One-Pot RT-LAMP CRISPR/Cas12b Platform for Rapid Detection of Tilapia Lake Virus

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Introduction

- Tilapia lake virus (TiLV) causes a serious viral disease in both cultured and wild tilapia, leading to high morbidity and mortality rates. It is now a reportable disease by the World Organisation for Animal Health (WOAH).
- TiLV belongs to the family *Amnoonviridae* and has 10 negative-sense RNA segments, with a pleiomorphic enveloped virion that measures 55-100 nm in diameter.
- Current diagnostic methods include RT-PCR, semi-nested RT-PCR, nested RT-PCR, and RT-qPCR.
- However, these tests require advanced equipment and costly reagents, making them impractical for field use and in resource-limited laboratories.

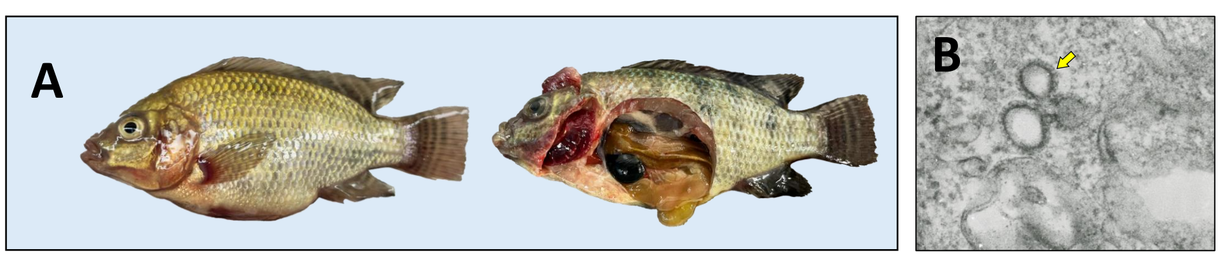
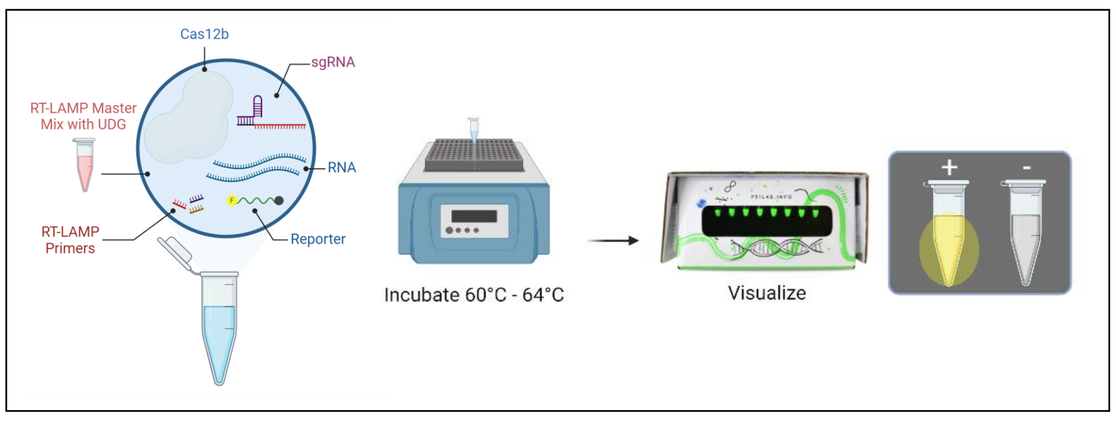
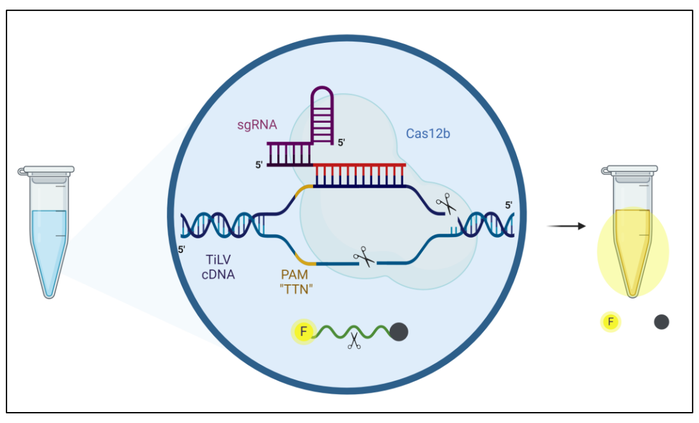


Figure 1. Infected tilapia; **1B.** TiLV virion

Methods

- *In silico* RT-LAMP primers and crRNA design.
- Generation of TiLV RNA standards.
- Determination of the analytic and diagnostic performance of TiLV one-pot assay.
- Comparison of lethal and non-lethal sampling methods for TiLV detection.





Results

Figure 2. Alignment of partial segment 4 (234 bp) sequences for 20 TiLV strains illustrating the *in-silico* specificity of the RT-LAMP primers and sgRNA.

Figure 3. TiLV one-pot assay amplification plot using a serial dilution of RNA standards ranging from 10^7-10^2, and 50 copies.



Figure 4. TiLV one-pot assay visualization using portable viewer (RNA standards from 10^7-10^2, and 50 copies. Last tube is negative control).

Table 1. Analytical sensitivity of TiLV one-pot assay. **95% Limit of Detection is 79.6 copies (CI: 48–132 copies)**

RNA standards	Positive wells (/60)
10^7	60
10^6	60
10^5	60
10^4	60
10^3	60
10^2	51
50	44

Table 2. Analytical specificity of TiLV one-pot assay against other viruses.

RNA Virus	Result	DNA Virus	Result
ISAV	Negative	CyHV1	Negative
SAV	Negative	CyHV2	Negative
Picorna virus	Negative	CyHV3	Negative
NNV	Negative	ISKNV	Negative
IHNV	Negative	LMBV	Negative
VHSV	Negative	EHNV	Negative
SVCV	Negative	CEV	Negative
Guppy Virus	Negative		

Table 3. Diagnostic performance of **151 positive** and **110 negative** samples. Overall diagnostic characteristics: **DSe of 92% (CI: 87–96%)** and **DSp of 100% (CI: 97–100%)**.

Population ID	Sample Description and Location	Positive Samples		Negative Samples	
		Positive (RT-qPCR)	Positive (one-pot)	Negative (RT-qPCR)	Negative (one-pot)
1	ICe injection 2023-Florida	30	27	30	30
2	ICe injection 2022-Florida	43	43	40	40
3	ICe injection R17-85-California	11	11	0	0
4	Cohabitation R17-85-California	1	1	0	0
5	ICe injection Caribbean	8	8	0	0
6	Cohabitation Caribbean	4	4	0	0
7	Field outbreak-Thailand	54	45	0	0
8	Surveillance-Florida	0	0	24	24
9	Surveillance-Thailand	0	0	16	16

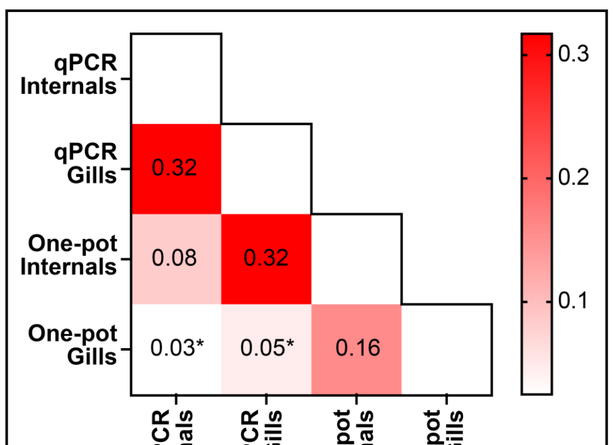


Figure 5. Heatmap of p-values from McNemar post-hoc pairwise comparison among four diagnostic tests, with Bonferroni corrections applied. Statistically significant differences were defined as $p < 0.05$.

Conclusions

- TiLV one-pot assay is a rapid, sensitive, specific, and economical method for detecting TiLV, validated according to stage 1 (Analytical characteristics) and stage 2 (Diagnostic characteristics) of the WOAH-Assay development pathways.
- TiLV one-pot assay offers a low risk of cross-contamination and can be employed in the field or laboratories with limited resources to detect TiLV.
- The use of gills as a sample with the TiLV one-pot assay needs careful consideration.

Future Studies

- Full validation can be completed following stage 3 (Reproducibility) and stage 4 (Implementation) based on WOAH guidelines.

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