

## Inter-laboratory Proficiency Test 2, 2016

Copenhagen, 27<sup>th</sup> September, 2016

Dear colleague(s),

The inter-laboratory Proficiency Test **2016** consists again this year of two separate tests, designated 1 and 2 respectively. **This letter provides information on proficiency test 2 (PT2), only.**

Therefore, in PT2 we would like participants to identify any of the notifiable fish viruses **KHV** and **ISAV** listed in [Council Directive 2006/88/EC](#) according to diagnostic procedures given in [Commission Implementing Decision \(EU\) 2015/1554](#) whereas for identification of **SAV** we recommend to follow the guidelines included in [OIE Manual of diagnostic tests for Aquatic animals 2016 Chapter 2.3.6 Infection with salmonid alphavirus](#). On the background of the positive experience from Inter-laboratory Proficiency test 2015, also for 2016 the EURL has decided that the panel of pathogens to be investigated should be expanded to include SAV-Salmonid Alpha virus. Thereby please consider that one of the ampoules of PT 2 2016 might contain SAV. As we are aware that this is not a listed disease in the European legislation, all participants are free to decide if the ampoules in PT2 will be tested for SAV or not. When delivering the spreadsheet results of PT2 therefore participants are asked to declare if ampoules are tested for SAV or not. The EURL will then take care of calculating the score accordingly.

PT2 consist of 4 ampoules labelled VI, VII, VIII and IX, respectively. Each ampoule contains 0.2 ml lyophilised cell culture supernatant/milliQ water mixed in equal volumes with 2% w/v lactalbumin hydrolysate solution. The ampoules should be handled as described in Annex 1 and stored in the dark at 4°C after receipt if the solving of the test is not started right away. Beware that the ampoules may contain high titered virus and as such there is a risk of contamination. Please acknowledge receipt of the parcel immediately on arrival (by email to [tevk@vet.dtu.dk](mailto:tevk@vet.dtu.dk)).

Furthermore, participants should be aware that the test ampoules may contain other viruses (e.g. other fish herpes-, rhabdo-, rana- or birna viruses).

Participants are encouraged to use their normal laboratory procedures following [Commission Implementing Decision \(EU\) 2015/1554](#) whereas for identification of SAV we recommend to follow guidelines included in [OIE Manual of diagnostic tests for Aquatic animals 2016 Chapter 2.3.6 Infection with salmonid alphavirus](#). It is not mandatory to grow KHV and ISAV on cell cultures in this test, but the viruses have not been inactivated and should thus be viable in cell cultures.

In order to obtain a uniform way of answering you will be requested to download a spreadsheet that will be available from the [EURL web page](#) and insert your results in the white cells.

A special sheet (Ampoule VI-IX) is available for the virus identification results of PT2. Please submit your results in the spreadsheet in an email.

**The results should be returned to us no later than November 25<sup>th</sup>, 2016.** Please be aware that the European Commission will be forwarded your results.

For ISAV we strongly encourage participants to sequence the HPR region of the virus, as ISAV HPR0 is no longer on the list of non-exotic diseases by e.g. using the primer set described in Mjaaland et al. (2002), Virology 304, 379-391 for amplification and sequencing of the HPR region. We would kindly ask laboratories to submit all sequencing results that have been used for genotyping of isolates. Please use the spreadsheet (sequencing results) for submission of any sequencing data.

All data will be compiled and a report produced and returned for your information, with the coding of each participating laboratory kept confidential.

We request that you do not forward any virus- isolate which may be present in the received samples of the proficiency tests to third parts without having contacted the EURL-Fish diseases for permission in advance.

For further information please do not hesitate to contact us by fax, telephone or e-mail.

Sincerely yours,

Teena Vendel Klinge, Niels Jørgen Olesen and Niccolò Vendramin

*Annex1: Inter-laboratory Proficiency Test 2, (year)2016*

The ampoules were produced at the National Veterinary Institute, Technical University of Denmark from May to September, 2016.

Each ampoule contains 0.2 ml cell culture supernatant/MilliQ water mixed in a 1:1 ratio with a 2% w/v lactalbumin hydrolysate solution in water (0.4 ml/ampoule).

Please store the ampoules in the dark at 4°C upon receipt, and proceed with the examination at your earliest convenience.

Please be aware, when opening the ampoules the content will tend to escape due to the vacuum in the closed ampoules. We recommend using the small saw that is included in the shipment, for opening the ampoules. Opening of an ampoule is done by making a mark/scratch in one side of the ampoule (only one cut with the saw). Then wrap the ampoule in paper (to minimize the risk of cutting your hands on the broken glass) and open by cracking the top of the ampoule in the direction away from the saw marks. As the saw only makes a scratch on the outside of the ampoule, a saw can be reused to scratch more ampoules without cross contaminating samples. Re-dissolve the lyophilized material carefully in 2.00 ml cell culture or dilution medium (e.g. Eagles MEM supplemented with 10% foetal or newborn bovine serum and Tris or Hepes buffer), filter the solution through 0.45 µm membrane filter and transfer the solution to sterile tubes. The dilution is to be used directly for PCR analyses.

Use gloves, disinfect and discard all used material carefully between each ampoule in order to prevent cross contamination.