European Union Reference Laboratory for Fish Diseases
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PvP

IMPROVEMENT OF MOLECULAR DIAGNOSTIC TOOLS FOR A BETTER CONTROL OF VIRAL FISH PATHOGENS

INTER-PROFICIENCY TEST FOR IHNV REAL-TIME RT-PCR

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With the participation of Reference Laboratories of Denmark, Italy, Norway, Scotland, Czech Republic, Croatia, Germany, France

Detection of IHNV by one-step RT-PCR

IHNV real time RT-PCR described by Purcell *et al*., 2013 targeting the N-gene

Procedure was fully validated and we wanted to include it in our panel of diagnostic methods

Adapted into a « one step » protocol: faster and less prone to contamination

We organized two PTs as part of the validation process at the EURL for fish diseases, and at the NRL France

PT organized for the EURL for detection of IHNV using one-step RT-PCR

Each lab received a set of FTA cards with 10 samples by duplicate

PT was shipped using regular post (2 – 10 days)

Lab H received PT one month later and stored them for several months before processing

Sample	Content	Ct value stock	Ct value after Inoculation	Ct value after storage
1	IHNV isolate BLk94	21.5	24.6	25.4
2	IHNV isolate LR80	20.0	26.7	27.2
3	VHSV	19.8	-	22.6
4	IPN SP	26.5	-	29.7
5	IHNV isolate C18	20.8	27.0	27.8
6	IHNV isolate 4008	23.5	29.5	30.3
7	MEM media	-	-	-
8	Perch Rhabdovirus	+	-	+
9	IHNV isolate Nag06a	18.1	24.6	25.3
10	SAV	25.4	-	28.0

Samples:
One of each IHNV genotype

Four heterologous virus and one sample with only MEM

60 µl of cell supernatant was inoculated in the FTA cards.

Results

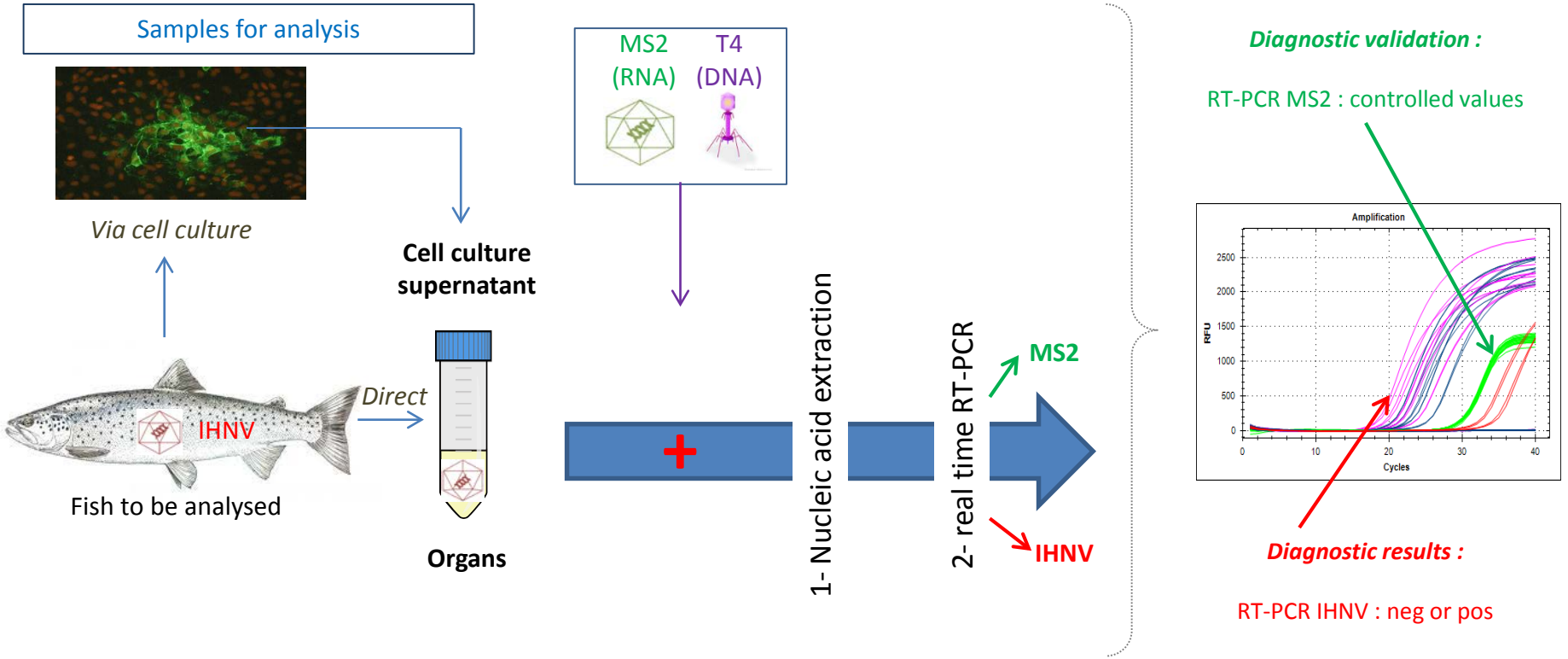
elution from FTA

sample	Laboratory							
	A	B	C[1]	D[2]	E[1]	F	G[3]	H
1- IHNV BIK94	21.9	22.7	23.54 24.84	27.78 27.83 27.66	27.90 25.84	20.6	20.7	22.1
2- IHNV LR80	22.6	23.7	23.60 22.89	29.12 29.51 29.59	29.14 26.00	21.9	22.6	23.0
3- VHSV	-	-	-	-	-	-	-	**Doubt 41.2**
4- IPN SP	-	-	-	-	-	-	-	-
5- IHNV C18	25.8	24.2	24.53 26.73	30.11 30.24 30.65			3.3	24.2
6- IHNV 4008	26.9	27.1	28.68 29.03	32.34 33.49 33.13	31.25 29.10	24.7	25.1	26.8
7- MEM	-	-	-	-	-	-	-	-
8 – Perch Rhabdovirus	-	-	-	-	-	-	-	**Doubt 39.6**
9 – IHNV Nag06a	23.1	21.6	21.82 21.37	27.92 27.60 28.01	27.47 24.74	19.7	20.8	22.7
10 - SAV	-	-	-	-	-	-	-	**Doubt 38.0**

Contamination?

Principle

- IHNV real time RT-PCR described by Purcell *et al.*, 2013 adapted into a « one step » protocol
- External exogenous control (bacteriophages) described by Ninove *et al.*, 2011



Preparation

- Sample preparation:
  - IHNV samples:
    - N61 (French isolate 1989, genotype E) at 3 dilution levels (low, medium and high)
    - PP14 (French isolate 2017, genotype E) at medium level
  - VHSV sample: OO61 (French isolate 2016, genotype Ia2))
  - Eagle medium

- Primers, probe and phage preparation

- Homogeneity and Stability tests
  → *CVs between 1.7 and 3%*

- Shipment to 5 labs (2 days at 5°C) and one parcel kept in our lab to mimic the shipment

Results

- Recommended protocol was sent to all participants but each laboratory could use its own protocol

- 7 sets of results with combination of :
  - 2 different enzymes
  - 4 different machines (including a Qiagen Rotor Gene)
  - 4 different elution volumes
  - 2 different primers and probe concentrations
  - 2 different volumes of RNA template
  - 2 different thermal conditions

→ Qualitatively, all labs gave some consistent results with the expected ones.

Results

- IHNV detection, analysis of 7 sets of results:

	Ct Mean (min – max)	Sd	CV%	Conformity
1	**21.21** (16.78 – 23.95)	2.50	**11.8%**	NC
2	0.00	0.00	/	C
3	**25.02** (20.89 – 28.25)	2.46	**9.8%**	C
4	0.00	0.00	/	C
5	**28.48** (23.91 – 31.13)	2.41	**8.5%**	C
6	**24.23** (16.62 – 27.73)	3.66	**15.1%**	NC

- IHNV detection (excluding the RotorGene cycler), Analysis of 6 sets of results:

	Ct Mean (min – max)	Sd	CV%	Conformity
1	**21.95** (19.90 – 23.95)	1.70	**7.8%**	C
2	0.00	0.00	/	C
3	**25.71** (24.39 – 28.25)	1.81	**7.0%**	C
4	0.00	0.00	/	C
5	**29.24** (28.01 – 31.13)	1.45	**5.0%**	C
6	**25.50** (23.90 – 27.73)	1.61	**6.3%**	C

All the CVs are below the 10% expected by the French NRL

Results

- MS2 bacteriophage detection, analysis of 7 sets of results :

	Ct Mean (min – max)	Sd	CV%	Conformity
1	**31.92** (27.5 – 35.0)	2.44	**7.7%**	C
2				
3				
4				
5				
6				

Compiling 42 Ct results (6 samples and 7 sets of results), CV is below the 10% expected by the French NRL

Conclusions

- The real time RT-PCR described by Purcell was adapted and validated in our lab
- The use of bacteriophage MS2 as an external exogenous control was adopted in our lab
- The French NRL was accredited in October 2017 for the IHNV diagnosis by real time RT-PCR
- At the EURL for fish diseases are starting the processes of the diagnostic validation aiming to be accredited soon
- We thank all the participants for their contribution and for these good results!