Outcome of proficiency test on EIA serology

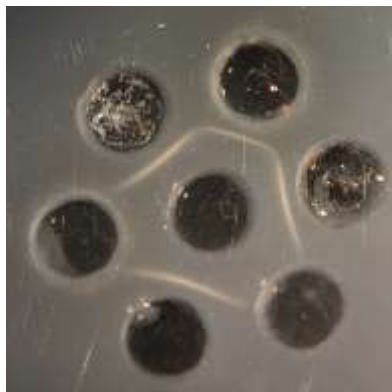## Anses Dozulé Laboratory for equine diseases

VIPARE unit

Delphine Gaudaire

EIA PT GENERAL INFORMATION

- **AIM OF THE PT**
  - Detect EIA antibodies:

    using Agar Gel Immuno-Diffusion Test (AGID test) as described in the OIE manual Chapter 2.5.6

    using Enzyme ImmunoSorbent Assay (ELISA)

EIA PT GENERAL INFORMATION

- **INFORMATION TO 34 LABS**

  - Information sent by e-mail on May 17th, 2018

- **SCHEDULE OF THE BOTH EIA PT**

  - Samples sent on June 12th, 2018

  - Results due on July 6th, 2018

  - Each participant received an outcome of the PT by e-mail:

    EIA AGID PT: September 3rd, 2018

    EIA ELISA PT: September 4th, 2018

  - Official report will be sent to each lab by the end of September

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EIA PT CODIFICATION

- Lab codification:

	EIA AGID lab code	EIA ELISA lab code
Participant 1	18-A	18-G
Participant 2	18-B	/
Participant 3	18-C	18-D

- Codification of samples :

Lab code	Sample #													
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**…..**	**20**	**21**	**22**	**23**	24
18-A	645	267	288	426	95	656	632	691	….	816	758	690	538	194
18-B	721	397	891	384	14	959	58	256	….	572	533	794	191	958
18-C	407	569	893	172	623	776	355	405	….	772	108	187	300	446

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2018 EIA AGID PT
18-A-645
Reconstitute with 0,5 ml of distilled water
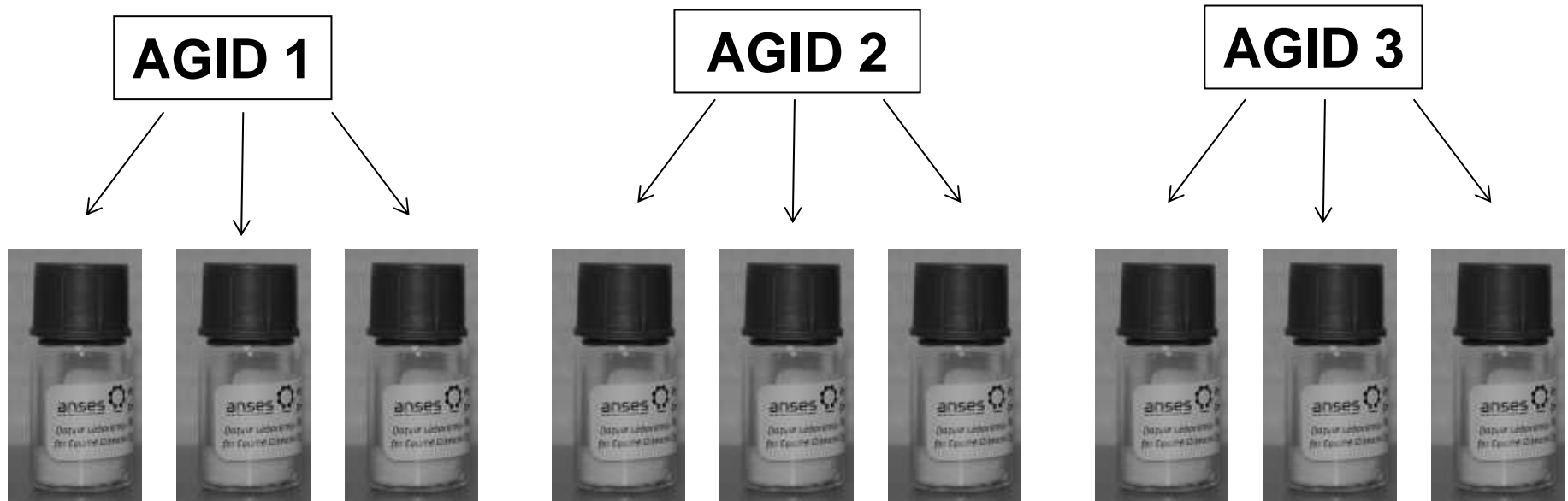
EIA PT sample validation

- Validation of each lyophilized serum:

Homogeneity

25 tested vials in 1 assay (FD ISO GUIDE 35) using ELISA

Reproducibility

3 vials tested in three independent AGID tests

EIA PT sample validation

Stability

Short-term stability (stability after reconstitution):

    3 days at room temperature

    1 week at +2-8° C

    1 month at -20° C

Long-term stability (accelerated conditions):

    3 weeks at +30° C before reconstitution

> **Homogeneity, reproducibility and stability have been validated for all the sera produced for the both PT**

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Outcome of EIA AGID PT

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- EIA AGID PT nb of participants: 32 (26 NRLs)

EU COUNTRIES	EIA AGID PT		
	2010	2014	2018
AUSTRIA	✓	✓	✓
BELGIUM	✓	✓	✓
BULGARIA	✓	✓	✓
CROATIA		✓	✓
CZECH REPUBLIC	✓	✓	✓
CYPRUS			✓
DENMARK	✓	✓	✓
ESTONIA	✓	✓	✓
FINLAND	✓	✓	✓
FRANCE	✓	✓	✓
GERMANY	✓	✓	✓
GREECE			✓
HUNGARY		✓	✓
IRELAND	✓	✓	✓
ITALY	✓	✓	✓
LATVIA	✓	✓	✓
LITHUANIA		✓	✓
LUXEMBOURG			
MALTE			
POLAND	✓	✓	✓
PORTUGAL	✓	✓	✓
ROMANIA	✓	✓	✓
SLOVAKIA	✓	✓	✓
SLOVENIA	✓	✓	✓
SPAIN	✓	✓	✓
SWEDEN	✓	✓	✓
THE NETHERLANDS	✓	✓	✓
UNITED KINGDOM	✓	✓	✓
	21/26	24/28	26/28

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EIA AGID PT samples

- Preparation of 9 batches of serum

Mix of 2 negative sera

1 naturally infected horse

2 experimentally infected horses

Serum 1

Serum 2

Sera 3 to 8

Serum 9

Lyophilization

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EIA AGID PT samples

- 24 samples/panel to be analyzed by AGID test :

Serum #	Sample #	Expected result
1	1 to 12	Negative
2	13 to 14	Positive
3	15 to 16	Positive
4	17	Positive
5	18	Positive
6	19 to 20	Positive
7	23	Positive
8	24	Positive
9	21 to 22	Positive

4 strong positive:
sample# 15-16-17-23

3 medium positive:
sample# 18-21-22

5 weak positive:
sample# 13-14-19-20-24

- ➢ 12 negative samples

- ➢ 12 positive samples

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EIA AGID PT results

- Criteria to evaluate the performance of each participant :

  - ✓ All negative samples should be found as such

  - ✓ All positive samples should be found as such
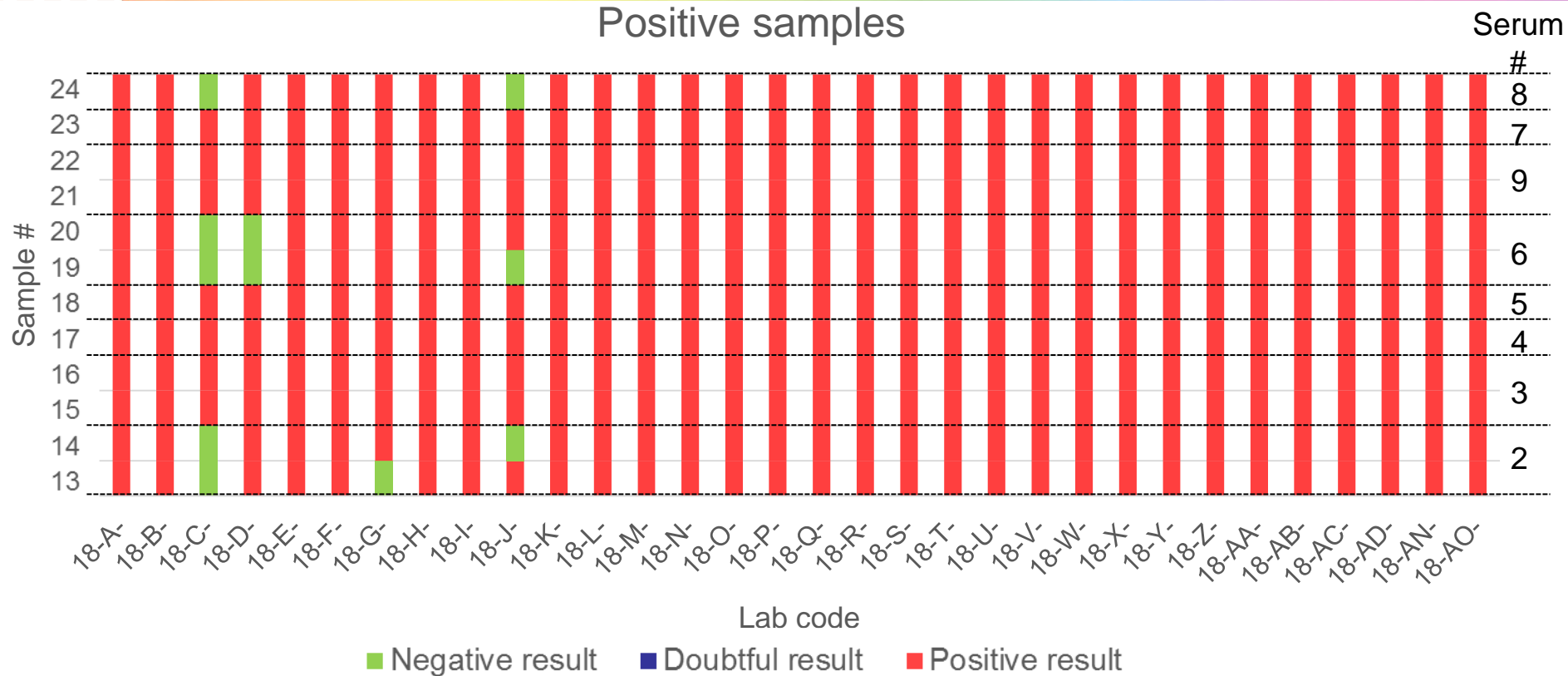
  - ✓ Only one mistake out of 24 samples was accepted
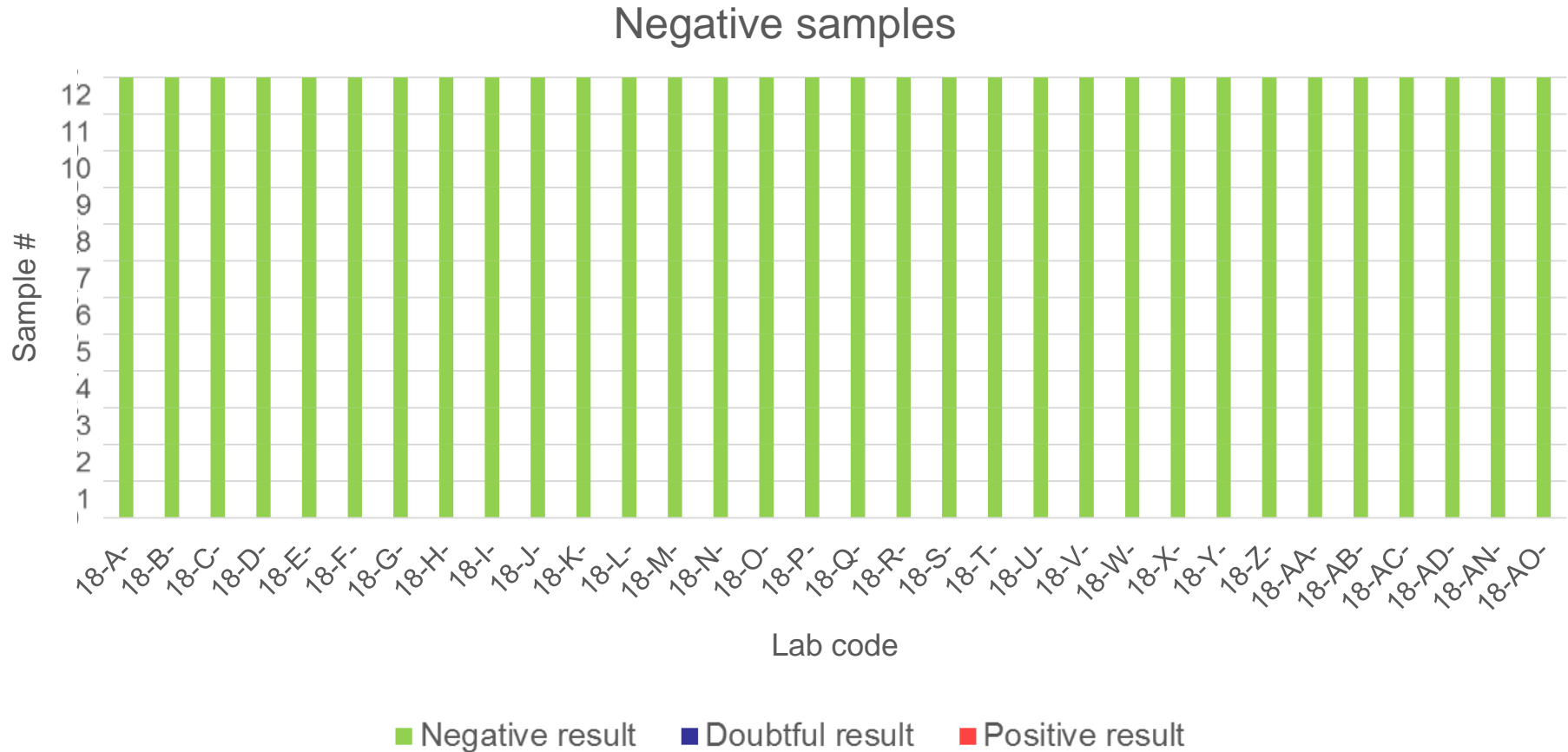
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EIA AGID PT results

Negative samples

- 100% of participants obtained satisfactory results
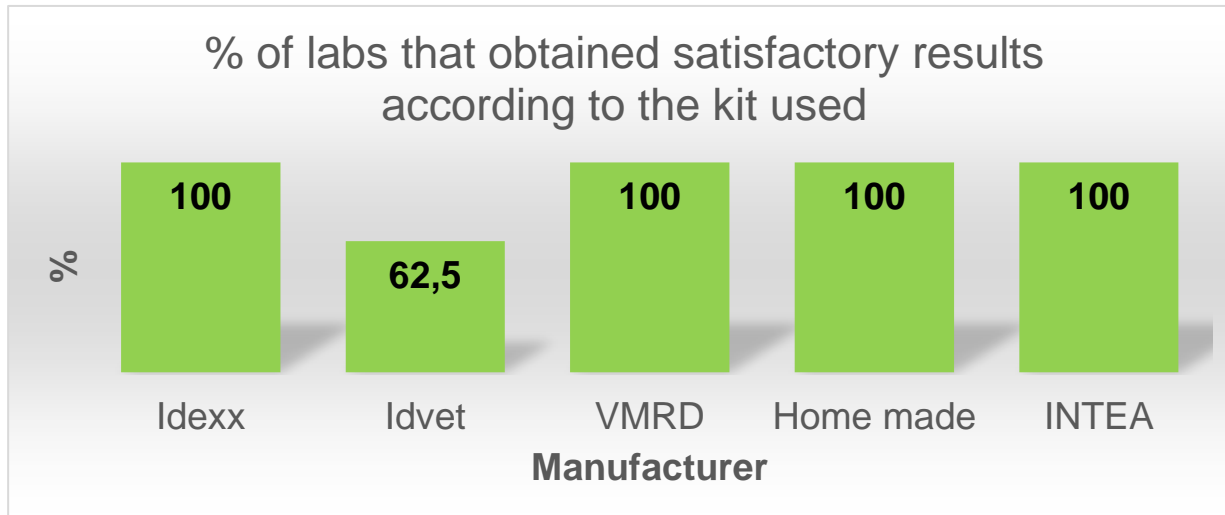
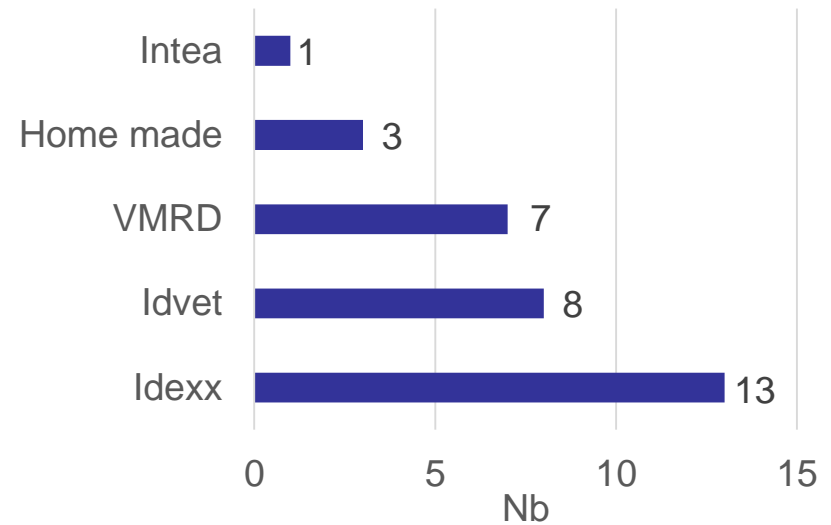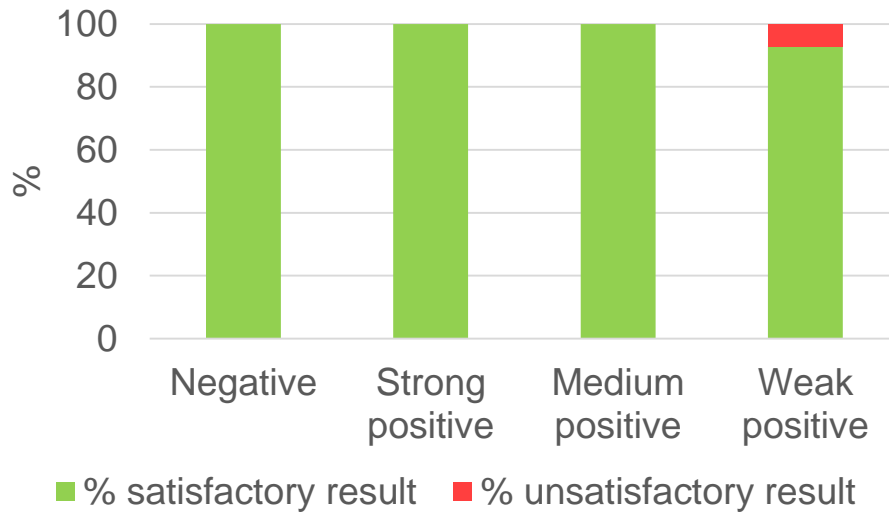**Each laboratory exhibited a very good specificity**

EIA AGID PT results

- 11 out of 372 positive samples have been analyzed as negative (specificity: 97%)

- 3 laboratories failed to detect positive samples

29 out of 32 participants obtained satisfactory results

EIA AGID PT results

% satisfactory result (green) vs % unsatisfactory result (red) across Negative, Strong positive, Medium positive, Weak positive categories.

Number of labs by manufacturer (Nb):
- Intea: 1
- Home made: 3
- VMRD: 7
- Idvet: 8
- Idexx: 13

% of labs that obtained satisfactory results according to the kit used

Manufacturer	%
Idexx	100
Idvet	62,5
VMRD	100
Home made	100
INTEA	100

Unsatisfactory results are not in relation with the kit used

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EIA AGID PT conclusion

- 29 out of the 32 labs obtained satisfactory results

- European NRL network exhibited a very good specificity
  26/26 detected all negative samples

- The sensitivity of European NRL network is 88,5%
  23/26 European NRL detected all positive samples

- 3 labs need to improve their sensitivity to detect all positive samples (especially weak positive sample)

- Training session will be organize by the EU-NRL

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Outcome of EIA ELISA PT

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EIA ELISA PT GENERAL INFORMATION

- EIA AGID PT nb of participants: 23

EU COUNTRIES	EIA ELISA PT 2018
AUSTRIA	■
BELGIUM	
BULGARIA	
CROATIA	■
CZECH REPUBLIC	■
CYPRUS	■
DENMARK	
ESTONIA	
FINLAND	■
FRANCE	■
GERMANY	■
GREECE	■
HUNGARY	■
IRELAND	■
ITALY	■
LATVIA	■
LITHUANIA	■
LUXEMBOURG	
MALTE	
POLAND	
PORTUGAL	■
ROMANIA	■
SLOVAKIA	
SLOVENIA	■
SPAIN	■
SWEDEN	
THE NETHERLANDS	■
UNITED KINGDOM	■

19/26

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EIA ELISA PT samples

- Preparation of 9 batches of serum

Mix of 2 negative sera	1 naturally infected horse	2 experimentally infected horses	
↓	↓	↙	↘
Serum 1	**Sera 2 to 3**	**Sera 4 to 6**	**Sera 7 to 9**

Lyophilization

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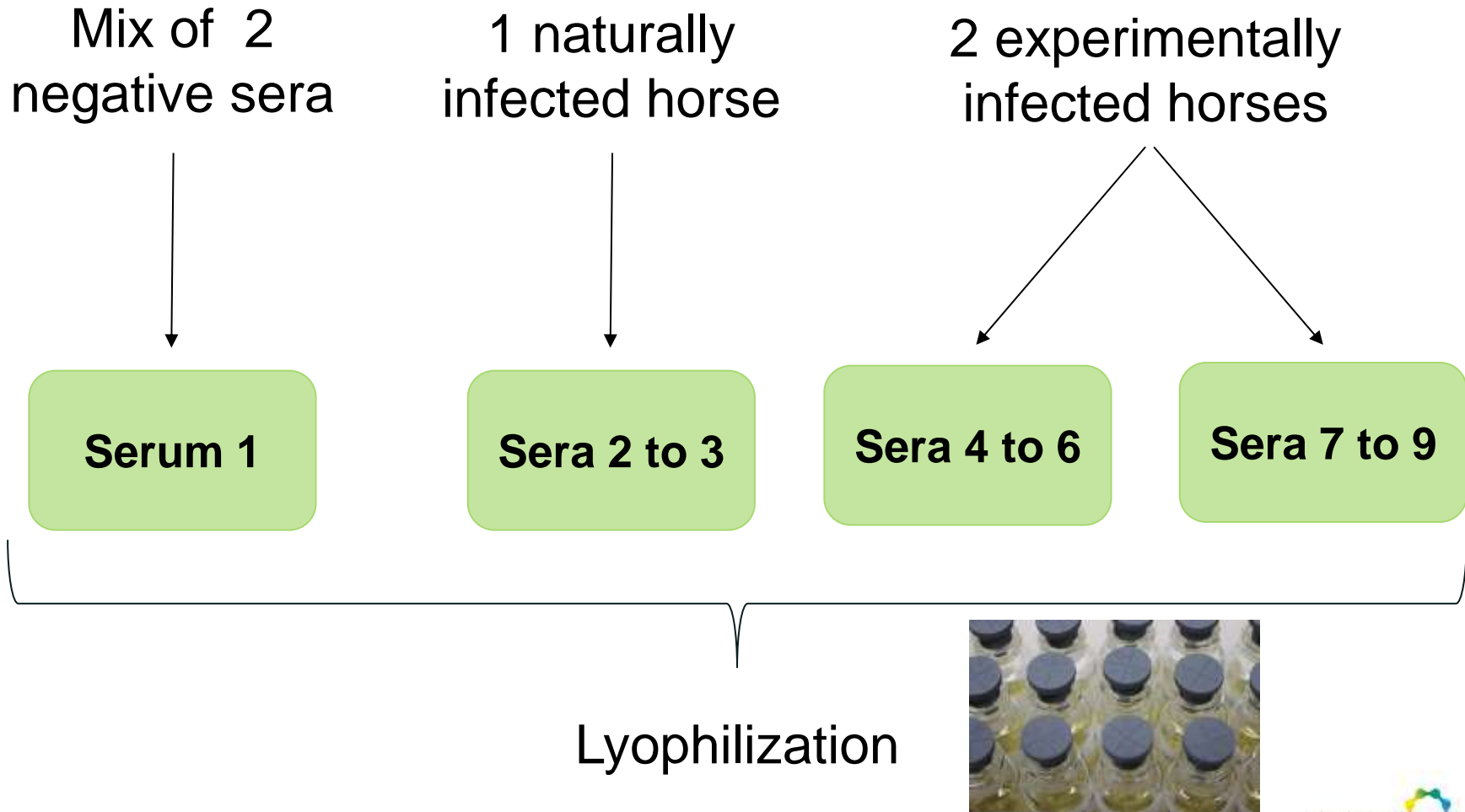
EIA ELISA PT samples

- 24 samples/panel to be analyzed by ELISA:

Serum #	Sample #	Expected result
1	1 to 8	Negative
2	9 to 11	Positive
3	12 to 14	Positive
4	15	Positive
5	16 to 18	Positive
7	19	Positive
6	20 to 21	Positive
8	22	Positive
9	23 to 24	Positive

- ➢ 8 negative samples
- ➢ 16 positive samples

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EIA ELISA PT results

- Criteria to evaluate the performance of each participant :

  - ✓ All negative samples should be analyzed as such

  - ✓ All positive samples should be analyzed as such
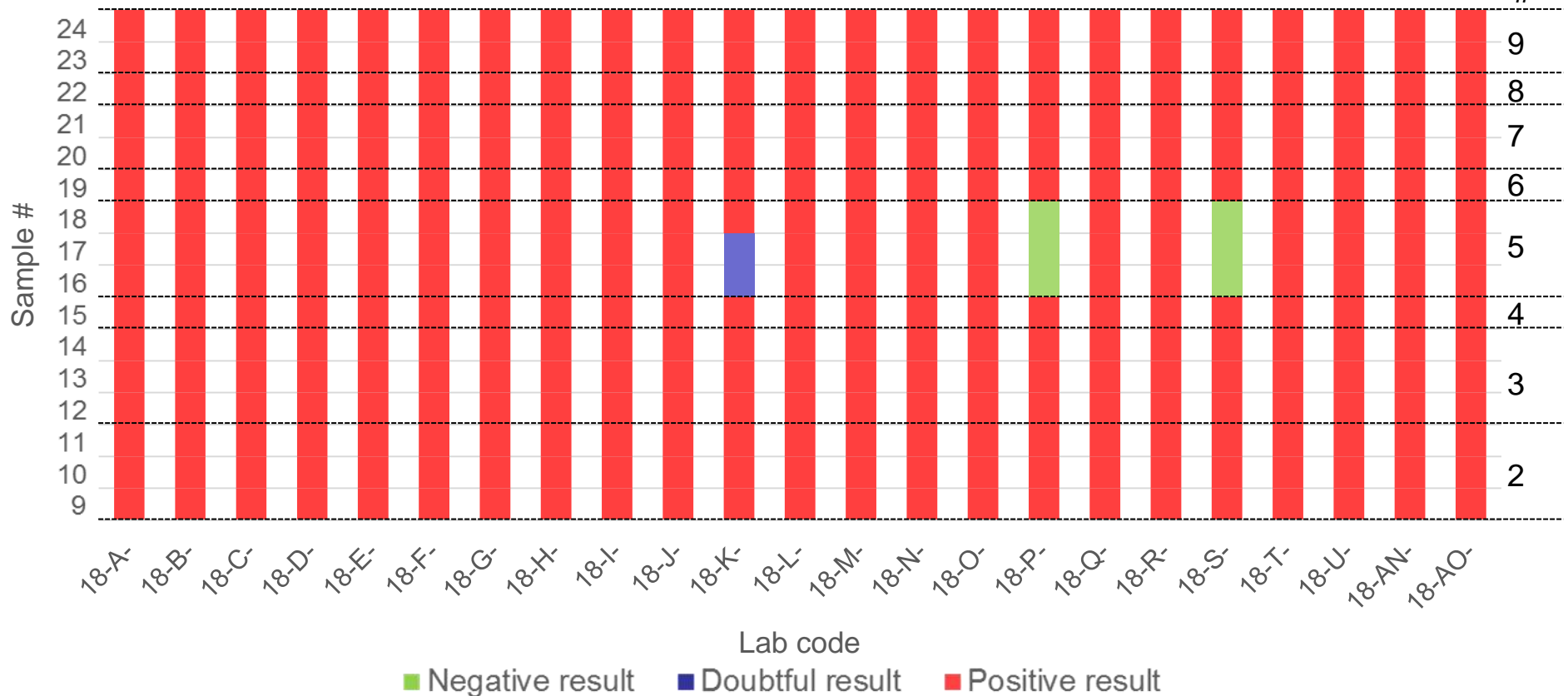
EIA ELISA PT results
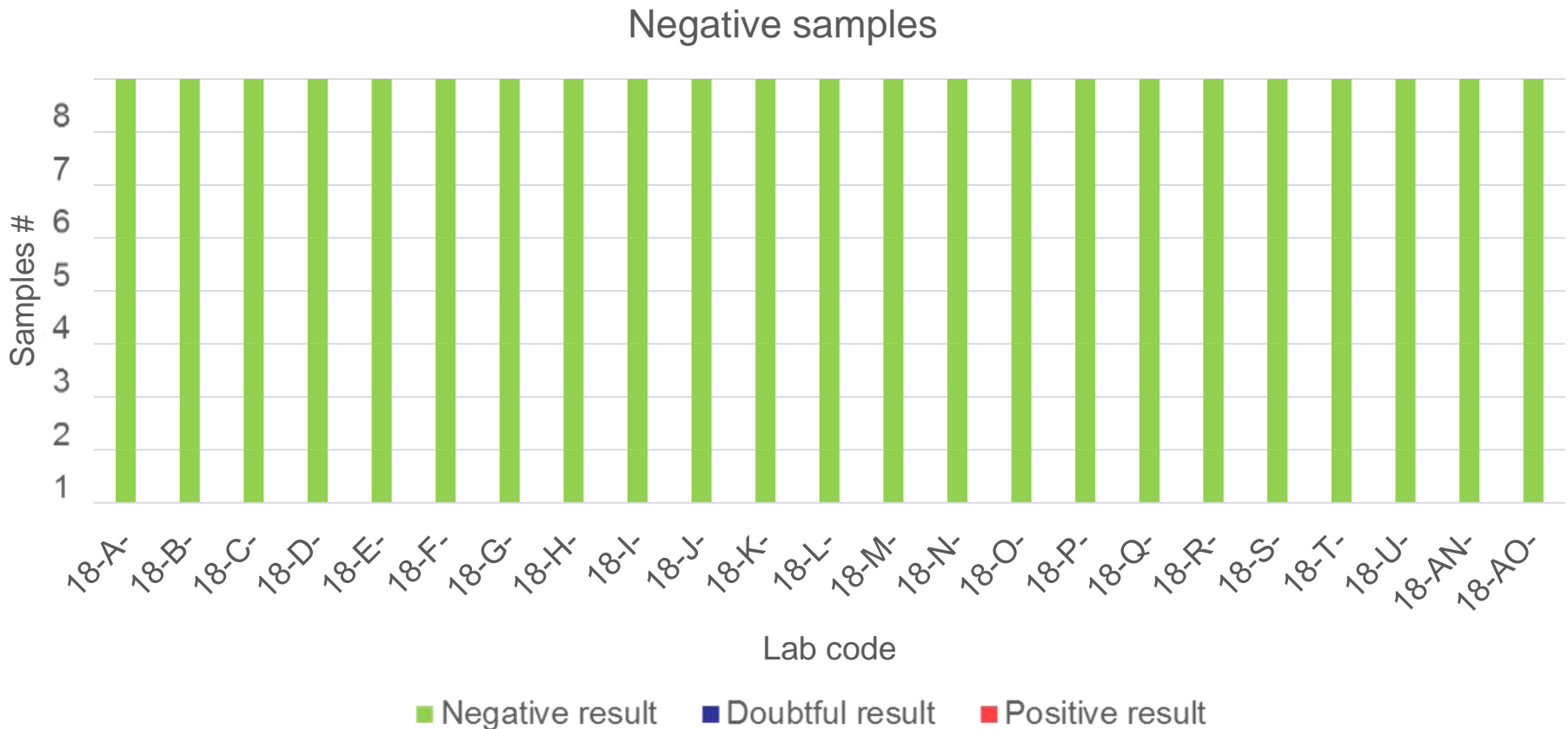
Negative samples

- 100% of participants obtained satisfactory results

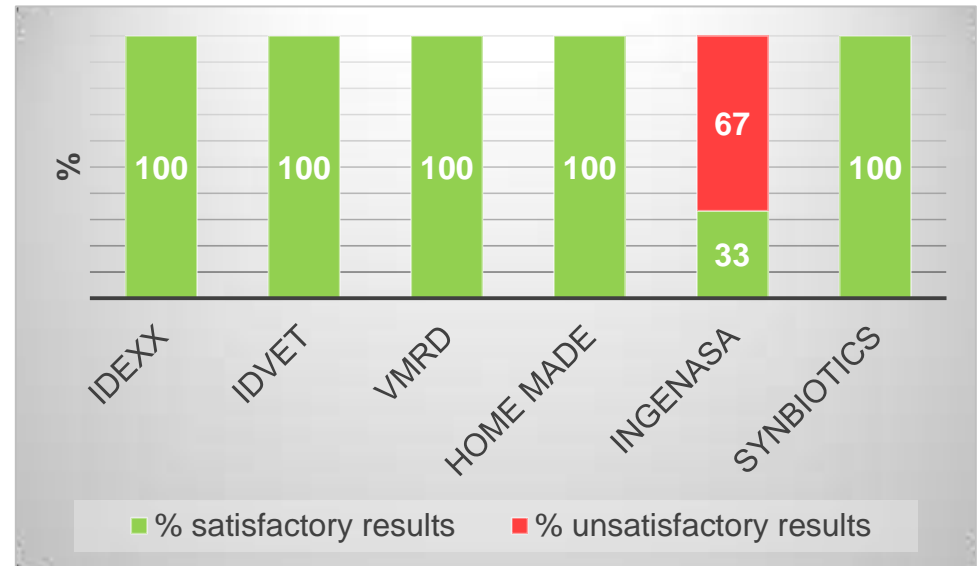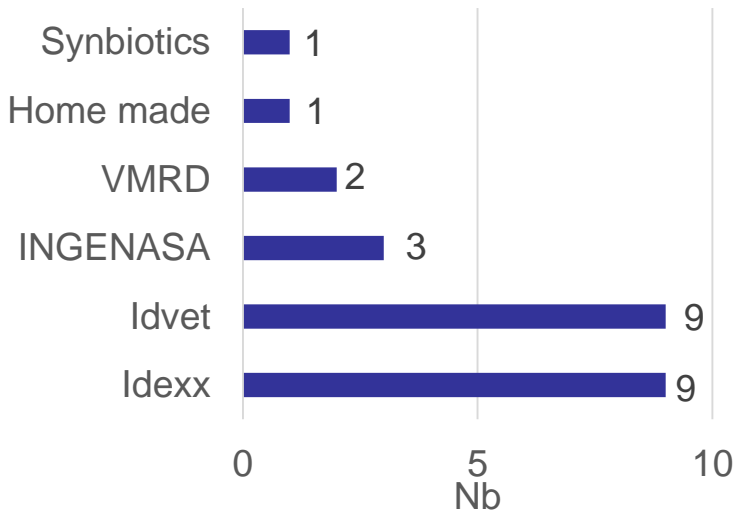**Each laboratory exhibited a very good specificity**

EIA ELISA PT results

- 2 laboratories failed to detect positive samples (sensitivity: 98,4%)

21 out of 23 labs obtained satisfactory results

EIA ELISA PT results

- Lab code 18-P and 18-S failed to detect the same serum (# 5)

3 labs used INGENASA kits and 2 out of 3 failed to detect the positive serum 5

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EIA ELISA PT results

- 21 out of the 23 labs obtained satisfactory results

- European NRL network exhibited a very good specificity

- The sensitivity of European NRL network is 89,5%
  17 out of 19 European NRL detected all positive samples

- 2 labs failed to detect all positive samples of the ELISA PT

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Thank for your attention

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