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February to August 2018

**REPORT OF THE ELECTRONIC *AD HOC* GROUP ON TILAPIA LAKE VIRUS<sup>1</sup>**  
**February to August 2018**

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The OIE electronic *ad hoc* Group on Tilapia lake virus (TiLV) was established in November 2017 to evaluate published and unpublished methods for detection of TiLV, describe the level of validation of each method and determine additional validation requirements, recommend any additional assays that may need to be developed, facilitate the sourcing and distribution of well-characterised positive control material for method evaluation, and implement inter-laboratory comparability studies.

This report covers activities and achievements of the *ad hoc* Group from February to August 2018. The list of participants and Terms of Reference are presented in Annexes I and II.

**Activities, achievements and next steps**

Following a request by the Aquatic Animal Health Standards Commission, in May 2018 countries who had reported the presence of Tilapia lake virus (TiLV) were contacted by the OIE Director General requesting them to consider providing positive TiLV control material to the OIE Collaborating Centre for New and Emerging Diseases and Diagnostic Test Validation, at the CSIRO Australian Animal Health Laboratory (AAHL) for molecular test evaluation and inter-laboratory comparability studies.

The *ad hoc* Group was pleased to report that to date, material containing infectious TiLV has been received (or is in the process of being organised) from Chinese Taipei, Israel, Peru and Thailand.

The *ad hoc* Group's work is currently focused on point 5. of the Terms of Reference which is being led by the OIE Collaborating Centre for New and Emerging Diseases, Australian Animal Health Laboratory, Australia, i.e.

**TOR 5: Develop and implement a work plan for inter-laboratory comparability studies** **Aim:** To develop and implement a plan for investigation of the inter-laboratory comparability of the following assays for the molecular detection of TiLV:

1. Conventional semi-nested assay (RT-nPCR) described by Dong *et al.* (2017).
2. Real-time SYBR assay (RT-qPCR) described by Tattiyapong *et al.* (2017).
3. Real-time probe-based assay (RT-qPCR) which is unpublished and has been provided by the *ad hoc* Group member Dr Prof. Hong Liu.

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<sup>1</sup> Note: This *ad hoc* Group report reflects the views of its members and may not necessarily reflect the views of the OIE. This report should be read in conjunction with the September 2018 report of the Aquatic Animal Health Standards Commission because this report provides its considerations and comments. It is available at <http://www.oie.int/en/standard-setting/specialists-commissions-working-ad-hoc-groups/aquatic-animals-commission-reports/meeting-reports/>

## **Methodology:**

### Receipt of materials at AAHL

Material to be used in the inter-laboratory comparability studies will be sourced by *ad hoc* Group members and sent to the CSIRO Australian Animal Health Laboratory (AAHL) in Geelong, Australia. Material Transfer Agreements will be established to ensure the material supplied is only used to support the activities of the *ad hoc* Group. On arrival at AAHL, the presence of TiLV in the materials will be confirmed by conventional PCR and sequence analysis, the virus will be amplified in E-II cell cultures and stored in liquid nitrogen.

### Amplification of TiLV and generation of positive control material

TiLV will be amplified in E-II cell cultures and the end-point dilution determined using 10-fold dilutions with each of the TiLV molecular tests to ensure the material is suitable for preparation of the inter-laboratory comparability panel. This will also enable comparison of the analytical sensitivity (ASe) of the molecular assays. Clarified cell culture supernatant will be gamma-irradiated at 50kGy and tested to determine the degree of degradation of the TiLV RNA by the gamma-irradiation. Previous work done at AAHL with other finfish viruses suggests this will not render the material unable to be used.

If the TiLV molecular tests perform as expected preliminary assessment of analytical specificity (ASp) will be undertaken for each of the TiLV molecular tests using a nucleic acid extracted from a number of finfish viruses held at AAHL.

### Inter-laboratory comparability panel

The inter-laboratory comparability panel would consist of 20 positive and 10 negative samples that would include:

1. 10-fold dilution series (7 samples) to enable estimates of efficiency of real-time molecular assays;
2. Strong positive (at least 2 samples);
3. Medium positive (at least 2 samples);
4. Weak positive (at least 2 samples);
5. 10-fold dilution of medium and low positive;
6. Positive samples with various viral concentrations to make up the 20 positive samples;
7. Negative samples consisting of supernatant of uninfected cell culture (10 samples).

Material will be provided as a gamma-irradiated cell culture supernatant with 50µL extracted and tested.

Not explicitly stating the planned composition of the panel is not to act as a test of the capability of the *ad hoc* Group member's laboratories, it is simply that it is good laboratory practice to provide "blinded" samples to participants undertaking this kind of test evaluation. Multiple aliquots of each of the different samples will be stored.

Homogeneity testing will be undertaken using 10 aliquots of each different concentration, with a coefficient of variation of <5% indicating homogeneity of the samples. Additional stability testing, using three aliquots of each sample will be undertaken at -20°C, 4°C and 22°C, at Day 0, Day 7 and Day 14, to make sure there are no stability issues with transport delays of the inter-laboratory comparability panel to *ad hoc* Group member's laboratories. Stability testing will also be undertaken when all laboratories have reported results to check the stability of stored aliquots at AAHL.

Participating laboratories will receive the samples as numbered tubes to test them blind with panels tested at least three times. Results will be reported back to the Chair of the *ad hoc* Group for collation and reporting back as uncoded results to the participating laboratories for discussion. Use of duplicate samples and 10-fold dilutions of samples will enable statistical analysis to determine repeatability and reproducibility.

Initially, the inter-laboratory comparability panel will use different dilutions of a single TiLV isolate. When additional TiLV isolates are obtained from different geographical locations, ASp and ASe will be determined.

Depending of the results new assays may need to be designed if not all isolates are detected by one, or more, of the molecular tests. Regardless, a second inter-laboratory comparability panel could be prepared as described above, but using several different geographical isolates of TiLV, to provide greater confidence on the robustness and repeatability of the tests.

The inter-laboratory comparability panel will also be provided to laboratories outside the *ad hoc* Group if these laboratories provide TiLV material can be used to support the activities of the *ad hoc* Group.

#### Result reporting

A result reporting sheet will be prepared which will request as much information as possible about the testing process in each laboratory. Information will include extraction methods/kits used and volume extracted, and volume eluted, molecular test reagents/kits used and reaction volume and template volume used and result recoding and interpretation, including threshold for real-time tests and cut-off determination. This will be provided prior to the shipment of the inter-laboratory comparability panel and a draft will be provided to *ad hoc* Group members for comment. It is highly desirable to have all testing and reporting submitted within one month of receipt of the panel.

#### **Next steps**

The *ad hoc* Group will continue their work and will report back progress to the next meeting of the Aquatic Animals Commission in February 2019.

#### **References:**

DONG, H., SIRIROOB, S., MEEMETTA, W., SANTIMANAWONG., W., GANGNONNGIW, W., PIRARAT, N., KHUNRAE, P., & RATTANAROJPONG, T., VANICHVIRIYAKIT, R. & SENAPIN, S. (2017). Emergence of tilapia lake virus in Thailand and an alternative semi-nested RT-PCR for detection. *Aquaculture*, **476**, 111–118.

TATTIYAPONG P., SIRIKANCHANA K. & SURACHETPONG W. (2017). Development and validation of a reverse transcription quantitative polymerase chain reaction for tilapia lake virus detection in clinical samples and experimentally challenged fish. *Journal of Fish Diseases*, **41(2)**, 255–261.

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.../Annexes



## REPORT OF THE OIE AD HOC GROUP ON TILAPIA LAKE VIRUS

### List of participants

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Annex I (contd)

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### **Terms of Reference**

The electronic *ad hoc* Group should:

1. Critically review the available literature regarding detection methods for TiLV and any unpublished methods that may also be available.
2. Provide recommendations on additional method development requirements.
3. Provide recommendations on method validation requirements.
4. Determine sources of well-characterised viable and non-viable positive control material for use in method evaluation and implementation in laboratories.
5. Develop a work plan for inter-laboratory comparability studies.
6. Draft a report by the end of January 2018 to be considered by the Commission when they meet in February 2019.

*Ad hoc* Group members should be familiar with Chapter 1.2. *Criteria for listing aquatic animal diseases* and the use of relevant glossary definitions in the *Aquatic Code*, and with the principles and methods of validation of diagnostic essays for infectious diseases in Chapter 1.1.2. of the *Aquatic Manual*.

