practical aquaculture, diseases are often preliminarily diagnosed based on external symptoms such as abnormal body coloration, appendage damage, white spots on the carapace, and empty gut. Post-mortem observations, including hepatopancreatic atrophy, gill damage, and muscle necrosis, are also used as supporting indicators. Parasitic infections and some bacterial pathogens can be identified morphologically using optical microscopy, while bacterial pathogens are typically isolated and characterized through culture and biochemical tests. These methods are technically mature and cost-effective, and they remain useful in small-scale farming operations and routine laboratory diagnostics.

However, conventional methods have clear limitations in terms of sensitivity, specificity, timeliness, and field applicability. Many important viral diseases lack specific clinical symptoms in the early stages, and some individuals may carry subclinical infections, making early detection based solely on visual observation unreliable (Zwetlana et al., 2023). In addition, morphological similarities among parasites, bacteria, and pathological changes can lead to misdiagnosis. For key viral pathogens such as WSSV, TSV, and DIV1, the lack of stable and applicable cell culture systems limits the effectiveness of traditional isolation and culture-based methods (Lee et al., 2023). Furthermore, culture-based techniques have limited ability to detect Vibrio spp. in the viable but non-culturable (VBNC) state, often underestimating the actual pathogen load (Bohara et al., 2023).

Conventional methods are also constrained by long detection cycles. Histopathology, microbial culture, and serological assays typically require several days to weeks and depend on specialized laboratories and trained personnel, which is inadequate in rapidly evolving disease scenarios (Bohara et al., 2023). In screening broodstock, seedstock, and asymptomatic carriers, these methods often fail to detect low-level infections, even though such carriers play a critical role in disease transmission (Zwetlana et al., 2023). Therefore, with the expansion of intensive aquaculture and global trade, traditional diagnostic approaches are no longer sufficient for precise diagnosis and early warning, driving the development of highly sensitive molecular and rapid detection technologies.

4.2 Molecular and immunological detection technologies

The widespread application of molecular biology has significantly advanced shrimp disease diagnostics from empirical observation to standardized and highly sensitive detection. Among these, nucleic acid amplification techniques have become the cornerstone of pathogen detection. Conventional PCR, nested PCR, quantitative real-time PCR (qPCR), loop-mediated isothermal amplification (LAMP), and recombinase polymerase amplification (RPA) have been widely applied for detecting major pathogens, including WSSV, IHHNV, DIV1, TSV, YHV, IMNV, as well as AHPND-causing Vibrio and EHP (Lee et al., 2023; Lou et al., 2025). These methods enable the detection of low-abundance nucleic acids, allowing early identification of pathogens during latent or initial infection stages, thereby supporting timely management decisions.

Among nucleic acid-based methods, qPCR has become the most widely used due to its high sensitivity, specificity, and quantitative capability. For example, standardized qPCR systems have been established for WSSV detection, while multi-target real-time PCR assays have been developed for emerging viruses such as DIV1, enabling dynamic monitoring and early warning (Lee et al., 2023). Similarly, PCR and qPCR have demonstrated high reliability and reproducibility in detecting bacterial and microsporidian pathogens such as AHPND-causing Vibrio and EHP (Lou et al., 2025).

Recent developments have further improved the efficiency and practicality of molecular detection. Direct PCR techniques eliminate the need for nucleic acid extraction, thereby shortening detection time and reducing contamination risks. In addition, multiplex qPCR enables simultaneous detection of multiple pathogens. For instance, a five-plex qPCR assay can detect WSSV, IHHNV, DIV1, AHPND-causing Vibrio, and EHP in a single reaction, with a detection limit of 10 copies/μL and high specificity validated in large-scale studies (Lou et al., 2025). These advances significantly enhance detection efficiency in complex disease systems involving multiple pathogens.