



Dear colleagues,

These instructions refers to the excel spreadsheet to be filled and delivered with PT results.

Since the management of data collection and analysis is made via an excel-based procedure, we kindly ask you to follow strictly the instructions. Please use the file exactly as we deliver it to you, don't make a copy and change the structure. Please fill in the white cells.

#### Concerning 'Accreditation situation':

- Insert country and name of laboratory - ones inserted data will automatically be transferred to the other sheets.

#### Concerning 'PT1 Ampoule I-V':

- Fill in the arrival date of the ampoules.
- As described in the example in the spreadsheet, for all tests except real-time (RT-) PCR: fill in the white cells with "+" (test performed and **positive**) and "-" (test performed and **negative**). Leave N/A if you did not perform the test.
- In case you performed a real-time (RT-) PCR, please insert Ct value in the column '*Real-time (RT-) PCR Ct value*'. NOTE: only include the Ct value if the test is positive according with the criteria followed in your laboratory. If the test is negative, fill with "-", even if a Ct value beyond your cut-off value was found. Please report in the comment box if test was found negative but a high Ct value was observed and please include the Ct value here. *Please note that we are asking for the Ct values obtained directly from the resuspended ampoule; not for the Ct values obtained from the isolates after cell culture isolation.*
- Concerning the column "Concluding results", use only the name of the virus proposed in table 1.
- Regarding the concluding results for Ranavirus, if present, use the name "EHNV" or "Ranavirus – NOT EHNV", this definition shall be corroborated by the sequencing results
- Please fill in white cells "date of inoculation", "date of final reading". "Days of inoculation" are calculated automatically.
- Do only use "X" or "x" when filling out the titer table and nothing else such as "+"
- Remember to tick which of the cell lines is used for titration (BF-2, EPC, FHM or RTG-2).
- Please insert detailed information about the isolate directly in the sequencing tab of the spreadsheet file.

#### Concerning 'PT2 Ampoule VI-IX':

- As described in the example in the spreadsheet, fill in the N/A prefilled white cells with "+" (test performed and **positive**); "-" (test performed and **negative**) and just leave the N/A if you did not perform the test.
- If Real-Time (RT-) PCR has been performed, please indicate with "+" in the 'Real-time (RT-) PCR' column if the sample is found positive or "-" if it is found negative. Insert the Ct.-value in the Ct-value column, also if the Ct value is higher than your cut-off value (and the sample therefore is negative).
- Concerning the column "Concluding results", use only the name of the virus as suggested in table 1 and remember to indicate if HPR-deleted ISAV or HPR0 ISAV was identified.
- In order to improve harmonization between procedure and protocols used in different fish disease laboratories, please insert in the table which kind of thermocycler you use, which software, the nucleic acid purification kit, the primers etc.
- Please insert detailed information about the isolate in the sequencing sheet.



**Concerning ‘Sequencing results’:**

- The column ‘Pathogen Identification’ will be filled in automatically, transferred from the sheets with the results on PT1 and PT2
- Concerning the column “Genotype”, type in only the genotype of the virus. See table 1.
- Feel free to type in all other relevant information such as serotype, possible isolate identification etc. in the column ‘Additional information’.

Table 1:

To be inserted in “Concluding results” for Ampoule	To be inserted in “Genotype” for Sequencing
VHSV	I (a-e), II, III, IV (a-d)
IHNV	U, M, L, E, J
EHNV	EHNV
Ranavirus – NOT EHNV	Not EHNV
SVCV	Genogroup 1 (a-d), 2, 3, 4
Birnavirus II (Telinavirus)	-
Perch Rhabdovirus	-
IPNV	Genogroup 1, 2, 3, 4, 5 Optionally: within genogroup 1 – genotype (1-4)
HPR-deleted ISAV <b>or</b> HPR0 ISAV	HPR-deleted / HPR0
KHV	CyHV (1-3)
SAV	1, 2, 3, 4, 5, 6

Do not hesitate to contact us if you need further information or explanation and remember that suggestions for improving are always welcome.

Teena Klinge and Niccoló Vendramin