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Review article

Diagnostic strategies for Tilapia lake virus: Comprehensive review

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Abstract

Tilapia Lake Virus (TiLV) is a highly contagious viral infection with a significant impact on aquaculture. TiLV has been reported worldwide and still occurs in many countries. Typically, TiLV can be diagnosed using various techniques that have been studied and verified through a wide range of methods, including gross pathology, histopathology, immunohistochemistry (IHC), immunofluorescence (IFA), in-situ hybridization (ISH), cell culture, reverse transcriptase polymerase chain reaction (RT-PCR), reverse transcriptase real-time polymerase chain reaction (RT-qPCR), nested RT-PCR, semi-nested RT-PCR, reverse transcriptase loop-mediated isothermal amplification (RT-LAMP), and indirect ELISA. Additional advance techniques that include reverse transcriptase recombinase polymerase amplification Cas12a (RT-RPA-Cas12a), RT-RPA lateral flow dipstick (RT-RPA-LFD), real-time RT-RPA (RT-qRPA), droplet digital polymerase chain reaction (ddPCR), and colorimetric LAMP (cLAMP) have been reported. Herein, we have identified and demonstrated various techniques for diagnosing TiLV that can be used in aquaculture research and future studies.

Keywords: Cell culture, Diagnostic techniques, RT-PCR, RT-qPCR, RT-LAMP, RT-RPA, ISH, IHC, ELISA, TiLV.

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INTRODUCTION

Tilapia Lake Virus (TiLV) is a highly contagious viral pathogen that has emerged as a major threat to global Tilapia aquaculture and industry food security worldwide. Recently, tilapia has been established as an important source of food and has become in high demand (FAO, 2025). The first report of TiLV was recorded in Israel by Eyngor (2014) and was subsequently followed by an incidence in Ecuador (Ferguson et al., 2014), both of which resulted in high mortality rates among existing Tilapia populations. TiLV has now spread rapidly across Asia, Africa, North America, and South America (FAO, 2018). The virus presents a formidable challenge to the aquaculture industry, causing high mortality rates among both farmed and wild tilapia populations, thereby threatening food security and the livelihoods of millions of people who rely on aquaculture worldwide. The virus primarily affects Nile tilapia (*Oreochromis niloticus*), red tilapia (*Oreochromis* sp.), grey tilapia (*O. niloticus* × *O. aureus*), hybrid tilapia (*O.niloticus* x *O.aureus*, or *O.niloticus* x *O.mossambicus*), blue tilapia (*O. aureus*), and other wild tilapia species (Machimbirike et al., 2019; Surachetpong et al., 2020). Other studies have also explored the susceptibility of ornamental African cichlids (Yamkasem et al., 2021a). In these past studies, the high mortality rate of TiLV infected Tilapia have often exceeded 90% (Dong et al., 2017b), especially among smaller fish popoulation (Surachetpong et al., 2017; Roy et al., 2021).

TiLV, *Tilapia tilapinevirus*, is an enveloped, negative-sense, single-stranded RNA virus belonging to the genus *Tilapinevirus* and family *Amnoonviridae* with 10 segmented genomes (Bacharach et al., 2016; Behera et al., 2017; Kembou Tsofack et al., 2017; Jaemwimol et al., 2018; Ahasan Mohammad et al., 2020; Surachetpong et al., 2020; Aich et al., 2021; Contreras et al., 2021; Thawornwattana et al., 2021; Abu Rass et al., 2022) and 55-80 nanometers of viral particle size (Eyngor et al., 2014; Tattiyapong et al., 2017; Li et al., 2022b). Accordingly, TiLV segment 1 shares some characteristics with the PB1 subunit of the influenza C virus (Bacharach et al., 2016; Kembou Tsofack et al., 2017). Notably, gross lesions observed during TiLV infection can be variable. Most fish exhibit a variety of systemic clinical signs, including ocular alterations that can involve opacity of the lens, as well as exophthalmia and buphthalmia (Eyngor et al., 2014; Surachetpong et al., 2017; Senapin et al., 2018; Yamkasem et al., 2019), lethargy (Ferguson et al., 2014), skin erosion and discoloration (Eyngor et al., 2014; Ferguson et al., 2014; Behera et al., 2017; Tattiyapong et al., 2017; Yamkasem et al., 2019), and lesions on various internal organs. Symptoms can also include liver necrosis, splenomegaly, and kidney swelling (Surachetpong et al., 2017; Tattiyapong et al., 2017; Senapin et al., 2018). The virus spreads horizontally and can occur through direct contact, as well as exposure to contaminated water (Dong et al., 2017b; Surachetpong et al., 2017; Tattiyapong et al., 2017) and equipment (Nicholson et al., 2020), all of which pose significant challenges to disease management in aquaculture systems.

Currently, there is no effective treatment for TiLV and there are no commercially available vaccines against TiLV. Therefore, research efforts have now been initiated into the development of vaccines that can mitigate or prevent TiLV infection. These would include the development of inactivated TiLV vaccines (Zeng et al., 2021b; Mai et al., 2022), recombinant protein vaccines (Chamtim et al., 2022; Sanyalukruechai et al., 2024; Thanapasuk et al., 2025), and DNA vaccines (Zeng et al., 2021a; Chamtim et al., 2022; Islam et al., 2022; Yu et al., 2022). Accordingly, rapid and accurate diagnostic methods remain the optimal approach to disease management and biosecurity protocols in Tilapia aquaculture. Therefore, a comprehensive understanding of the available diagnostic techniques that encompass both presumptive and confirmatory methods would be essential for practitioners and researchers who engage in TiLV surveillance and outbreak investigations.

For the diagnosis of TiLV, typical identification methods include gross pathology, histopathology examinations, viral isolation in cell cultures, molecular

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biology, immunological methods, and rapid testing that relies on a combine diagnostic method (Figure 1). Regarding molecular biology, reverse transcriptase polymerase chain reaction (RT-PCR) and reverse transcriptase real-time polymerase chain reaction (RT-qPCR) are the most widely utilized methods, as they have been associated with high sensitivity and specificity for viral RNA identification.

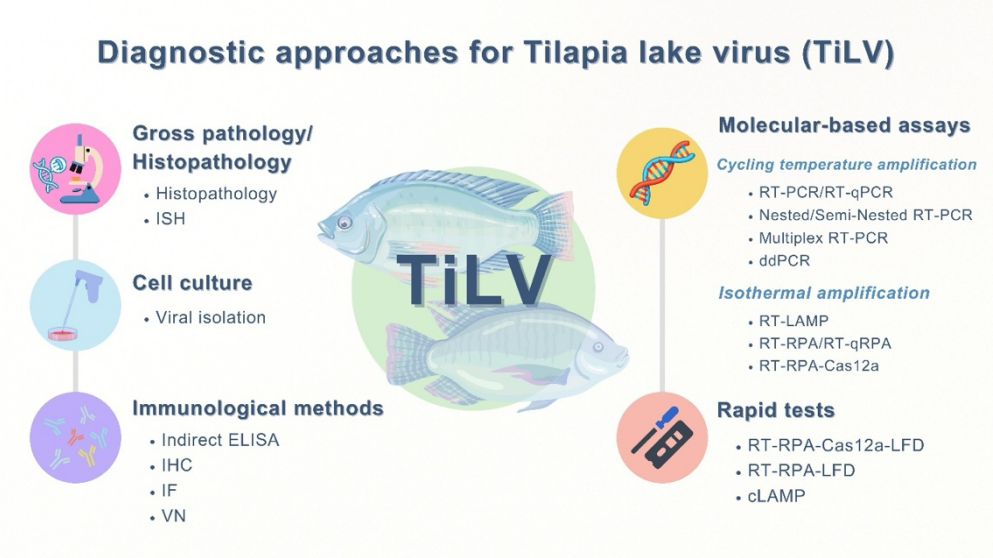


Figure 1 Overview of diagnostic approaches for Tilapia Lake virus (TiLV). This figure summarizes the major diagnostic methods currently used for the detection of Tilapia Lake virus (TiLV). The diagnostic approached have been categorized into five main groups: gross pathology and histopathology, cell culture, immunological methods, molecular-based assays, and rapid diagnostic tests. Gross pathology and histopathology involve the examination of tissue lesions and cellular alterations associated with TiLV infection. Histopathological analysis identifies characteristic tissue damage, while in situ hybridization (ISH) allows localization of viral nucleic acids within infected tissues. Cell culture refers to viral isolation techniques used to propagate TiLV in susceptible cell lines for the confirmation of infection. Immunological methods detect viral antigens or host immune responses, including indirect enzyme-linked immunosorbent assay (ELISA), immunohistochemistry (IHC), immunofluorescence (IF), and virus neutralization (VN) assays. Molecular-based assays are divided into two main categories: cycling temperature amplification and isothermal amplification. Cycling temperature amplification includes reverse transcriptase polymerase chain reaction (RT-PCR), reverse transcriptase real-time PCR (RT-qPCR), nested/semi-nested RT-PCR, multiplex RT-PCR, and droplet digital PCR (ddPCR). Isothermal amplification methods include reverse transcriptase loop-mediated isothermal amplification (RT-LAMP), reverse transcriptase recombinase polymerase amplification (RT-RPA), real-time RT-RPA (RT-qRPA), and RT-RPA coupled with CRISPR-Cas12a detection (RT-RPA-Cas12a). Rapid diagnostic tests provide field-applicable detection methods. These include RT-RPA-Cas12a combined with lateral flow detection (RT-RPA-Cas12a-LFD), RT-RPA with lateral flow detection (RT-RPA-LFD), and colorimetric loop-mediated isothermal amplification (cLAMP).

Importantly, histopathology examinations remain a method for confirming clinical cases through gross lesions, as well as various histopathological and immunohistochemistry findings such as skin erosions and discoloration (Eyngor et al., 2014; Tattiyapong et al., 2017; Yamkasem et al., 2019), liver necrosis (Surachetpong et al., 2020), syncytial hepatitis (Ferguson et al., 2014; del-Pozo et al., 2016; Behera et al., 2017; Debnath et al., 2020), ocular alterations (Eyngor et al., 2014; Yamkasem et al., 2019), lethargy (Eyngor et al., 2014), and nervous system damages (Eyngor et al., 2014; Tattiyapong et al., 2017; Jaemwimol et al.,

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2018; Dinh-Hung et al., 2021). Alternatively, although localized TiLV infection can be determined from immunohistochemistry (IHC) (Piewbang et al., 2021) and in-situ Hybridization (ISH) (Bacharach et al., 2016; Dong et al., 2017b; Jaemwimol et al., 2018; Pierezan et al., 2020; Dinh-Hung et al., 2021; Yamkasem et al., 2021a), the speed that is required for the management of rapid outbreaks is lacking. Accordingly, viral isolation in cell cultures has been used for the successful propagation of TiLV in various studies and has been suggested as an effectively sensitive method (Kembou Tsofack et al., 2017). In immunological examinations, such as those involve Indirect Enzyme-Linked Immunosorbent Assay (iELISA), the detection of viral antigens with moderate to high degrees of sensitivity has been reported in previous filed evaluations (Hu et al., 2019; Valsalam et al., 2023). More advancements in molecular biology technologies, such as those involving RT-PCR (Eyngor et al., 2014; Behera et al., 2017; Dong et al., 2017b; Kembou Tsofack et al., 2017; Liamnimitr et al., 2017; Nicholson et al., 2017; Tattiyapong et al., 2017; Mugimba et al., 2018; Senapin et al., 2018; Acharya et al., 2019; Nanthini et al., 2019; Pulido et al., 2019; Yin et al., 2019; Pierezan et al., 2020; Contreras et al., 2021; Dinh-Hung et al., 2021; Kampeera et al., 2021; Skornik et al., 2021; Yamkasem et al., 2021b; Chamtim et al., 2022; Delamare-Deboutteville et al., 2023), RT-qPCR (Kembou Tsofack et al., 2017; Liamnimitr et al., 2017; Jaemwimol et al., 2018; Mugimba et al., 2018; Tattiyapong et al., 2018; Phusantisampan et al., 2019; Thammatorn et al., 2019; Nicholson et al., 2020; Pierezan et al., 2020; Rakus et al., 2020; Taengphu et al., 2020; Skornik et al., 2021; Waiyamitra et al., 2021; Wang et al., 2021; Yamkasem et al., 2021a; Chengula et al., 2022; He et al., 2022; Li et al., 2022a; Megarani et al., 2022; Delamare-Deboutteville et al., 2023; Turner et al., 2023), semi-nested RT-PCR (Dong et al., 2017b; Kembou Tsofack et al., 2017; Senapin et al., 2018; Dinh-Hung et al., 2021; Li et al., 2022b), RT-LAMP (Phusantisampan et al., 2019; Yin et al., 2019; Kampeera et al., 2021), RT-RPA, RT-RPA-Cas12a (Sukonta et al., 2022), RT-qRPA (Wang et al., 2021), RT-RPA-LFD (Srivorakul et al., 2025), and ddPCR (Shahi et al., 2023) are being further developed to improve the speed and accuracy associated with the effective identification of TiLV.

The objective of this review is to synthesize the full range of diagnostic methods currently used for the detection of TiLV, with specific attention being paid to both established techniques and recent advancements. By drawing together findings obtained from gross pathology and histopathology examinations, cell culture systems, molecular diagnostics, immunological assays, and rapid tests, this manuscript aims to clarify how each method contributes to TiLV detection and illustrates the strengths and limitations that influence their practical application in aquaculture. This study was conducted as a comprehensive review aimed at synthesizing the excisteing knowledge comprising on the various diagnostic approaches used in the management of TiLV outbreaks. Relevant literature was identified through searches across a variety of scientific databases including PubMed, ScienceDirect, and Scopus, using the following key terms: (TITLE-ABS-KEY (Tilapia lake virus) OR TITLE-ABS-KEY (TiLV) AND TITLE-ABS-KEY (diagnosis) OR TITLE-ABS-KEY (cell culture) OR TITLE-ABS-KEY (pcr) OR TITLE-ABS-KEY (histopathology) OR TITLE-ABS-KEY (tilapia)) AND PUBYEAR > 2013 AND PUBYEAR < 2026 AND (LIMIT-TO (LANGUAGE , "English")). Publications were selected based on their relevance to the diagnostic approaches for TiLV, mitigation including molecular, immunological, histopathological, and cell culture techniques. Articles published in English between 2014 and 2025 were considered. The selected literature was qualitatively evaluated and organized according to the diagnostic approaches and the thematic areas. This strategy enabled a comprehensive and integrative synthesis of the currently employed diagnostic tools, along with their applications and inherent limitations. However, it should be acknowledged that the absence of a formal systematic methodology may introduce potential selection bias and limit the reproducibility of the review process.

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TiLV PATHOGENESIS AND TARGET CELL INFECTION

The pathogenesis of TiLV involves multifactorial interactions among incidences of TiLV, the fish population and relevant environmental factors. Infection is primarily initiated through horizontal transmission, occurring via immersion or cohabitation in virus-contaminated water (Eyngor et al., 2014; Liamnimitr et al., 2017; Yamkasem et al., 2022), and vertical transmission that can be present in broodstock and progeny (Yamkasem et al., 2019). These multiple transmission mechanisms, combined with the optimal replication conditions that involve environmental water temperatures between 25°C and 31°C (Eyngor et al., 2014; Fathi et al., 2017; Kembou Tsofack et al., 2017), can facilitate the rapid spread of the disease . The infection begins when the virus enters the host through the gills, skin, or by the oral route via ingestion of contaminated water or infected fish (Surachetpong et al., 2020) and can be spread to various internal organs via hematogenous spreading (Kembou-Ringert et al., 2024). Ultimately, this can then results in systemic infection and severe disease manifestations (Kembou-Ringert et al., 2023). It has been acknowledge that the virus exhibits strong broad tissue tropism, that may include the brain, liver, kidneys, spleen, gills, intestines, heart, muscle connective tissue, pancreas, eyes, mucus, ovaries, and testis (Tattiyapong et al., 2017; Pierezan et al., 2020; Piewbang et al., 2021; Kembou-Ringert et al., 2023). Among these tissues, the liver, intestines, gill, and brain are considered optimal for diagnostic purposes due to their high viral burden and consistent pathological changes (Tattiyapong et al., 2017; Pierezan et al., 2020). The resulting clinical manifestations include lethargy, reduced feeding, external lesions, ulcers, and high mortality rates, particularly among juveniles. The disease progresses rapidly, often resulting in death within days (Tattiyapong et al., 2017; Pierezan et al., 2020), especially under poor biosecurity or stressful conditions.

TiLV SAMPLE COLLECTION

Accurate diagnosis of TiLV relies on proper tissue collection, which must be conducted suitably from organs where the virus is known to replicate. As TiLV exhibits a strong tropism for specific tissues, such as those found in the liver, brain, gill, and intestines (Eyngor et al., 2014; Dong et al., 2017b; Tattiyapong et al., 2017; Jaemwimol et al., 2018; Pierezan et al., 2020), targeted sampling has become critical for effective detection. The liver is the primary organ for diagnosis due to its high viral load and characteristic necrosis (Dong et al., 2017b; Tattiyapong et al., 2017; Jaemwimol et al., 2018; Pierezan et al., 2020; Surachetpong et al., 2020). The brain, which is essential for identifying encephalitis, particularly in fish, is known to display neurological symptoms (Eyngor et al., 2014; Dong et al., 2017b; Tattiyapong et al., 2017; Yamkasem et al., 2019; Pierezan et al., 2020). Secondary organs, such as the spleen, intestines, gills, and skin, may also present lesions, especially in cases of advanced infections such as those exhibiting skin hemorrhages and ulcerations (Eyngor et al., 2014; Behera et al., 2017; Tattiyapong et al., 2017; Yamkasem et al., 2019). The reliable diagnosis of TiLV requires a careful alignment of sample type, tissue selection, and diagnostic methodology. Because TiLV exhibits tissue tropism and induces multisystemic pathology, the selection of the specimen must consider the pathogenesis of the virus, the stage of the disease, and the analytical sensitivity of each diagnostic platform. Sample matching ensures that tissues with the highest viral load or most characteristic lesions are paired with the most suitable detection technique, thereby improving diagnostic accuracy and reproducibility across laboratories. Table 1 presents the recommended sample sources for each diagnostic approach.

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Table 1 Sample matching methods

Diagnostic methods	Sample types	Antigenic detection	Serologic detection	Target tissues
Necropsy and gross pathology	Live animal or carcass	✓	-–	–
Histopathology examination	Formalin-fixed paraffin embedded tissue (FFPE)	✓	–	Brain, liver, kidney, and gill (Eyngor et al., 2014; Dong et al., 2017b; Tattiyapong et al., 2017)
ISH	Formalin-fixed paraffin embedded tissue (FFPE)	✓	–	Liver, kidney, and gill (Dong et al., 2017b) Brain and liver (Jaemwimol et al., 2018)
IHC	Formalin-fixed paraffin embedded tissue (FFPE)	✓	–	Liver, pancreas, kidney, gill, intestines, brain, and spleen (Piewbang et al., 2021)
iELISA	Fresh homogenate tissues (Valsalam et al., 2023), fish serum (Hu et al., 2019)	✓	✓	–
Cell culture	Fresh homogenate tissues	✓	–	Brain and liver (Tattiyapong et al., 2017)
Molecular-based assays	Fresh homogenate tissues	✓	–	Brain and liver (Eyngor et al., 2014; Tattiyapong et al., 2017) Liver, kidney, and gill (Dong et al., 2017a)

Note: ✓, suitable/applicable for detection; –, not suitable/not applicable for detection

TiLV DIAGNOSTIC TECHNIQUES

Gross pathology and histopathology examination

Clinical and gross pathological signs serve as initial presumptive indicators for TiLV infection, though they are insufficient for more definitive diagnosis due to an overlap with other tilapia diseases. However, the pathological findings obtained from the gross necropsy of TiLV infected fish usually present with tissue impairment, inflammation and hemorrhages in various organs, such as in those exhibiting opacity of the lens and exophthalmia (Eyngor et al., 2014; Surachetpong et al., 2017; Senapin et al., 2018; Yamkasem et al., 2019), lethargy (Ferguson et al., 2014), and skin erosion and discoloration (Figure 2A) (Eyngor et al., 2014; Ferguson et al., 2014; Behera et al., 2017; Tattiyapong et al., 2017; Yamkasem et al., 2019). Lesions on certain internal organs may also be present, as well as liver necrosis (Figure 2B), splenomegaly, and kidney swelling (Surachetpong et al., 2017; Tattiyapong et al., 2017; Senapin et al., 2018).

TiLV can produce a widespread pathological response, with degenerative, inflammatory, and necrotic changes occurring across multiple organ systems. The virus shows a marked preference for the liver, brain, spleen, kidneys, and gills (Eyngor et al., 2014; del-Pozo et al., 2016; Behera et al., 2017; Tattiyapong et al., 2017; Pierezan et al., 2019; Debnath et al., 2020). With regard to histopathology examination, the following may be present; cellular degeneration, hemorrhages, syncytial cell formation of the liver (Figure 3A, 3B) (del-Pozo et al., 2016; Behera et al., 2017; Pierezan et al., 2019; Debnath et al., 2020; Mugimba et al., 2020; He et al., 2022), encephalitis with neuronal degeneration and perivascular cuffing (Eyngor et al., 2014; Behera et al., 2017; Tattiyapong et al., 2017; Pierezan et al., 2019; Debnath et al., 2020; Mugimba et al., 2020), as well as tubular necrosis (Figure 3C), and infiltration of the inflammatory cells (Figure 3D), Additionally, increased Melano macrophage center (MMC) in the kidneys and spleen can also be observed (Tattiyapong et al., 2017; Mugimba et al., 2020; He et al., 2022). All of the above are associated with intracytoplasmic inclusion body (ICIB) presentation in the liver and brain (Pierezan et al., 2019; Debnath et al., 2020; Mugimba et al., 2020). Moreover, an ultrastructural study involving electron microscopy (EM) revealed viral particles in infected cells from 55-80 nm (Eyngor et al., 2014; Tattiyapong et al., 2017; Li et al., 2022b).

ISH represents a molecular-anatomical technique for detecting and confirming the present of TiLV under the direct visualization of viral nucleic acids within tissue samples and histopathological lesions. The ISH development for TiLV detection in tissues (Dong et al., 2017b; Jaemwimol et al., 2018; Pierezan et al., 2020; Dinh-Hung et al., 2021; Yamkasem et al., 2021a) was demonstrated, while Bacharach (2016) presented ISH coupled with a fluorescence probe in both tissue

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samples and cell cultures. Table 2 demonstrates primer sequences for generating TiLV-specific DIG-labelling probes in ISH.

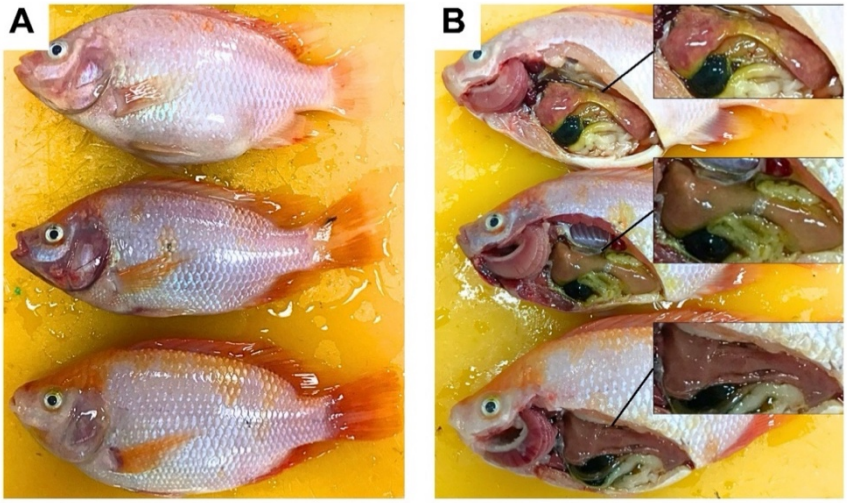


Figure 2 Gross lesions of TiLV infection in Tilapia. (A) Skin hemorrhage and discoloration. (B) The livers were demonstrated with pale, edema, necrosis, and hemorrhage.

Table 2 Primer lists of ISH for TiLV detection

Segment	Primer	Primer/probe sequence (5′- 3′)	Target size	References
1	TiLV/nSeg1F	TCTGATCTATAGTGTCTGGGCC	274	(Dinh-Hung et al., 2021)
	TiLV/nSeg1RN	CCACTTGTGACTCTGAAACAG		
3	ME1	GTTGGGCACAAGGCATCC TA	250	(Dinh-Hung et al., 2021)
	7450/150R	TATCACGTGCGTACTCGTTCAGT		
3	TiLV-112F	CTGAGCTAAAGAGGCAATATGGATT	112	(Pierezan et al., 2020)
	TiLV-112R	CGTGCGTACTCGTTCAGTATAAGTTCT		

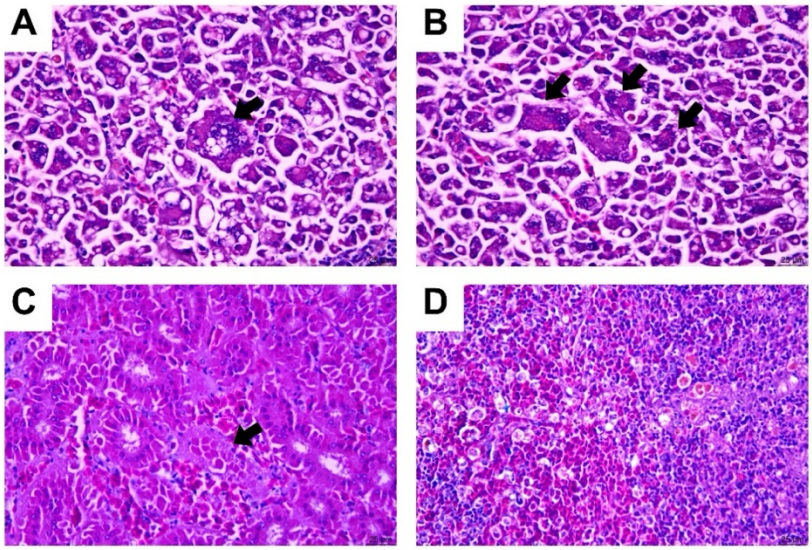


Figure 3 Histopathological findings obtained from TiLV-infected tissues of Tilapia with Hematoxylin and Eosin (H&E) stains. (A-B) Syncytial giant cells, displayed in multiple nuclei (arrow). (C) Tubular necrosis (arrow) of posterior kidney. (D) Multifocal necrotic areas in the spleen.

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Cell culture

Viral cell culture is an accepted method of viral isolation, propagation, and diagnosis. Several fish cell lines have been shown to support TiLV replication, with the E-11 cell lines being among the most widely used (Eyngor et al., 2014). Most viruses need cells for infection, while cell cultures are based on fish cell lines that typically exhibit cytopathic effects (CPE) (Figure 4) within a few days, including cell rounding, enlargement, vacuolization, and eventual detachment from the monolayer. Table 3 summarizes the cell cultures that have been used in TiLV isolation.

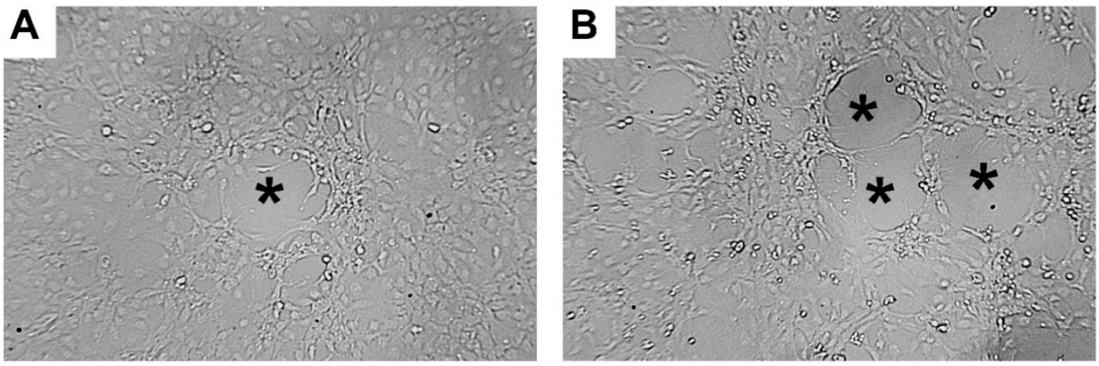


Figure 4 Cytopathic effect (CPE) in TiLV-infected cells. (A) TiLV-infected E-11 cells are demonstrated by plaque formations (asterisk) at 4 dpi. (B) TiLV-infected E-11 cells are demonstrated by plaque formations (asterisk) at 5 dpi.

Table 3 Summary of TiLV in cell culture

Species	Cell culture name	Cell origin/catalog number	Culture condition	CPE (dpi)	References
Striped snakehead (*Ophicephalus striatus*)	E-11	ECACC (01110916), SSN-1, Striped snakehead fry	- L-15, 5% FBS, 25°C - L-15, 10% FBS, 25°C - L-15, 2% FBS, 25°C - L-15, 10% FBS, 27°C - L-15, 10% FBS, 28°C - L-15, 5% FBS, 26°C - L-15, 2% FBS, 26°C - L-15, 10% FBS, 26°C	- Plaque formation and vacuolated cell on 5 dpi (Fig. 4A) - Syncytial formation, round, and swollen cell on 3-7 dpi	(Eyngor et al., 2014; Liamnimitr et al., 2017; Tattiyapong et al., 2017; Jaemwimol et al., 2018; Chengula et al., 2019; Dong et al., 2019; Thammatorn et al., 2019; Yamkasem et al., 2019; Rakus et al., 2020; Tattiyapong et al., 2020; Lertwanakarn et al., 2021; Mojzesz et al., 2021; Piewbang et al., 2021; Roy et al., 2021; Widziolek et al., 2021; Abdullah et al., 2022; Adamek et al., 2022; Chamtim et al., 2022; He et al., 2022; Li et al., 2022a; Mai et al., 2022; Piewbang et al., 2022; Tattiyapong et al., 2022; Tran et al., 2022; Yamkasem et al., 2022; Lertwanakarn et al., 2023; Raksaseri et al., 2023; Turner et al., 2023)
Hybrid snakehead (*Chana argus* x *Chaba maculata*)	**CAMK**	Kidney	- M199, 10% FBS, 28°C	Cell shrinkage, cell detachment, ballooning cell, and cell aggregation on 3 dpi	(Li et al., 2022a)
	CAMB	Brain	M199, 10% FBS, 27°C	N/A	(Wang et al., 2020)
Nile tilapia (*Oreochromis niloticus*)	**TiB**	Brain	- L-15, 10% FBS, 27°C - M199, 10% FBS, 28°C	- Swollen and rounding cell on 3 dpi - Round and swollen cell, and	(Wang et al., 2018; Hu et al., 2019; Yin et al., 2019; Wang et al., 2021; Zeng et al., 2021b; Li et al., 2022a;

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			- M199, 10% FBS, 27°C - M199, 4% FBS, 28°C - L-15, 10% FBS, 25°C	cell accumulation on 2 dpi	Piewbang et al., 2022; Lertwanakarn et al., 2023)
	On1L (primary cell lines)	Liver	- L-15, 20% FBS, 28°C - L-15, 10% FBS, 28°C	- Syncytial cell formation, cell shrinkage, rounding cell, and cell destruction of monolayer on 3-5 dpi - Cell elongation (Fig. 4B), gapping monolayer of cell on 10 dpi - Plaque formation, cellular extension	(Thangaraj et al., 2018; Li et al., 2022a; Valsalam et al., 2024)
	On1B (primary cell lines)	Brain	- L-15, 20% FBS, 28°C	- Plaque formation, increase granularity, elongation of cell, rounding cell, and destruction monolayer on 3-5 dpi - Syncytial formation, cell shrinkage, cell detachment	(Thangaraj et al., 2018; Li et al., 2022a)
	OnH (primary cell lines)	Heart	L-15, 20% FBS, 28-32°C	Elongate cells, rounding cells, and cell detachment on 4-8 dpi	(Yadav et al., 2021)
	TA-02 (Tilapia Astrocyte clone 02 cell line)	Brain	L-15, 10% FBS, 28°C	Multivacuolated, monolayer disintegration on 2-4 dpi	(Li et al., 2022b)
Mozambique tilapia (*Oreochromis mossambicus*)	**OmB** (primary cell lines)	Brain	- L-15, 2% FBS, 25°C - L-15, 10% FBS, 25°C	4 and 8 dpi	(Kembou Tsofack et al., 2017; Pierezan et al., 2020; Abu Rass et al., 2022)
	TmB (primary cell lines)	Bulbus arteriosus	- MEM, 2% FBS, 25°C - L-15, 10% FBS, 25°C	4, 5, and 6 dpi	(Kembou Tsofack et al., 2017; Pierezan et al., 2019; Soto et al., 2019)
	TG (primary cell lines)	Gill	L-15, 15% FBS, 28°C	No CPE	(Nanthini et al., 2019)
	TH (primary cell lines)	Heart	L-15, 15% FBS, 28°C	No CPE	(Nanthini et al., 2019)
	TE (primary cell lines)	Eye	L-15, 15% FBS, 28°C	No CPE	(Nanthini et al., 2019)
Hybrid tilapia (*Oreochromis niloticus* x *Oreochromis aureus*)	**TFC#10** (primary cell lines)	Caudal fin, Tilapia fin cell 10	- L-15, 20% FBS, 28°C - L-15, 10% FBS, 28°C	4-7 dpi, 7-8 dpi	(Mugimba et al., 2020; Chengula et al., 2022)
Hybrid tilapia (*Oreochromis niloticus* x *Oreochromis mossambicus*)	**TO-2** (primary cell lines)	Ovary	N/A	No CPE, cell does not support TiLV replication	(Kembou Tsofack et al., 2017)
	TK-1	Kidney	L-15, 10% FBS, 27°C	Swollen cell, vacuolated cell, detachment, and granulated cell on 5 dpi	(Chamtim et al., 2022)
Common carp (*Cyprinus carpio*)	**CCB**	Brain	M199, 10% FBS, 28°C	Cell shrinkage, cell detachment, and ballooning cell on 3 dpi	(Li et al., 2022a)
	CCKF	Fin	L-15, 15% FBS, 28°C	No CPE	(Thangaraj et al., 2018)
	EPC	ATCC CRL 2872	L-15 , 5% FBS, 25°C	No CPE	(Eyngor et al., 2014)
	KF-1		L-15, 5% FBS, 25°C	No CPE	(Eyngor et al., 2014)

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Grass carp (*Ctenopharyngodon idella*)	**CIK**	Kidney	M199, 10% FBS, 28°C	Cell aggregation, cell mass on 3 dpi	(Li et al., 2022a)
Goldfish (*Carassius auratus*)	**CrCB**	Brain	M199, 10% FBS, 28°C (Li et al., 2022a)	Cell shrinkage, cell detachment, and ballooning cell on 3 dpi	(Li et al., 2022a)
Fantail goldfish (*Carassius auratus auratus*)	**FtFG**	Fin	N/A	No CPE	(Thangaraj et al., 2018)
Chinook salmon (*Oncorhynchus tshawytscha*)	**CHSE-214**	ATCC CRL 1681	L-15, 5% FBS, 25°C	No CPE	(Eyngor et al., 2014)
Rainbow trout (*Salmo gairdneri*)	**RTG-2**	ATCC CCL 55	L-15, 5% FBS, 25°C	No CPE	(Eyngor et al., 2014)
Yellow catfish (*Horabagrus brachysoma*)	**HBF**	Fin	L-15, 15% FBS, 28°C	No CPE	(Thangaraj et al., 2018)
Fathead minnow (*Pimephales promelas*)	**FHM**	Fin	M199, 10% FBS, 28°C	Cell shrinkage, cell detachment, and ballooning cell on 3 dpi	(Li et al., 2022a)
		ATCC CCL 42 (Eyngor et al., 2014)	L-15, 5% FBS, 25°C	No CPE	(Eyngor et al., 2014)
Mandarin fish (*Siniperca chuatsi*)	**SS**	Brain	M199, 10% FBS, 28°C	Round and swollen cell on 2 dpi	(Li et al., 2022a)
Rare gudgeon (*Gobiocypris rarus*)	**GrE**	Epithelioid	M199, 10% FBS, 28°C	Cell shrinkage, cell detachment, and ballooning cell on 3 dpi	(Li et al., 2022a)
Zebrafish fry (*Danio rerio*)	**ZF4**		M199, 10% FBS, 28°C	Cell shrinkage, cell detachment, and ballooning cell on 5 dpi	(Li et al., 2022a)
Largemouth Bass (*Micropterus salmoides*)	**MSF**	Fin	M199, 10% FBS, 28°C	Cell shrinkage, cell detachment, and ballooning cell on 3 dpi	(Li et al., 2022a)
Bluegill (*Lepomis micropterus*)	**BF-2**	ATCC CCL 91	L-15, 5% FBS, 25°C	No CPE	(Eyngor et al., 2014)
Brown bullhead (*Ictalurus nebulosus*)	**BB**	ATCC CCL 59	L-15, 5% FBS, 25°C	No CPE	(Eyngor et al., 2014)
Malayan Leaffish (*Pristolepis fasciatus*)	**CFF**	Caudal fin	- L-15, 10% FBS, 28°C - L-15, 2% FBS, 28°C	- Cell elongate, rounding cell, cell detachment, and destruction of monolayer on 5-6 dpi - Elongate cell, rounding cell, and destruction cell on 3-7 dpi	(Behera et al., 2017; Thangaraj et al., 2018)
Pearlspot (*Etroplus suratensis*)	**PSF**	Caudal fin	L-15, 10% FBS, 28°C	No CPE	(Thangaraj et al., 2018)
Red-line torpedo (*Puntius denisonii*)	**RTF**	Fin	L-15, 10% FBS, 28°C	No CPE	(Thangaraj et al., 2018)
Freshwater angel fish (*Pterophyllum scalare*)	**AFF**	Caudal fin	L-15, 10% FBS, 28°C	Rounding cell, detachment of monolayer on 5-6 dpi	(Thangaraj et al., 2018)
Oscar (*Astronotus ocellatus*)	**AOF**	Fin	N/A	Plaque formation, cell granulation, elongated cell, rounding cell, and cell detachment on 5-6 dpi	(Thangaraj et al., 2018)

Molecular biology

Cycling temperature amplification

Cycling temperature amplification techniques remain the most widely established method of conventional molecular diagnostics for TiLV detection. These approaches utilize successive cycles of denaturation, primer annealing, and extension to exponentially amplify specific target viral nucleic acids. RT-PCR serves as one of the fundamental tools in molecular diagnostics. This method has been developed with a wide range of primer sets to detect all TiLV segments. Typically, after RT-PCR yielded amplification products, it would need to be visualized by agarose gel electrophoresis. Table 4 presents a summary of the

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primer lists of RT-PCR for TiLV detection that have been reported in previous studies. Moreover, RT-qPCR represents a significant advancement over conventional RT-PCR methods by allowing real-time monitoring of nucleic acid amplification and providing quantitative assessment of the viral load. Both SYBR® green-base and probe-base were used to identify TiLV in a previous study (Table 5). Additionally, nested and semi-nested RT-PCR assays have been widely employed to enhance the sensitivity and specificity of TiLV. In a nested RT-PCR system, the reaction is carried out over two rounds of amplification. The first round amplifies a broader region of the viral genome, and the second round uses a different set of primers that bind to sites within the initial product. While semi-nested RT-PCR follows a similar concept, it uses one primer from the first round together with a new internal primer for the second amplification. Both formats have been applied to conserved regions of the TiLV genome, segments 1 and 3 (Table 6), and have consistently shown similarly improved degrees of sensitivity over nested RT-PCR and exhibited better results than RT-qPCR (Dong et al., 2017b). Interestingly, multiplex RT-PCR has been explored as a practical approach for detecting TiLV with other important pathogens that commonly affect tilapia (Delamare-Debouteville et al., 2023). This method combines multiple primer sets into a single reaction and allows simultaneous amplification of several targets, which can reduce both the amount of time and the reagents that are required. Table 7 presents a list of primers that have been developed for TiLV detection by multiplex RT-PCR. Lastly, ddPCR has also gained attention as a powerful method for quantitative TiLV detection without the need of a standard sample (Mazaika and Homsy, 2014). A recent study has demonstrated that ddPCR could detect TiLV in both tissues and water samples with greater accuracy than traditional molecular assays of 100% sensitivity and specificity (Shahi et al., 2023).

Table 4 Summary of primer lists used in RT-PCR for TiLV detection

TiLV segment	Primer name	Primer sequence (5' - 3')	Product size (bp)	References
1	TiLV-Seg1-F	TCATTCGCCTATATAGTTAC	1614	(Dong et al., 2017b)
	TiLV-Seg1-R	TTAATTACGCACTATTACTG		
	Segment-1-FWD	CCTCATTCCTCGTTGTGTAAGT	1000	(Mugimba et al., 2018)
	Segment-1-REV	AGGAGTTGCTGTTGGGTTATAG		
	TiLV-S1-F	ATGTGGGCATTTCAAGAA	1560	(Pulido et al., 2019)
	TiLV-S1-R	TTAGCACCCAGCGGTGGGCT		
	SpecificTiLV-F	CAGGGAGAAAGCCCACTGAG	500	(Chamtim et al., 2022)
	SpecificTiLV-R	CTCCAATTGACCCCATGCCT		
	TiLV-F3-1	CTGACTTGGGATTGCCACC	203	(Yin et al., 2019)
	TiLV-B3-1	TGCCTAGCTGCTCTGATCT		
	CS1F	GCAAATATTTCTCTCATTCGCCTAT	1641	(Delamare-Debouteville et al., 2023)
	CS1R	CCAAACGTTATCTCTTAATTACGCA		
2	Segment-2-FWD	GTCCAGGGCGGTATGTATTG	834	(Mugimba et al., 2018)
	Segment-2-REV	CTTACGGCTGACAAGTCTCTAAG		
	TiLV-S2-F	ATGAGTCAGTTTGGGAAA	1374	(Pulido et al., 2019)
	TiLV-S2-R	CTACTGATTTAGATCCATAT		
	CS2F	GCAAATCTTTCTCATTATTACCATA	1471	(Delamare-Debouteville et al., 2023)
	CS2R	CCAAATTTTACTCTCTATTACCAAA		
3	ME1	GTTGGGCACAAGGCATCCTA	250	(Eyngor et al., 2014; Behera et al., 2017; Kembou Tsofack et al., 2017; Nicholson et al., 2017; Senapin et al., 2018; Acharya et al., 2019; Dinh-Hung et al., 2021)
	7450/150R	TATCACGTGCGTACTCGTTCAGT		
	Nested ext-1	TATGCAGTACTTTCCCTGCC	491	(Eyngor et al., 2014; Behera et al., 2017; Kembou Tsofack et al., 2017; Tattiyapong et al., 2017; Nanthini et al., 2019; Nicholson et al., 2020; Pierezan et al., 2020; Contreras et al., 2021; Yamkasem et al., 2021b)
	Nested ext-2	TTGCTCTGAGCAAGAGTACC		
	Segment-3-FWD	GTCGAGGCATTCCAGAAGTAAG	834	(Mugimba et al., 2018)
	Segment-3-REV	GAGCTAAGGGAACGGCTATTG		
	TiLV-112F	CTGAGCTAAAGAGGCAATATGGATT	300	(Liamnimitr et al., 2017)

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	Nested ext-1	TATGCAGTACTTTCCCTGCC		
	TiLV-S3-F	ATGGACTCGCGGTTCGCA	1260	(Pulido et al., 2019)
	TiLV-S3-R	TTATCTCGCAAATGGGTGTA		
	Nested ext-1	TATGCAGTACTTTCCCTGCC	415	(Skornik et al., 2021)
	ME1	GTTGGGCACAAGGCATCCTA		
	CS3F	GCAAATTTTTCCCATAATCCTCTAT	1371	(Delamare-Debouteville et al., 2023)
	CS3R	CCAAATATTACCCCTTAATCCTTAA		
4	S4-F or TiLV-D-F	AGCAGCAGCAGGAGAAAGAG	358	(Nicholson et al., 2017; He et al., 2022)
	S4-R or TiLV-D-R	ACCGTCCTGTTTCTGAATGG		
	Segment-4-FWD	GCCTACTTCGTTGCCTATCTC	524	(Mugimba et al., 2018)
	Segment-4-REV	GCCCAATGGTTCCCATATCT		
	TiLV-S4-F	ATGGTGAGAACTACAAAG	1065	(Pulido et al., 2019)
	TiLV-S4-R	CTATCTTCCAACAGCCCCCG		
	CS4F	GCAAATCTTTCTCCAATTACCGTCT	1250	(Delamare-Debouteville et al., 2023)
	CS4R	CCAAAGTTTACTCCTATTACCCAGA		
5	TiLV-Seg5-F	TTTTTCTCAGTTTACCACTC	1073	(Dong et al., 2017b)
	TiLV-Seg5-R	TTATCTCAGACTCCAATAGC		
	Segment-5-FWD	CAACTCTTAGCCTCCGGAATAC	696	(Mugimba et al., 2018)
	Segment-5-REV	CGTTCTGCACTGGGTTACA		
	TiLV-S5-F	ATGTTTCTCTTATCTCAG	1032	(Pulido et al., 2019)
	TiLV-S5-R	TCAAGATAATGGAAGCAGAG		
	CS5F	GCAAATTTTACTCTTTTTCTCAGTT	1099	(Delamare-Debouteville et al., 2023)
	CS5R	CCAAATGTTTCTCTTATCTCAGACT		
6	Segment-6-FWD	CCCACACGACAGGACATATAG	948	(Mugimba et al., 2018)
	Segment-6-REV	GAGTTGGCTTAGGGTGATAAGA		
	TiLV-S6-F	ATGCATTTTTATCTACAG	954	(Pulido et al., 2019)
	TiLV-S6-R	TCACATGTATTTATTGATTT		
	CS6F	GCAAATATTTCTCTCAATCAAGCAC	1044	(Delamare-Debouteville et al., 2023)
	CS6R	CCAAATTTTACCTCTCGCATGCATT		
7	Segment-7-FWD	TCCTTTAGGGATTGGCACTAAC	486	(Mugimba et al., 2018)
	Segment-7-REV	TTCCATCGACTGCTCCTAGA		
	TiLV-S7-F	ATGTCCTACAAGATTGGT	588	(Pulido et al., 2019)
	TiLV-S7-R	TTAGAGTTCAAATGTGATCC		
	CS7F	GCAAATCTTTCTCTCATGCTACCAT	777	(Delamare-Debouteville et al., 2023)
	CS7R	CCAAATTTTACTCTCTTTGCATTGC		
8	Segment-8-FWD	CTTAAGGGCCATCCTGTCATC	476	(Mugimba et al., 2018)
	Segment-8-REV	TGGCTCAAATCCCAACACTAA		
	TiLV-S8-F	ATGGCTCAAATTCCAACA	525	(Pulido et al., 2019)
	TiLV-S8-R	TTATTTAAGCATTTCACGGA		
	CS8F	GCAAATTTTTCTCATCATTACACAA	657	(Delamare-Debouteville et al., 2023)
	CS8R	CCAAATATTACCTCATCTACACTAA		
9	S9-F	TTGGTGATGTCACGATGGATA	351	(Nicholson et al., 2017)
	S9-R	AGTTCTATCGCCAGCCATGT		
	TiLV-Seg9-F	ACGTCCTTAAAGTCATACTT	522	(Dong et al., 2017b; Kampeera et al., 2021)
	TiLV-Seg9-R	ACAAGTCCGATTACTTTTTC		
	Segment-9-FWD	GATATCCTCCACATGACCCTTC	261	(Mugimba et al., 2018)
	Segment-9-REV	GTACGTCACTTTGTGCCATTAC		
	TiLV-S9-F	ATGTCACGATGGATAGAA	351	(Pulido et al., 2019)
	TiLV-S9-R	TCATAAAGTTTTATCGCCAG		
	CS9F	GCAAATCTTTCTCACGTCCTTAAAG	548	(Delamare-Debouteville et al., 2023)
	CS9R	CCAAATTTTACTCACAAGTCCGATT		
10	Segment-10-FWD	TCCTCTCTGTCCCTTCTGTT	276	(Mugimba et al., 2018)
	Segment-10-REV	CAGGATGAGTGTGGCAGATTAT		
	TiLV-S10-F	ATGAGTGTGGCAGATTAT	342	(Pulido et al., 2019)
	TiLV-S10-R	CTAAGACTGCACGTCAAGAG		
	CS10F	GCAAATCTTTCCCTCTGACACC	465	(Delamare-Debouteville et al., 2023)
	CS10R	CCAAATTTTAACCCTACTAACACCA		

Isothermal amplification

Isothermal amplification techniques have gained considerable attention for TiLV detection that avoid thermal cycling. These techniques can be performed with simpler equipment and are suited for on-farm or low-resource settings. RT-LAMP is one of the earliest isothermal methods that has been applied to TiLV (Phusantisampan et al., 2019; Yin et al., 2019; Kampeera et al., 2021). Typically, it uses four to six primers to amplify the viral genome at a single temperature, specifically 63°C (Yin et al., 2019; Kampeera et al., 2021) or 65°C (Phusantisampan et al., 2019). RT-LAMP can produce large quantities of DNA within one hour and can be visualized by turbidity or colorimetric changes. In addition, a colorimetric LAMP (cLAMP) has been generated for visual detection via both the naked eye and

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through AI-based technology (Jaroenram et al., 2023). A summary of primer lists has been presented in Table 8.

RT-RPA represents another rapid and low-temperature system that typically operates between 37-42°C (Zhao et al., 2024), which can detect target genomic DNA in less than 20 minutes. A more recent adaptation, RT-qRPA has been previously studied (Wang et al., 2021). It was developed to allow researchers to compare RT-qRPA with RT-qPCR in the quantification of TiLV. A summary of primer lists of RT-RPA and RT-qRPA are presented in Table 9. To further enhance specificity, CRISPR-assisted methods such as RT-RPA-Cas12a, have been introduced for TiLV detection (Sukonta et al., 2022). By combining the rapid amplification of RPA with the sequence-discriminating ability of CRISPR, RT-RPA-Cas12a minimizes false positives and provides researchers with a highly sensitive diagnostic platform (Sukonta et al., 2022).

Table 5 Summary of primer lists used in RT-qPCR for TiLV detection.

TiLV segment	Primer name	Primer sequence (5' - 3')	Product size (bp)	References
SYBR Green-based RT-qPCR				
1	TiLV-SG1-F1	GTCGCTGAAAGACAGGAATCT	97	(Chengula et al., 2022)
	TiLV-SG1-R1	AGTAGAGTGTCCAGGAGGTATG		
2	TiLV-SG2-F1	GAACCTGGCCCACAACATA	101	
	TiLV-SG2-R1	GGGTACACTTTGGCTGATAGAG		
3	TiLV-112F	CTGAGCTAAAGAGGCAATATGGATT	112	(Liamnimitr et al., 2017; Jaemwimol et al., 2018; Tattiyapong et al., 2018; Phusantisampan et al., 2019; Thammatorn et al., 2019; Nicholson et al., 2020; Pierezan et al., 2020; Skornik et al., 2021; Waiyamitra et al., 2021; Yamkasem et al., 2021a; Yamkasem et al., 2021b; Turner et al., 2023; Valsalam et al., 2024)
	TiLV-112R	CGTGCGTACTCGTTCAGTATAAGTTCT		
	ME1	GTTGGGCACAAGGCATCCTA	250	(Kembou Tsofack et al., 2017)
	7450/150R/ME2	TATCACGTGCGTACTCGTTCAGT		
	TiLV-SG3-F4 or TiLV seg3	TCCAGATCACCCTTCCTACTT	109	(Mugimba et al., 2018; Chengula et al., 2019; Mugimba et al., 2020; Chengula et al., 2022)
	TiLV-SG3-R4	ATCCCAAGCAATCGGCTAAT		
4	TiLV-SG4-F4	GCGCAGTATTCAGTCCATCT	96	(Chengula et al., 2022)
	TiLV-SG4-R4	GAGGGAGAACGCTAAGAAATCA		
5	TiLV-SG5-F2	GATAATGGAAGCAGAGGGACTT	110	
	TiLV-SG5-R2	GTATTCCGGAGGCTAAGAGTTG		
6	TiLV-SG6-F3	ACGCTTGACGTGTAGTTTGA	119	
	TiLV-SG6-R3	ATGAGTTGGCTTAGGGTGATAAG		
7	TiLV-SG7-F1	CCAGGTTGTGCCATTTGTATTG	96	
	TiLV-SG7-R1	TTCCATCGACTGCTCCTAGA		
8	TiLV-SG8-F2	GCCTTTCCTCTATCCGCTTTC	110	
	TiLV-SG8-R2	TCCGGATTTGCTTTCCCTAAC		
9	TiLV-SG9-F3	CCCTATCACCATTGCACTCTC		
	TiLV-SG9-R3	CATCACAACCTGGTCAAGTCT	119	
10	TiLV-SG10-F2	CAGAATCACAGTCGTCCATCTC	101	
	TiLV-SG10-R2	GCGAAGTTGGAAGAATGAAAGG		
NA	TiLV-Q-F	AGTGTGACAGTCGACGCAAT	128	(He et al., 2022)
	TiLV-Q-R	TCGACGCAGTTAATCCCAGG		
Probe-based RT-qPCR				
1	TiLV-S1-qF	CAA GTC AGT AGA GAT TGA AAG CT	N/A	(Delamare-Deboutteville et al., 2023)
	TiLV-S1-qR	CCC ACT TAC ACA ACG AGG AAT		
	TiLV-S1-qProbe	6-FAM-TTC CCT CCC AGG CAT GTG GGG A-BHQ-1		
3	TiLV-93F	AGCCTGCCACACAGAAG	N/A	(Waiyamitra et al., 2018)
	TiLV-93R	CTGCTTGAGTTGTGCTTCT		
	TiLV-93Probe	FAM-CTCTACCAGCTAGTGCCCCA-Iowa Black		
3	TiLV-F	AGCCTGCCACACAGAAG	N/A	(Rakus et al., 2020)
	TiLV-R	CTGCTTGAGTTGTGCTTCT		
	TiLV-P	FAM-CTCTACCAGCTAGTGCCCCA-BHQ		
9	TiLV-S9-qF	CTAGACAATGTT TTCGATCCAG	N/A	(Taengphu et al., 2020; Delamare-Deboutteville et al., 2023)
	TiLV-S9-qR	TTCTGTGTCAGTAATCTTGACAG		
	TiLV-S9-qP	FAM-TGCCGCCGCAGCACA AGCTCCA-BHQ		

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N/A	TiLV-F	GGCAAGAAAGCTGCTTCAAAGA	64	(Megarani et al., 2022)
	TiLV-R	CGCTCTCGTCAGCACCATAC		
	TiLV-P	CGAAGTTGGAAGAATG		
N/A	F10	AATGCTTCTCATCTCATGTAGTCCAGGCAC	147	(Wang et al., 2021)
	R10	TTTTGCCAAGAAACCTACTGATTCAGATCC		
	T1	GGTGGCAGTGCAGGAGGATCGCTTTAAGGA(BHQ)(THF)(FAM)GAGGGTTGAGAATTT		
N/A	Primer1	GACTGCAGCTATGTTATCTGG	N/A	(Li et al., 2022a)
	Primer2	TGGTGTAAGTGGGGTTGTT		
	Probe	AAGCAGCAGGAATGTGCCTAT		

Table 6 Summary of primer lists used in Nested and Semi-Nested RT-PCR for TiLV detection.

TiLV segment	Round	Primer name	Primer sequence (5' - 3')	Product size (bp)	References
Nested RT-PCR					
3	1	Nested ext-1	TATGCAGTACTTTCCCTGCC	491	(Kembou Tsofack et al., 2017)
		Nested ext-2	TTGCTCTGAGCAAGAGTACC		
	2	ME1	GTTGGGCACAAGGCATCCTA	250	
		7450/150R/ME2	TATCACGTGCGTACTCGTTCAGT		
Semi-nested RT-PCR					
1	1	TiLV/nSeg1F	TCTGATCTATAGTGTCTGGGCC	620	(Dong et al., 2017b; Debnath et al., 2020; Taengphu et al., 2020)
		TiLV/nSeg1R	AGTCATGCTCGCTTACATGGT		
	2	TiLV/nSeg1F	TCTGATCTATAGTGTCTGGGCC	274	
		TiLV/nSeg1RN	CCACTTGTGACTCTGAAACAG		
3	1	Nested ext-1	TATGCAGTACTTTCCCTGCC	415	(Dong et al., 2017b; Senapin et al., 2018; Chiamkunakorn et al., 2019; Pulido et al., 2019; Basri et al., 2020; Debnath et al., 2020; Li et al., 2022b; Tran et al., 2022; Valsalam et al., 2023)
		ME1	GTTGGGCACAAGGCATCCTA		
	2	7450/150R/ME2	TATCACGTGCGTACTCGTTCAGT	250	
		ME1	GTTGGGCACAAGGCATCCTA		

Table 7 Summary of primer lists used in Multiplex RT-PCR for TiLV detection

TiLV segment	Primer name	Primer sequence (5' - 3')	Product size (bp)	References
Multiplex RT-PCR (mPCR)-Ta 52°C				
1	CS1F	GCAAATATTTCTCTCATTCGCCTAT	1641	(Delamare-Deboutteville et al., 2023)
	CS1R	CCAAACGTTATCTCTTAATTACGCA		
2	CS2F	GCAAATCTTTCTCATTATTACCATA	1471	
	CS2R	CCAAATTTTACTCTCTATTACCAAA		
3	CS3F	GCAAATTTTTCCCATAATCCTCTAT	1371	
	CS3R	CCAAATATTACCCCTTAATCCTTAA		
4	CS4F	GCAAATCTTTCTCCAATTACCGTCT	1250	
	CS4R	CCAAAGTTTACTCCTATTACCCAGA		
5	CS5F	GCAAATTTTACTCTTTTTCTCAGTT	1099	
	CS5R	CCAAATGTTTCTCTTATCTCAGACT		
8	CS8F	GCAAATTTTTCTCATCATTACACAA	657	
	CS8R	CCAAATATTACCTCATCTACACTAA		
Multiplex RT-PCR (mPCR)-Ta 60°C				
6	CS6F	GCAAATATTTCTCTCAATCAAGCAC	1044	(Delamare-Deboutteville et al., 2023)
	CS6R	CCAAATTTTACCTCTCGCATGCATT		
7	CS7F	GCAAATCTTTCTCTCATGCTACCAT	777	
	CS7R	CCAAATTTTACTCTCTTTGCATTGC		
9	CS9F	GCAAATCTTTCTCACGTCCTTAAAG	548	
	CS9R	CCAAATTTTACTCACAAGTCCGATT		
10	CS10F	GCAAATCTTTCCCTCTGACACC	465	
	CS10R	CCAAATTTTAACCCTACTAACACCA		

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Table 8 Summary of primer lists used in RT-LAMP for TiLV detection

Segment	Primer name	Primer sequence (5' - 3')	Length (bp)	References
1	TiLV-F3-1	CTGACTTGGGATTGCCACC	19	(Yin et al., 2019)
	TiLV-B3-1	TGCCTAGCTGCTCTGATCT	19	
	TiLV-FIP-1	CTTCTGCGAGTCTTTGAGGCACCTGATGGCCCAGACACTA	40	
	TiLV-BIP-1	AGAACCCGACTAGAGGCTCTCTCTGACACGAGGAGCCTATG	41	
	TiLV-LF-1	AGGACAGACAGGACTTTCTGATC	23	
	TiLV-LB-1	CAGACTTGGACCCCCTGGT	19	
3 (external)	TiLV-F3	GGGCACAAGGCATCCTAC	18	(Phusantisampan et al., 2019)
	TiLV-B3	AGACCACACTCCTCACCG	18	
3 (internal)	TiLV-FIP	CGATACAAGGCTTCGGGCCGGATGCTGAGCTGAGGGAAC	39	
	TiLV-BIP	GGTGGCACCACCCAGACTTGAGAG CACTCGAAGAACCCA	39	
9	F3	TTCCGTGCTCCGTTACTA	18	(Kampeera et al., 2021; Jaroenram et al., 2023)
	B3	TTGACAGAGATGAGTCGGT	19	
	FIP	GAGTTAATGACACTGAGTCAGCCA/TTTT/GGACCCAGCTCCCTATC	47	
	BIP	CAGACTTGACCAGGTTGTGATGT/TTTT/TCACTTTGTGCCATTACCC	48	
	LF	GCAGGAGAGTGCAATGGT	18	
	LB	AGCACAAGCTCCAAGGG	17	

Table 9 Summary of primer lists used in RT-RPA and RT-qRPA TiLV detection

TiLV segment	Primer name	Primer sequence (5' - 3')	Product size (bp)	References
RT-RPA				
9	F2	GAGATGAGTCGGTCGTACGTCACTTTGTGC	130	(Sukonta et al., 2022)
	R1	AGATCTCTGAACATTCTAGACAATGTTTTC		
RT-qRPA				
N/A	F10	AATGCTTCTCATCTCATGTAGTCCAGGCAC	147	(Wang et al., 2021)
	R10	TTTTGCCAAGAAACCTACTGATTCAGATCC		
	T1	GGTGGCAGTGCAGGAGGATCGCTTTAAGGA(BHQ)(THF)(FAM)GAGGGTTGAGAATTT		

Immunological methods

Indirect Enzyme-Linked Immunosorbent Assay (iELISA)

Accordingly, iELISA has been applied as an immunological approach to detect antibodies against TiLV. The study conducted by Hu (2019) reported on the determination of an anti-TiLV antibody from the S8 coated protein that had been obtained from serum samples with 100% sensitivity and specificity. Recently, a study conducted by Valsalam (2023) reported on the development of iELISA to detect the TiLV antigen in tilapia tissues and mucus with a degree of accuracy of 73.28%.

Immunohistochemistry (IHC)

Immunological diagnostic approaches have been used to exploit antibody-antigen interactions to detect viral components in clinical specimens, particularly those present in tissue sections. This technique, combined with labeled antibodies to TiLV viral proteins and positive tissues samples, will show the immunopositively signals that are visible under light microscopy in relevant chromogenic color. Furthermore, this technique typically uses 3,3-diaminobenzidine (DAB). According to a study conducted by Piewbang (2021), the production of the anti-TiLV antibody was demonstrated and used to identify TiLV protein in histopathological tissue sections under IHC, while the results confirm the tissue tropism of TiLV in the liver, pancreas, kidneys, gill, intestines, brain, and spleen.

Immunofluorescence (IF)

This technique employs fluorescently labeled antibodies directed against TiLV viral proteins. It is an immunological technique used to detect and localize TiLV within infected cells and tissues. IF has been used to successfully identify in TiLV infections in certain cell cultures, such as TiB cell (Wang et al., 2018), S8 protein (Hu et al., 2019), CAMB cells (Wang et al., 2020), and protein 4 in OmB cells (Abu Rass et al., 2022).

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Viral neutralization assay (VN)

This immunoassay method is based on the principle that neutralized antibodies can bind to viral particles and can then be used in the determination of the quantitation of antibodies (Savini et al., 2024). Recently, the development of invitro neutralization assay using IgY antibodies against TiLV segment 4 protein has been reported (Setthawong et al., 2025)

Rapid tests

Rapid diagnostic methods have become increasingly important for the detection of TiLV. RT-RPA has emerged as one of the most promising rapid amplification platforms due to its ability to operate at low temperatures and produce a detectable target genome within minutes. When combined with LFD detection, RT-RPA provides a simple, equipment-light assay that is suitable for on-site use and avoids the need for electrophoresis, making it practical for field laboratories or hatcheries with limited diagnostic capacity. Moreover, CRISPR-based methods such as RT-RPA-Cas12a-LFD have been indicated for more specificity. This combination of rapid amplification and CRISPR-mediated sequence recognition helps reduce the false-positive results that can commonly be associated with isothermal methods. In addition, RT-PCR-LFD has been developed along with RT-RPA-LFD and exhibited 100% sensitivity and specificity (Srivorakul et al., 2025). Recently, Jaroenram (2023) published a report on a new cLAMP assay that has been mentioned in isothermal molecular biology for the detection of TiLV DNA by naked eye visualization under an AI-based model. This study carried 96% sensitivity accuracy, while the results could be readout within 1 hour. Presently, RT-RPA-Cas12a-LFD (Sukonta et al., 2022), RT-PCR-LFD, RT-RPA-LFD (Srivorakul et al., 2025), and cLAMP (Jaroenram et al., 2023) represent alternative choices for rapid diagnosed TiLV.

DISCUSSION AND CONCLUSIONS

A broad spectrum of diagnostic tools has been developed for TiLV, reflecting the complexity of this disease and the diverse needs of aquaculture systems (Table 10). In Table 11, previous research studies conducted on TiLV diagnosis have been grouped and have demonstrated the progressive development of relevant research. This would include the initial discovery of, and the basic diagnostic approaches for, various advanced technologies and their integration in field applications. Traditional diagnostic approaches, including gross pathology and histopathology, continue to play an important role in initial outbreak investigations. Gross pathology offers a rapid, low-cost assessment that can reveal characteristic external and internal lesions, such as those appearing as incidences of skin erosion, ocular abnormalities, and hepatic necrosis. However, these lesions are not specific to TiLV and may overlap with bacterial and parasitic diseases, limiting their value for confirmation. Histopathology provides deeper insight into tissue-level damage, such as syncytial hepatitis, neuronal degeneration, and renal tubular necrosis, but it remains a time-consuming process and requires experienced personnel. Although these techniques are indispensable for characterizing disease manifestations, they cannot independently confirm TiLV infection. Accordingly, other diagnostic tools may need to be confirmed.

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Table 10 TiLV diagnosis methodology comparison

Diagnostic methods	Sensitivity	Specificity	LOD	Approximate operating time	Advantages	Limitation
Gross pathology	-	-	-	1-2 hours	Fast, low cost	Require differential diagnosis, non-specific lesion, cannot confirm TiLV infection, well-trained personnel
Histopathology	-	-	-	1-2 days	Revealed cellular changes of TILV infected tissues	Require tissue processing, suggestive diagnosis, cannot confirm TiLV infection, well-trained personnel
ISH	-	Positive signal of ISH to infected tissues	-	1-2 days	Localized TiLV tissue tropism	Well-trained personnel, time consuming, high-cost equipment and reagent
IHC	-	Positive signal of IHC to infected tissues	-	1-2 days	Localized TiLV tissue tropism	Well-trained personnel, require antibody optimization, time consuming, high-cost equipment and reagent, cannot confirm TiLV infection (Piewbang et al., 2021)
iELISA	80.8-100% (Hu et al., 2019), 85.33% (Valsalam et al., 2023)	51.22% (Valsalam et al., 2023)	-	2-3 hours	Immunological screening in farm, invasive sample screening (Valsalam et al., 2023), low-cost	Well-trained personnel, antibody/antigen optimization
IF	92.6% (Hu et al., 2019)	Positive signal of IF to infected tissues	-	1-2 days	Localized TiLV tissue tropism	Well-trained personnel, time consuming, high-cost equipment and reagent
Cell culture	Lower than Rt-qPCR 10,000 times (Tattiyapong et al., 2018)	-	-	4-12 dpi (Tattiyapong et al., 2017)	High sensitivity, viral propagation	Well-trained personnel, time consuming
RT-PCR	Lower than RT-qPCR 100 times (Tattiyapong et al., 2018) Lower that cLAMP 10 times (Jaroenram et al., 2023)	-	70,000 copies (Kembou Tsofack et al., 2017)	4-5 hours	High sensitivity, high specificity	High-cost equipment and reagent, well-trained personnel, primer optimization, time consuming, require heavy TiLV infected tissues
RT-qPCR	100% (Phusantisampan et al., 2019; Wang et al., 2021), 78.8% (Shahi et al., 2023)	100% (Wang et al., 2021)	62 copies (Wang et al., 2021), 70 copies/reaction (Dong et al., 2017b), 2 copies /ul (Tattiyapong et al., 2018)	3-4 hours	High sensitivity, high specificity, quantitative genome	High-cost equipment and reagent, well-trained personnel, primer optimization, primer-dimer formation
Nested RT-PCR	-	-	7 copies/reaction (Dong et al., 2017b)	4-5 hours	High sensitivity, high specificity, can detect TiLV in low molecules (Dong et al., 2017b)	High-cost equipment and reagent, well-trained personnel, primer optimization
Semi-nested RT-PCR	95.6% (Hu et al., 2019)		7.5 copies/reaction (Dong	4-5 hours	High sensitivity, high specificity	High-cost equipment and reagent, well-trained personnel, primer optimization

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			et al., 2017b)			
Multiplex RT-PCR	-	-	-	4-5 hours	Detection of several target genomes in one reaction (Delamare-Debouttéville et al., 2023)	Effective for high TiLV load samples (Delamare-Debouttéville et al., 2023), need separate gel electrophoresis due to different target size (Delamare-Debouttéville et al., 2023), primer optimization, high-cost equipment and reagent, limit concentration of cDNA template, well-trained personnel
ddPCR	100% (Shahi et al., 2023)	100% (Shahi et al., 2023)	3.3 copies/reaction (Shahi et al., 2023)	3-4 hours	High sensitivity, quantitative genome	High-cost equipment and reagent, limit concentration of cDNA template, well-trained personnel
RT-LAMP	100% (Yin et al., 2019), 80.95% (Phusantisampan et al., 2019)	100% (Yin et al., 2019)	1.6 copies/reaction (Yin et al., 2019)	45-60 minutes (Phusantisampan et al., 2019; Yin et al., 2019)	Fast, high sensitivity, on-site application, naked eye visualization	Require four to six primers
RT-RPA	-	-	-	30-40 minutes	Fast, high sensitivity, simple equipment for amplification	Require primer optimization
RT-qRPA	93.33% (Wang et al., 2021)	100% (Wang et al., 2021)	620 copies (Wang et al., 2021)	30 minutes (Wang et al., 2021)	Fast, high sensitivity	Require primer optimization, high-cost equipment and reagent
RT-RPA-Cas12a	-	No cross-react with other diseases (Sukonta et al., 2022)	200 copies (Sukonta et al., 2022)	1 hour	Fast, simple equipment for amplification, naked eye visualization	Require primer optimization, false-negative results, less sensitivity than nested RT-PCR, RT-qPCR, and RT-LAMP
RT-RPA-Cas12a-LFD	-	No cross-react with other diseases (Sukonta et al., 2022)	200 copies (Sukonta et al., 2022)	1 hour	Fast, on-site application, simple equipment for amplification, naked eye visualization, suitable for resource-limited post amplification detection	Require primer optimization, false-negative results, less sensitivity than nested RT-PCR, RT-qPCR, and RT-LAMP
cLAMP	96% (Jaroenram et al., 2023)	No cross-react with other diseases (Jaroenram et al., 2023)	1 copy/reaction (Jaroenram et al., 2023)	1 hour	Fast, high sensitivity, high specificity, simple equipment for amplification, large-scale operation, naked eye visualization, suitable for resource-limited post-amplification detection	Cannot distinguish target from off-target DNA due to TqB pH sensitivity, high-resource settings
RT-RPA-LFD	100% (Srivorakul et al., 2025)	100% (Srivorakul et al., 2025)	3.19 copies/ul (Srivorakul et al., 2025)	1 hour	Fast, high sensitivity, simple equipment for amplification, naked eye visualization, suitable for resource-limited post-amplification detection	Require primer optimization, non-specific amplification

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Table 11 Clustering of TiLV research and diagnostic development

TiLV diagnostic development	Diagnostic approaches	Research focus
Initial characterization of TiLV	Gross pathology, histopathology, viral isolation on cell culture, RT-PCR	Discovery of a novel virus, characterization of TiLV, viral propagation, basic clinical signs, gross lesions and histopathology, basic molecular methods
Investigation of pathogenesis and basic diagnostics	Histopathology, ISH, IHC, RT-PCR, semi-nested PCR	Tissue tropism, route of transmission, improvement of basic detection methods
Advancement of diagnostic technologies	RT-qPCR, ddPCR, ELISA, IHC, RT-LAMP	Improvement of sensitivity, specificity, and rapid detection, development of quantitative high-sensitivity assays, validation of molecular and immunological methods
Application of diagnostic technologies in field settings	RT-RPA, RT-qRPA, cLAMP, RT-LAMP, CRISPR-Cas systems, LFD	Rapid, portable, and cost-effective detection methods, application of diagnostics in on-site aquaculture settings,

Diagnostic specificity can be strengthened through ISH, IHC, and IF, which enable visualization of TiLV within affected tissues. These methods clearly represent tissue tropism, particularly in the liver, brain, gills, and kidneys (Eyngor et al., 2014; del-Pozo et al., 2016; Behera et al., 2017; Tattiyapong et al., 2017; Pierezan et al., 2019; Debnath et al., 2020; Piewbang et al., 2021). Importantly, these methods require specialized equipment, high-cost reagents, and extensive assay optimization, making them less suited for rapid or field-based applications. Cell culture remains the gold standard for viral isolation and biological characterization by successfully propagating TiLV in certain cell cultures, such as E-11 (Eyngor et al., 2014) and TiB (Wang et al., 2018; Li et al., 2022b). Although they are invaluable for pathogenicity studies, vaccine development, and the confirmation of live viruses, the cell culture process is labor-intensive, slow (4-12 dpi) (Tattiyapong et al., 2017), and impractical for routine surveillance. These limitations hinder timely intervention during outbreaks and reduce feasibility for many diagnostic laboratories.

Molecular diagnostics continue to form the core of TiLV confirmatory testing. RT-PCR and RT-qPCR assays demonstrate high analytical performance, with RT-qPCR providing quantitative viral loads and detection limits as low as 2 copies/μL (Tattiyapong et al., 2018). Nested and semi-nested RT-PCR further increase sensitivity, enabling detection in samples with low viral burden (Dong et al., 2017b); however, this can occur at the expense of additional handling steps and a longer processing time. The emergence of multiplex RT-PCR has expanded diagnostic utility by enabling the simultaneous amplification of multiple viral segments within a single reaction. This strategy reduces reagent consumption and processing time, but assay performance depends heavily on primer compatibility and balanced amplification efficiency, particularly across diverse segment sizes. Multiplex RT-PCR performs best in samples with high viral loads and still requires downstream electrophoresis, limiting its overall rapidity. The ddPCR represents a significant advancement by offering absolute quantification without standard curves and perfect analytical specificity. With a limit of detection at approximately 3.3 copies/reaction (Shahi et al., 2023), ddPCR surpasses most conventional molecular methods. Despite its high precision, the cost of ddPCR platforms and the need for specialized laboratory infrastructure can restrict access to advanced facilities. The development of isothermal amplification methods has markedly accelerated progress toward field-deployable TiLV diagnostics. RT-LAMP is known for high sensitivity and rapid amplification within 45-60 minutes (Phusantisampan et al., 2019; Yin et al., 2019). However, the technique requires multiple primers when compared with other molecular methods. RT-RPA functions at lower isothermal temperatures (37-42°C) than RT-LAMP (63°C or 65°C) and can produce results within 20-30 minutes while requiring minimal equipment. However, nonspecific products and primer-dimer formation remain concerns. RT-qRPA improves quantification and maintains rapid performance, although the requirement for a portable fluorometer can add expense. CRISPR-enhanced methods such as RT-

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RPA-Cas12a and RT-RPA-Cas12a-LFD have introduced higher specificity by coupling RPA with Cas12a-mediated reporter cleavage. These assays allow naked-eye visualization, reduce incidences of false positives, and are adaptable to resource-limited settings. However, their sensitivity still falls below that of RT-qPCR, nested RT-PCR, and RT-LAMP, with reported detection limits of around 200 copies (Sukonta et al., 2022). Newer rapid tests such as RT-RPA-LFD and cLAMP further expand point-of-care applicability, offering high sensitivity, simple workflows, and results within one hour. However, TiLV, as a segmented RNA virus, exhibits sequence variation among geographically distinct isolates, which can lead to primer-template mismatches and reduced amplification efficiency. Therefore, assays targeting conserved regions or multiple genome segments are more suitable for surveillance and early detection. Notably, continuous updating and validation of primer sets across diverse strains are essential for maintaining diagnostic accuracy.

Effective control and surveillance of TiLV in aquaculture systems is very important. Diagnostic methods play a pivotal role in supporting biosecurity measures, particularly for the early detection and screening of broodstocks and fry populations. Immunological assays contribute to important complementary capabilities. The iELISA allows moderate-to-high throughput screening for exposure and immune responses, offering sensitivity values between 80.8% and 100% when compared with IFA and semi-nested RT-PCR, which had yielded value of 92.6% and 95.6%, respectively (Hu et al., 2019). Despite its advantages in sero-surveillance and herd-level monitoring, iELISA requires careful antigen standardization to avoid cross-reactivity. Other immunodiagnostics, including IHC, IF, and VN assays, have demonstrated significant value for tissue localization, antibody response characterization, and functional immunity assessment, yet they remain better suited to research laboratories due to specialized requirements.

Overall, the diagnostic landscape for TiLV illustrates a continuing shift from complex laboratory-based assays toward rapid, field-deployable molecular platforms. While each method has inherent strengths and limitations, ranging from sensitivity, specificity to cost, turnaround time and the practicality needed to allow each one to be integrated across multiple approaches, accurate diagnoses during outbreaks remain crucial for long-term surveillance programs. Therefore, the selection of appropriate diagnostic approaches should consider not only analytical performance but also their practicality and applicability within broader disease management frameworks.

In conclusion, the accurate and timely diagnosis of TiLV remains essential for effective disease control in Tilapia aquaculture. Conventional methods such as gross pathology, histopathology, and cell culture provide foundational insights into disease expression and viral biology, yet their diagnostic power is constrained by low specificity, long processing times, and high labor demands. Molecular assays including RT-PCR, RT-qPCR, nested/semi-nested RT-PCR, multiplex PCR, and ddPCR are continuing to anchor confirmatory diagnosis due to their high analytical sensitivity and specificity. Herein, ddPCR demonstrated excellent performance for quantitative detection. At the same time, rapid isothermal platforms such as RT-LAMP, RT-RPA, RT-qRPA, and CRISPR-assisted RT-RPA-Cas12a are expanding diagnostic accessibility by enabling faster detection with minimal equipment, making them well suited for farm-level screening and early outbreak response. Integrating multiple diagnostic tools, that are supported by proper sample selection, and a clear understanding of each method's capabilities, can offer the most reliable strategy for TiLV detection across different epidemiological contexts. Continued refinement of rapid tests, coupled with improved standardization of molecular and serological protocols, will be critical for strengthening global TiLV surveillance, thereby mitigating the disease's potential impact on aquaculture.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this manuscript.

AUTHOR CONTRIBUTIONS

Saralee Srivorakul: Conceptualization (lead); Writing – Original Draft (lead); Data Curation (equal); Writing – Review and Editing (equal).
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