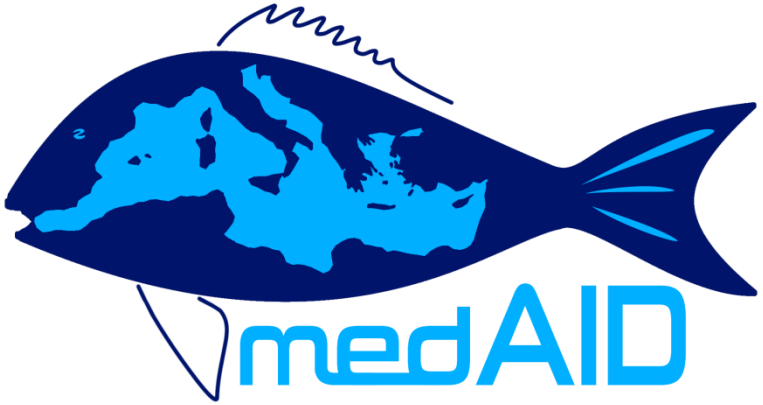


Results of the 2nd VER Inter-laboratory Proficiency Test (VER-IPT)

Toffan A., Pascoli F., Buratin A., Toson M., Panzarin V.

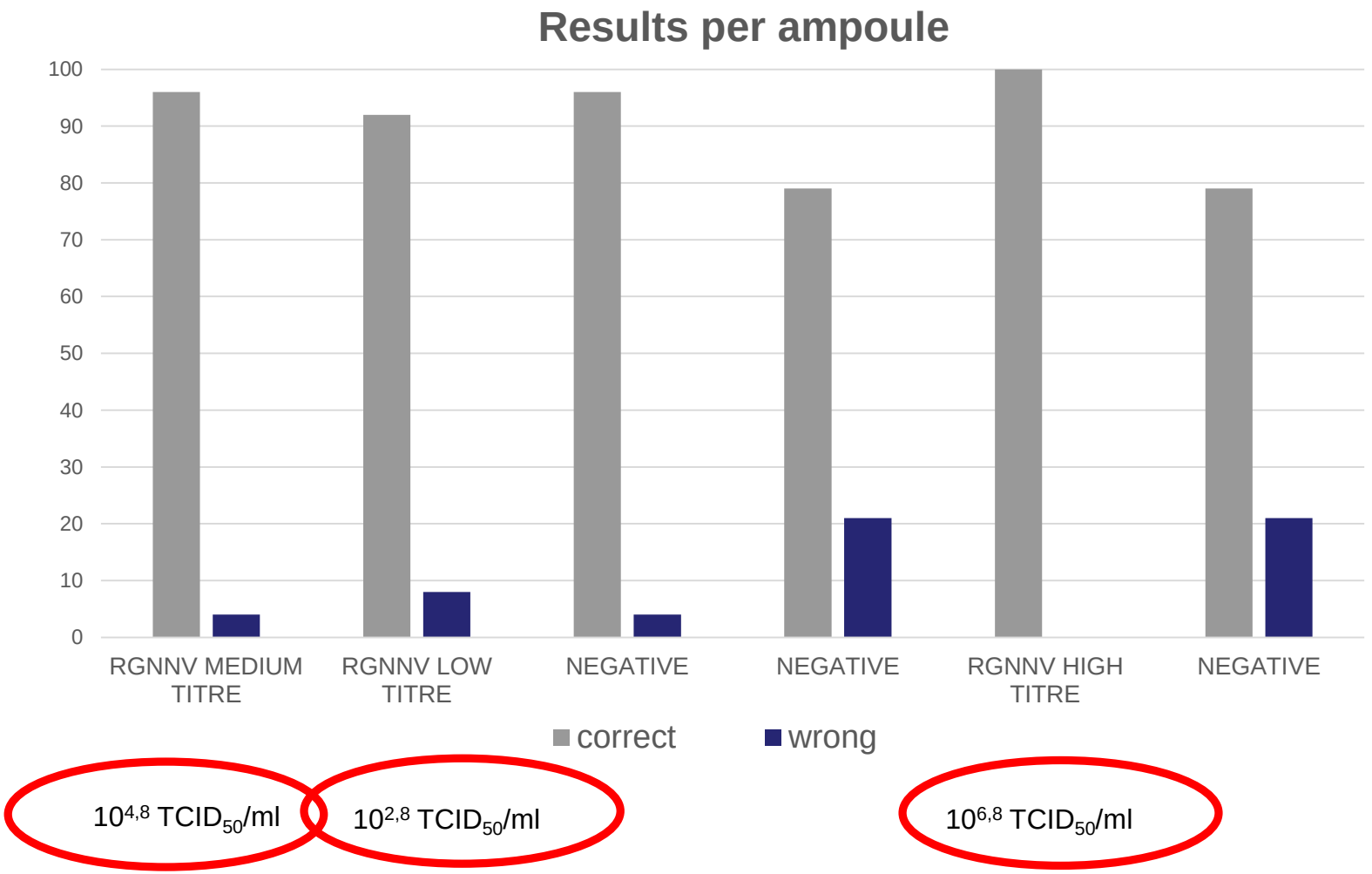
Istituto Zooprofilattico Sperimentale delle Venezie, OIE reference laboratory for Viral Encephalopathy and Retinopathy, National Reference Centre for Fish, Molluscs and Crustacean Diseases, Legnaro, Padova, Italy

1° VER-IPT in brief

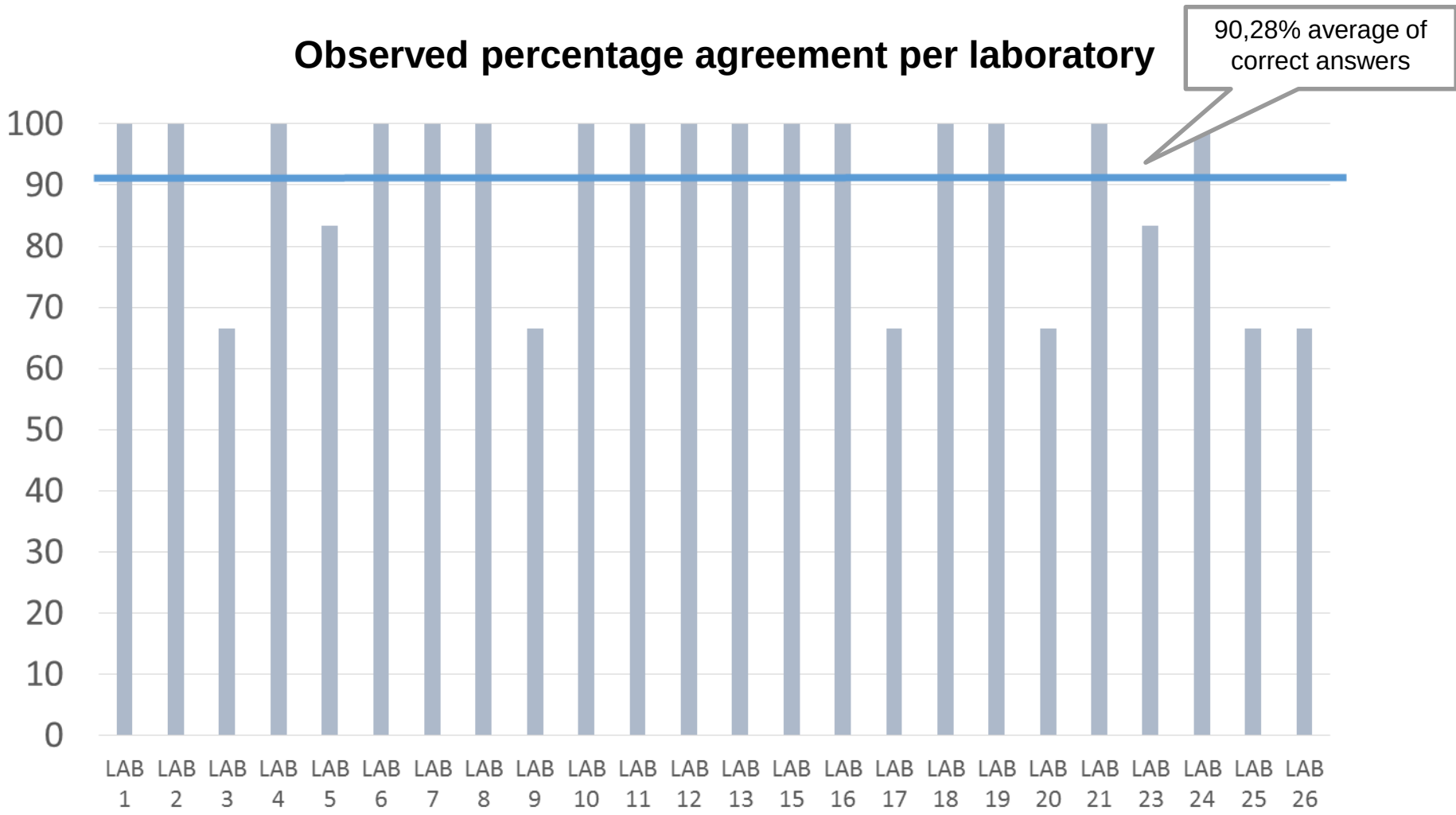
- The first VER-IPT was organized in 2016
- Only 1 genotype of betanodavirus included (RGNNV)
- 6 samples
- Real time RT-PCR only
- No sequence requested

26 applications, 24 participants

1° VER-IPT in brief



1° VER-IPT in brief



Results & Discussion of the 1° VER-IPT

- ✓ 16 out of 24 laboratories scored 100% correct results
- ✓ 8 laboratories produced a % of correct answers ranging between 66.67 - 83.33%.
- ✓ Overall agreement (K) 0.674

- ✓ Laboratories performing Real-Time RT-PCR for VER showed an overall **good sensitivity**
- ✓ Test **specificity** appeared to be the **major problem**
- ✓ Setting the diagnostic cut-off value is both a difficult and important task

- ✓ The **positive feedback** of participants emphasizes the importance of such initiatives to improve VER diagnosis

2° VER-IPT: Background

The **2nd Annual Inter-laboratory Proficiency Test (VER IPT)** for the molecular detection of betanodavirus was organized in the framework of

MedAID (Mediterranean Aquaculture Integrated Development) project,

a four-year project, funded by the EU in the frame of Horizon 2020

The goal of MedAID is to increase the overall competitiveness and sustainability of the Mediterranean marine fish-farming sector throughout the whole value chain.

- WP4: Health management, disease and fish welfare
- Task 4.2: Strengthening diagnostic capacities by harmonizing competences

2° VER-IPT: Aims

Aims:

Make an inventory of:

- laboratories performing diagnostic/research activities for betanodaviruses;
- laboratories able to genoptype betanodaviruses;
- molecular methods in use;
- collect other relevant epidemiologic data.

New features:

- **Different VER genotypes** included
- **Both real time RT-PCR and end-point RT-PCR** could be used
- Optionally, the **identification of the viral species** was requested

2° VER-IPT: Timetable

Activity	2017				2018			
	Aug	Sept	Oct	Nov-Dec	Jan	Feb-Mar	April	May
Preparation								
Stability testing								
Applications collection								
Shipping								
Testing time								
Results analysis								
Final report								

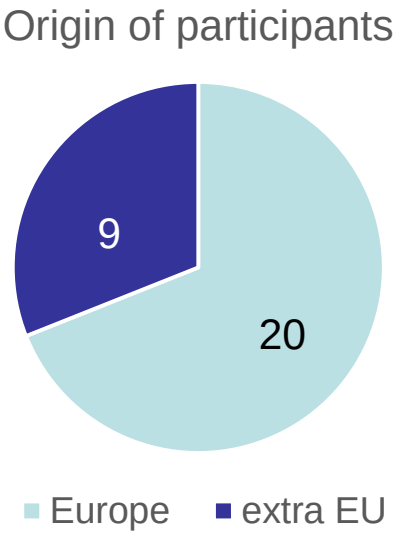
Deadline for results 23/03/2018

2° VER-IPT: Results

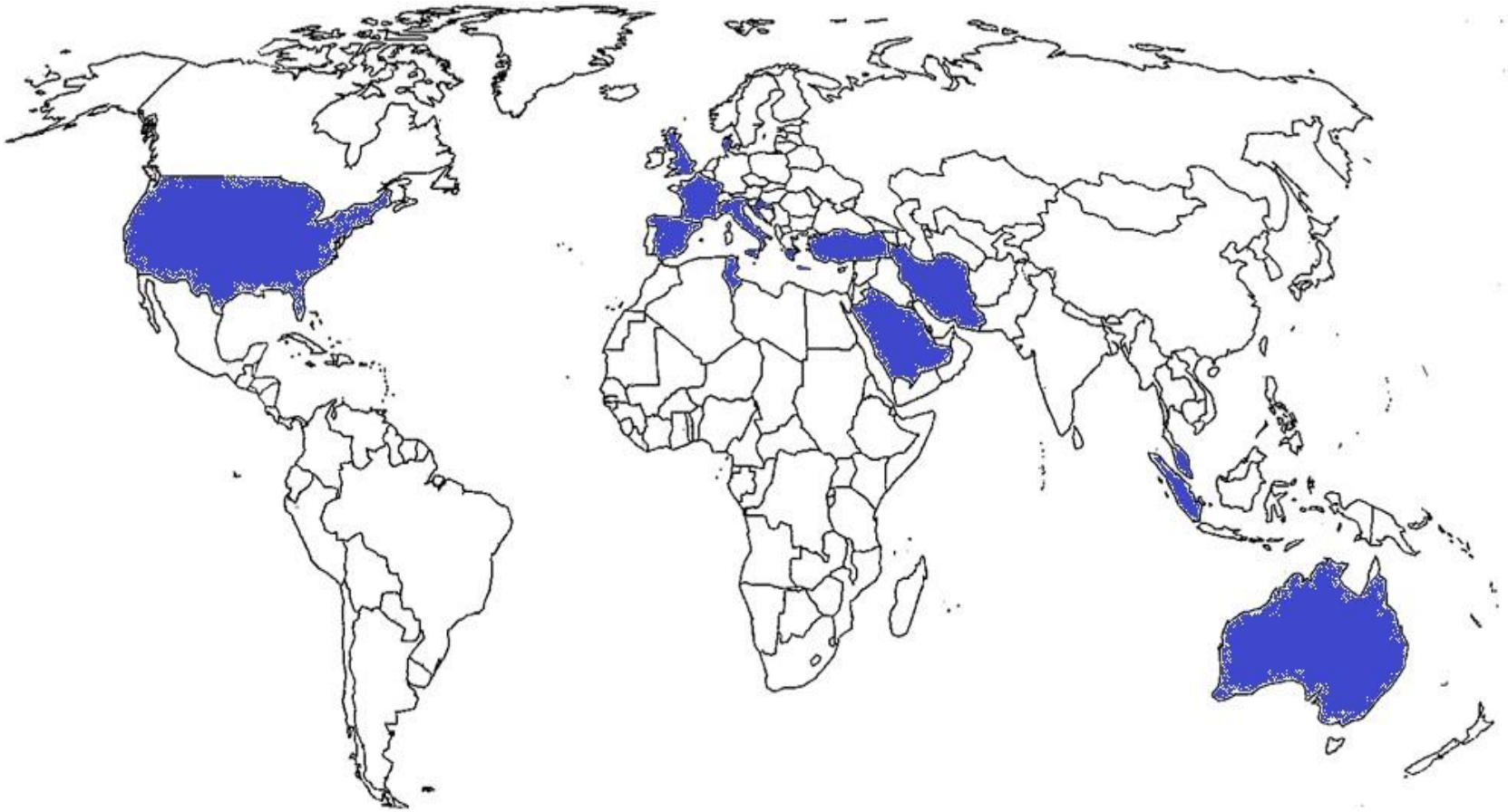
- 32 applications received
- 32 sets of samples shipped to 18 different countries

29 laboratories sent back results within the deadline

6 MedAID partners
4 PerformFISH partners



2° VER-IPT: Countries participating

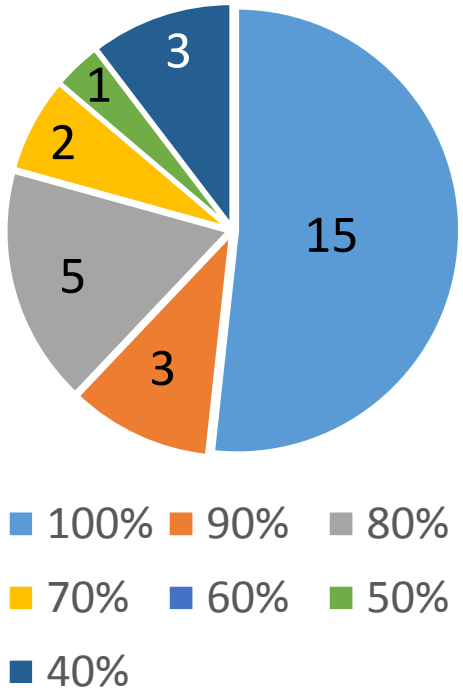


2° VER-IPT: Ampoules contents

Vial n°	Result	Contents (genotype)	Viral titre (TCID50/ml)	Reference
1	Negative	Sterile MEM	-	-
2	**Positive**	**389/I96 (SJ/RG)**	10^6,30	Vendramin et al. 2014
3	**Positive**	**283.2009 (RGNNV)**	10^3,60	Panzarin et al. 2012
4	Negative	Sterile MEM	-	-
5	Negative	Rainbow trout negative serum	-	-
6	**Positive**	**283.2009 (RGNNV)**	10^3,60	Panzarin et al. 2012
7	**Positive**	**484.2.2009 (SJNNV)**	10^6,55	Panzarin et al. 2012
8	**Positive**	**367.2.2005 (RG/SJ)**	10^4,55	Panzarin et al. 2012
9	Negative	Sterile MEM	-	-
10	**Positive**	**389/I96 (SJ/RG)**	10^6,30	Vendramin et al. 2014

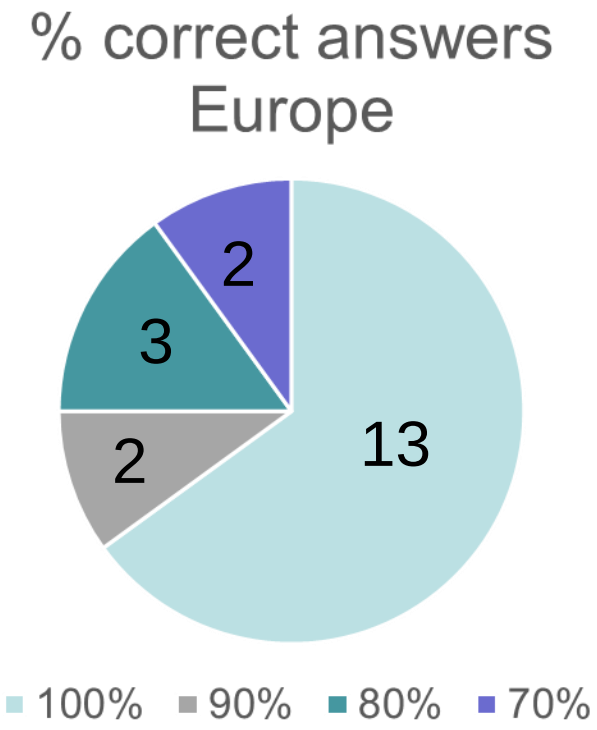
2° VER-IPT: Results

Detection of the presence of a betanodavirus in a sample



Score	n° of labs	%
100%	15	51,7
90%	3	10,3
80%	5	17,2
70%	2	6,9
60%	0	0,0
50%	1	3,4
40%	3	10,3

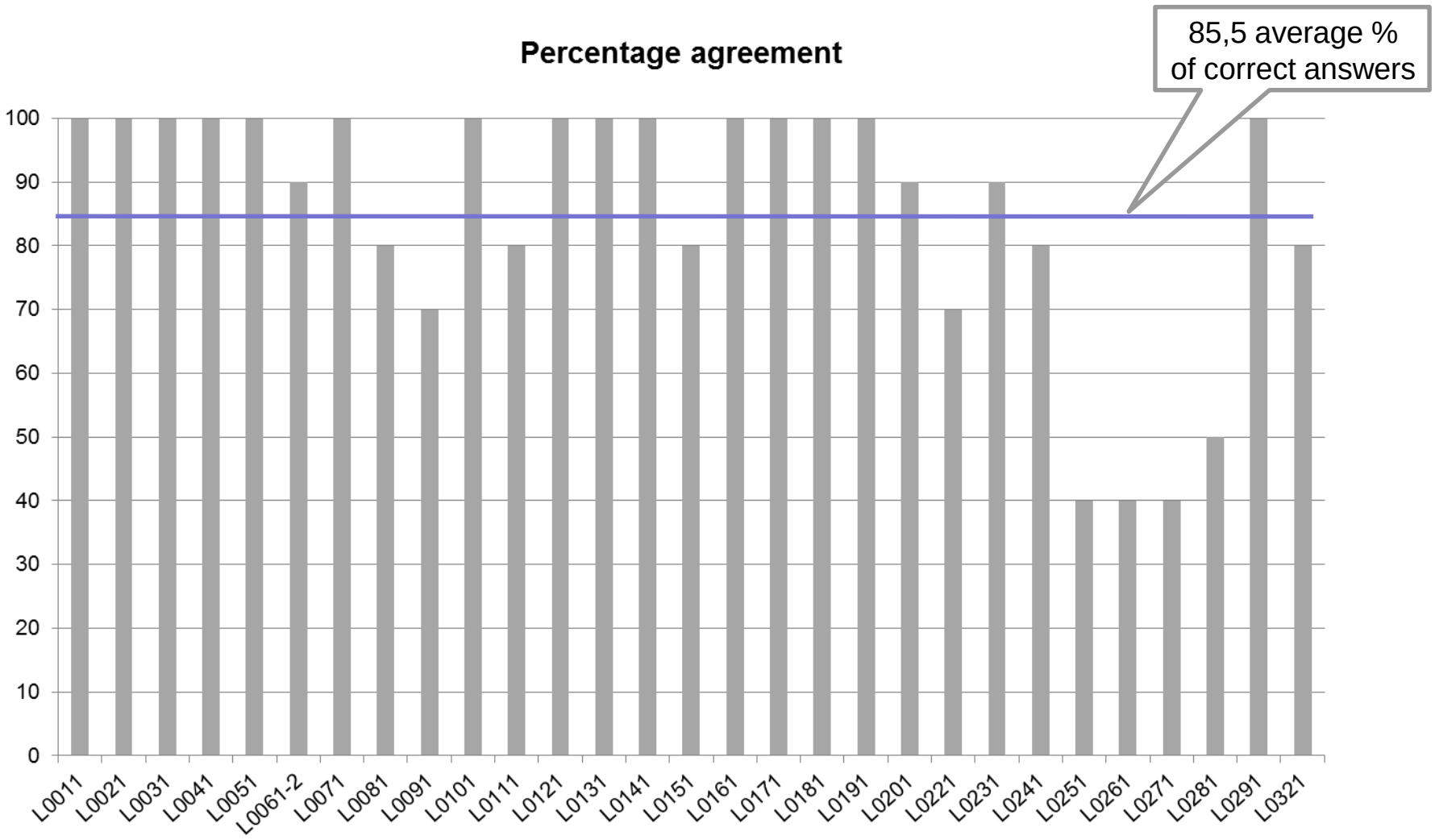
2° VER-IPT: Results of Med Countries



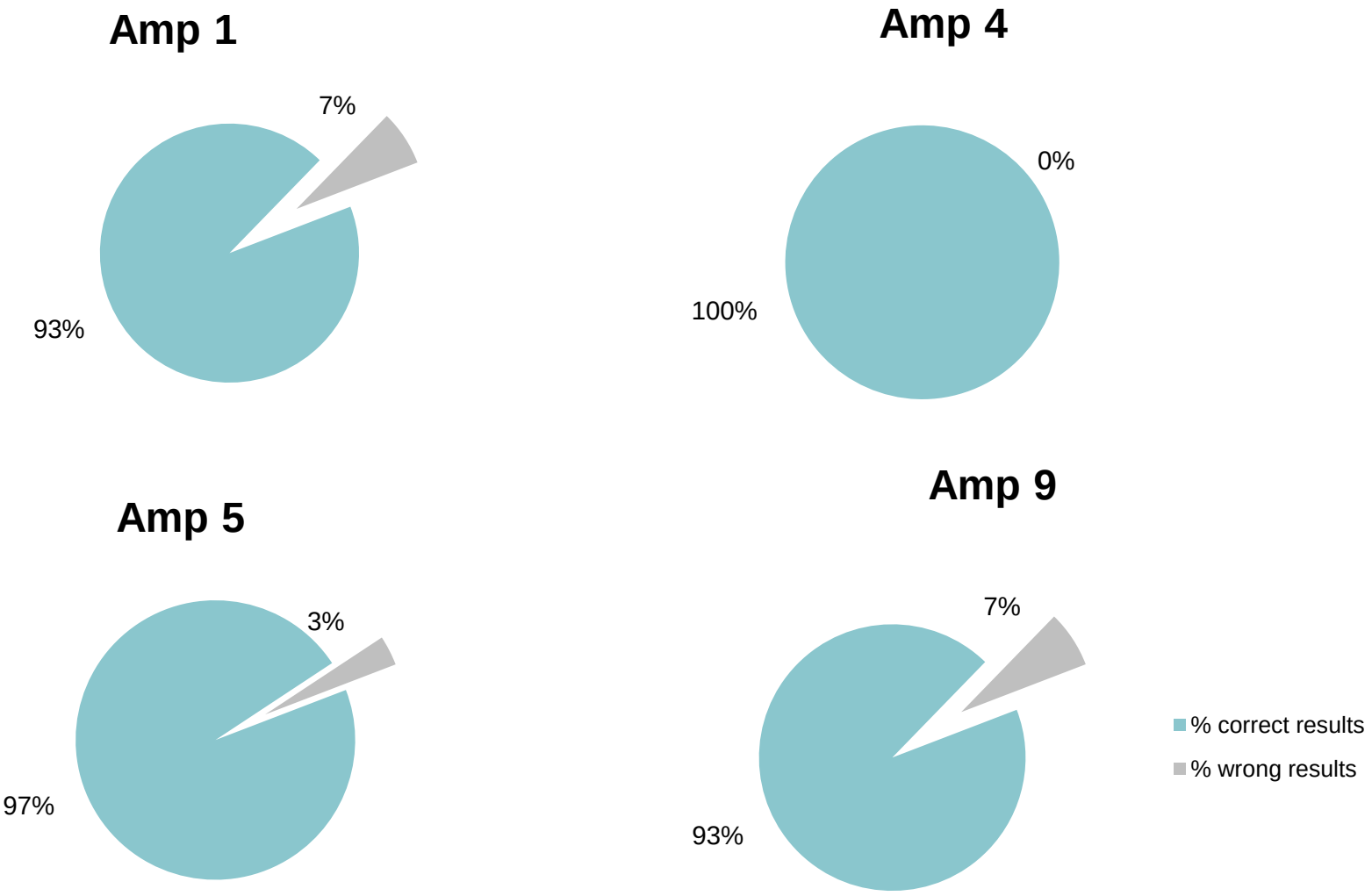
% Europe (20/29; 68,9%)		
correct answers	n° of labs	% out of EU participants
100%	13	65,0
90%	2	10,0
80%	3	15,0
70%	2	10,0
60%	0	0,0
50%	0	0,0

European labs have a better diagnostic capacity than those outside the EU

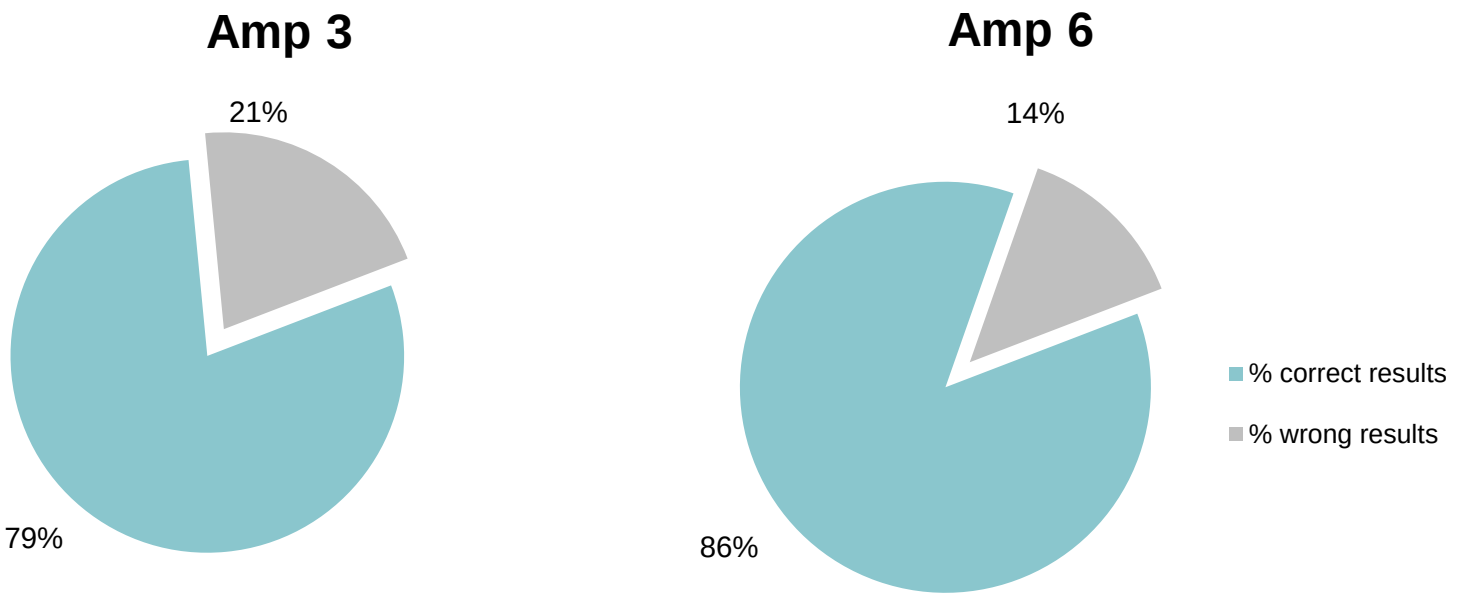
2° VER-IPT: Results



2° VER-IPT: Results per ampoules- Negative



2° VER-IPT: Results per ampoules- RGNNV

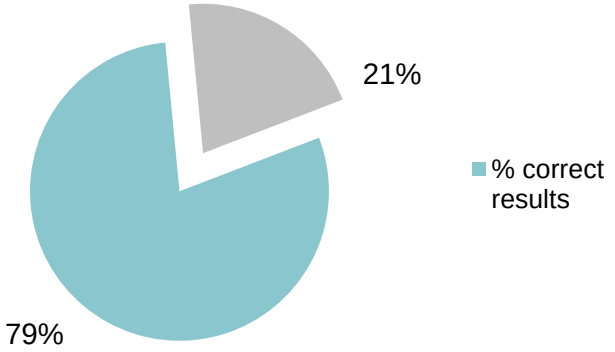


283.2009 (RGNNV)	10^3,60 TCID50/ml

2° VER-IPT Results per ampoules SJNNV & reassortants

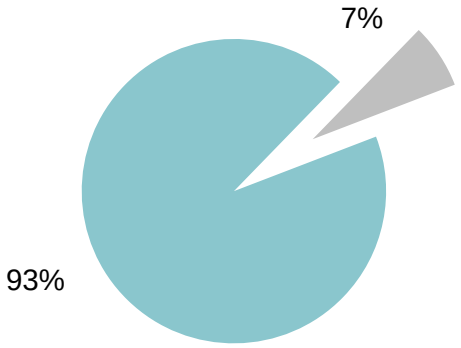
Amp 7: SJNNV

10^6,55 TCID50/ml

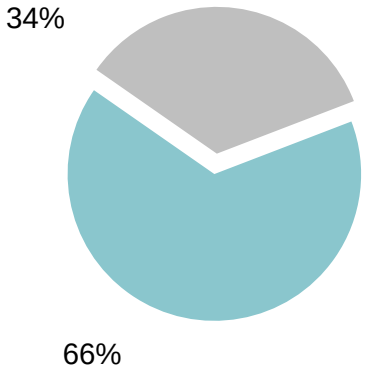


Amp 8: RG/SJ

10^4,55 TCID50/ml

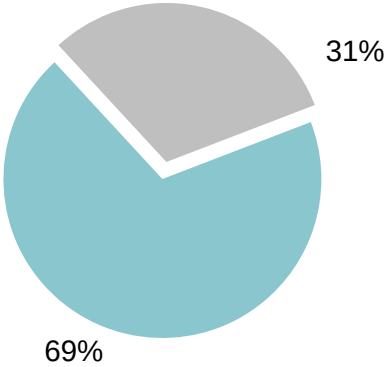


Amp 2: SJ/RG



Amp 10: SJ/RG

10^6,30 TCID50/ml



2° VER-IPT: Real time protocol used

23 out of 29 laboratories chose real time RT-PCR methods to complete the exercise

Most widely used protocols :

- 8/29 Panzarin et al 2010
- 8/29 Baud et al 2015
- 5 other published protocols (Hick & Whittington 2010, Dalla Valle et al. 2010, Olveira et al. 2013)
- 2 commercially available kits

- 6 RT-PCR or unpublished methods or data not reported

2° VER-IPT: Viral species identification

- Only 13 laboratories out of 29 (44,8%) performed the complete/partial characterization of the positive samples
- 12 by RT-PCR and sequencing
- 1 by RT-PCR only

RNA1	N°	%
	10	76,9
100%	7	70,0
90%	1	10,0
80%	1	10,0
0%	1	10,0

RNA2	N°	%
	13	100,0
100%	3	23,1
90%	2	15,4
80%	5	38,5
70%	2	15,4
0%	1	7,7

RNA2 appears to cause more problems for identification (SJNNV and reassortant strains resulted particularly challenging)

2° VER-IPT: Viral species identification

Only 2 out of 13 labs performed a complete and correct identification of viral genotype

RNA1&RNA2 correct answers	N° of lab	% out of 13
100%	2	15,4
91,7%	1	7,7
83,3%	4	30,8
75,0%	2	15,4
50,0%	1	7,7
33,3%	1	7,7
25,0%	1	7,7

There is room for improvements!

2° VER-IPT: Conclusion

- ✓ 15 out of 29 laboratories scored 100% correct results
- ✓ Overall agreement (K) 0.5387

- ✓ Laboratories performing diagnosis for VER showed an overall **good specificity**
- ✓ Test **sensitivity** with some **reassortant strain SJ/RG and the SJNNV** appeared to be the **major problem**
- ✓ Few laboratories performed complete and correct viral species identification

medAID

Mediterranean Aquaculture Integrated Development

Thank you for your attention!