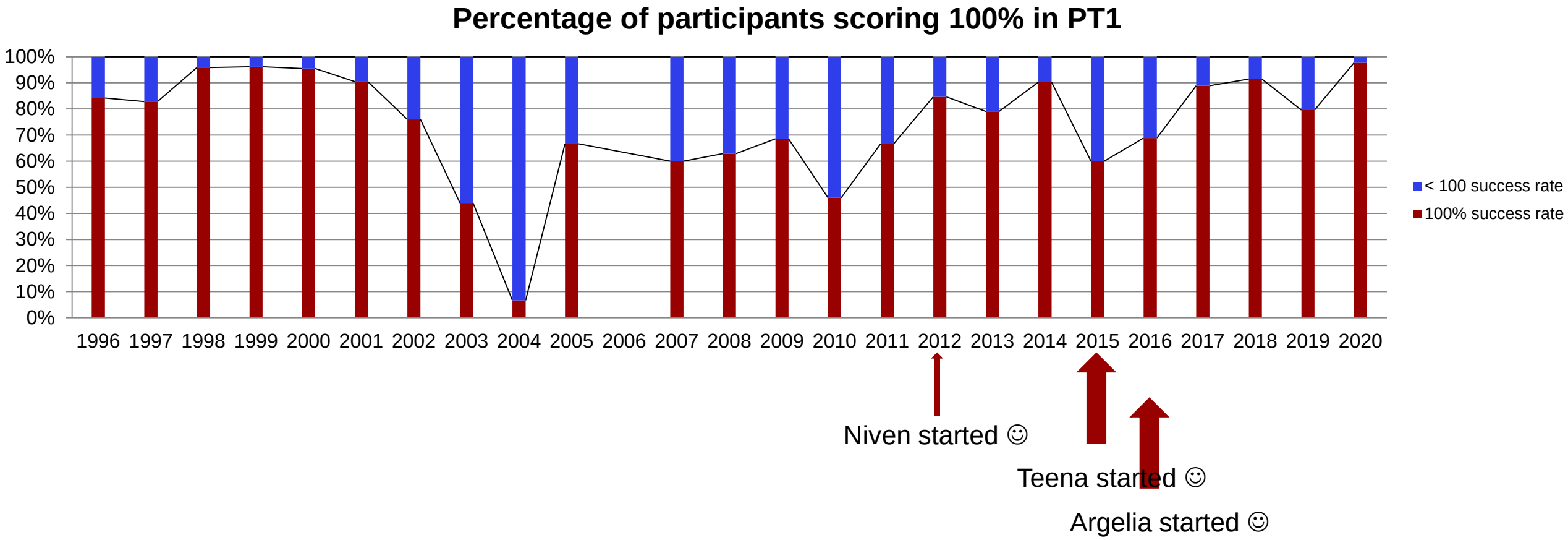
Results from ILPT 2020 for listed fish diseases

25.03.2021 – Zoom meeting between EURL for fish diseases and participants to the ILPT 2020 for fish diseases

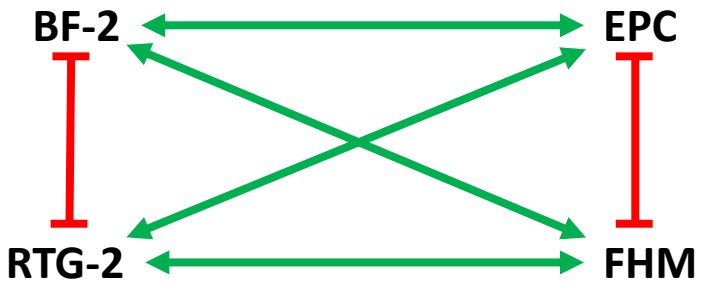

Percentage of participants scoring 100% in PT1
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1996 1997 1998 1999 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015 2016 2017 2018 2019 2020
< 100 success rate
100% success rate
Niven started ☺
Teena started ☺
Argelia started ☺

Testing PT1 the use of cell lines

- The proficiency test was prepared under DS/EN ISO/IEC 17043

- The reductions of the titres after lyophilisation were between 1-3 log in the same cell line.

This highlights the importance of using two heterologous cell lines which allow optimal replication for viral isolates.



Use of cell culture

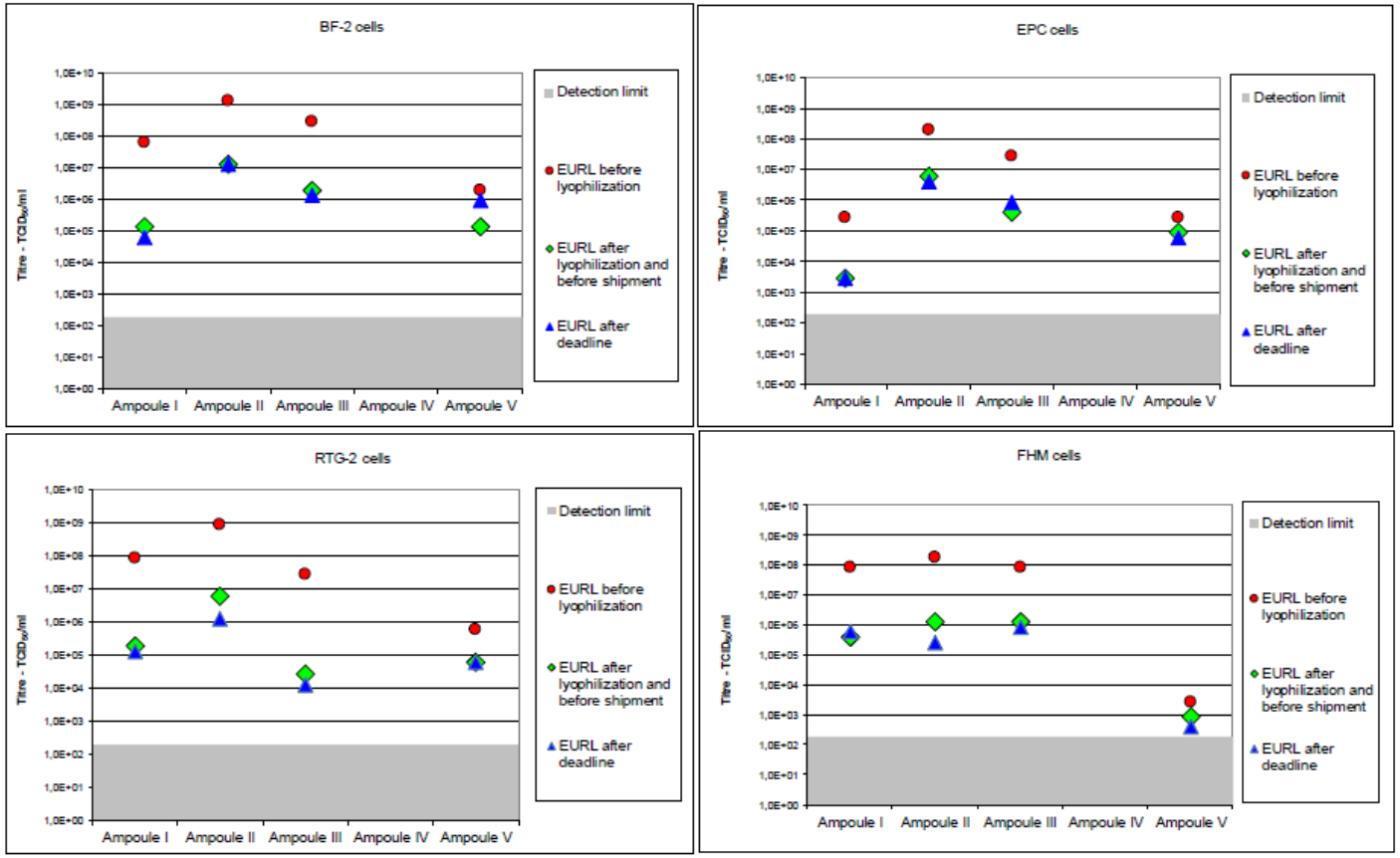
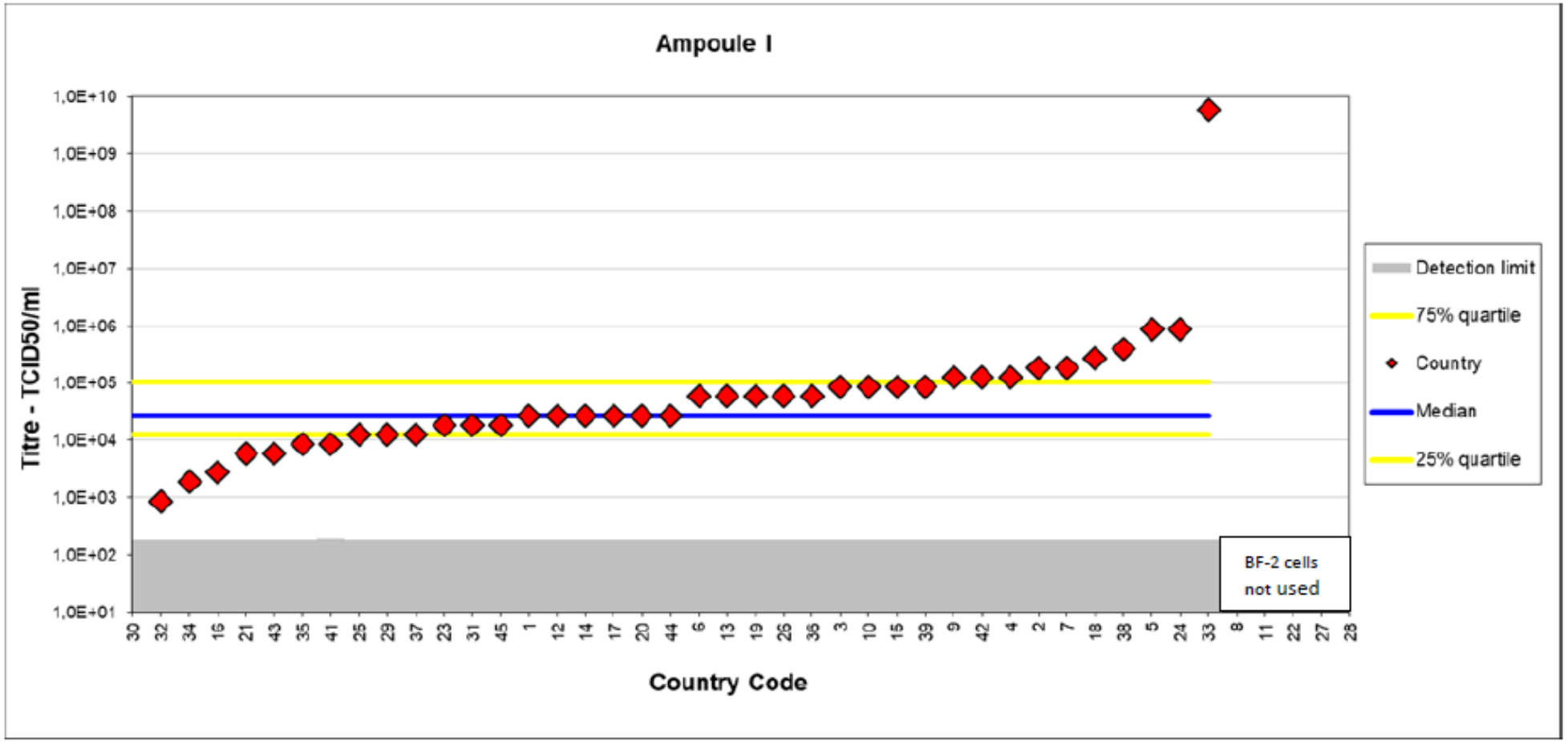
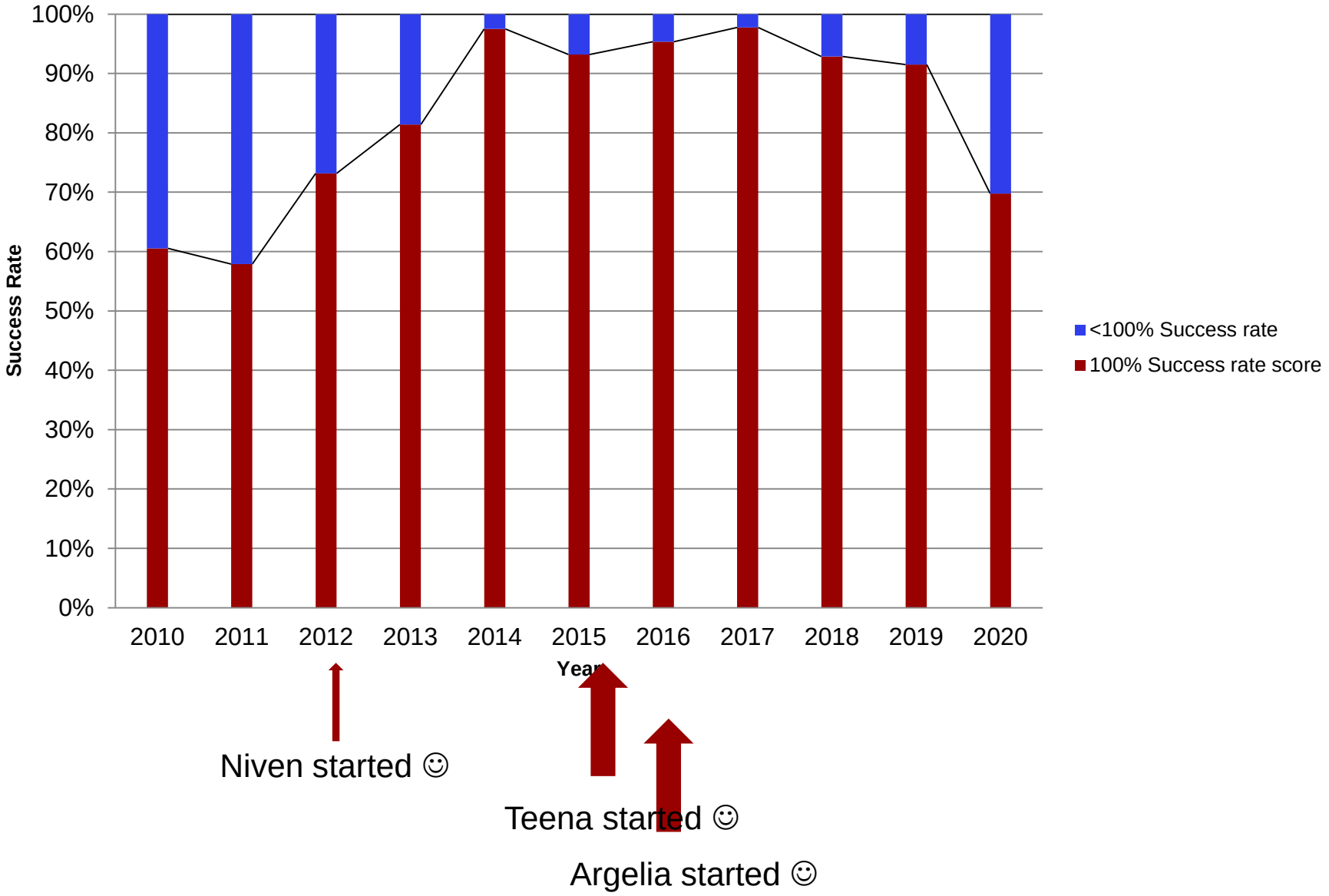


Figure 4. Virus titres in different cell lines: before lyophilisation, before shipment and after deadline for handling in results (storage 4°C in the dark).

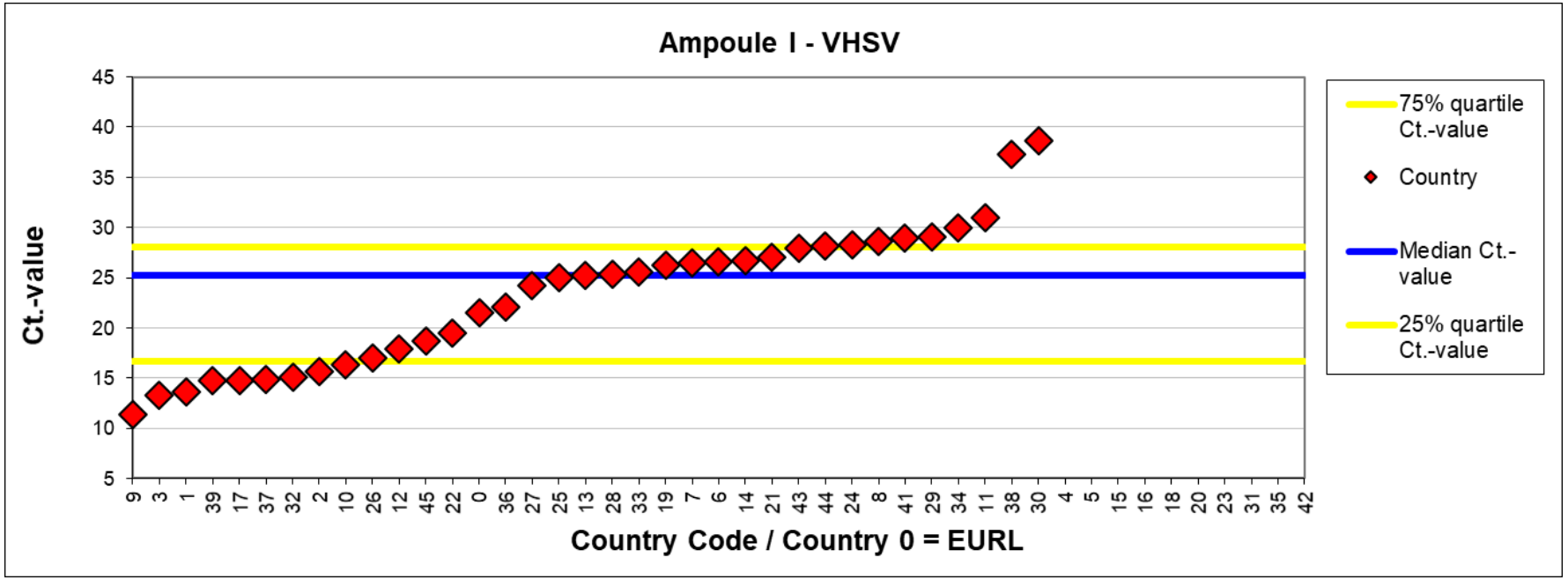
Comparing the titration results - poll



Percentage of participant scoring 100% in PT2



Comparing the ct results - poll



Median Ct.-value	25,3
Maximum Ct.-value	38,7
Minimum Ct.-value	11,3
25% quartile Ct.-value	16,7
75% quartile Ct.-value	28,1

1- from ampoule or from cell culture isolate?
2- differences in purification kit
3- different machines
4- different set up (threshold)
5- hopefully similar primers/probe

Scoring – PT1

Scores

Starting with proficiency test 2003 we have provided a scoring system for the identification part of the proficiency tests. We have assigned a score of 2 for each correct answer/identification, giving the possibility for obtaining a maximum score of 10 (Table 3).

- **Ampoule I:** VHSV identification was given the score 2.
- **Ampoule II:** IHNV and IPNV identification was given the score 2, identification of only IHNV would have given 1 point and identification of IPNV only would have given 0 points to this ampoule.
- **Ampoule III:** SVCV identification was given the score 2.
- **Ampoule IV:** Identification “No virus”, “Blank”, “Not IHNV, not VHSV, not IPNV, not SVCV, not EHNV” or similar answer was given the score 2
- **Ampoule V:** EHNV identification was given the score 2. No identification of the Ranavirus by sequencing or REA was given the score 1.

Underperfomances – PT1

- Linked to Ranavirus- Specifically discrimination of EHNV (currently exotic pathogen – to be included in list A) and endemic ranavirus (ESV-ECV)
  - Discrimination has to be performed either by sequencing MCP or Restriction Enzyme Analysis (REA) . According to OIE diagnostic manual

One laboratory reported use of commercial specific pathogen qPCR assay, however the provider does not make available documentation, and no further validation was performed by the laboratory. General topic for discussion

Comments – PT1

- 1 laboratory reported CPE in the titration plate of Ampoule IV – concluding remarks – Negative

Scoring – underperfomances Sequencing PT1

2 points per correct isolate genotype were given (eg. Amp.1 Vhsv gen. Ia = 2 points ; Amp.1 Vhsv gen. 1 = 1 point)

- Amp. 1 VHSV 3 labs which provided incomplete genotype (Gen. I instead of Ia) and 2 labs that provided wrong genotype (gen. II or gen.III)

- Amp.2 IHNV + IPNV. Various define genotype M for IHNV (difficult to discriminate M and E in old isolate – so full score for the M)
- Still a few laboratory use Serotype Sp or SP for IPNV, but we are asking Genotype NOT serotype

- Amp.3 SVCV 2 incorrect genotype Id

- Amp. 5 - EHNV/RANA sequencing or REA (Restriction Enzyme Analysis) is obligatory to fully discriminate exotic EHNV (from april 2021 IN LIST A disease)

- AMP 1,2,3 in the scoring of Genotyping
- AMP 5 in the official score of the certificate

Scoring – PT2

A bit more complicate because of co-infection

We have assigned a score of 2 points for each ampoule (Table 13), giving the possibility for obtaining a maximum score of 8. Identifying the correct pathogen or the correct combination of pathogens in one ampoule gives score of 2 points.

Special criteria were applied in ampoule VIII with co-infection of SAV and ISAV:

- Detecting both pathogens and correctly identifying ISAV as HPRΔ gave full score of 2.
- Not detecting SAV and correctly detecting and typing ISAV as HPRΔ gave 1 point.
- Detecting SAV and ISAV but incorrectly typing ISAV as HPR0 gave 1 point.
- Detecting SAV only and not ISAV gave 0 point.
- Detecting only ISAV but incorrectly typing ISAV as HPR0 gave 0 point.

Underperfomances – PT2

- For PT2 the underperfomances related to:
- ISAV HPR0 /HPR del – 2 laboratories identified HPR0 instead of HPR del.

ISAV HPR Del is requested in the concluding results . For this **year for the last time**, if “ISAV” only was included in concluding results we cross-check with Genotype cell and or Sequence provided.

SO for ISAV in concluding results if it is “ISAV-HPR deleted” or “ISAV HPR 0” – if not specified in the concluding results score reduced

In genotyping cells, refer to HPR only, no G Europe /North America etc. for the sequencing protocol reference to the new diagnostic manual (on EURL website from mid April 2021)

If one laboratory sequence ISAV but do not assign genotype in the genotype cells reduced score

SAV this year was SAV3 – 1 laboratory provided SAV 4 instead of 3

For KHV – we request to indicate CyHV-3 or KHV in the genotyping cells – this has to be corroborated by a sequence to count for the score (not obligatory).

Thanks for your attention ☺

- Question – comments – inputs?