



Inter-laboratory Proficiency Test 2, 2023

Kgs. Lyngby, 29th September 2023

Dear colleague(s),

The inter-laboratory Proficiency Test **2023** consists again this year of two separate tests, designated 1 and 2 respectively. **This letter provides information on proficiency test 2 (PT2), only.**

The test consists of **4 ampoules** (labelled VI –IX) lyophilized cell culture supernatant. 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.

Please acknowledge receipt of the parcel immediately on arrival by email to Thomas Weise thomwei@aqua.dtu.dk

In PT2 we would like the participants to identify any of the notifiable fish viruses **ISAV and KHV** if present in the ampoules, bearing in mind that the test ampoules can contain other viruses (e.g. other fish alpha-; herpes-, rhabdo-, rana- and birna viruses), please consider that, at least one of the ampoules of PT 2 2023, might contain SAV. As we are aware that this is still not a listed disease in European legislation, each participant has voluntarily decided and declared beforehand whether PT2 ampoules will be tested for SAV or not. The EURL will take care of calculating the score accordingly. Beware that the ampoules may contain high amount of virus and therefore there is a risk of contamination.

Participants are encouraged to use their normal laboratory procedures following the following [the diagnostic manuals made available from the EURL](#) whereas for identification of SAV we recommend to follow the guidelines in the [WOAH Manual of diagnostic tests for Aquatic animals 2021 Chapter 2.3.8 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 in theory be viable in cell cultures.

In order to obtain a uniform way of answering, please submit your results in the Excel spread sheet (Pt. 2 Ampoule VI-IX) available for download at the EURL web page, <http://www.eurl-fish-crustacean.eu/> under 2023. Remember to fill the form according to related instructions available on the website. Kindly remember: only fill in the virus name under ‘Concluding Results’ and only fill in the genotype under ‘Genotype’. Please fill in Ct. values in the column named “Ct value”. Ct.-values provided from all participants will be compiled and analysed.



We would kindly ask laboratories to submit all sequencing results that have been used for genotyping of isolates by using the spread sheet 'Sequencing results'.

The genotyping results provided from all participants will be compiled and analysed.

Please submit the filled out spreadsheet in an email to Teena Klinge tevk@aqua.dtu.dk

The Proficiency test is meant to be a tool for each laboratory to assess its own capacity of detecting and identifying fish pathogens; therefore the content of the ampoules and the results obtained during the examination has to be kept confidential by each participant until information on the content in each ampoule have been released from the EURL.

The results should be returned to us no later than December 15th, 2023. Please be aware that we will forward the results from each laboratory to the EURL desk officer at the European Commission.

In regards to the **ISAV isolate, it is mandatory to sequence the HPR region of the virus**, as HPR0 ISAV is not listed. The sequencing protocol recommended is the one provided by [Mjaaland et al. \(2002\), Virology 304, 379-391](#) for amplification and sequencing of the HPR region. Not providing the description of HPR type in the concluding result will lead to reduction in score for the specific ampoule.

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 telephone or e-mail.

Sincerely yours,

Teena Vendel Klinge, Niccolò Vendramin



Annex1: Inter-laboratory Proficiency Test 2, 2023

The ampoules were produced at the National Institute for Aquatic Resources, Technical University of Denmark in May and June, 2023.

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! For opening the ampoules, we recommend you to use the small saw that is included in the shipment. Opening of an ampoule is done by marking with the blade you receive in the kit, the thin neck of the glass ampoule. You can see a video tutorial on how to open the ampoules at <https://www.eurl-fish-crustacean.eu/fish/proficiency-test>. 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.