UNITED STATES OF AMERICA – COMMENTS

Annex 25. Item 9.2.1. – Chapter 2.3.11. 'Infection with Tilapia lake virus'

United States	**Category:** general We thank the Commission for its work in developing this new manual chapter. Many of our proposed edits are intended to enhance clarity and to ensure that the level of detail provided is consistent with that of other pathogen-specific chapters in the Manual.

CHAPTER 2.3.11.

INFECTION WITH TILAPIA LAKE VIRUS (TILV)

1. Scope

Infection with Tilapia lake virus (TiLV) means infection with the pathogenic agent *Tilapinevirus* of the family Amnoonviridae.

United States	**Category:** change **Proposed amended texts (or precise suggested deletion):** Please see the proposed change in blue – "Infection with ~~Tilapia~~ tilapia lake virus (TiLV) means infection with the pathogenic agent *Tilapinevirus tilapiae* of the family Amnoonviridae." **Rationale:** These revisions are necessary because "tilapia" should not be capitalized in the virus's common name, and the scientific name should be updated to "*Tilapinevirus tilapiae*" because the ICTV adopted the requirement that all viral species names follow a binomial format (Genus species) around 2021-2023; therefore, the correct species name should be *tilapiae* (https://ictv.global/taxonomy/taxondetails?taxnode_id=202505391&taxon_name=Tilapinevirus%20tilapiae).

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

First described by Eyngor *et al.* (2014), TiLV was originally identified as an orthomyxo-like virus tentatively assigned, because of its similarity, to the family Orthomyxoviridae (Bacharach *et al.,* 2016). ICTV (2023) classified it as species *Tilapinevirus tilapiae*, genus Tilapinevirus, within a new family, *Amnoonviridae* (Realm Riboviria, kingdom Orthornavirae, phylum Negarnaviricota, class Insthoviricetes, order Articulavirales).

United States	**Category:** change

	Proposed amended texts (or precise suggested deletion): Please see the proposed changes in blue – "First described by Eyngor *et al.* (2014), TiLV was originally identified as an orthomyxo-like virus tentatively assigned, because of its similarity, to the family Orthomyxoviridae (Bacharach *et al.,* 2016). The International Committee on Taxonomy of Viruses (ICTV) ~~(2023)~~ formally classified it as ~~species~~ *Tilapinevirus tilapiae*, ~~genus Tilapinevirus,~~ within a new family, *Amnoonviridae* (2023)~~(Realm Riboviria, kingdom Orthornavirae, phylum Negarnaviricota, class Insthoviricetes, order Articulavirales)~~." **Rationale:** We consider these revisions necessary to enhance clarity and to ensure that the level of detail remains consistent with other pathogen-specific chapters in the Manual. The current text provides a level of taxonomic detail (for example, realm, kingdom, etc.) that is not included in other chapters.

Each genomic segment encodes a single open reading frame (ORF). RNA1 (1641 nt) encodes a protein with an RNA-dependant RNA polymerase (RdRP) domain homologous to the PB1 of influenza C virus (Acharya *et al.,* 2019; Bacharach *et al.,* 2016; Ortiz-Baez *et al.,* 2020). RNA2 (1471 nt) and RNA3 (1371 nt) ORFs encode the PB2 and PA polymerase subunit homologs of orthomyxoviruses (Arragain *et al.,* 2023) and RNA4 (1250 nt) encodes a nucleoprotein (Abu Rass *et al.,* 2022a). The identities and functions of proteins encoded by the remaining segments RNA5: 1099 nt; RNA6: 1044 nt; RNA7: 777 nt; RNA8: 657 nt; RNA9: 548 nt; and RNA10: 465 nt (Bacharach *et al.*, 2016) are unknown.

Virions are spherical, pleomorphic, enveloped, of 55–100 nm in diameter and contain 10 segments of linear negative sense RNA of varying lengths totalling approximately 10.3 kb (Bacharach *et al.,* 2016; Eyngor *et al.,* 2014).

The replication cycle of TiLV is not currently known. All segments have conserved, complementary sequences at their 5'- and 3'-ends, similar to those found in orthomyxoviridae genome sequences (Bacharach *et al.,* 2016). TiLV does not hemagglutinate red blood cells, suggesting that entry into host cells is different to orthomyxoviruses which do (Chengula *et al.,* 2019). It is also known that entry occurs via dynamin-mediated endocytosis in a cholesterol-dependent, cytoskeleton-dependent manner that is independent of clathrin and pH (Abu Rass *et al.,* 2022b). Reassortment of genomic segments has been observed on several occasions (Abbadi *et al* 2023; Chaput *et al.,* 2020; Verma *et al.,* 2022).

United States	**Category:** change **Proposed amended texts (or precise suggested deletion):** Please see the proposed changes to the above 3 paragraphs in blue – "Virions are spherical, pleomorphic, enveloped, of 55–100 nm in diameter and contain 10 segments of linear negative sense RNA of varying lengths totalling approximately 10.3 kb (Bacharach *et al.,* 2016; Eyngor *et al.,* 2014). All segments have conserved, complementary sequences at their 5'- and 3'-ends, similar to those found in orthomyxoviridae genome sequences (Bacharach *et al.*, 2016). Reassortment of genomic segments has been reported on several occasions (Abbadi *et al* 2023; Chaput *et al.*, 2020; Verma *et al.*, 2022). Each genomic segment encodes a single open reading frame (ORF). RNA segments are numbered based on segment size with the largest segment being segment 1. Segment 1 ~~(1641 nt)~~ encodes a protein with an RNA-dependant RNA polymerase (RdRP) domain homologous to the PB1 of influenza C virus (Acharya *et al.*, 2019; Bacharach *et al.*, 2016; Ortiz-Baez *et al.*, 2020). ~~RNA2 (1471 nt) and RNA3~~Segment 2 and segment 3 ~~(1371 nt)~~ ORFs encode the PB2 and PA polymerase subunit homologs of orthomyxoviruses (Arragain *et al.*, 2023). ~~RNA4~~ Segment 4 ~~(1250 nt)~~ encodes a nucleoprotein (Abu Rass *et al.*, 2022a). The identities and functions of proteins encoded by the remaining segments 5 through 10~~RNA5: 1099 nt; RNA6:~~

	~~1044 nt; RNA7: 777 nt; RNA8: 657 nt; RNA9: 548 nt; and RNA10: 465 nt~~ (Bacharach *et al*., 2016) are unknown. ~~Virions are spherical, pleomorphic, enveloped, of 55–100 nm in diameter and contain 10 segments of linear negative-sense RNA of varying lengths totalling approximately 10.3 kb (Bacharach *et al*., 2016; Eyngor *et al*., 2014).~~ The replication cycle of TiLV is not currently known.~~ All segments have conserved, complementary sequences at their 5'- and 3'-ends, similar to those found in orthomyxoviridae genome sequences (Bacharach *et al*., 2016). TiLV does not hemagglutinate red blood cells, suggesting that entry into host cells is different to orthomyxoviruses which do (Chengula *et al*., 2019). It is also known that entry occurs via dynamin-mediated endocytosis in a cholesterol-dependent, cytoskeleton-dependent manner that is independent of clathrin and pH (Abu Rass *et al*., 2022b). Reassortment of genomic segments has been observed on several occasions (Abbadi *et al* 2023; Chaput *et al*., 2020; Verma *et al*., 2022).~~" **Rationale:** These revisions enhance clarity and ensure that the level of detail remains consistent with other pathogen-specific chapters in the Manual. For example, text was moved for readability and also to align with the flow of other chapters that start by describing the whole virus, then parts of the virus and later molecular makeup, etc. The specific nucleotides were removed because this information is not routinely provided in other chapters and there are instances when a given segment was reported in GenBank to have varying lengths.

2.1.2. Survival and stability in processed or stored samples

Genomic RNA and viable virus, capable of infecting cell cultures, were shown to persist for up to 28 days in tilapia fillets at –20°C. Genomic RNA was detectable by PCR methods but not able to infect cell cultures when the fillets were from subclinical fish or when clinically affected material was stored frozen for 90 and 120 days. Subclinically affected fillets failed to transmit disease after 14 days frozen storage (Thammatorn *et al.,* 2019). Sample preservation studies by Rawiwan *et al*. (2021), indicated genomic RNA will persist for at least 1 year under most common sample storage methods (–20°C, 70% and 95% ethanol, RNAlater, Whatman FTA cards) for subsequent detection by molecular methods. Genomic quantity was hardly changed in RNALater or frozen storage over the period, compared with a rapid loss of up to approximately two logs in the first 30 days followed by limited further loss for the other methods.

United States	**Category:** change **Proposed amended texts (or precise suggested deletion):** Please see the proposed changes in blue – "~~Genomic RNA and viable~~Infectious virus, capable of infecting cell cultures, ~~were~~ was shown to persist for up to 28 days in tilapia fillets stored at –20°C. Genomic RNA was detectable by PCR ~~methods~~in fillets collected from both non-clinical and clinical fish stored frozen for 90 to 120 days but this material ~~not able to~~did not infect cell cultures. ~~when the fillets were from subclinical fish or when clinically affected material was stored frozen for 90 and 120 days. Subclinically~~Fillets from non-clinical PCR positive fish ~~affected fillets~~ failed to transmit disease after 14 days frozen storage (Thammatorn *et al.,* 2019). Sample preservation studies by Rawiwan *et al*. (2021), indicated genomic RNA will persist for at least 1 year under most common sample storage methods (–20°C, 70% and 95% ethanol, RNAlater™, Whatman FTA cards) for subsequent detection by molecular methods. ~~Genomic quantity~~Genome quantification was not impacted for ~~hardly changed in~~samples preserved in RNALater™ or ~~frozen~~ stored ~~storage~~ at -20°C ~~over the period,~~for one year. ~~compared with a~~ A rapid loss of up to approximately two logs occurred in the first 30 days for

	samples stored in ethanol or on Whatman FTA cards. ~~followed by limited further loss for the other methods.~~" **Rationale:** These revisions enhance clarity and ensure that the level of detail remains consistent with other pathogenspecific chapters in the Manual. Also, genomic RNA is negative sense RNA which, in the absence of viral proteins, cannot infect cell cultures or fish.

2.1.3. Survival and stability outside the host

TiLV genomic RNA remained detectable in faeces and water samples collected from experimental challenge tanks 10 days post infection (Pierezan *et al.*, 2019). In studies using spiked water samples incubated at 27°C, TiLV genomic RNA was shown to persist for at least 14 days in sterile distilled water, with 1 log reduction during the period whilst 4 log reductions to below detectable levels were seen after 3 and 7 days respectively in freshwater from fish rearing tanks or natural pond water (Yamkasem *et al.,* 2022). In the same studies the virus was shown to lose infectivity in E-11 cells after 1 day of incubation at 27°C in each of the water types.

For inactivation methods, see Section 2.4.5.

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with Tilapia lake virus according to Chapter 1.5 of *Aquatic Animal Health Code* (*Aquatic Code*) are:

United States	**Category:** change **Proposed amended texts (or precise suggested deletion):** Please see the proposed change in blue – "…infection with ~~Tilapia~~ tilapia lake virus…" **Rationale:** The common name of the virus does not capitalize "tilapia".

Family	Scientific name	Common name
Cichlidae	*Oreochromis aureus × O. niloticus*	blue-Nile tilapia hybrid
	Sarotherodon galilaeus	mango tilapia
	Oreochromis mossambicus	Mozambique tilapia
	Oreochromis niloticus	Nile tilapia
	Oreochromis niloticus × O. mossambicus	red hybrid tilapia

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to infection with TiLV according to Chapter 1.5 of the *Aquatic Code* are:

Family	Scientific name	Common name
Cyprinidae	*Barbonymus schwanenfeldii*	tinfoil barb

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following species, but an active infection has not been demonstrated:

Family	Scientific name	Common name
Latidae	*Lates calcarifer*	barramundi

2.2.3. Likelihood of infection by species, host life stage, population or sub-populations

Fingerlings are mainly affected, particularly when juveniles are stocked into grow out ponds and cages, although infection and mortality can occur across all life stages. Dong *et al.* (2017) reported approximately 90% mortality in red tilapia fingerlings within one months of stocking into cages. Mortality just over 9% in medium to large sized Nile tilapia was noted by Fathi *et al*. (2017). Experimental evidence demonstrates that TiLV susceptibility is weight-dependent; smaller fish are significantly more vulnerable than larger individuals, with higher mortality, earlier clinical onset, and more severe pathology in the liver and spleen Roy *et al.* (2021).

United States	**Category:** editorial **Proposed amended texts (or precise suggested deletion):** Please see the proposed change in blue – "…fingerlings within one month~~s~~ of stocking into cages." **Rationale:** Grammatical edit.

2.2.4. Distribution of the pathogen in the host

The main organ consistently affected is the liver. Kidney, brain, spleen and gills are also commonly affected and are organs the virus can be observed and detected (Dong *et al.,* 2017; Eyngor *et al.,* 2014; Jansen *et al.,* 2019 for reviews).

2.2.5. Aquatic animal reservoirs of infection

Low levels of viable virus and genomic RNA has been detected in asymptomatic fish (Senapin *et al.* 2018).

2.2.6. Vectors

To date, no living organisms have been identified as vectors for TilV.

2.3. Disease pattern

2.3.1. Mortality, morbidity and prevalence

Though morbidity and mortality vary significantly across both wild and farmed populations, ranging from 10% to over 90% depending on environmental conditions and management practices, evidence suggests that fish with no prior exposure to TiLV are highly susceptible with high mortality. Field data and experimental studies suggest that smaller fingerlings (0.5–50 g) are more vulnerable, though larger, older fish that are naïve will succumb.

2.3.2. Clinical signs, including behavioural changes

Disease was observed in both natural and cultured conditions with a range of signs and behaviours currently not pathognomonic. These include lethargy, seclusion, anorexia, poor body condition, skin discoloration, severe anaemia, ocular alterations including opacity, bilateral exophthalmia, skin abrasion and congestion, scale protrusion, and abdominal swelling (Eyngor *et al.,* 2014; Jaemwimol *et al*., 2018; Kembou Tsofack *et al*., 2017; Surachetpong *et al*., 2017). Affected animals can show loss of appetite, pale coloration of the body, aggregation near the bottom, sluggish movement, abnormal swimming, and isolation (Dong *et al*., 2017).

2.3.3 Gross pathology

Gross pathologies often observed include protrusion of scales, exophthalmia, paleness of gill and liver, clear ascitic fluid in abdomen and congestion of brain liver and sometimes spleen. Other lesions included

skin erosions and haemorrhages in the leptomeninges(Eyngor *et al.,* 2014; Fergusson *et al.,* 2014; Surachetpong *et al*., 2017).

2.3.4. Modes of transmission and life cycle

TiLV is transmitted horizontally between fish via waterborne exposure, physical transfer or ingestion of infected material potentially including shed mucus (Eyengor *et al.,* 2014; Liamnimitr *et al.,* 2018; Pierezan *et al.,* 2019). Vertical transmission of TiLV has been suggested based on the detection of viral RNA in broodstock reproductive tissues and early life stages (Aich *et al.,* 2022; Dong *et al.,* 2020; Yamkasem *et al.,* 2019).

2.3.5. Environmental factors

Environmental factors and seasonality are drivers of TiLV outbreaks, which peak during the summer months at water temperatures between 22–32°C. Outbreak severity is further exacerbated by high stocking densities, frequent production cycles, and low stocking weights (Kabuusu *et al.,* 2018). In recirculating aquaculture systems (RAS), the water column may act as a viral reservoir; without sufficient UV disinfection, viral particles can accumulate and significantly increase the impact on infected farms (Sresung *et al.,* 2026).

2.3.6. Geographical distribution

TiLV was first reported in Israel in 2014 from mortality events that were ongoing since 2009 (Eyngor *et al*., 2014). Infection with TiLV has since been reported from South America (Ferguson *et al*., 2014; Kembou Tsofack *et al*., 2017), Africa (Fathi *et al*., 2017; Mugimba *et al*., 2018), Asia (Abdulla *et al.,* 2018, Amal *et al*., 2018; Behera *et al*., 2018; Chaput *et al*., 2020; Debnath *et al.,* 2020; Dong *et al*., 2017; Surachetpong *et al*., 2017; Tran *et al*., 2022) and North America (Ahasan *et al*., 2020).

See WAHIS (https://wahis.woah.org/#/home) for recent information at the country level.

2.4. Biosecurity and disease control strategies

2.4.1. Vaccination

There are currently no commercially available vaccines for controlling TiLV.

2.4.2. Chemotherapy including blocking agents

There are currently no chemotherapeutics for controlling TiLV disease.

2.4.3. Immunostimulation

There are no available recognised probiotics for TiLV.

2.4.4. Breeding resistant strains

There are currently no recognised TiLV resistant tilapia commercially available. Heritable variation in resistance to TiLV disease has been demonstrated in various natural and farmed tilapia populations (Adamek *et al*., 2022; Barria *et al*., 2021; Ferguson *et al*., 2014) indicating selective breeding for resistance to TiLV should be possible.

2.4.5. Inactivation methods

Common disinfectants are likely to be effective for TiLV inactivation. Viral reductions of >4 $\log_{10}$ median tissue culture infective dose ($TCID_{50}$)/ml were obtained with ≥20 ppm free chlorine and ≥50 ppm available iodine after 30 min contact time (temperature and soiling levels not indicated, Soto *et al*., 2019). Reductions of >5 $\log_{10}$ $TCID_{50}$/ml were observed by Jaemwimol *et al*. (2019).

2.4.6. Disinfection of eggs and larvae

Disinfection of eggs according to standard procedures is suggested as an important control measure (see chapter 4.4 of the *Aquatic Code*) and iodophore disinfectants are likely to be effective (Soto *et al*., 2019).

2.4.7. General husbandry

Introducing live fish without adequate quarantine increases the risk of outbreaks. There is currently no definitive report on the transmission dynamics between wild and farmed fish, nor on the impact of processing facilities on disease spread. Biosecurity practices can be used to reduce the risk of TiLV infection including adequate quarantine, efficient disinfection, optimal water quality and proper carcass disposal.

In recirculating aquaculture systems (RAS), the continuous reuse of water may allow the virus to accumulate in both the water column and sediment, which intensifies infection pressure and sustains prolonged outbreaks (Sresung *et al.,* 2026). Decreased stocking densities may be beneficial to reduce environmental viral loads.

3. Specimen selection, sample collection, transportation and handling

This section draws on information in Sections 2.2, 2.3 and 2.4 to identify populations, individuals and samples that are most likely to be infected.

3.1. Selection of populations and individual specimens

Routine sampling should prioritise the testing of sick or recently dead fish, in accordance with Chapter 2.3.0. General information (diseases of fish).

3.2. Selection of organs or tissues

TiLV replication and transcription occurs at sites of pathology with a generally broad tissue tropism. Liver, kidney, spleen and gills are suitable organs to target for diagnostic sampling. In clinically acute and symptomatic fish (spiral swimming) the brain can be targeted (Aich *et al.,* 2022; Jansen *et al.,* 2019).

3.3. Samples or tissues not suitable for pathogen detection

TiLV is not consistently detected in faeces (Liamnimitr *et al.,* 2018), and in asymptomatic fish the brain is frequently not positive, making this unsuitable target for surveillance (Mugimba *et al.,* 2018).

United States	**Category:** editorial **Proposed amended texts (or precise suggested deletion):** Please see the proposed change in blue – "…making this an unsuitable target for surveillance…" **Rationale:** Grammatical edit.

3.4. Non-lethal sampling

Mucus but not faeces can be taken as a non-lethal sample for detection of TiLV with both viral RNA and viable virus capable of causing cytopathic effect (CPE) in cell culture detected in mucus of experimental and field samples at similar diagnostic sensitivities to liver samples (Liamnimitr *et al.,* 2018).

3.5. Preservation of samples for submission

For guidance on sample preservation methods for the intended test methods, see Chapter 2.3.0 General information (diseases of fish).

3.5.1. Samples for pathogen isolation

The results of bioassay depend strongly on the quality of samples (time since collection and time in storage). Fresh specimens should be kept on ice and preferably sent to the laboratory within 24 hours of collection. To avoid degradation of samples, use alternate storage methods only after consultation with the receiving laboratory. For recommendations on transporting samples for virus isolation to the laboratory, see Section B.2.4 of Chapter 2.3.0 *General information* (diseases of fish).

United States	**Category:** deletion **Proposed amended texts (or precise suggested deletion):** Please see the deletion proposed in blue – "~~The results of bioassay depend strongly on the quality of samples (time since collection and time in storage).~~ Fresh specimens…" **Rationale:** This language isn't necessary for readability or clarity.

3.5.2. Preservation of samples for molecular detection

Tissue samples for PCR testing should be preserved in 80% (v/v) analytical-grade ethanol. The recommended ratio of ethanol to tissue is 10:1 based on studies in terrestrial animal and human health. The use of lower grade (laboratory or industrial grade) ethanol is not recommended. If material cannot be fixed it may be frozen.

Standard sample collection, preservation and processing methods for molecular techniques can be found in Section B.2.5 of Chapter 2.3.0 *General information* (diseases of fish).

The best preservation of TiLV genomic RNA in tissue samples for PCR testing was observed with RNALater and storage at –20°C (Rawiwan *et al.,* 2021). Ethanol 70% or 95% and Whatman FTA cards were inferior with approximately a 3 log drop in viral copy number after 30 days with much of that occurring within the first 7 days. They do however represent suitable options for high titre clinical samples if expense of proprietary storage reagents and cold chain difficulties are a factor.

The recommended ratio of analytical/reagent-grade (undenatured) ethanol to tissue is 10:1. Samples in RNA stabilisation reagents should be prepared according to the recommendation from the manufacturers and placed in at least five volumes of the reagent. Samples in RNA stabilising reagents can be shipped frozen or on ice or at room temperature if transport time does not exceed 24 hours.

Samples may also be frozen at –80°C and kept frozen until assayed.

United States	**Category:** change **Proposed amended texts (or precise suggested deletion):** Please see the proposed changes in blue – "Tissue samples for PCR testing ~~should be~~can be preserved in molecular preservative or 80% (v/v) ~~analytical~~molecular-grade ethanol. The recommended ratio of ethanol to tissue is 10:1 based on studies in terrestrial animal and human health. The use of lower grade (laboratory or industrial grade) ethanol is not recommended. If material cannot be fixed it may be frozen. Standard sample collection, preservation and processing methods for molecular techniques can be found in Section B.2.5 of Chapter 2.3.0 *General information* (diseases of fish). ~~The best preservation of TiLV genomic RNA in tissue samples for PCR testing was observed with RNALater and storage at –20°C (Rawiwan *et al.*, 2021). Ethanol 70% or 95% and Whatman FTA cards were inferior with approximately a 3 log drop in viral copy number after 30 days with much of that occurring within the first 7 days. They do however represent suitable options for high titre clinical samples if expense of proprietary storage reagents and cold chain difficulties are a factor.~~ ~~The recommended ratio of analytical/reagent-grade (undenatured) ethanol to tissue is 10:1. Samples in RNA stabilisation reagents should be prepared according to the recommendation from the manufacturers and placed in at least five volumes of the~~

	~~reagent. Samples in RNA stabilising reagents can be shipped frozen or on ice or at room temperature if transport time does not exceed 24 hours.~~ Samples may ~~also~~ be frozen at –80°C and kept frozen until assayed." **Rationale:** The recommended changes are designed to improve readability and the ensure level of detail provided is consistent with other Manual chapters. Also, section 2.1.2. provides information on survival and stability of the virus in processed or stored samples, noting a 2 log drop in viral copy, which is inconsistent with the 3 log drop referenced in this section.

3.5.3. Samples for histopathology, immunohistochemistry or *in-situ* hybridisation

Standard sample collection, preservation and processing methods for histological techniques can be found in Section B.2.2 of Chapter 2.3.0 *General information* (diseases of fish).

3.5.4. Samples for other tests

Viral nucleic acids can be detected in blood samples using molecular methods, while serological testing for antibodies can be used to confirm prior exposure in fish (Tattiyapong *et al.,* 2020).

United States	**Category:** change **Proposed amended texts (or precise suggested deletion):** Please see the proposed changes in blue – "~~Viral nucleic acids can be detected in blood samples using molecular methods, while s~~Serological testing for antibodies ~~can be used to~~has been used in some situations to confirm prior exposure in fish…" **Rationale:** Since blood is not a routine sample used for TilV testing, we recommend leaving serological testing as an option because it is being conducted for research etc., while condensing the language for readability.

3.6. Pooling of samples

Pooling of samples from more than one individual animal for a given purpose is only recommended where robust supporting data on diagnostic sensitivity and diagnostic specificity have been evaluated and found to be suitable. If the effect of pooling on diagnostic sensitivity has not been thoroughly evaluated, larger specimens should be processed and tested individually. Small life stages can be pooled to obtain the minimum amount of material for virus isolation or molecular detection.

4. Diagnostic methods

The methods currently available for pathogen detection that can be used in i) surveillance of apparently healthy animals, ii) presumptive diagnosis in clinically affected animals and iii) confirmatory diagnostic purposes are listed in Table 4.1. by animal life stage.

Ratings for purposes of use. For each recommended assay a qualitative rating for the purpose of use is provided. The ratings are determined based on multiple performance and operational factors relevant to application of an assay for a defined purpose. These factors include appropriate diagnostic performance characteristics, level of assay validation, availability cost, timeliness, and sample throughput and operability. For a specific purpose of use, assays are rated as:

+++ = Methods are most suitable with desirable performance and operational characteristics.

++ = Methods are suitable with acceptable performance and operational characteristics under most circumstances.

+ = Methods are suitable, but performance or operational characteristics may limit application under some circumstances.

Shaded boxes = Not appropriate for this purpose.

Validation stage. The validation stage corresponds to the assay development and validation pathway in chapter 1.1.2. The validation stage is specific to each purpose of use. Where available, information on the diagnostic performance of recommended assays is provided in Section 6.3.

WOAH Reference Laboratories welcome feedback on diagnostic performance of recommended assays, in particular PCR methods. Of particular interest are any factors affecting expected assay sensitivity (e.g. tissue components inhibiting amplification) or expected specificity (e.g. failure to detect particular genotypes, detection of homologous sequences within the host genome). These issues should be communicated to the WOAH Reference Laboratories so that advice can be provided to diagnostic laboratories and the standards amended if necessary.

Table 4.1. *WOAH recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals*

Method	A. Surveillance of apparently healthy animals				B. Presumptive diagnosis of clinically affected animals				C. Confirmatory diagnosis[1] of a suspect result from surveillance or presumptive diagnosis			
	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV
Wet mounts												
Histopathology						+	+	1				
Cell culture	+	+	+	1	++	++	++	1				
Real-time RT-PCR	+	+++	+++	3	+	+++	+++	3	+	+++	+++	3
Conventional RT-PCR	+	+	+	1	++	++	++	2				
Nested conventional RT-PCR	+	+	+	1	++	++	++	2				
Conventional RT-PCR followed by amplicon sequencing									+++	+++	+++	2
In-situ hybridisation										+	+	NA
Bioassay												
LAMP												
Ab-ELISA		+	+	NA								
Ag-ELISA												
Other antigen detection methods: immunohistochemistry										+	+	NA
Other methods: transmission electron microscopy										+	+	NA

LV = level of validation, refers to the stage of validation in the WOAH Pathway (chapter 1.1.2); NA = not available; PCR = polymerase chain reaction; LAMP = loop-mediated isothermal amplification; Ab- or Ag-ELISA = antibody or antigen enzyme-linked immunosorbent assay, respectively.

[1]For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). [2]Susceptibility of early and juvenile life stages is described in Section 2.2.3.

Shading indicates the test is inappropriate or should not be used for this purpose.

4.1. Wet mounts

Not applicable

4.2. Histopathology and cytopathology

The brains of TiLV infected fish reveal multifocal haemorrhages, edema, proliferative glial cells and capillary congestion (Amal *et al.,* 2018; Eyngor *et al.,* 2014; Kembou Tsofack *et al.,* 2017). In the liver of infected fish, syncytial cell formation and massive hepatocellular necrosis with pyknotic and karyolitic nuclei are most commonly observed (Behera *et al.,* 2018; Dong *et al.,* 2017; Ferguson *et al.,* 2014; Jaemwimol *et al.,* 2018; Kembou Tsofack *et al.,* 2017; Senapin *et al.,* 2018). Eosinophilic intracytoplasmic inclusion bodies were present in the liver cells and splenic cells of infected fish. In the kidney, multiple necrotic foci were observed in the anterior kidney with increased melanomacrophage centres (MMCs), and in spleen dispersion of melanin granules were observed (Tattiyapong *et al.,* 2017).

4.3. Cell culture for isolation

Suitable cell lines For TiLV isolation include the established E-11 (from snakehead fish) and newly developed cell lines OnlL, OnlB, OnH, OmB, TmB, and RHTiB, from liver, brain and heart tissues of *Oreochromis niloticus* or related hybrids (reviewed in Aich *et al.,* 2022). E-11 is recognised for quick development of observable CPE. Primary tilapia brain cell cultures are also effective but more time consuming and less user-friendly. The optimal temperature range is 25–30°C with 28°C most often cited as optimum using enriched media such as L-15 supplemented with 10% FBS.

Cell lines should be monitored regularly (e.g. every 6 months) to ensure that susceptibility to targeted pathogens has not changed.

4.4. Nucleic acid amplification

PCR assays should always be run with the controls specified in Section 2.5 *Use of molecular techniques for surveillance testing, confirmatory testing and diagnosis* of Chapter 2.3.0 *General information* (diseases of fish). Each sample should be tested in duplicate.

Extraction of nucleic acids

Numerous different kits and procedures can be used for nucleic acid extraction. The quality and concentration of the extracted nucleic acid is important and should be checked using a suitable method as appropriate to the circumstances, for example optical density or running a gel.

A reverse transcriptase PCR (RT-PCR) has been developed (Eyngor *et al*., 2014), followed by a more sensitive nested RT-PCR suitable for detection of TiLV in clinical cases (Kembou Tsofack *et al*., 2017), a semi-nested RT-PCR with an improved analytical sensitivity (7.5 viral copies per reaction) (Dong *et al*., 2017), real-time SYBR assay, with analytical sensitivity of two copies of plasmid (Tattiyapong *et al*., 2018), and a real-time probe-based assay (Waiyamitra *et al.*, 2018), for which diagnostic sensitivity has not yet been determined.

All the above assays were used in an inter-laboratory panel testing, together with two, at the time unpublished, assays from the laboratories of the WOAH Reference Laboratories for SVCV (WOAH *ad hoc* Group reports 2018a, b and 2021). The three real-time probe-based PCR assays had the highest sensitivity, repeatability and robustness of all assays evaluated and were the recommended assays for detecting TiLV. The SYBR-based PCR assay (Tattiyapong RT-qPCR) produced acceptable results but would require optimisation of reaction components and conditions and definition of assay interpretation criteria, prior to implementation for routine use. However, this assay still exhibited acceptable sensitivity, repeatability and robustness and would be an appropriate assay for use in laboratories that do not have access to real-time probe-based assays.

The conventional semi-nested assay (RT-nPCR) described (Dong *et al*., 2017; Eyngor *et al*., 2014; Kembou Tsofack *et al*., 2017) was the least sensitive assay, which is not unusual when conventional and real-time assays are compared. The Dong RT-nPCR assay is of sufficient sensitivity for confirmatory testing of samples screened positive by real-time PCR, for the purpose of confirmation of TiLV by sequence analysis, when investigating clinical disease or as follow-up testing of samples screened positive by real-time PCR from surveillance testing of apparently healthy animals.

United States	**Category:** change **Proposed amended texts (or precise suggested deletion):**

	Please see the proposed changes in blue – "~~Numerous different kits and procedures can be used for nucleic acid extraction.~~ Commercial kits should be validated or undergo equivalence testing with current validated extraction procedures prior to routine use.~~The quality and concentration of the extracted nucleic acid is important and should be checked using a suitable method as appropriate to the circumstances, for example optical density or running a gel.~~ Multiple reverse transcription PCR (RT-PCR) assays have been developed for detection of TiLV. These include a~~A reverse transcriptase PCR (~~ RT-PCR~~) has been developed~~ (Eyngor *et al*., 2014), ~~followed by a morea~~ ~~sensitive~~ nested RT-PCR (RT-nPCR) suitable for detection of TiLV in clinical cases (Kembou Tsofack *et al*., 2017), a semi-nested RT-PCR ~~with an improved analytical sensitivity (7.5 viral copies per reaction)~~ (Dong *et al*., 2017), a real-time SYBR assay~~, with analytical sensitivity of two copies of plasmid~~ (Tattiyapong *et al*., 2018), and a real-time probe-based assay (Waiyamitra *et al*., 2018)~~. for which diagnostic sensitivity has not yet been determined.~~ All the above assays were used in an inter-laboratory ~~panel testing~~comparison, together with two additional, at the time unpublished, assays from ~~the laboratories of~~ the WOAH Reference Laboratories for SVCV (WOAH *ad hoc* Group reports 2018a, b and 2021). The three real-time probe-based PCR assays had the highest sensitivity, repeatability and robustness of all assays evaluated and were the recommended assays for detecting TiLV. The SYBR-based PCR assay (Tattiyapong RT-qPCR) produced acceptable results but would require optimisation of reaction components and conditions and definition of assay interpretation criteria, prior to implementation for routine use. However, this assay still exhibited acceptable sensitivity, repeatability and robustness and would be an appropriate assay for use in laboratories that do not have access to real-time probe-based assays. The RT-nPCR~~conventional semi-nested assay (RT-nPCR) described (Dong *et al*., 2017; Eyngor *et al*., 2014; Kembou Tsofack *et al*., 2017)~~ was the least sensitive assay, which is not unusual when conventional and real-time assays are compared. The Dong semi RT-nPCR assay is of sufficient sensitivity for confirmatory testing…" **Rationale:** The recommended changes are designed to improve readability and the ensure level of detail provided is consistent with other Manual chapters. Additionally, the last sentence of the first paragraph above should be removed because this language is already covered in section 2.5. above. The USDA APHIS National Veterinary Services Laboratory also participated in this inter-laboratory comparison.

4.4.1. Real-time RT-PCR

Virus nucleic acid can be purified from: aliquots of cell culture medium from infected monolayer cells; tissue/organs homogenised in media specified in Section 3.5.1, tissue samples in RNA stabilising reagent, fresh or frozen tissue samples. In the case of culture medium from infected monolayer cells, or in tissue homogenised in MEM, aliquots should be centrifuged at 1000 ***g*** for 5 minutes to remove cell debris.

Two real-time RT-PCR assays targeting the putative polymerase acidic (PA) protein gene encoded by segment 3 have been validated to stage 1 and are described herein (assays 3 and 4). The sensitivity of two additional real-time RT-PCR assays targeting the putative RNA dependant RNA polymerase (RdRP/PB1) gene encoded by segment 1 and the putative polymerase acidic (PA) protein gene encoded by segment 3 respectively, (assays 1 and 2) compared favourably and have been validated to stage 2 and in part to stage 3 (repeatability and

reproducibility by interlaboratory test) are also described. Finaly a recent sensitive probe-based real-time RT-PCR assay targeting the hypothetical protein gene encoded by segment 10 and validated to stage 2 (stage 3 in part) is described in assay 5 (Megarani *et al.*, 2022). Assays 1, 3 or 5 are recommended based on sensitivity, level of validation and target conservation supporting ability to detect multiple geographical strains.

Positive and negative controls should be included.

Total RNA from infected cells and/or tissues is extracted using a phase-separation method or by use of a commercially available RNA isolation kit used according to the manufacturer's instructions.

In one-step RT-PCR gene-specific primers are used both to generate a cDNA transcript and for real-time RT-PCR. Both reactions occur in the same tube, which minimises the risk of contamination. Primers are used at a final concentration of 900 nM and the final probe concentration is 250 nM. 2.5 µl of extracted RNA (50 ng–2 ug) is added to each 25 µl RT-PCR reaction.

Alternatively, a two-step assay can be performed

Step 1: Reverse-transcription: extracted RNA is reverse transcribed non-discriminately into cDNA using random primers. The cDNA synthesis reactions and cycling conditions are best performed using manufacturer's instructions for commercially available kits which have been extensively tested with a variety of RNA templates, including GC- and AU-rich targets and RNAse expressed at low levels.

Step 2: Real-time PCR: the TaqMan real-time PCR assay uses forward primer, reverse primer and probe Primers are used at a final concentration of 400 nM, and the final concentration of the probe is 200 nM. 2.5 µl of cDNA product is added to each 25 µl PCR reaction. Thermal cycling conditions are 50°C for 2 minutes, 95°C for 10 minutes followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute (Garver *et al*., 2011). A sample is negative if no Ct (threshold cycle) is recorded, while samples with a Ct of 1-40 are considered positive for TiLV. Samples with Ct values of >40 are considered inconclusive.

***Primers and probes* (*sequence*)**

United States	**Category:** change **Proposed amended texts (or precise suggested deletion):** Please see the proposed changes in blue – "The primers and probes for five real-time assays are shown in table 4.4.1.1. Two real-time RT-PCR assays (method 3 and method 4) targeting the putative polymerase acidic (PA) protein gene encoded by segment 3 have been validated to stage 1 and are described herein. ~~(assays 3 and 4). The sensitivity of two additional~~The real-time RT-PCR assays targeting the putative RNA dependant RNA polymerase (RdRP/PB1) gene encoded by segment 1 (method 1) and the assay targeting the putative polymerase acidic (PA) protein gene encoded by segment 3 (method 2) ~~respectively, (assays 1 and 2) compared favourably and~~ have been validated to stage 2 and in part to stage 3 (repeatability and reproducibility by interlaboratory test) are also described. Finaly a ~~recent~~ sensitive probe-based real-time RT-PCR assay, not evaluated in the interlaboratory comparison, targeting the hypothetical protein gene encoded by segment 10 and validated to stage 2 (stage 3 in part) is described in ~~assay~~ method 5 (Megarani *et al.*, 2022). ~~Assays~~ Methods 1, 3 or 5 are recommended for TiLV detection based on sensitivity, level of validation and target conservation ~~supporting ability~~vital to detecting multiple ~~geographical~~ strains of TiLV. Controls should be run with each assay: negative extraction control; positive control; no template control; internal RT-PCR control. Ideally, the positive control should be distinguishable from viral genomic sequence, thus allowing detection of

	any cross-contamination leading to false positive results. ~~Positive and negative controls should be included.~~ ~~Total RNA from infected cells and/or tissues is extracted using a phase separation method or by use of a commercially available RNA isolation kit used according to the manufacturer's instructions.~~ ~~In~~When performing a one-~~step~~ tube RT-PCR ~~gene-specific primers are used both to generate a cDNA transcript and for real-time RT-PCR. Both~~ both~~reactions~~ reverse transcription and target ~~occur~~amplification occurs in the same tube, which minimises the risk of contamination. Gene-specific primers are used both to generate a cDNA transcript and for amplification of the cDNA. Primers and probe are ~~used at a final concentration of 900 nM and the final probe concentration is 250 nM. 2.5 µl of extracted RNA (50 ng–2 ug) is~~ added to each 25 µl RT-PCR reaction. Alternatively, a two-step assay can be performed. Step 1: Reverse-transcription: extracted RNA is reverse transcribed non-discriminately into cDNA using random primers. The cDNA synthesis reactions and cycling conditions are best performed using manufacturer's instructions for commercially available kits ~~which have been extensively tested with a variety of RNA templates, including GC- and AU-rich targets and RNAse expressed at low levels~~. Commercial kits should be evaluated for use prior to adopting." … "***Table 4.4.1.1. Primers and probes* (*sequence*)"** **Rationale:** The recommended changes are designed to improve readability and the ensure level of detail provided is consistent with other Manual chapters.

Pathogen/ target gene	Primer/probe (5–3)	Concentration	Cycling parameters[(a)]
Method 1. Stone *et al.* (in WOAH 2021 and Soto *et al.,* 2024 Supplement) 73 bp, GenBank Accession No.: MN687695.1			
TiLV Segment 1 putative RdRP [PB1]	TiLV CEFAS qFor2: AGA-CAC-ACT-GCT-AAA-AGT-GTG TiLV CEFAS qRev: GCG-CAC-CAT-GTA-AGC-GAG-C TiLV CEFAS qProbe 6FAM AAT-AGG-CAT-GGG-GTC-AAT-YGG-AGT-TAMRA	900 nM (primers) 250 nM (probe) for 1-step or 400 nM 200 nM (respectively) for 2-step	48°C/30 min followed by 95°C/10 min, followed by 40 cycles of 95°C/15 sec and 60°C/60 sec
Method 2: Liu *et al.* (in WOAH 2021 and Soto *et al.,* 2024 Supplement), 67 bp, GenBank Accession No.: OQ437056.1			
TiLV Segment 3 putative polymerase acidic [PA] protein	TiLV-F1: CGA-ACT-GTT-GCC-TTT-GGA-AAT-T TiLV-R1: TGA-AGA-ATA-AGT-GGA-TTG-CCT-TTG TiLV-P1-6FAM CCG-CGG-CTG-GCC-TTC-CAG-Iowa Black FQ	900 nM 250 nM or 400 nM 200 nM	48°C/30 min followed by 95°C/10 min, followed by 40 cycles of 95°C/15 sec and 60°C/60 sec
Method 3: Waiyamitra *et al.* (2018); 93 bp, GenBank Accession No.: MN939374.1			
TiLV Segment 3 putative polymerase acidic [PA] protein	TiLV-93F: AGC-CTG-CCA-CAC-AGA-AG TiLV-93R: CTG-CTT-GAG-TTG-TGC-TTC-T TiLV-93Probe: F6AM-CTC-TAC-CAG-CTA-GTG-CCC-CA-Iowa Black FQ	900 nM 250 nM or	48°C/30 min followed by 95°C/10 min, followed by 40 cycles of 95°C/15 sec and 60°C/60 sec

Pathogen/ target gene	Primer/probe (5–3)	Concentration	Cycling parameters[a]
		400 nM 200 nM	
Method 4: Tattiyapong *et al.* (2018); 112 bp, GenBank Accession No.: PP600911.1			
TiLV Segment 3 putative polymerase acidic [PA] protein	TiLV-112F: CTG-AGC-TAA-AGA-GGC-AAT-ATG-GAT-T TiLV-112R: CGT-GCG-TAC-TCG-TTC-AGT-ATA-AGT-TCT	900 nM	48°C/30 min followed by 95°C/10 min, followed by 40 cycles of 95°C/15 sec and 60°C/60 sec
Method 5: Megarani *et al.* (2022); 91 bp, GenBank Accession No.: MN687724.1			
TiLV Segment 10 Hypothetical protein	TiLV-F: GGC-AAG-AAA-GCT-GCT-TCA-AAG-A TiLV-R: CGC-TCT-CGT-CAG-CAC-CAT-AC TiLV -P: 6FAM-CGA-AGT-TGG-AAG-AAT-G-MGB	900 nM 250 nM	50°C/5 min followed by 95°C/20 sec, followed by 40 cycles of 95°C/3 sec and 62°C/30 sec (fast protocol)

[a]A denaturation step prior to cycling has not been included.

4.4.2. Conventional RT-PCR

***Primers* (*sequence*)**

United States	**Category:** addition **Proposed amended texts (or precise suggested deletion):** Please see the proposed addition in blue – "The primers listed in Table 4.4.2.1 will detect TiLV and can be used to generate PCR amplicon for sequence confirmation. However, real-time RT-PCR (see section 4.4.1) is the method recommended for TiLV detection based on sensitivity, level of validation and target conservation vital to detecting multiple strains of TiLV. ***Table 4.4.2.1. Primers* (*sequence*)**" **Rationale:** The additional text proposed is designed to improve readability and ensure the level of detail provided is consistent with other Manual chapters.

Pathogen/ target gene	Primer (5–3)	Concentration	Cycling parameters[a]
Method 1. Dong *et al.* (2017); product size 415 bp in first round, 250 bp in second round; GenBank Accession No.: MK392374.1			
TiLV Segment 3 putative polymerase acidic [PA] protein	TiLV-Nested ext-1: TAT-GCA-GTA-CTT-TCC-CTG-CC TiLV-ME1: GTT-GGG-CAC-AAG-GCA-TCC-TA	180 nM	50°C/30 min followed by 94°C/2 min followed by 30–35 cycles* of 94°C/30 sec, 60°C/30 sec, 68°C/30 sec then 68°C/7 min
TiLV Segment 3 putative polymerase acidic [PA] protein	TiLV-7450/150R/ME2: TAT-CAC-GTG-CGT-ACT-CGT-TCA-GT TiLV-ME1: GTT-GGG-CAC-AAG-GCA-TCC-TA	360 nM	94°C/15 min followed by 30–35 cycles* of 94°C/30 sec, 60°C/30 sec, 68°C/30 sec then 68°C/7 min
Method 2: Stone *et al.* (in WOAH 2021 and Soto *et al.,* 2024 Supplement), 689 bp, GenBank Accession No.: MN687695.1			

Pathogen/ target gene	Primer (5–3)	Concentration	Cycling parameters[(a)]
TiLV Segment 1 putative RdRP [PB1]	TiLV For: AGA-GAC-GCA-CGC-TAG-ATC-TG TiLV Rev: TGT-AAG-TGG-GGT-TGT-TAT-ACC	100 nM	50°C/30 min followed by 94°C/2 min followed by 35 cycles of 94°C/30 sec, 60°C/30 sec, 68°C/30 sec then 68°C/7 min
TiLV Segment 1 putative RdRP [PB1]	TiLV Fint: GGT-CTC-AGC-AGT-GTA-CAC-CG TiLV Rint: ATC-TGG-AAC-AGA-CGT-TCT-GC	100 nM	94°C/15 min followed by 35 cycles of 94°C/30 sec, 60°C/30 sec, 68°C/30 sec then 68°C/7 min

*Data not shared in the paper – trail in-house 30–35 recommended.

4.4.3. Other nucleic acid amplification methods

There are many various molecular detection methods that have been developed for TiLV in recent years, some more research orientated. A rapid, one-step reverse transcription, loop-mediated, isothermal amplification (RT-LAMP) method for the detection of TiLV in fish tissues was developed by Phusantisampan *et al*. (2020). Although the assay was capable of detecting virus from different geographical locations in Thailand, it has not been evaluated using the full range of global isolates. A droplet digital PCR has been developed by Shahi *et al.* (2023) * consistent with TiLV morphology in infected tissues or cell culture preparations. However, due to limited specificity and sensitivity, TEM should only be used in combination with molecular identification methods and is not recommended as a standalone diagnostic assay.

5. Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations

For surveillance to demonstrate freedom from infection with TiLV in apparently healthy populations, real-time RT-PCR is the recommended test due to its high analytical sensitivity, specificity, and suitability for high-throughput screening across susceptible life stages. Surveillance testing should target appropriate tissues and be conducted in accordance with the principles outlined in the *Aquatic Code*. Positive screening results should be followed by confirmatory testing as described in Section 6 to support official disease status determination.

If real-time PCR apparatus is not available, nested or semi-nested PCR can be used for surveillance acknowledging and accommodating (e.g. by increased sample numbers and appropriate controls) the lower sensitivity and greater risk of amplicon contamination and false positive results associated with these methods.

6. Corroborative diagnostic criteria

This section only addresses the diagnostic test results for detection of infection in the absence (Section 6.1.) or in the presence (Section 6.2.) of clinical signs but does not evaluate whether the infectious agent is the cause of the clinical event.

The case definitions for a suspect and confirmed case have been developed to support decision making related to trade and confirmation of disease status at the country, zone or compartment level. Case definitions for disease confirmation in endemically affected areas may be less stringent. If a Competent Authority does not have the capability to undertake the necessary diagnostic tests it should seek advice from the appropriate WOAH Reference Laboratory, and if necessary, refer samples to that laboratory for confirmatory testing of samples from the index case in a country, zone or compartment considered free.

When two molecular methods are used for confirmation of a case, it is preferable to use two species-specific methods targeting non-overlapping regions of the pathogen genome.

6.1. Apparently healthy animals or animals of unknown health status[1]

Apparently healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link(s) to an infected population. Hydrographical proximity to, or movement of animals or animal products or equipment, etc., from a known infected population equate to an epidemiological link. Alternatively, healthy populations are sampled in surveys to demonstrate disease freedom.

1 For example transboundary commodities.

6.1.1. Definition of suspect case in apparently healthy animals

The presence of infection with TiLV shall be suspected in apparently healthy animals if at least one of the following criteria is met:

i) Positive result by conventional RT-PCR

ii) Positive result by nested conventional RT-PCR

iii) Positive result by real-time RT-PCR

iv) TiLV-typical CPE in cell culture observed in a susceptible fish cell line, without confirmatory identification of the virus

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with TiLV is considered to be confirmed in apparently healthy animals if at least one of the following criteria is met:

i) Positive result on tissue preparations by real-time RT-PCR and positive result by conventional RT-PCR followed by sequencing of the amplicon

ii) TiLV-typical CPE in cell culture followed by virus identification by conventional RT-PCR and sequencing of the amplicon

6.2 Clinically affected animals

Clinical signs are not pathognomonic for a single disease; however they may narrow the range of possible diagnoses, mortality may be the only or most dominant observation.

6.2.1. Definition of suspect case in clinically affected animals

The presence of infection with TiLV shall be suspected in clinically affected animals if at least one of the following criteria is met:

i) Gross pathology or clinical signs associated with the disease as described in this chapter

ii) Histo- or cytopathological changes consistent with the presence of the pathogen or the disease

iii) Positive result by a real-time RT-PCR

iv) Positive result by a conventional RT-PCR

v) Positive result by a recommended antigen or antibody detection test

vi) TiLV-typical CPE in cell culture

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with TiLV is considered in to be confirmed in clinically affected animals if at least at least one of the following criteria is met:

i) Positive result on tissue preparations by real-time RT-PCR and positive result by conventional RT-PCR followed by sequencing of the amplicon

ii) TiLV-typical CPE in cell culture followed by virus identification by conventional RT-PCR and sequencing of the amplicon

6.3. Diagnostic sensitivity and specificity for diagnostic tests

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with TiLV are provided in Tables 6.3.1. and 6.3.2 (no data are currently available). Data are only presented where tests are validated to at least level 2 of the validation pathway described in Chapter 1.1.2. and the information is available within published diagnostic accuracy studies.

6.3.1. For presumptive diagnosis of clinically affected animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation

Real-time RT-PCR	Diagnosis	Clinically diseased fish from farms and processing plants	Liver, spleen, kidney, brain, mucus and blood for nonlethal samples	*Oreochromis niloticus,* hybrid, other *Oreochormis* and susceptible species	96.8 (278)	100 (278)	Conventional RT-PCR and alternative real-time RT-PCR	Megarani *et al.,* 2022

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = number of samples used in the study,
RT-PCR: = reverse-transcriptase polymerase chain reaction.

United States	**Category:** editorial **Proposed amended texts (or precise suggested deletion):** Please see the proposed change in blue – "*Oreochromis niloticus*, hybrid, other *Oreochro~~r~~mis* and susceptible species" **Rationale:** Correction to the spelling of the Genus.

6.3.2. For surveillance of apparently healthy animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = number of samples used in the study,
PCR: = polymerase chain reaction.

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NB: Currently (2026) there is no WOAH Reference Laboratory for infection with Tilapia lake virus (please consult the WOAH web site for the list: https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3).

NB: First adopted in 20XX.