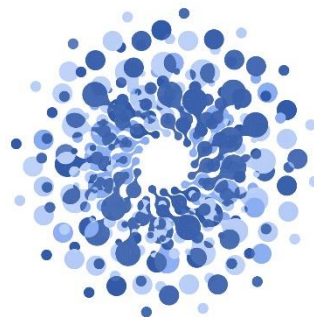




# PROFICIENCY TESTING 2023 AVIAN INFLUENZA AND NEWCASTLE DISEASE

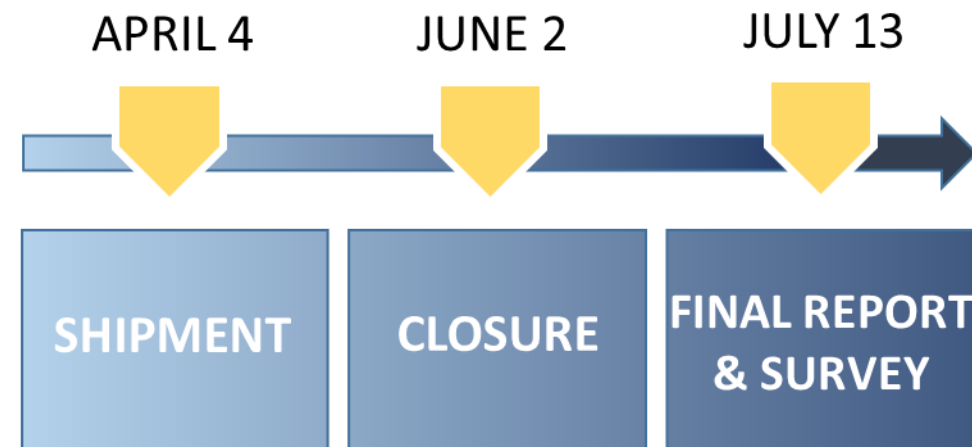
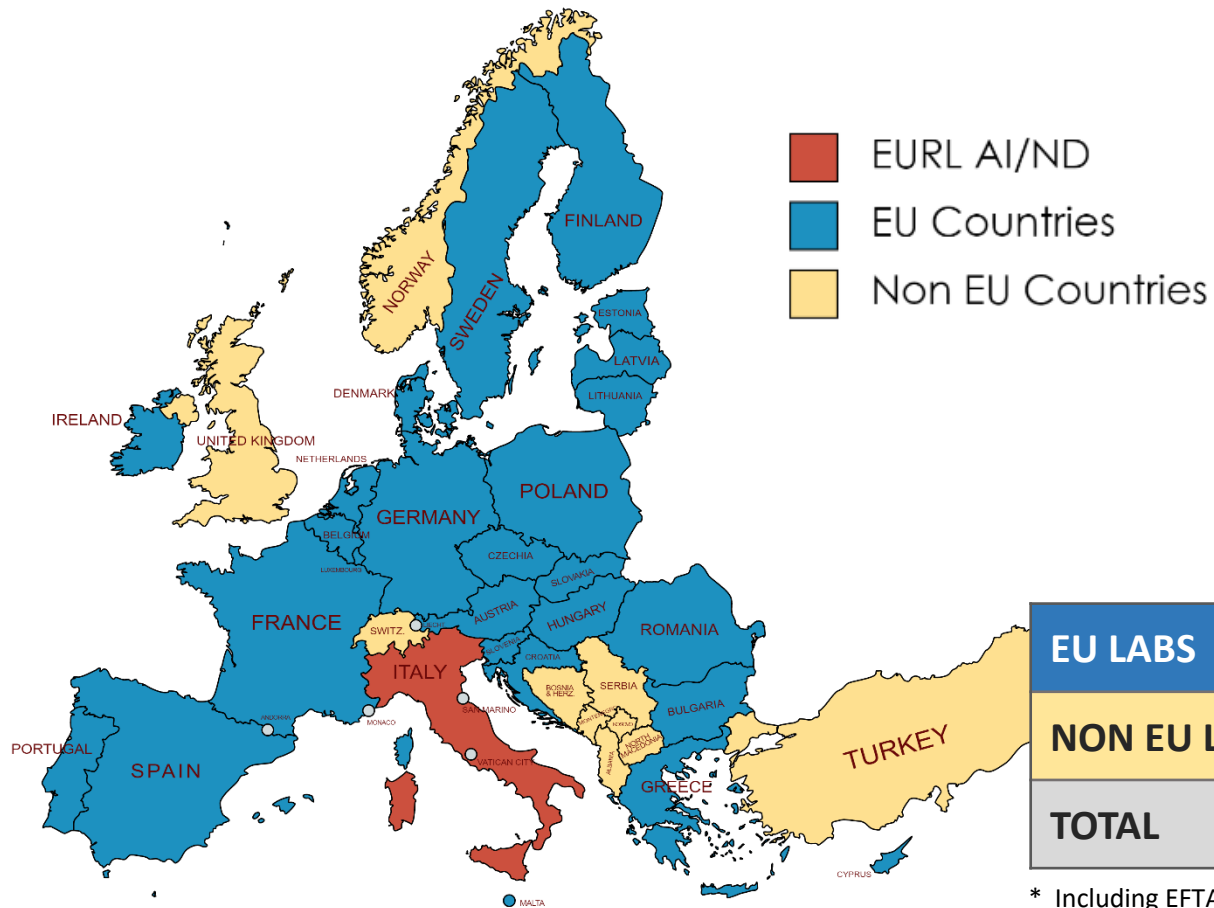


**29<sup>th</sup> Annual Meeting**

of the National Reference Laboratories for Avian Influenza  
and Newcastle Disease of European Union Member States

**Viviana Valastro**  
IZSVe-EURL for AI/ND

# TIMELINE AND PARTICIPATING LABORATORIES



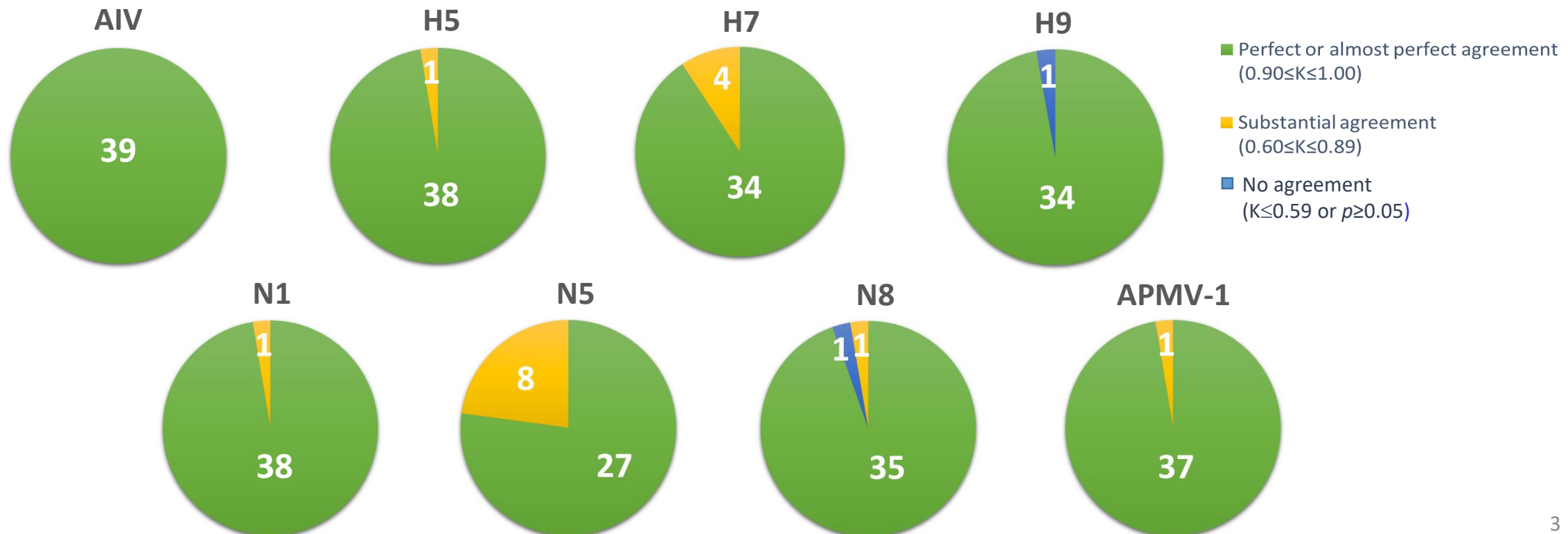
	PT SCHEME		
	VIRUS GENOME DETECTION AI/ND	VIRUS ANTIGEN DETECTION AI/ND	ANTIBODIES DETECTION AI/ND
<b>EU LABS</b>	25	24	25
<b>NON EU LABS*</b>	14	10	12
<b>TOTAL</b>	<b>39</b>	<b>34</b>	<b>37</b>

\* Including EFTA laboratories

# RESULTS – ASSESSMENT OF PARTICIPANTS

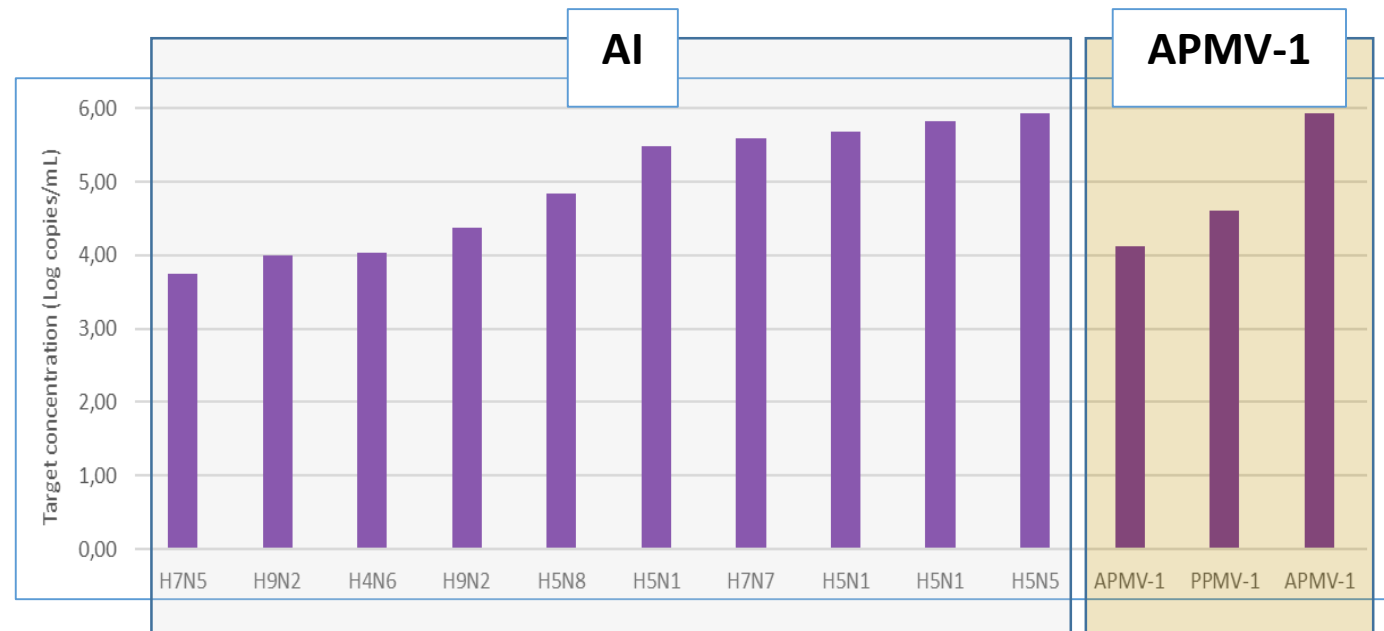
- 39 Participating Labs
- Success rate = 99.6%

- 25 LABS: 100% correct results
- 11 LABS: Substantial agreement with the EURL only for 1 PARAMETER
- 1 LAB: Substantial agreement with EURL for 2 PARAMETERS
- 1 LAB: Substantial agreement with the EURL for 1 PARAMETER and No agreement for 1 PARAMETER
- 1 LAB: Substantial agreement with the EURL for 2 PARAMETERS and No agreement for 1 PARAMETER



# PANEL COMPOSITION

- **15 Samples** in liquid format including:
  - 13 AI/APMV-1 POSITIVES**
  - 2 AI/APMV-1 NEGATIVES**




Sample	Strain	Type	Pathotype	Lineage/Clade/Genotype	RT-ddPCR GC/ml
M01	A/Anas platyrhynchos/Belgium/467_H191864_19VIR8799-66/2017	H7N5	LPAI	Eurasian	10 <sup>3.75</sup>
M02	A/turkey/Italy/16VIR8643-60/2016	H9N2	LPAI	Y439	10 <sup>4.00</sup>
M03	PPMV1/collared dove/Cyprus/20VIR3543-13/2020	APMV-1	Virulent	VI.2.1.1.2.2*	10 <sup>4.61</sup>
M04	AOAV-1/chicken/Spain/2278-54_22VIR7253-24/2022	APMV-1	Virulent	VII.2*	10 <sup>5.94</sup>
M05	A/goose/Italy/20VIR7660-8/2020	H5N8	HPAI	Eurasian, 2.3.4.4b	10 <sup>4.84</sup>
M06	A/turkey/Italy/21VIR9117-15/2021	H5N1	LPAI	Eurasian	10 <sup>5.48</sup>
M07	NDV V4-vaccine like	APMV-1	Avirulent	I.1.2.1*	10 <sup>4.12</sup>
M08	A/mallard/Italy/19VIR7018-9/2019	H4N6	LPAI	-	10 <sup>4.03</sup>
M09	A/pheasant/Italy/21VIR2284-22/2021	H9N2	LPAI	Y439	10 <sup>4.38</sup>
M10	A/mallard/Italy/22VIR4781-2/2022	H7N7	LPAI	Eurasian	10 <sup>5.60</sup>
M11	A/mute swan/Romania/11981-1_21VIR3163-5/2021	H5N5	HPAI	Eurasian, 2.3.4.4b	10 <sup>5.94</sup>
M12	Negative	-	-	-	-
M13	A/avian/Italy/22VIR8877-44/2022	H5N1	HPAI	Eurasian, 2.3.4.4b	10 <sup>5.83</sup>
M14	A/duck/Italy/22VIR8870-11/2022	H5N1	LPAI	Eurasian	10 <sup>5.68</sup>
M15	Negative	-	-	-	-

\* According to Dimitrov et al., 2019

## RESULTS - DETECTION

Sample	Strain	Type	Success rate and False results (No.)								
			M	H5	H7	H9	N1	N5	N8	APMV-1	
M08	A/mallard/Italy/19VIR7018-9/2019	H4N6									
M03	PPMV1/collared dove/Cyprus/20VIR3543-13/2020	APMV-1									
M04	AOAV-1/chicken/Spain/2278-54_22VIR7253-24/2022	APMV-1									
M07	NDV V4-vaccine like	APMV-1									
M06	A/turkey/Italy/21VIR9117-15/2021	H5N1									
M13	A/avian/Italy/22VIR8877-44/2022	H5N1									
M14	A/duck/Italy/22VIR8870-11/2022	H5N1		97% (1)				97% (1)			
M11	A/mute swan/Romania/11981-1_21VIR3163-5/2021	H5N5							97% (1)		
M05	A/goose/Italy/20VIR7660-8/2020	H5N8								97% (1)	
M01	A/ <i>Anas platyrhynchos</i> /Belgium/467_H191864_19VIR8799-66/2017	H7N5								80% (7)	
M10	A/mallard/Italy/22VIR4781-2/2022	H7N7			90% (4)						
M09	A/pheasant/Italy/21VIR2284-22/2021	H9N2					97% (1)				
M02	A/turkey/Italy/16VIR8643-60/2016	H9N2					97% (1)				
M12	Negative										97% (1)
M15	Negative									97% (1)	

 100% success rate

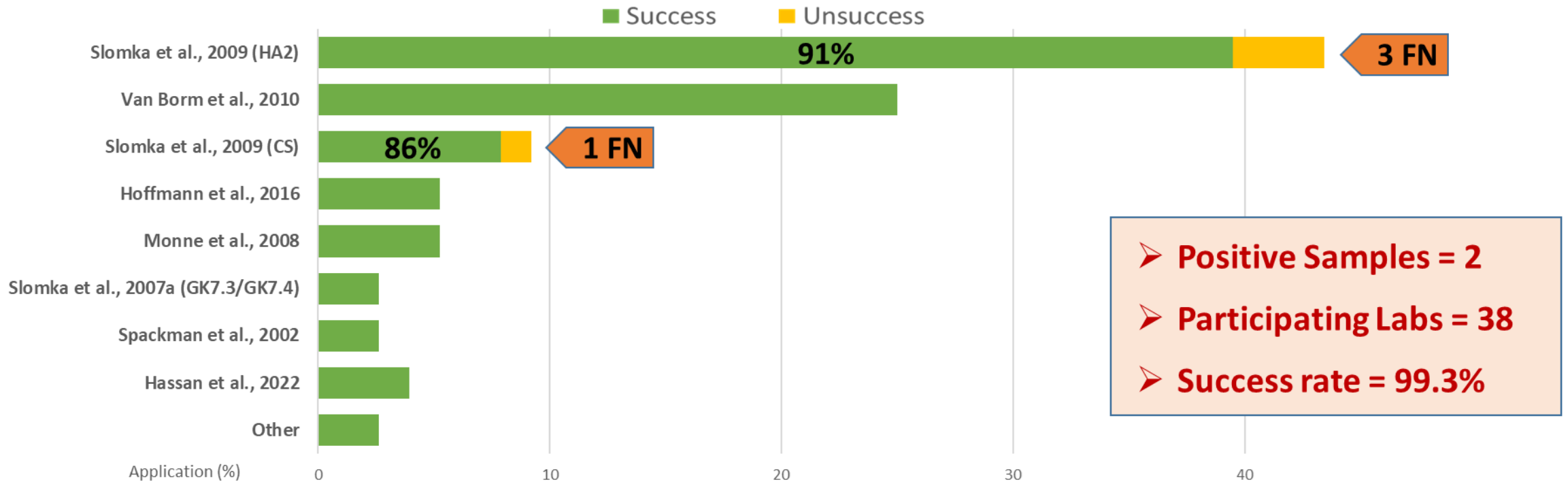
1 out of 4 failing labs performed additional analyses and gave the correct CS and pathotype

# INTRA-METHODS RESULTS – DETECTION OF H7 STRAINS

Sample	Strain	Type	RT-ddPCR GC/ml	EURL				Participants	
				AIV (M)	H7			Median Ct	Detection
				Heine 2015	Slomka 2009	Hassan 2022	Slomka 2007		
M01	<i>A/Anas platyrhynchos/Belgium/467_H191864_19VIR8799-66/2017</i>	H7N5	10 <sup>3.75</sup>	32.92	35.94	33.32	Positive	34.38	100%
M10	<i>A/mallard/Italy/22VIR4781-2/2022</i>	H7N7	10 <sup>5.60</sup>	26.56	Negative	28.11	Positive	29.30	90% (4)

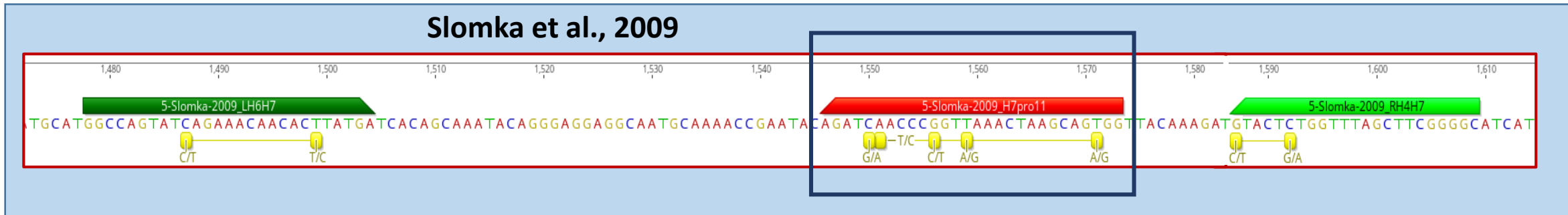
( ) Number of false results

≈ 2 Log



# OLIGOS *IN SILICO* CHECK

Sample M10      H7N7      A/mallard/Italy/22VIR4781-2/2022



**geneious**  
<https://www.geneious.com>

PRIMER FOR

PROBE

PRIMER REV



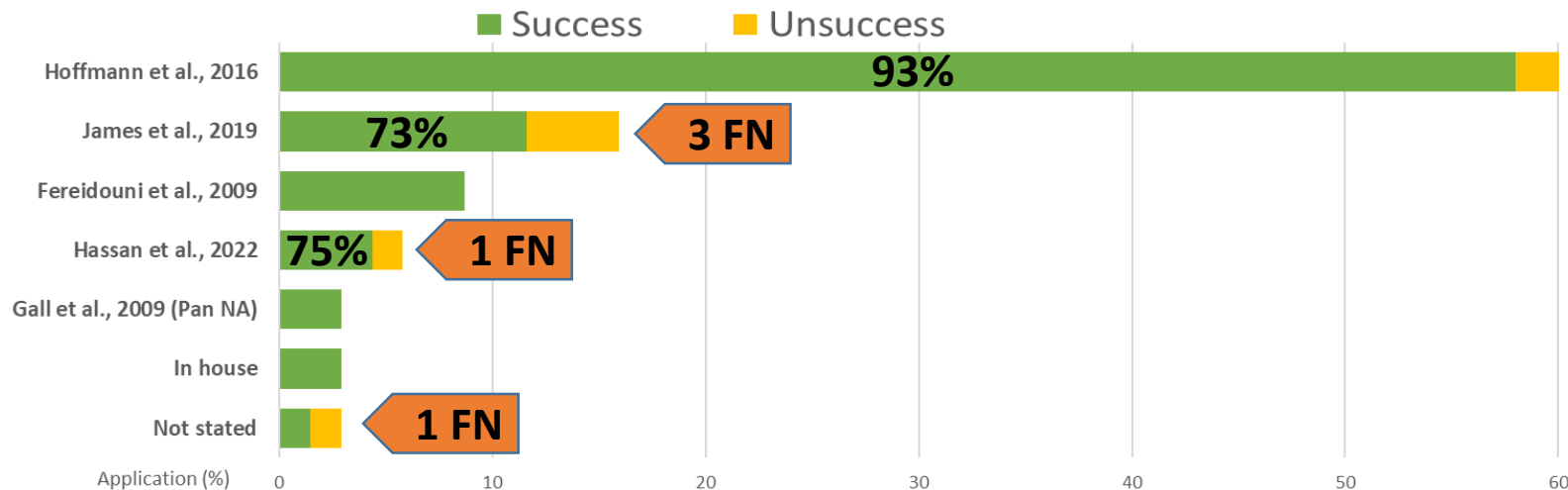
# INTRA-METHODS RESULTS – DETECTION OF N5 STRAINS

➤ Participating Labs = 35 ➤ Positive samples = 2 ➤ Success rate = 98.5%

Sample	Strain	Type	RT-ddPCR GC/mL	EURL Ct*	Labs Ct	Detection
M01	<i>A/Anas platyrhynchos/Belgium/467_H191864_19VIR8799-66/2017</i>	H7N5	$10^{3.75}$	31.90	34.22	80% (7)
M11	<i>A/mute swan/Romania/11981-1_21VIR3163-5/2021</i>	H5N5	$10^{5.94}$	26.05	27.90	97% (1)

( ) Number of false results; \* Hassan et al., 2022

≈ 2 Log

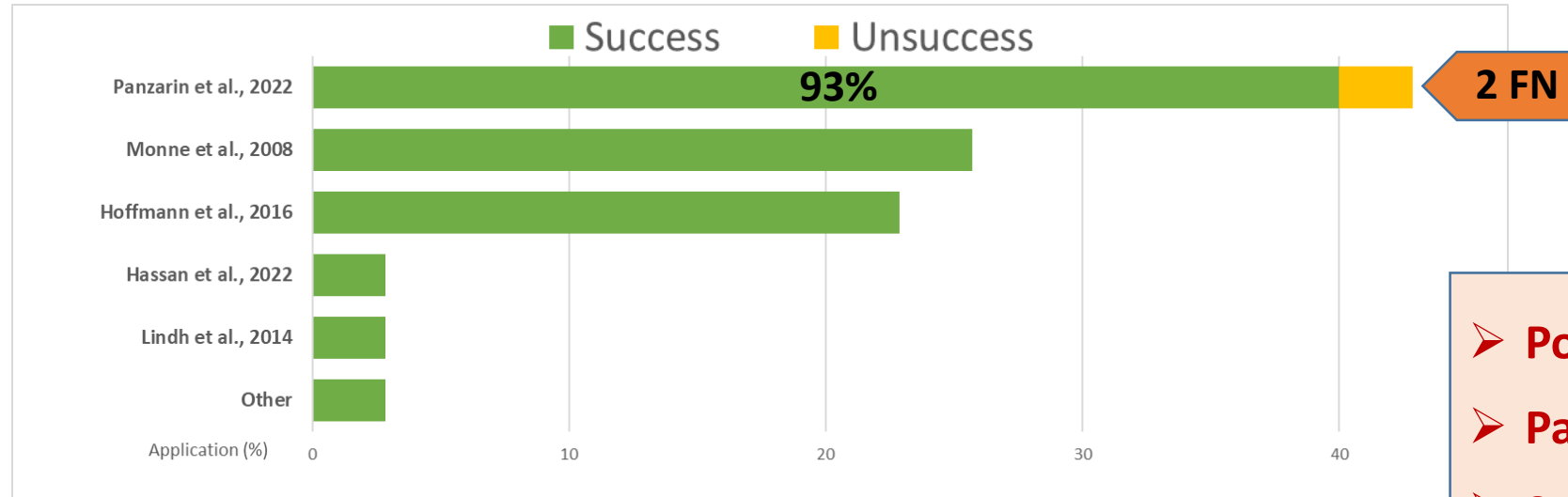


Method	Detection	
	M01	M11
Hoffmann et al., 2016	86% (3)	100%
James et al., 2019	60% (2)	83% (1)
Fereidouni et al., 2009	100%	100%
Hassan et al., 2022	50% (1)	100%
Gall et al., 2009 (Pan NA)	100%	100%
In house	100%	100%
Not stated	0% (1)	100%

100% success rate; ( ) Number of false results



# INTRA-METHODS RESULTS – DETECTION OF H9 STRAINS



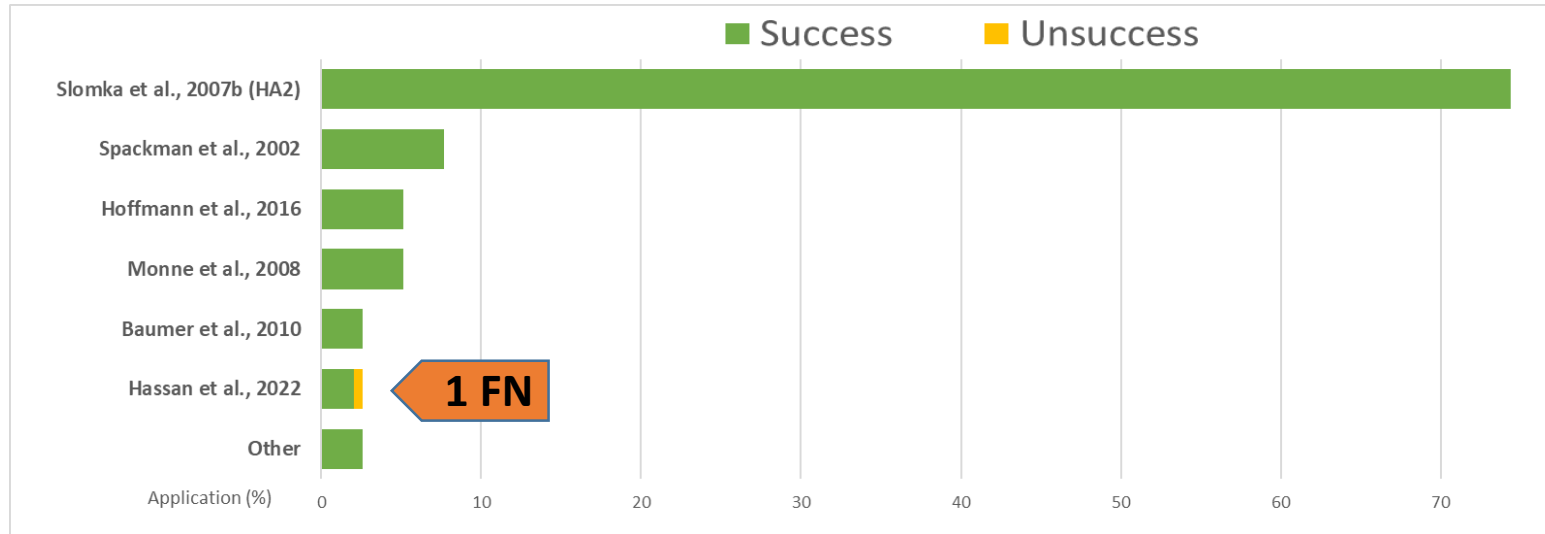
- Positive Samples = 2
- Participating Labs = 35
- Success rate = 99.6%

Sample	Type	RT-ddPCR GC/ml	EURL Ct Panzarin 2022*	Participants	
				Median Ct	Detection
M02	H9N2 A/turkey/Italy/16VIR8643-60/2016	$10^{4.00}$	28.97	32.20	97% (1)
M09	H9N2 A/pheasant/Italy/21VIR2284-22/2021	$10^{4.38}$	27.53	31.00	97% (1)

\* IZSve SOP VIR 014 – ed. 01

( ) Number of false results

# INTRA-METHODS RESULTS – DETECTION OF H5 STRAINS



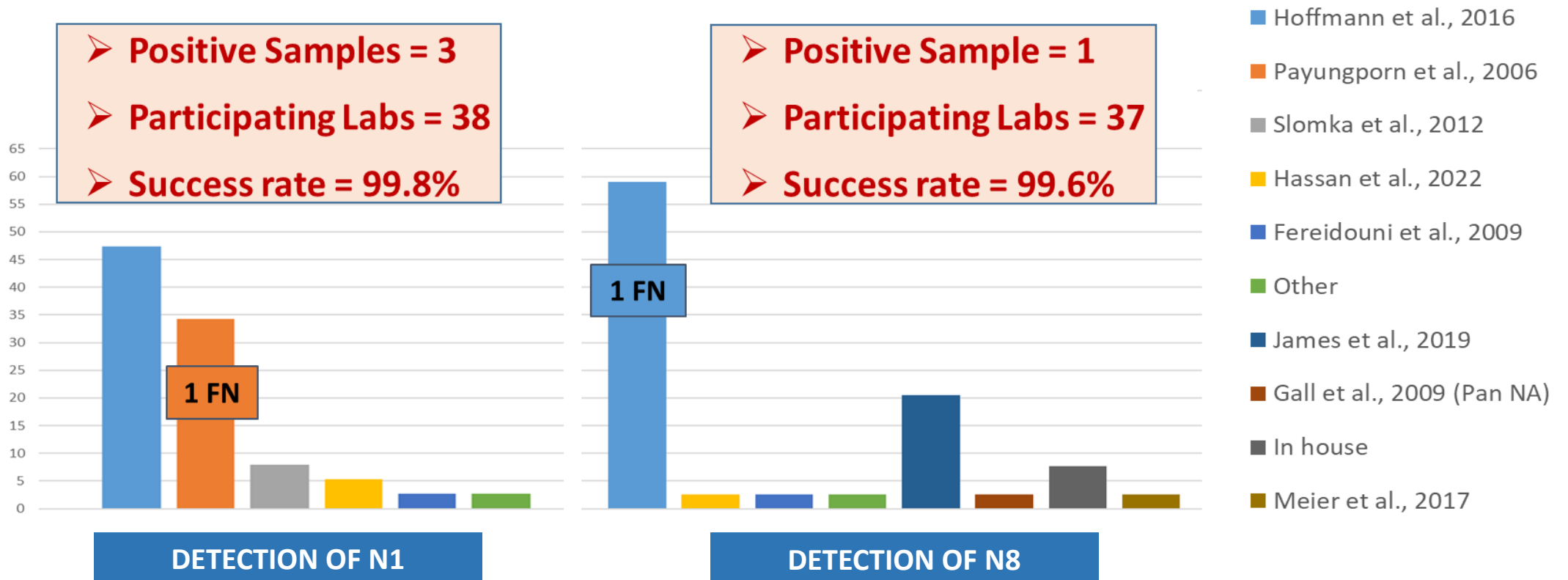
- Positive Samples = 5
- Participating Labs = 39
- Success rate = 99.8%

Sample	Strain	Type & Pathotype	EURL Ct		Participants	
			Hassan 2022	Slomka 2007	Median Ct	Detection
M05	A/goose/Italy/20VIR7660-8/2020	H5N8 HPAI	27.91	29.46	30.55	100%
M06	A/turkey/Italy/21VIR9117-15/2021	H5N1 LPAI	30.39	31.13	30.73	100%
M11	A/mute swan/Romania/11981-1_21VIR3163-5/2021	H5N5 HPAI	23.99	25.35	26.60	100%
M13	A/avian/Italy/22VIR8877-44/2022	H5N1 HPAI	24.28	26.29	27.16	100%
M14	A/duck/Italy/22VIR8870-11/2022	H5N1 LPAI	Negative	28.02	27.66	97% (1)

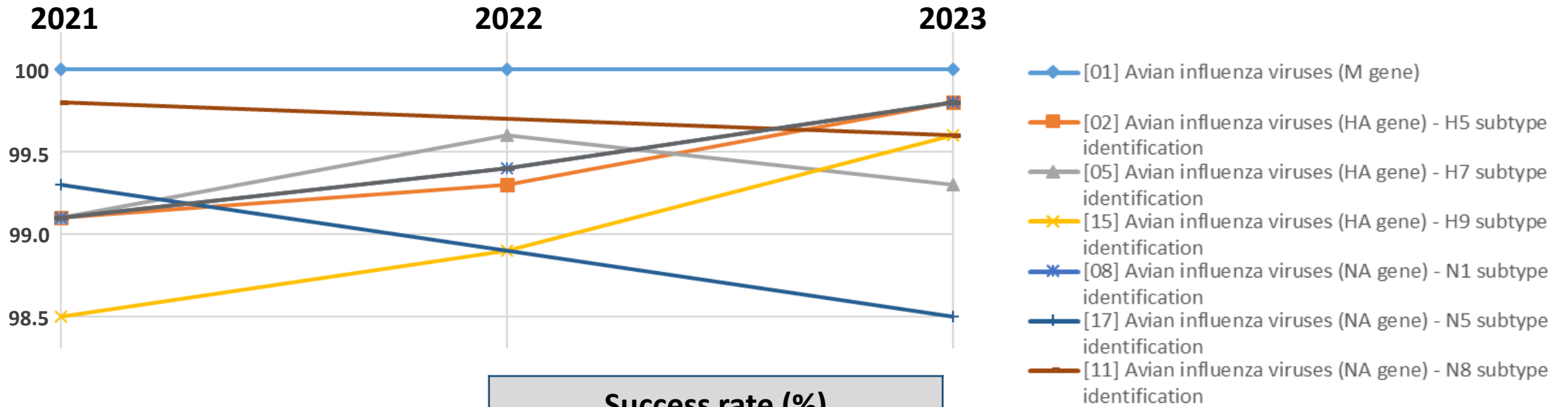
# INTRA-METHODS RESULTS – DETECTION OF N1 AND N8 STRAINS

Sample	Strain	Type	EURL Ct*	Labs Ct	Detection
M06	A/turkey/Italy/21VIR9117-15/2021	H5N1	28.33	28.55	100%
M13	A/avian/Italy/22VIR8877-44/2022	H5N1	27.08	28.10	100%
M14	A/duck/Italy/22VIR8870-11/2022	H5N1	26.85	28.02	97% (1)
M05	A/goose/Italy/20VIR7660-8/2020	H5N8	27.74	30.92	97% (1)

\* Hassan et al., 2022; ( ) Number of false results



# MONITORING PERFORMANCE



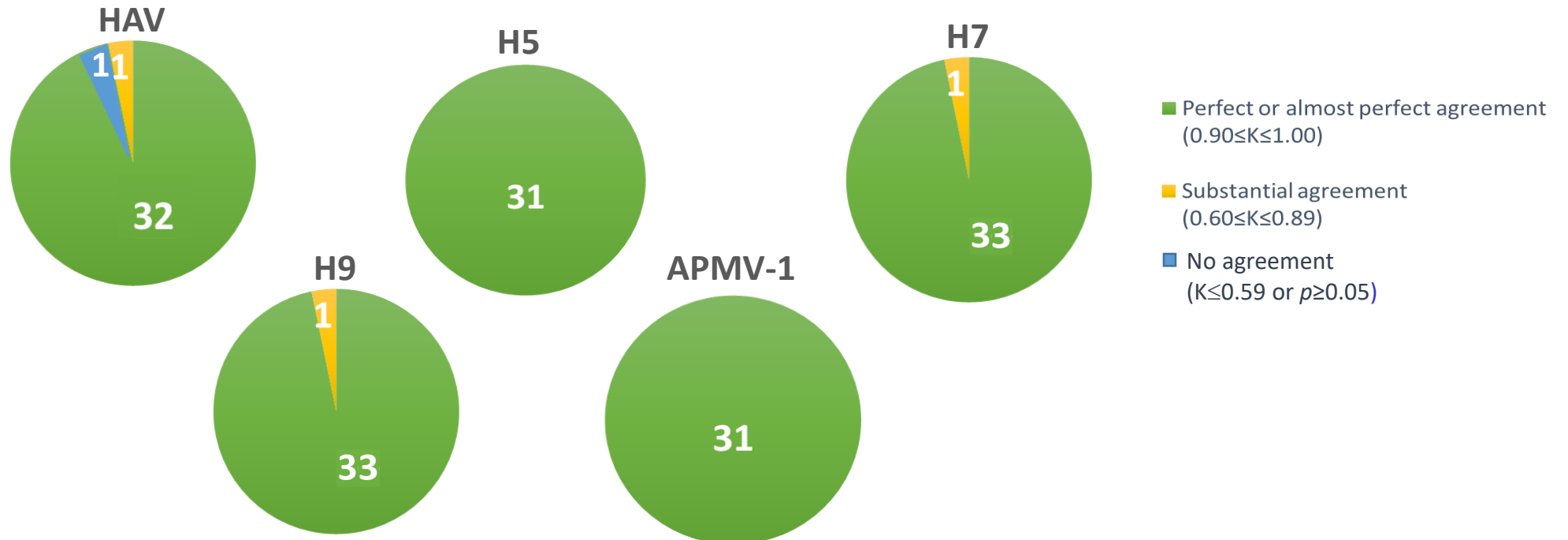
Parameter	Success rate (%)		
	2023	2022	2021
[01] AIV (M/NP)	100	100	100
[02] AIV-H5	99.8	99.3	99.1
[05] AIV-H7	99.3	99.6	99.1
[15] AIV-H9	99.6	98.9	98.5
[08] AIV-N1	99.8	99.4	99.1
[17] AIV-N5	98.5	-	99.3
[11] AIV-N8	99.6	-	99.8
[12] APMV-1	99.8	99.4	99.1

← ≈ 1%

# RESULTS – ASSESSMENT OF PARTICIPANTS

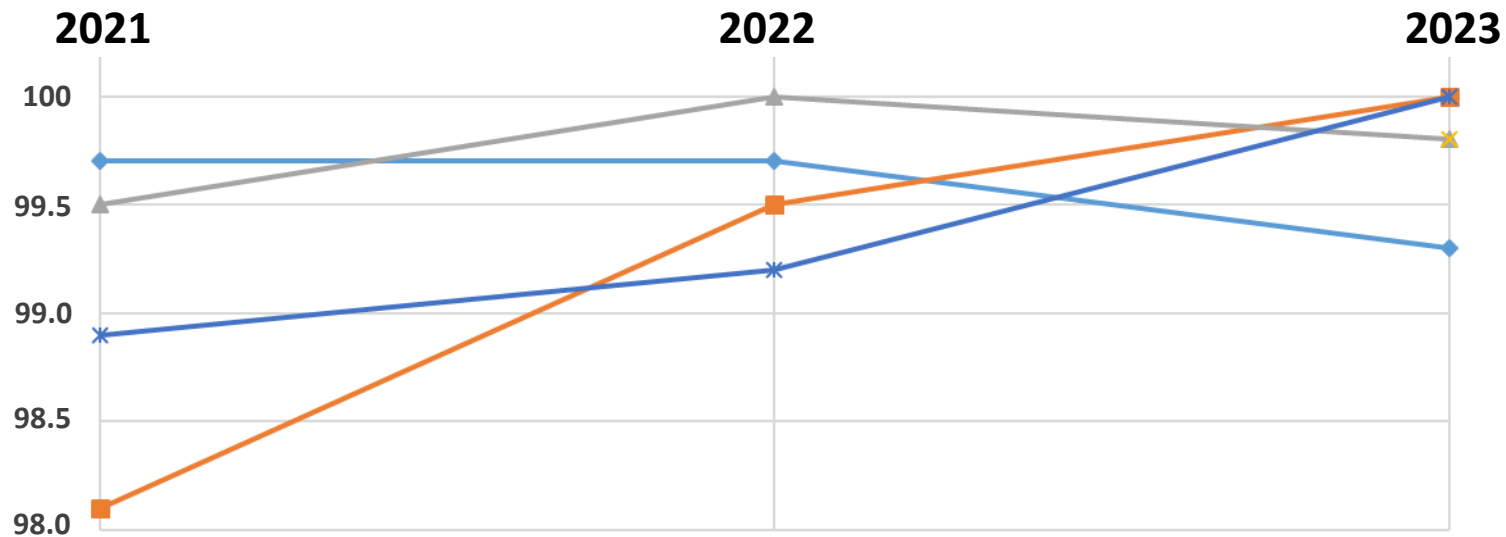
- **34 Participating Labs**
- **Success rate = 99.8%**

- **31 LABS** submitted results in **Perfect agreement** with the EURL for **ALL PARAMETERS**
- **1 LAB** submitted results in **Partial agreement** with the EURL only for **ONE PARAMETER**
- **1 LAB** submitted results in **Partial agreement** with EURL for **TWO PARAMETERS**
- **1 LAB** submitted results **with no agreement** with EURL for **ONE PARAMETER**



# MONITORING PERFORMANCE

Parameter	Success rate (%)			
	2023	2022	2021	
◆ [20] Haemagglutinating viruses (HAVs)	99.3	99.7	99.7	←
■ [21] Avian influenza viruses of H5 subtype	100	99.5	98.1	← ≈ 2%
▲ [22] Avian influenza viruses of H7 subtype	99.8	100	99.5	
✱ [24] Avian influenza viruses of H9 subtype	99.8	99.7	100	
* [23] Avian paramyxoviruses type 1	100	99.2	98.9	← ≈ 1%



# VIROLOGICAL PANEL COMPOSITION AND RESULTS

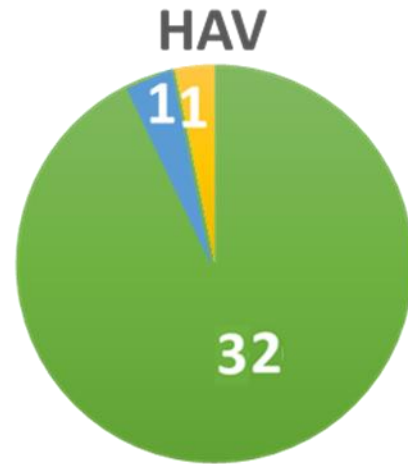
➤ **12 lyophilized samples including:**  
**10 AI/APMV-1 POSITIVES**  
**2 AI/APMV-1 NEGATIVES**

Sample	Strain	Type	Pathotype	Lineage/Clade/Genotype	HA Titer <sup>^</sup>	Success rate and False results (No.)				
						HAV	H5	H7	H9	APMV-1
V01	Negative	-	-	-	-	94% (2)			97% (1)	
V02	A/pheasant/Italy/21VIR2284-22/2021	H9N2	LPAI	Y439	1:128					
V03	A/mallard/Italy/22VIR9219-6/2022	H5N1	HPAI	Eurasian, 2.3.4.4b	1:128					
V04	A/Anas platyrhynchos/Belgium/8777_003_22VIR1107-26/2021	H7N8	LPAI	Eurasian	1:64					
V05	NDV V4-vaccine like	APMV-1	Avirulent	I.1.2.1*	1:256					
V06	PPMV-1/pigeon/Belgium/2449_22VIR1107-27/2021	APMV-1	Virulent	ND	1:128					
V07	A/mallard/Italy/20VIR4911-83/2020	H5N3	LPAI	Eurasian	1:128					
V08	APMV-1/chicken/Vushtrri-Kosovo/94_21VIR5162-29/2021	APMV-1	Virulent	VII.2	1:512					
V09	A/goose/Italy/20VIR7660-8/2020	H5N8	HPAI	Eurasian, 2.3.4.4b	1:256					
V10	A/common teal/Italy/21VIR49-88/2021	H7N3	LPAI	Eurasian	1:128					
V11	Negative	-	-	-	1:2	97% (1)				
V12	A/duck/Ireland/PV21-020707_21VIR7049-6/2021	H3N8	LPAI	-	1:256			97% (1)		

■ 100% success rate; HAV = Haemagglutinating virus; <sup>^</sup> Mode value of the HA titers obtained during the quality assessment tests and determined as the highest dilution of antigen causing complete agglutination of the RBCs; \* According to Dimitrov et al., 2019



# HA TITER RESULTS



- Perfect or almost perfect agreement (0.90 ≤ K ≤ 1.00)
- Substantial agreement (0.60 ≤ K ≤ 0.89)
- No agreement (K ≤ 0.59 or p ≥ 0.05)

HA TITER	PARTICIPANTS (no.)					EURL HA TITER
	1:1	1:2	1:4	1:16	1:64	
V01	2	8	3	1	1	-
V11	1	11	1	1	1	1:2



# SEROLOGICAL PANEL COMPOSITION AND RESULTS

- **37 Participating Labs**
- **Success rate = 99.5%**

- **12 lyophilized sera including:**  
 9 AI/APMV-1 POSITIVE SERA  
 3 AI/APMV-1 NEGATIVE SERA of which 1 APMV-2

Sample	Immunizing antigen				HI titer <sup>^</sup>	Success rate and False results (No.)				
	Strain	Type	Pathotype	Lineage/Clade/Genotype		AIV	H5	H7	H9	APMV-1
S01	A/poultry/Niger/ET1_21VIR2131-45/2021	H9N2	LPAI	G1	1:2048				<b>84% (5)</b>	
S02	Negative	-	-	-	-					
S03	APMV2/chicken/Yucaipa/56	APMV-2	-	-	1:128					
S04§	A/teal/Italy/16VIR345/2016	H7N7	LPAI	Eurasian	1:128					
S05	A/mute swan/England/AVP-18-1986/17	H5N6	HPAI	Eurasian, 2.3.4.4.b	1:64	97% (1)	<b>94% (2)</b>			
S06	NDV vaccine B1	APMV-1	Avirulent	II	1:256					
S07§	A/teal/Italy/16VIR345/2016	H7N7	LPAI	Eurasian	1:256					
S08§	A/duck/Italy/21VIR8024-20/2021	H5N3	LPAI	Eurasian	1:64					
S09§	A/duck/Italy/21VIR8024-20/2021	H5N3	LPAI	Eurasian	1:128		97% (1)	97% (1)		
S10	Negative	-	-	-	-					
S11	APMV-1/bassette chicken/Belgium/4096/2018	APMV-1	Virulent	VII.2	1:128					
S12	A/common teal/Italy/21VIR49-88/2021	H7N3	LPAI	Eurasian	1:512		97% (1)			

100% success rate; § Sample pairs S04 and S07 as well as S08 and S09 represent different dilution steps performed with the same stock serum; ^ Against homologous antigen

# CROSS REACTIONS RESULTS

## H9N2 A/poultry/Niger/ET1\_21VIR2131-45/2021 (G1-like lineage)

HAEMAGGLUTINATING ANTIGEN		EURL HI TITER TO TEST SAMPLE
Strain	Lineage	
H9N2-A/poultry/Niger/ET1_21VIR2131-45/2021	Eurasian, G1-like	<b>1:2048</b>
H9N2-A/mallard/Italy/3817-34/05	Eurasian, Y439-like	1:32
H9N2-A/turkey/Wisconsin/66	North America, WI/66-like	1:8

LABS HI TITER TO TEST SAMPLE
<b>1:16 (41)</b>

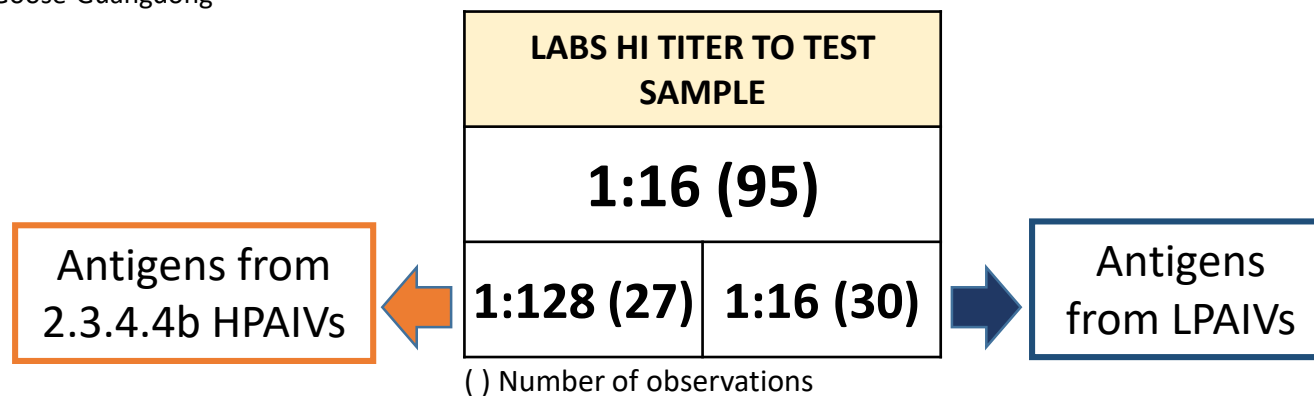
( ) Number of observations

# CROSS REACTIONS RESULTS

## H5N6 A/mute swan/England/AVP-18-1986/17 (Eurasian lineage, clade 2.3.4.4.b)

HAEMAGGLUTINATING ANTIGEN		EURL HI TITER TO TEST SAMPLE
Strain	Pathotype & Lineage	
H5N6-A/mute swan/England/AVP-18-1986/17	HPAI, Eurasian, 2.3.4.4b	<b>1:64</b>
H5N8-A/turkey/Italy/7898/2014	HPAI, Eurasian, 2.3.4.4c	1:16
H5N1-A/chicken/Scotland/1/59	HPAI, EA-nonGsGD*	1:16
H5N3-A/teal/England/7394-2805/06	LPAI, Eurasian	1:2

\* Eurasian avian nonGoose-Guangdong



EURL HI TITER TO TEST SAMPLE
<b>1:512</b>
1:128
1:16
1:32

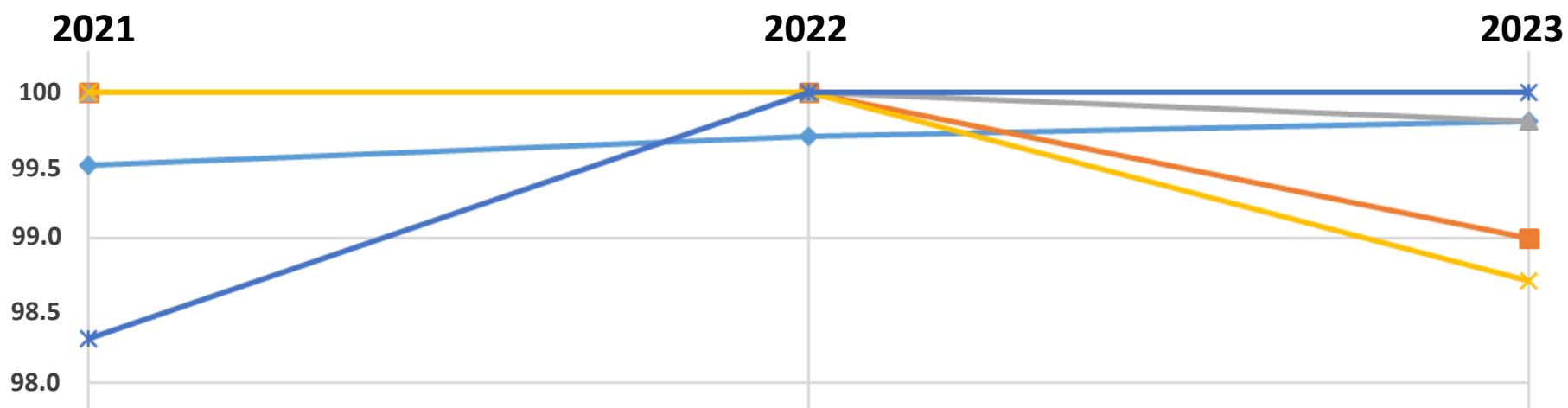
LABS HI TITER TO TEST SAMPLE
<b>1:64 (25)</b>

**2020 PT**

# MONITORING PERFORMANCE

Parameter	Success rate (%)		
	2023	2022	2021
◆ [30] Antibodies to type A avian influenza viruses	99.8	99.7	99.5
■ [31] Antibodies to H5 subtype of avian influenza viruses	99	100	100
▲ [32] Antibodies to H7 subtype of avian influenza viruses	99.8	100	100
✕ [34] Antibodies to H9 subtype of avian influenza viruses	98.7	100	100
* [33] Antibodies to avian paramyxoviruses type 1	100	100	98.3

≥ 1 %



# HIGHLIGHTS

➤ ≥ 99.5% success rate in each scheme

➤ EU-Recommended molecular methods were recognized fit for purpose

➤ To improve the identification of the H7 strains and avoid false negative results the adoption of more than one molecular method is recommended

➤ As for the serological scheme, we recommend including currently circulating H5 antigens of clade 2.3.4.4b in the cross HI tests as this may avoid failure in the detection of antibodies against such viruses in low or relatively low titer sera

## HIGHLIGHTS

➤ We deeply encourage you to share viruses and positive serum samples recently detected in your country.

Other than for future PTs, an expanded, up-to date and well-representative collection of strains and field serum samples from all over Europe is extremely important to us and would allow us to monitor the suitability of the reference reagents currently in use for the AI and NDV surveillance or to evaluate new strains to be selected as the best candidates for replacement



## ● ACKNOWLEDGMENT

- Laura Boscarato
- Lorenza Boscolo
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- Francesca Ellero
- Alessandra D'Anna
- Silvia Maniero
- Crispina Veggiato



**NEXT EDITION  
MARCH 2024**

# THANK YOU!