careful primer design minimizes non-specific amplification and enhances the sensitivity and specificity of molecular assays, enabling early and reliable detection of fish pathogens.

Table1. Characteristics of an Ideal PCR Primer

Parameter	Recommended Value
Primer length	18–30 nucleotides
GC content	40–60%
Melting temperature	55–65°C
Amplicon size	100–500 bp (diagnostic PCR)
Secondary structures	Avoid hairpins and dimers
Specificity	Target pathogen-specific gene

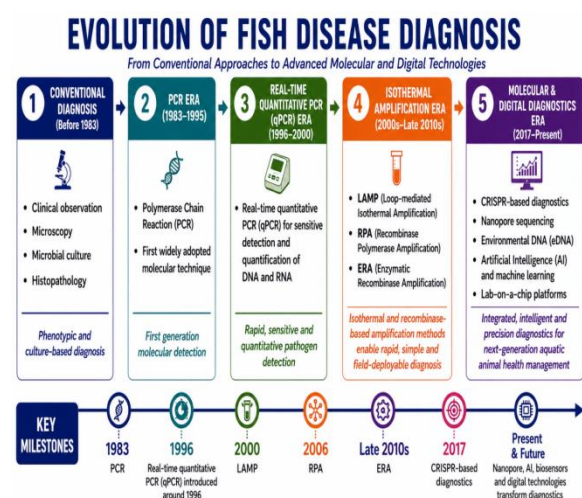


Figure 1: Diagrammatic representation of evolution of disease diagnosis in fish

Major Molecular Diagnostic Tools Used in Fish Pathology

1. Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) is the foundation of molecular diagnostics in fish pathology. It amplifies specific DNA sequences and offers high sensitivity and specificity. Its application can be extended to reverse transcription PCR for the RNA viruses by converting viral RNA into complementary DNA (cDNA) before amplification. It is widely used for diagnosing Tilapia Lake Virus (TiLV), Viral Nervous Necrosis Virus (VNNV) and Infectious Salmon Anaemia Virus (ISAV), providing reliable confirmation but the process is time consuming, requires a thermal cycler and well equipped laboratory.

2. Quantitative PCR (qPCR)

Quantitative PCR (qPCR), also known as real-time PCR, combines amplification with fluorescent detection to quantify pathogen load. Besides confirming infection, qPCR helps monitor disease progression, evaluate treatment efficacy and detect pathogens before clinical signs appear, making it a preferred tool for disease surveillance programmes.

3. Droplet Digital PCR (ddPCR)

Droplet digital PCR (ddPCR) partitions a sample into thousands of microscopic droplets, allowing absolute quantification of nucleic acids without requiring standard curves. Its exceptional sensitivity makes it valuable for detecting low-copy pathogens, latent infections and carrier fish that may escape conventional PCR detection.

4. Loop-Mediated Isothermal Amplification (LAMP)

Loop-mediated isothermal amplification (LAMP) amplifies DNA under a constant temperature (60–65°C), eliminating the need for a thermal cycler. Results are generated within an hour and can often be interpreted visually through colour changes. Because of its simplicity and affordability, LAMP has become an attractive tool for rapid diagnosis of diseases caused by *Aeromonas hydrophila*, KHV and

Streptococcus spp. in hatcheries and fish farms (Rolando et al., 2024).

5. Recombinase Polymerase Amplification (RPA)

Recombinase polymerase amplification (RPA) is another isothermal technique that operates at relatively low temperatures (37–42°C) and produces results within 20–30 minutes. Its portability and rapid turnaround make RPA highly suitable for on-site diagnosis, particularly when combined with portable detection devices (Bohara et al., 2024).

6. CRISPR-Based Diagnostics

CRISPR-Cas systems was originally developed for genome editing but in recent years have emerged as next-generation diagnostic platforms. Cas12 and Cas13 enzymes recognize pathogen specific nucleic acids with remarkable precision and generate detectable fluorescent or lateral flow signals. When integrated with LAMP or RPA, CRISPR assays provide ultra-sensitive, rapid and field deployable diagnostics for emerging fish diseases (Sahalet al., 2024).

Table 2. Molecular diagnostic techniques used for disease diagnosis in aquaculture

Technique	Target	Time Required	Sensitivity	Equipment Required
PCR	DNA	3–4 h	High	Thermal cycler
Reverse Transcriptase-PCR	RNA	3–4 h	High	Thermal cycler
qPCR	DNA/RNA	1.5–2 h	Very High	Real-time PCR system
ddPCR	DNA/RNA	3–4 h	Extremely High	Droplet digital PCR system
LAMP	DNA	30–60 min	High	Heating block/water bath
RPA	DNA/RNA	15–30 min	High	Portable incubator
CRISPR-Cas	DNA/RNA	30–60 min	Very High	Portable detection platform

7. Recombinase-Aided Amplification (RAA)

Recombinase-Aided Amplification (RAA) employs recombinase proteins to facilitate primer binding to homologous target DNA, followed by strand displacement and amplification at a constant temperature of 37–42°C. The assay provides results within 15–30 minutes with high sensitivity and specificity. RAA has been applied for detecting bacterial and viral pathogens in aquaculture, enabling early disease diagnosis. Its simple operation, minimal equipment requirement and compatibility with portable detection devices make RAA particularly suitable for on-site disease surveillance in fish farms.

8. Enzymatic Recombinase Amplification (ERA)

Enzymatic Recombinase Amplification (ERA) is an improved recombinase based isothermal amplification method designed to enhance reaction efficiency and robustness. By optimizing the enzyme formulation, ERA enables rapid amplification of target nucleic acids with excellent sensitivity under constant temperature conditions. Recent development of One pot ERA-CRISPR/Cas12a based rapid diagnostic kit for *Lactococcus garvieae* (Wang *et al.*, 2026) is one of the example of increasingly — coupled ERA-CRISPR-Cas

9. DNA Barcoding

DNA barcoding uses conserved genetic markers such as the cytochrome c oxidase subunit I (COI) and 16S rRNA genes to accurately identify fish species, parasites and seafood products. It also supports biodiversity assessment, species authentication and traceability within fisheries and aquaculture.

10. Fluorescence In Situ Hybridization (FISH)

Fluorescence in situ hybridization (FISH) combines molecular biology with histopathology by using fluorescent probes that bind directly to pathogen nucleic acids within infected tissues. This technique simultaneously visualizes pathogens and associated tissue

damage, thereby improving diagnostic accuracy and understanding of disease pathology (Jaies *et al.*, 2024).

11. Molecular Biosensors

Recent advances have led to the development of electrochemical, optical, hydrogel and CRISPR integrated biosensors capable of detecting pathogens within minutes. These portable platforms require minimal sample preparation and have great potential for real-time disease monitoring directly at aquaculture farms (Bohara *et al.*, 2024).

Advantages of Molecular Diagnostics

Laboratory-based techniques such as PCR, RT-PCR, qPCR, ddPCR and DNA sequencing remain the gold standard for confirmatory diagnosis due to their high analytical accuracy and comprehensive data output. In contrast, field-deployable technologies including LAMP, RPA, CRISPR-based assays and molecular biosensors offer rapid, affordable and user-friendly diagnosis with minimal equipment. Recent advances have enabled the integration of multiple technologies, such as LAMP-CRISPR, RPA-CRISPR and RPA-lateral flow assays, to enhance diagnostic accuracy, speed and portability.

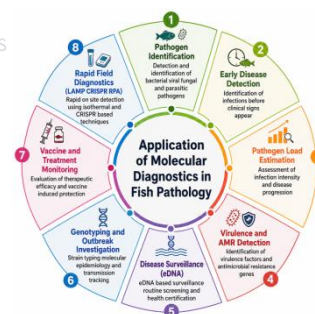


Figure 2: Diverse Applications of Molecular Diagnostics in Fish Pathology
Challenges Associated with Molecular Diagnostics in Aquaculture

Despite their remarkable advantages, molecular diagnostic techniques face several challenges that limit their widespread adoption in aquaculture. Advanced platforms such as qPCR, ddPCR and next-generation sequencing require expensive instruments, costly reagents and skilled personnel, making them inaccessible to many small scale laboratories and fish farms,

particularly in developing countries. Diagnostic accuracy can also be compromised by contamination, improper sample handling and the absence of standardized protocols across laboratories. Furthermore, many molecular assays require validation under diverse field conditions before routine application, while regulatory approval and quality assurance remain important concerns.

Future Perspectives

The future of fish disease diagnostics lies in the integration of advanced molecular technologies with digital aquaculture. The digital revolution in aquaculture, fuelled by new technologies such as IoT sensors and AI-based systems, has huge potential (Bardola and Khati, 2024) which can be supplemented with CRISPR-based diagnostics, nanopore sequencing, lab-on-a-chip devices and smartphone assisted platforms to deliver rapid, portable and point of care pathogen detection. Simultaneously, integrated biosensors, environmental DNA (eDNA) monitoring and metagenomics enabled surveillance will facilitate real-time disease monitoring, predictive outbreak analysis and precision health management. Together, these innovations will enhance biosecurity, reduce production losses and accelerate the transition toward sustainable and precision aquaculture over the coming decade.

Conclusion

Molecular diagnostic technologies have fundamentally transformed fish pathology. Beyond improving diagnostic accuracy, these tools support proactive disease management through early detection, effective surveillance, enhanced biosecurity and informed health interventions, thereby reducing production losses and improving aquaculture sustainability. As emerging diseases, climate change and global trade continue to challenge aquatic animal health, integrating molecular diagnostics into routine monitoring programmes has become increasingly important. The convergence of molecular techniques with innovations will further enhance real time disease detection and decision making. Continued investment in affordable, field deployable diagnostic

platforms, standardized protocols and capacity building will accelerate their adoption worldwide. By strengthening aquatic animal health management, molecular diagnostics will play a pivotal role in ensuring sustainable aquaculture, protecting aquatic biodiversity and contributing to global food and nutritional security.

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