National Veterinary Institute
EUROPEAN UNION REFERENCE LABORATORY FOR FISH DISEASES

Inter-laboratory Proficiency Test 1, 2016

Copenhagen, 27th *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 1 (PT1), only**.

PT1 consist of 5 ampoules labelled I, II, III, IV, and V, respectively. Each ampoule contains 0.2 ml lyophilised cell culture supernatant mixed in equal volumes with 2% w/v lactalbumin hydrolysate solution. The ampoules should be handled as described in Annex 1 and should be 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).

In **PT1**, participants are asked to identify all pathogen listed in Council Directive 2006/88/EC) according to the diagnostic procedures included in Commission Implementing Decision (EU) 2015/1554 or in the OIE Aquatic Manual. This means that participants are asked to **identify any of the notifiable fish viruses VHSV and IHNV** and **to isolate any Rana-virus if present; furthermore the rana-virus isolate has to be further characterized in order to determine whether it is the listed pathogen EHNV or another representative of the ranavirus family**. Participants should be aware that ampoules can also contain other viruses (e.g. other fish rhabdoviruses, ranaviruses, or birnaviruses). Participants are encouraged to use their normal standard laboratory procedures. However, the identification should be performed according to the procedures laid down in Commission Decision (EU) 2015/1554 using monolayered cell cultures followed by e.g. neutralisation test, immunofluorescence, ELISA or PCR. If ranaviruses should be present in any of the ampoules, it is mandatory to perform a sequence analysis of the isolate in order to determine if the isolate is EHNV.

Since monolayered cell cultures are important tools to be used for fish health surveillance for VHS, IHN and EHN, and for isolation of the respective viruses, we ask each participant to perform a titration of the content of each ampoule according to the procedures described in Annex 2. This ensures that the same procedures are used in all laboratories, making a comparison of the obtained titres – and the sensitivity of the fish cell cultures used - possible. Please record titration and identification results, and make a note on the date of arrival as well as the initial date of testing. In order to obtain a more uniform way of answering, please download the spreadsheet from the EURL web page and insert your results in the white cells.

Please submit the filled out spreadsheet in an email.

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We encourage all laboratories to identify VHSV and IHNV isolates as far as possible by means of genotyping in addition to the standard methods. Please use the genotype notification described in Einer-Jensen et al. (2004), Journal of General Virology 85, 1167–1179 for VHSV and for IHNV, either method as mentioned in the IHN chapter of the 2013 version of the OIE manual on Aquatic Animal Diseases (Emmenegger et al. (2000), Diseases of Aquatic Organisms 40 (3), 163-176 or Kurath et al. (2003), Journal of General Virology 84, 803-814) We would kindly ask laboratories to submit all sequencing results that have been used for genotyping of isolates. Please use the spreadsheet for submission of sequencing data.

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

Within the spreadsheet to be downloaded from the EURL web page is included a questionnaire on the accreditation status of the laboratory. We kindly ask all participants to fill the questionnaire and send it to us together with the results of the proficiency test. Please use the Excel spreadsheet to be downloaded from the EURL web page to register the results:

- Ampoule I-V: Virus identification results and virus titration results
- Sequencing results: Sequencing data
- Accreditation situation: Questionnaire on accreditation status

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.

Yours sincerely,

Teena Vendel Klinge, Niels Jørgen Olesen and Niccolò Vendramin

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***Annex 1: Inter-laboratory Proficiency Test 1,* 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 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 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 glass 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 titration. We recommend that the dilution is also used for inoculation onto cell cultures in 24-well plates for identification and characterisation.

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

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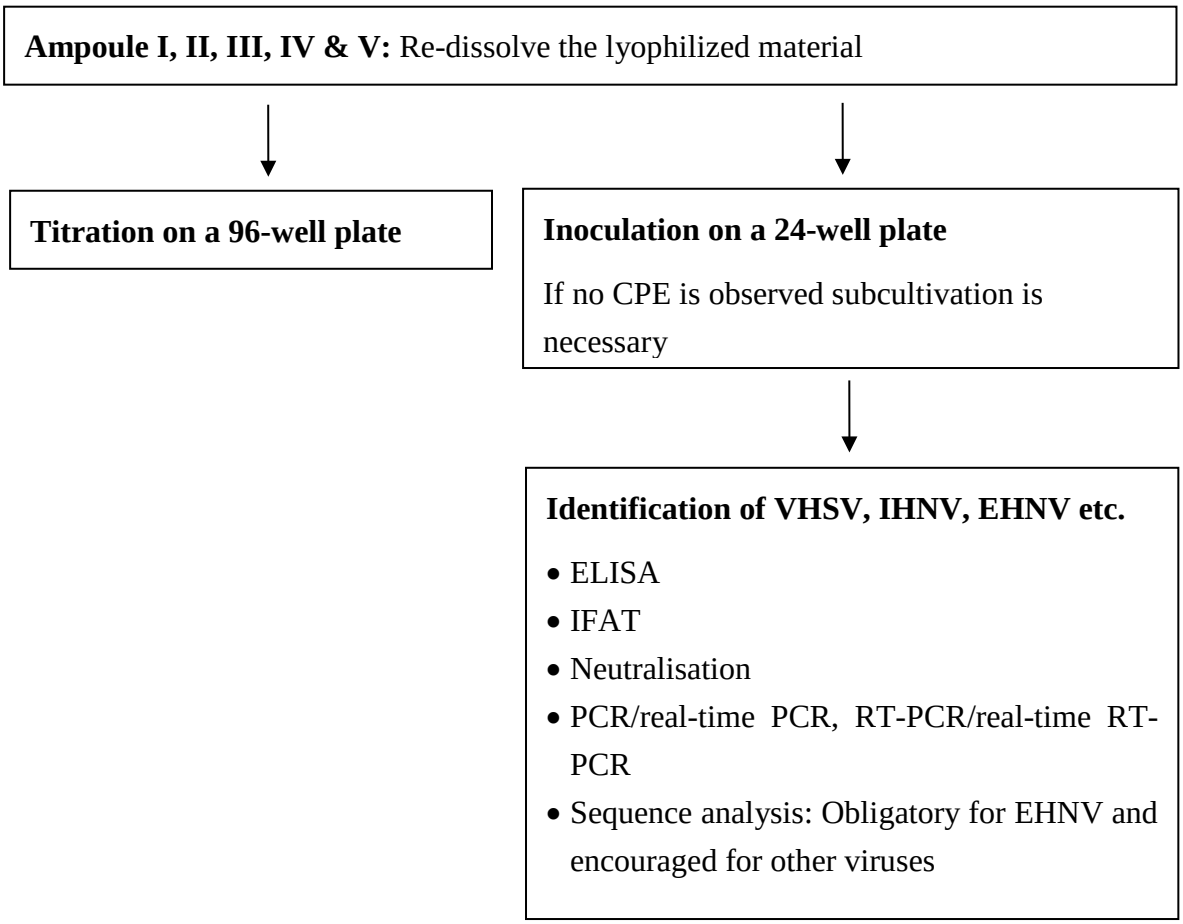
Recommendations for solving the proficiency test PT1



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Annex 2.1 PT1: Titration procedure

Re-dissolve the lyophilized material carefully in 2.00 ml cell culture medium supplemented with 10% foetal (FCS) or newborn calf serum and Tris or Hepes buffer (dilution medium), filter through 0.45 µm membrane filters and transfer the solution to sterile tubes.

For dilution of the content of the ampoules, use one 96-well micro titration plate for each ampoule (e.g. Micro well Plates 262170, Life Technologies).

1. Per cell line to be used for titration: Transfer 180 µl dilution medium into 7 wells in one column (e.g. well B1 to well H1 for BF-2 cells, B2 to H2 for EPC etc., please consult annex 2-2).

2. Transfer 200 µl re-dissolved virus into the appropriate number of wells in row A (e.g., transfer the content of ampoule I to well A1, A2, A3 and A4 if 4 cell lines is used for titration). Ampoule II, ampoule III, ampoule IV and ampoule V is diluted in a similar manner on separate plates.

3. Use a multi-channel pipette for 20 µl volumes for making the dilution. Lower the tip into the undiluted virus in row A, mix 20 times and transfer 20 µl to the surface of the medium in the wells of row B. Put on new tips, lower into wells in row B, and mix 20 times and transfer 20 µl to the surface of the medium in wells in row C. Put on new tips, an continue as described above.

For each ampoule, use a 96-well cell culture plate with 24 hours old BF-2 or RTG-2 cells, and EPC or FHM cells. A seeding density of 50.000 to 100.000 cells per well, in our laboratory, results in approximately 80% confluence after 24 hours incubation at 20°C, but this may vary from laboratory to laboratory. Use normal cell culture medium (e.g. Eagles MEM with 10% FCS, antibiotics, & Tris-buffer), 150 µl per well.

Inoculate 25 µl/well of each virus dilution into 6 replicate wells for each cell line, using a multi-channel pipette. The dilution and inoculation procedure is illustrated on the following page.

Incubate at 15°C until final reading 7 days after inoculation, where wells with CPE are registered.

Please use the Excel spreadsheet to be downloaded from the EURL web page to register the results. Please register the following:

- The titration results (X for those wells with CPE and – for those without, see explanation in the spreadsheet),
- Details of the cell cultures used for the titration and
- Identification results and methods in the tables provided in the spreadsheet.

In order to assure a uniform calculation of titres, all titres will be calculated at the National Veterinary Institute, Technical University of Denmark (DTU Vet) based on the raw data presented in spreadsheet to be downloaded from the EURL web page

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Annex 2.2 PT1

1. Dilution plate

One plate for each ampoule

			Ampoule I											
Cell line			BF-2	EPC	RTG-2	HFM								
10 fold virus dilution			1	2	3	4	5	6	7	8	9	10	11	12
200µl re-dissolved	10^{0}	A												
20µl re-dissolved	10^{-1}	B												
20µl 10^{-1}	10^{-2}	C												
20µl 10^{-2}	10^{-3}	D												
20µl 10^{-3}	10^{-4}	E												
20µl 10^{-4}	10^{-5}	F												
20µl 10^{-5}	10^{-6}	G												
20µl 10^{-6}	10^{-7}	H												

96-well plate with 24-hour old cell monolayer		BF-2/RTG-2						EPC/FHM					
		1	2	3	4	5	6	7	8	9	10	11	12
10^{0}	A												
10^{-1}	B												
10^{-2}	C												
10^{-3}	D												
10^{-4}	E												
10^{-5}	F												
10^{-6}	G												
10^{-7}	H					C	C					C	C

C = Control well, cell suspension without virus

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