

## Inter-laboratory Proficiency Test 2, 2013

Copenhagen, October 2<sup>nd</sup>, 2013

Dear colleague(s),

The inter-laboratory Proficiency Test **2013** 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** (all listed in [Council Directive 2006/88/EC](#)), even though the oomycete *Aphanomyces invadans* was delisted, it has been included this year as well. PT2 consist of 5 ampoules labelled VI, VII, VIII IX, and X respectively. Each ampoule contains 0.2 ml lyophilised cell culture supernatant/milliQ water mixed in equal volumes with 20% 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 active virus or large amounts of inactivated *Aphanomyces invadans* spores and as such there is a risk of cross contamination. 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 expected to use their normal PCR or real-time PCR methods for detection of KHV, their normal RT-PCR or real-time RT-PCR methods for detection of ISAV and their normal PCR method for detection of *Aphanomyces invadans*. 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 coloured boxes.

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.

If present, only **inactivated** *Aphanomyces invadans* will be found in the ampoules.

**The results should be returned to us no later than November 29<sup>th</sup>, 2013.** Please be aware that the European Commission will be forwarded your results.

For **ISAV** we encourage participants to sequence the **HPR region of the virus**, 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- or *Aphanomyces invadans* isolate which may be present in the received samples of the proficiency tests to third parts without having contacted the EURL for permission in advance.

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

Sincerely yours,

Anemone Ojala, Niels Jørgen Olesen and Niccolò Vendramin

*Annex1: Inter-laboratory Proficiency Test 2, (year)2013*

The ampoules were produced at the National Veterinary Institute, Technical University of Denmark from **May - August**, 2013.

Each ampoule contains 0.2 ml cell culture supernatant/MilliQ water mixed in a 1:1 ratio with a 20% 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.