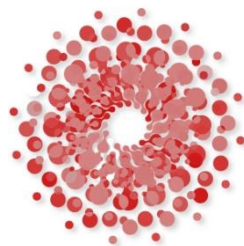




EURL PROFICIENCY TEST 2022 AVIAN INFLUENZA AND NEWCASTLE DISEASE



28th 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



ORGANIZATION

VIRUS GENOME DETECTION AI/ND

AIV (M gene)	APMV-1
AIV H5	
AIV H7	
AIV H9	
AIV N1	
AIV N3	
AIV N4	

VIRUS ANTIGEN DETECTION AI/ND

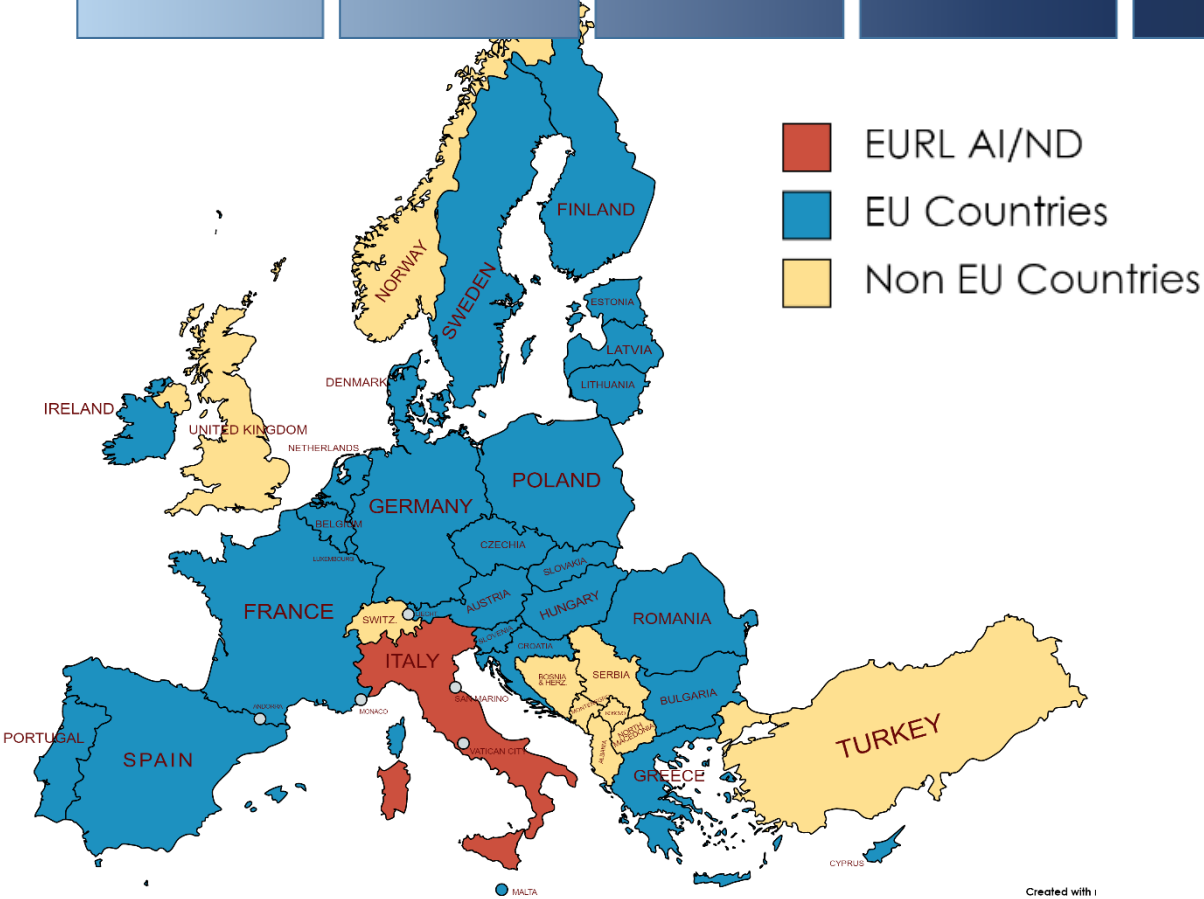
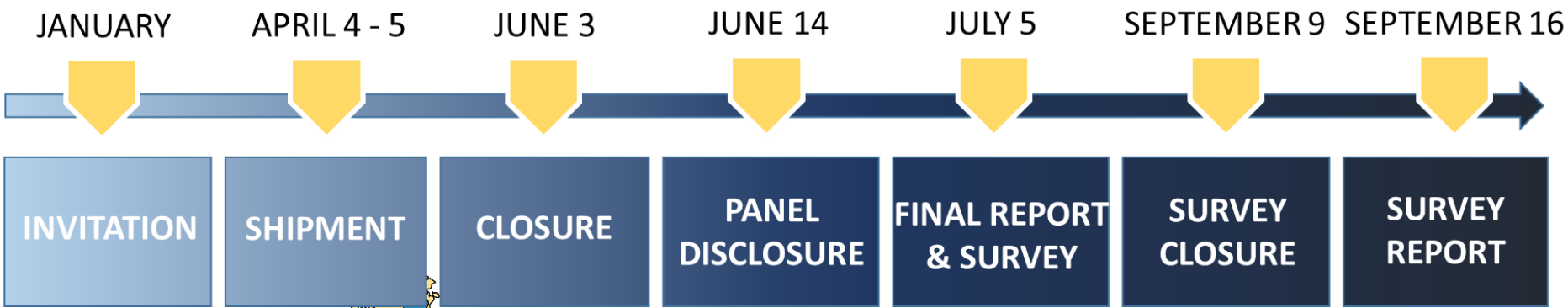
AIV H5	APMV-1
AIV H7	
AIV H9	

ANTIBODIES DETECTION AI/ND

AIV	APMV-1
AIV H5	
AIV H7	
AIV H9	



TIMELINE AND PARTICIPATING LABORATORIES



	PT SCHEME		
	Genome Detection	Antigen Detection	Antibodies Detection
EU LABS	25	24	25
NON EU LABS*	12	7	9
TOTAL	37	31	34

* Including EFTA laboratories

MOLECULAR PANEL COMPOSITION

➤ **15 Samples** in liquid format including **14 AI/ND Positives** and **1 Negative**

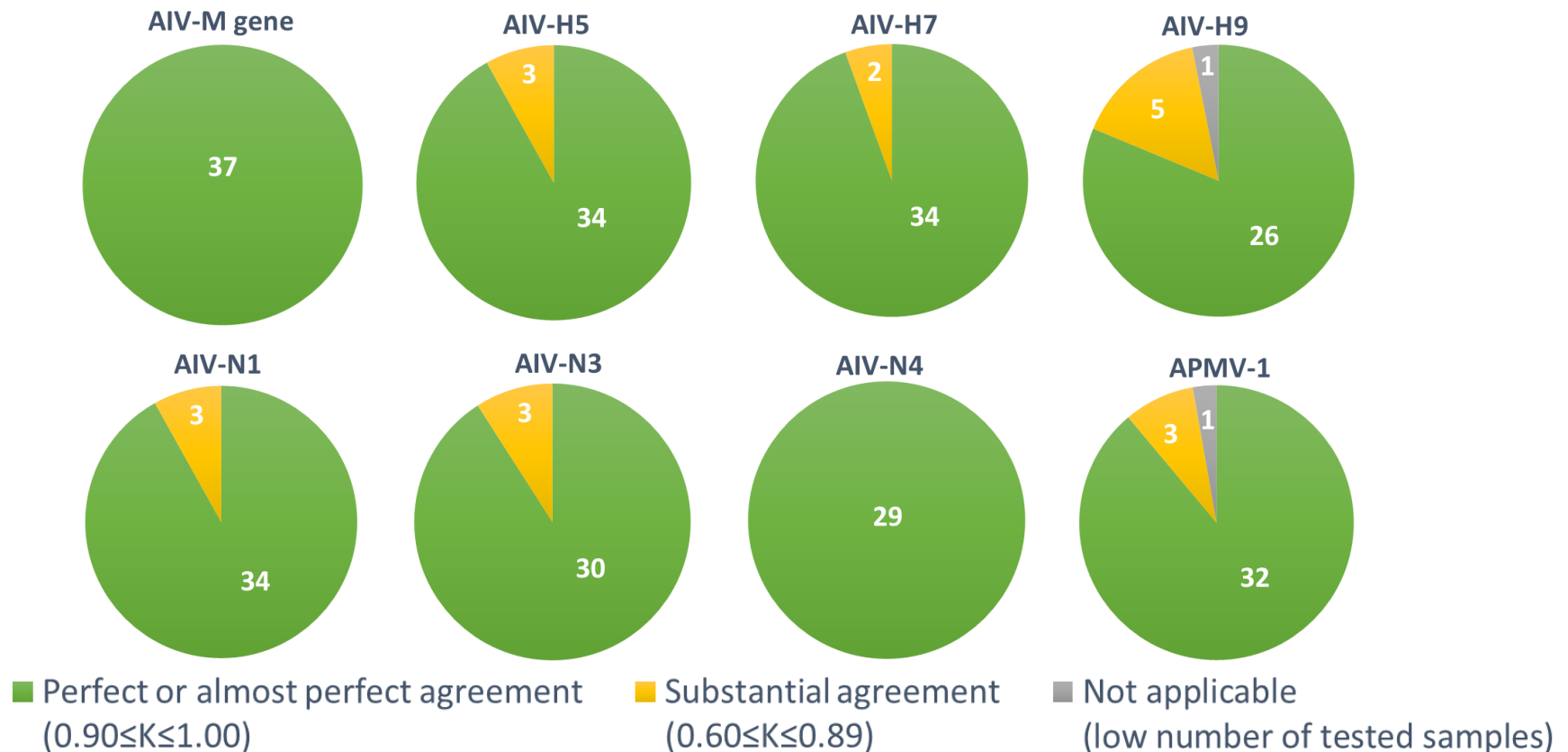
		Virus	Type	Lineage/ Genotype	Pathotype
APMV-1		NDV vaccine B1	APMV-1	II*	Avirulent
		NDV/chicken/Vushtrri-Kosovo/94_21VIR5162-29/2021	APMV-1	VII.2*	Virulent
		PPMV1/feral pigeon/Slovenia/PER1406_20_21VIR959-6/2020	PPMV-1	VI.2.1.1.2.2*	Virulent
AI	Other	A/chicken/Ireland/005930_20VIR5487-1/2020	H6N1	-	LPAI
	H5	A/chicken/Senegal/21VIR1084-21/2021	H5N1	2.3.4.4B	HPAI
		A/poultry/Niger/ET1_HALAL_21VIR2131-43/2021	H5N1	2.3.4.4B	HPAI
		A/knot wader/Ireland/PV21-000472_21VIR2956-11/2021	H5N3	2.3.4.4B	HPAI
		A/duck/Italy/21VIR8024-20/2021	H5N3	Eurasian	LPAI
		A/yellow legged gull/Switzerland/15_2021_0039_21VIR3035-9/2021	H5N4	2.3.4.4B	HPAI
		A/laying hen/Slovakia/Pah10_21VIR1086-7/2021	H5N5	2.3.4.4B	HPAI
	H7	A/chicken hen/Italy/15VIR1670-21/2015	H7N2	Eurasian	LPAI
		A/chicken hen/Italy/13VIR4527-11/2013	H7N7	Eurasian	HPAI
	H9	A/duck/Italy/21VIR8691-13/2021	H9N2	Y439	LPAI
		A/poultry/Niger/ET1_21VIR2131-45/2021	H9N2	G1	LPAI

* According to Dimitrov et al., 2019

ASSESSMENT

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

- **22 LABS** submitted **100% correct results**
- **11 LABS** submitted results in **Partial agreement** with the EURL only for **ONE PARAMETER**
- **4 LAB** submitted results in **Partial agreement** with EURL for **TWO out of EIGHT PARAMETERS**



RESULTS - DETECTION

Sample	Specie	Country	Year	Type	Clade/genotype	Success rate and False results (No.)							
						M	H5	H7	H9	N1	N3	N4	APMV-1
M02	Gull	Switzerland	2021	H5N4	2.3.4.4B								
M07	Poultry	Niger	2021	H5N1	2.3.4.4B								
M09	Chicken hen	Slovakia	2021	H5N5	2.3.4.4B								
M12	Knot wader	Ireland	2021	H5N3	2.3.4.4B								
M11	Chicken hen	Italy	2015	H7N2	Eurasian								
M15	Chicken hen	Italy	2013	H7N7	Eurasian								
M05	Pigeon	Slovenia	2020	PPMV-1	VI.2.1.1.2.2*								
M08	Negative												
M03	Poultry	Niger	2021	H9N2	G1								
M14	Duck	Italy	2021	H9N2	Y439								
M01	Chicken	Ireland	2020	H6N1	-								
M06	Chicken	Senegal	2021	H5N1	2.3.4.4B								
M13	Duck	Italy	2021	H5N3	Eurasian								
M04	Chicken	Kosovo	2021	APMV-1	VII.2*								
M10	NDV vaccine B1			APMV-1	II*								

 100% success rate

() Number of false results

* According to Dimitrov et al., 2019

88% (4)

97% (1) 97% (1)

97% (1) 95% (2)

97% (1) 97% (1)

92% (3)

91% (3)

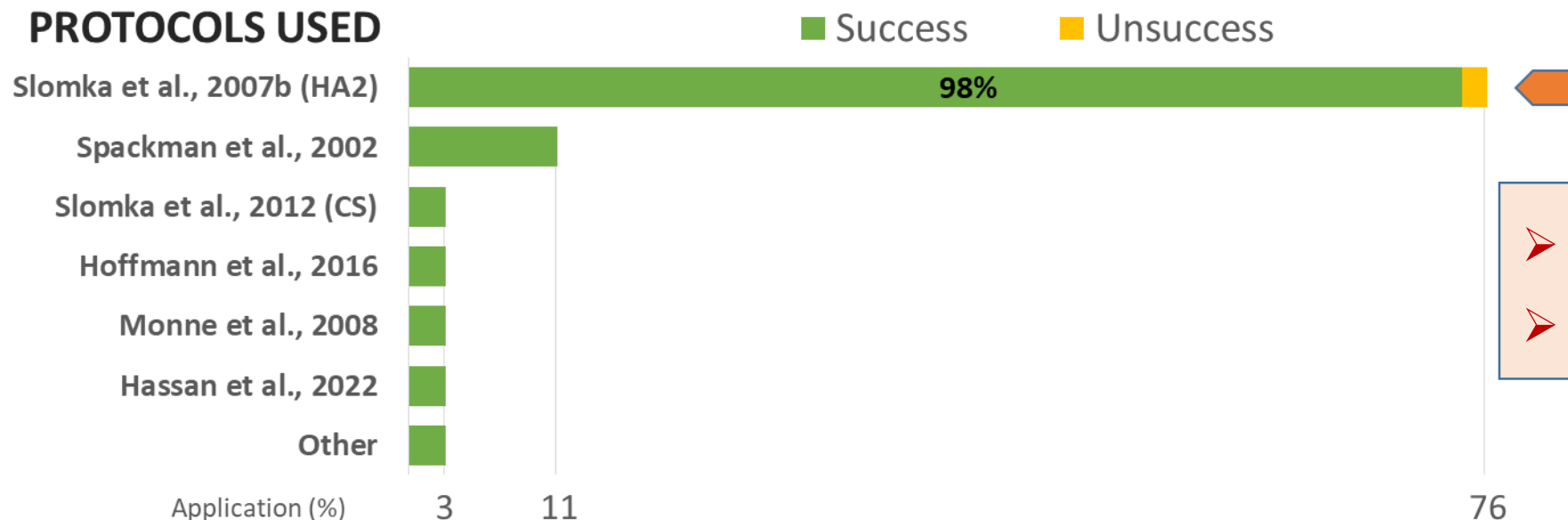
94% (2)

97% (1)

1 out of 3 failing labs gave the correct result as optional

INTRA-METHODS RESULTS – DETECTION OF H5 STRAINS

PROTOCOLS USED



➤ Participating Labs = 37
➤ Success rate = 99.3%

Sample	Specie	Country	Year	Type	Pathotype	GC/ml	Detection
M13	Duck	Italy	2021	H5N3	LPAI	$10^{4.35}$	92% (3)
M06	Chicken	Senegal	2021	H5N1	HPAI	$10^{4.15}$	97% (1)
M02	Gull	Switzerland	2021	H5N4	HPAI	$10^{5.19}$	100%
M12	Knot wader	Ireland	2021	H5N3	HPAI	$10^{5.20}$	100%
M09	Chicken hen	Slovakia	2021	H5N5	HPAI	$10^{5.73}$	100%
M07	Poultry	Niger	2021	H5N1	HPAI	$10^{5.91}$	100%

() Number of false results

INTRA-METHODS RESULTS – DETECTION OF H5 STRAINS

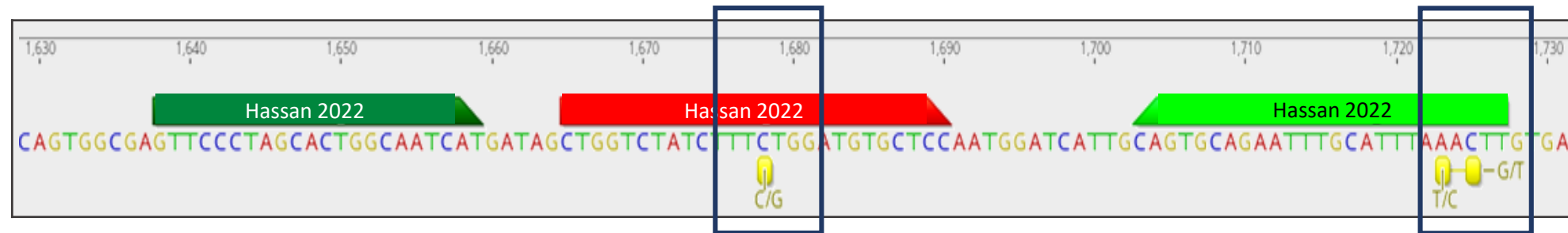
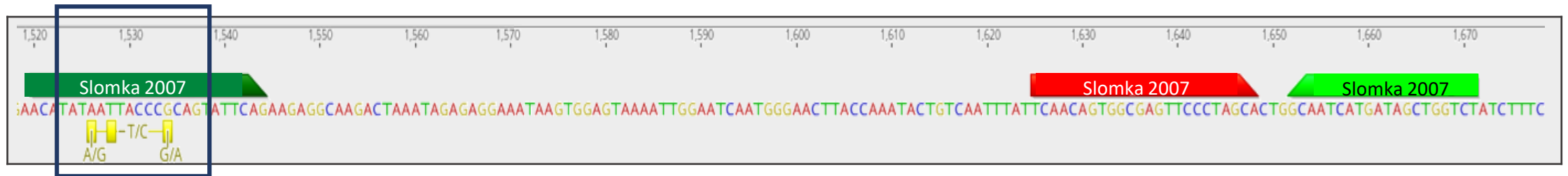
Sample	GC/ml	EURL Ct – H5				EURL Ct – M gene
		Slomka 2007	Hassan 2022	IZSVe SOP VIR 1005*		Heine 2015
M13 (H5N3)	10 ^{4.35}	35.52	33.47	32.18		30.21
M06 (H5N1)	10 ^{4.15}	31.23	30.18	31.28	32.14	31.28
M02 (H5N4)	10 ^{5.19}	28.60	27.27	29.07	29.14	27.43
M12 (H5N3)	10 ^{5.20}	29.81	28.51	30.15	30.67	27.14
M09 (H5N5)	10 ^{5.73}	26.91	24.77	26.40	27.12	26.69
M07 (H5N1)	10 ^{5.91}	25.78	27.20	25.65	26.27	25.98

* *Molecular pathotyping and phylotyping of Eurasian H5 avian influenza virus by real-time RT-PCR* (unpublished and for EU-NRLs internal use only). In light orange the duplex “H5-HPAI/clade 2.3.4.4” RRT PCR and in light blue the simplex “H5-LPAI” RRT PCR

OLIGOS *IN SILICO* CHECK

Sample	GC/ml	EURL Ct – H5			EURL Ct – M gene
		Slomka 2007	Hassan 2022	IZSve SOP VIR 1005*	Heine 2015
M13 (H5N3)	10 ^{4.35}	35.52	33.47	32.18	30.21

* Molecular pathotyping and phylotyping of Eurasian H5 avian influenza virus by real-time RT-PCR (unpublished and for EU-NRLs internal use only)



PRIMER FOR

PROBE

PRIMER REV

INTRA-METHODS RESULTS – DETECTION OF N3 STRAINS

- **Positive Samples = 2**
- **Participating Labs = 33**
- **Success rate = 99.4%**
96% sensitivity
100% specificity

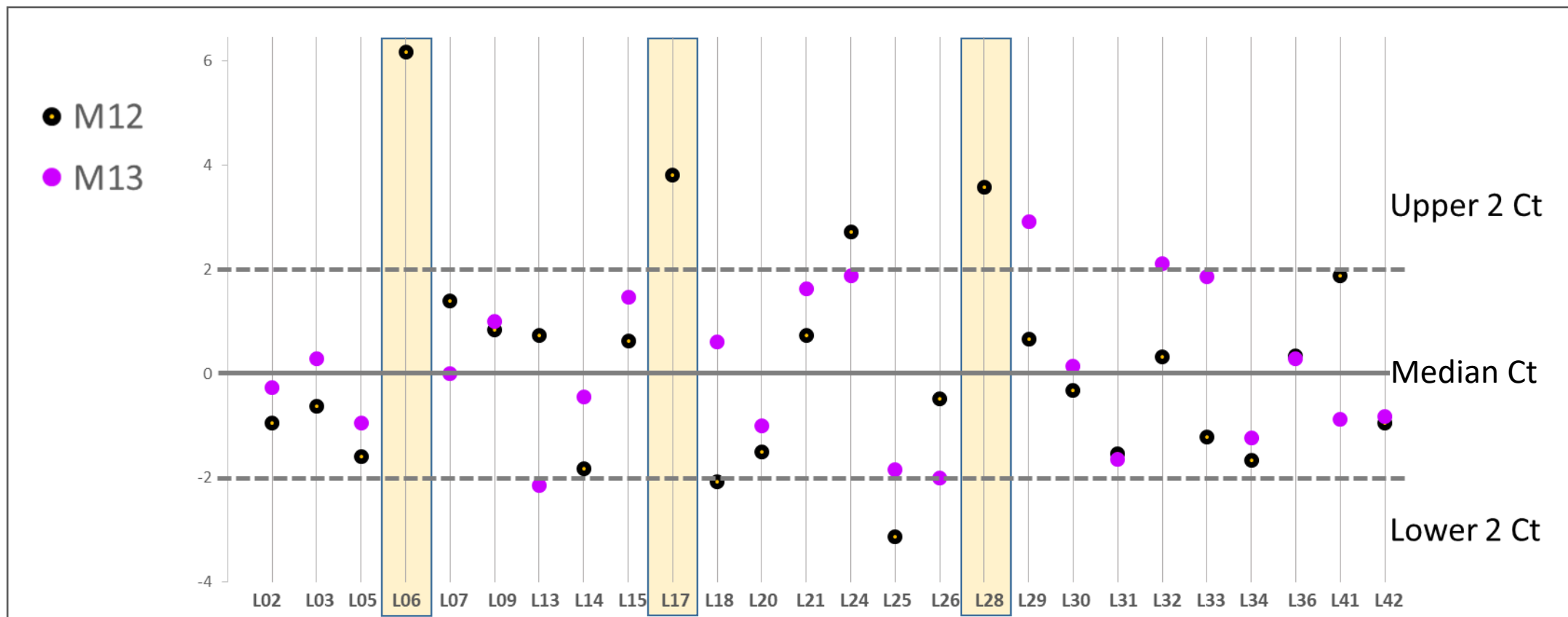
Method	Application (No. Labs)	M12	M13
Hoffmann et al., 2016	26		89% (3)
Fereidouni et al., 2009	4		
Hassan et al., 2022	1		
Gall et al., 2009	1		
Other	1		

 100% success rate
 () Number of false results

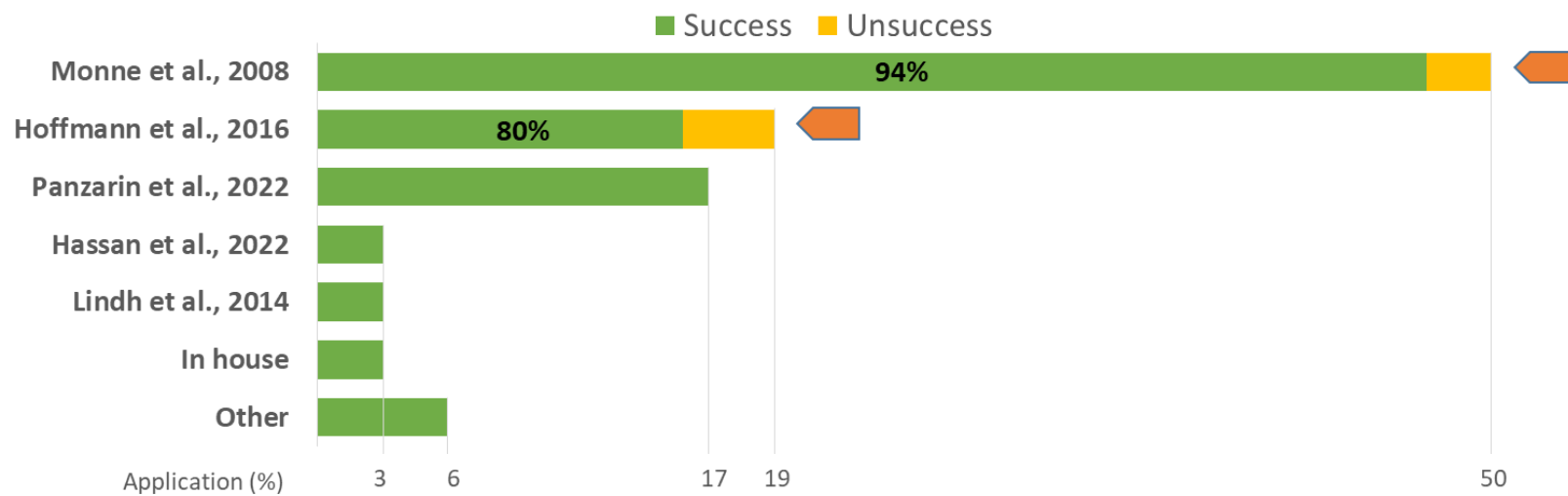
INTRA-METHODS RESULTS – DETECTION OF N3 STRAINS

Sample	Specie	Country	Year	Type	GC/mL	EURL Ct		Labs	
						Hoffmann	2016 Hassan 2022	Median Ct Hoffmann	Detection
M13	Duck	Italy	2021	H5N3	$10^{4.35}$	30.76	31.12	32.14	91% (3)
M12	Knot wader	Ireland	2021	H5N3	$10^{5.20}$	29.22	28.40	31.02	100%

() Number of false results



INTRA-METHODS RESULTS – DETECTION OF H9 STRAINS



➤ **Participating Labs = 32**

➤ **Success rate = 99%**
92.2% sensitivity
100% specificity

Sample	Specie	Country	Year	Type	Lineage	GC/ml	EURL Ct		Detection		
							Panzarin 2022*	Monne 2008	Monne 2008	Hoffmann 2016	Total
M03	Poultry	Niger	2021	H9N2	G1	$10^{4.66}$	26.84	N	94% (17/18)	57% (4/7)	88%
M14	Duck	Italy	2021	H9N2	Y439	$10^{3.44}$	30.78	33.73	94% (17/18)	100% (8/8)	97%

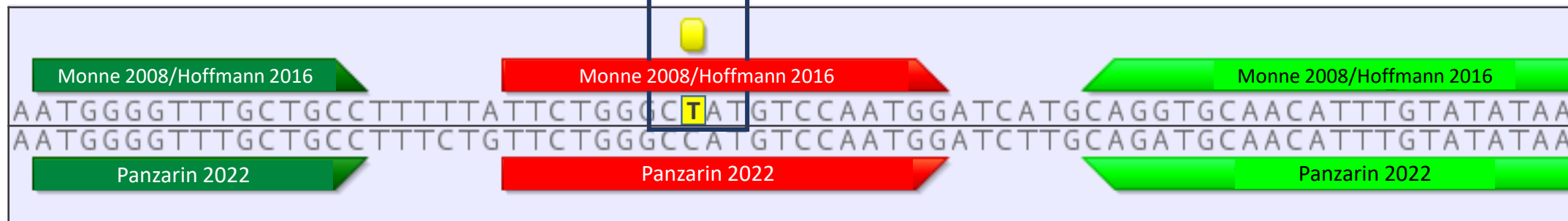
N = Negative

* IZSve SOP VIR 014 – ed. 01

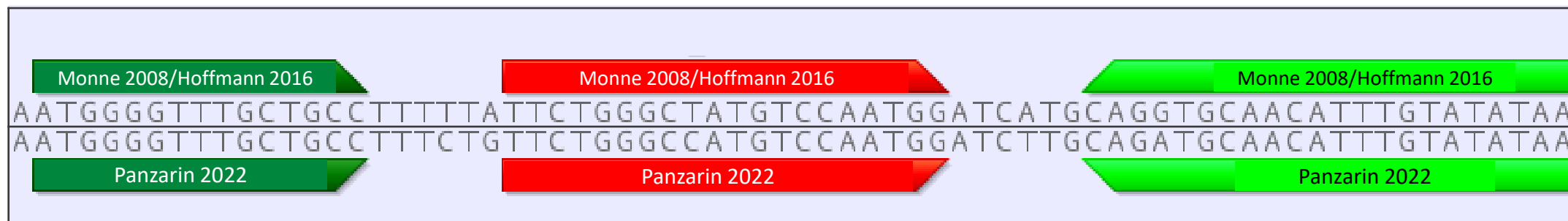
() Number of correct results/Number of total results

OLIGOS *IN SILICO* CHECK – H9 STRAINS

M03 A/poultry/Niger/ET1_21VIR2131-45/2021



M14 A/duck/Italy/21VIR8691-13/2021



PRIMER FOR

PRIMER REV

PROBE

geneious[®]
<https://www.geneious.com>

INTRA-METHODS RESULTS – DETECTION OF N1 STRAINS

Sample	Specie	Country	Year	Type	GC/mL	Labs	
						Median Ct	Detection
M01	Chicken	Ireland	2020	H6N1	$10^{3.81}$	34.95	95% (2)
M06	Chicken	Senegal	2021	H5N1	$10^{4.15}$	32.94	97% (1)
M07	Poultry	Niger	2021	H5N1	$10^{5.91}$	27.66	100%

() Number of false results

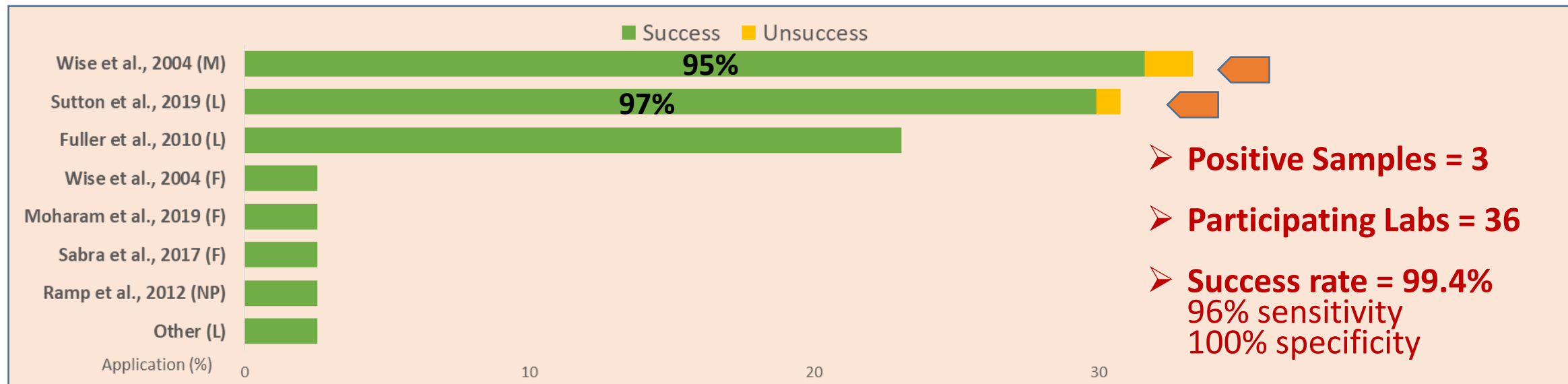
Method	Application (No. Labs)	M01 H6N1	M06 H5N1	M07 H5N1
Hoffmann et al., 2016	15	93% (1)		
Payungporn et al., 2006	14		93% (1)	
Slomka et al., 2012	3	67% (1)		
Agüero et al., 2007	1			
Fereidouni et al., 2009	1			
Hassan et al., 2022	1			
Other	2			

 100% success rate

() Number of false results

- **Participating labs = 37**
- **Success rate = 99%**
97% sensitivity
100% specificity

INTRA-METHODS RESULTS – DETECTION OF APMV-1 STRAINS

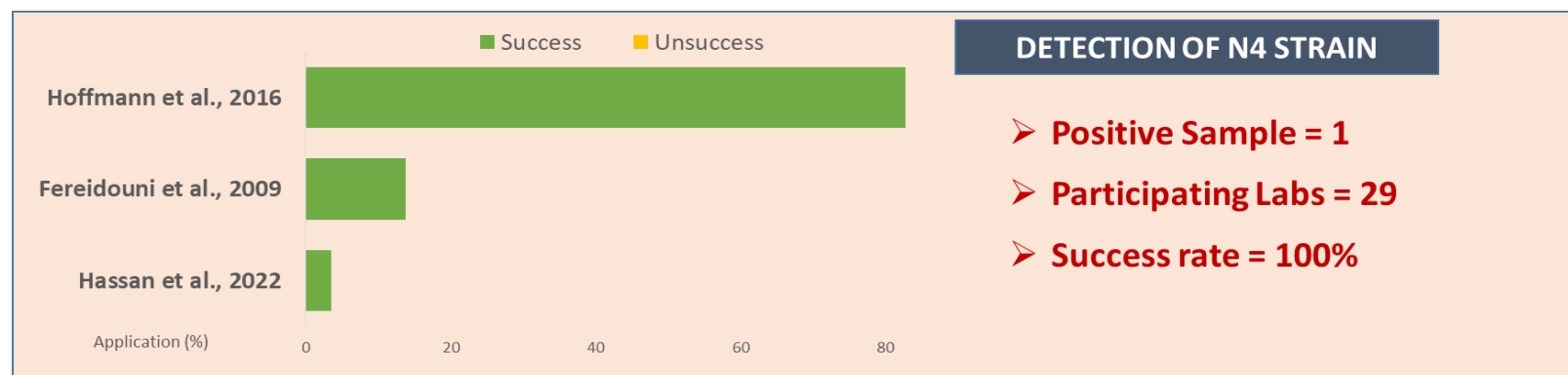
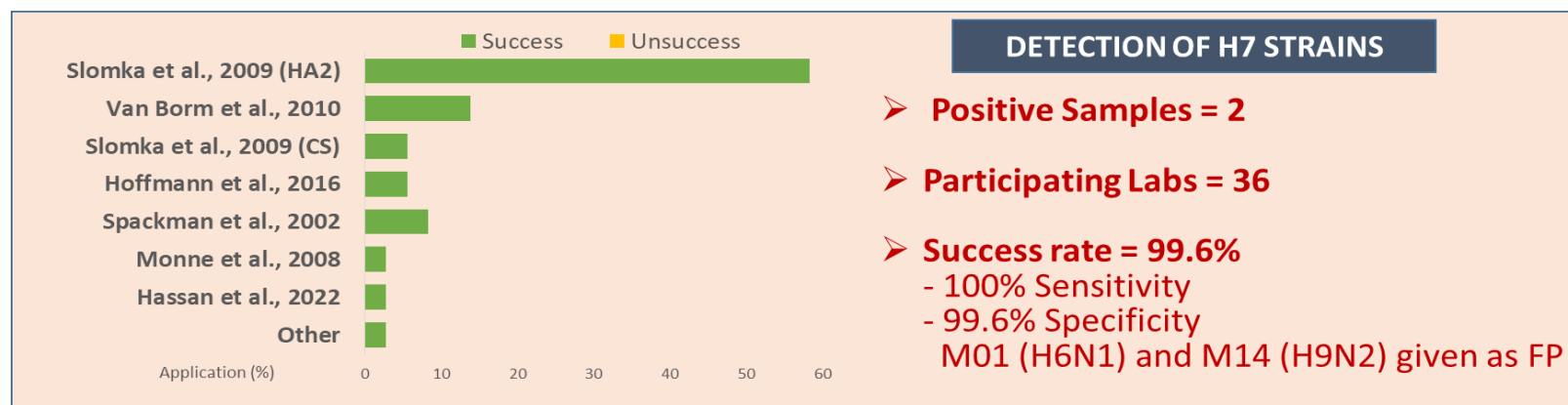
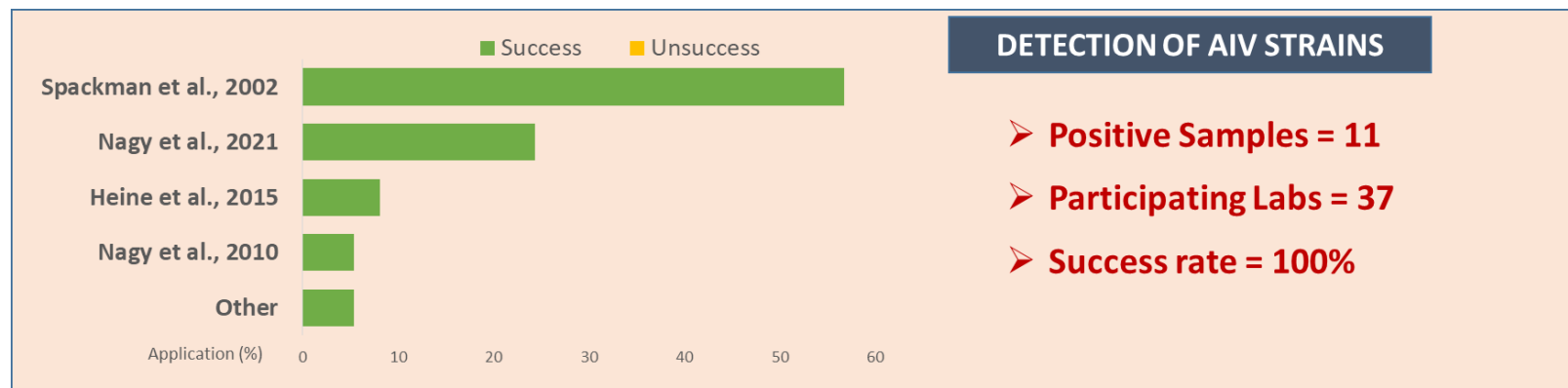


Sample	Virus	Genotype*	GC/ml	EURL Ct	Detection		
				Sutton 2019	Wise 2004	Sutton 2019	Total
M04	NDV/chicken/Vushttri-Kosovo/94_21VIR5162-29/2021	VII.2	10 ^{5.15}	25.15	92% (12/13)	92 (11/12)	94%
M10	NDV vaccine B1	II	10 ^{4.60}	26.52	92% (12/13)	100% (12/12)	97%
M05	PPMV1/feral pigeon/Slovenia/PER1406_20_21VIR959-6/2020	VI.2.1.1.2.2	10 ^{5.26}	25.21	100% (13/13)	100% (12/12)	100%

* According to Dimitrov et al., 2019

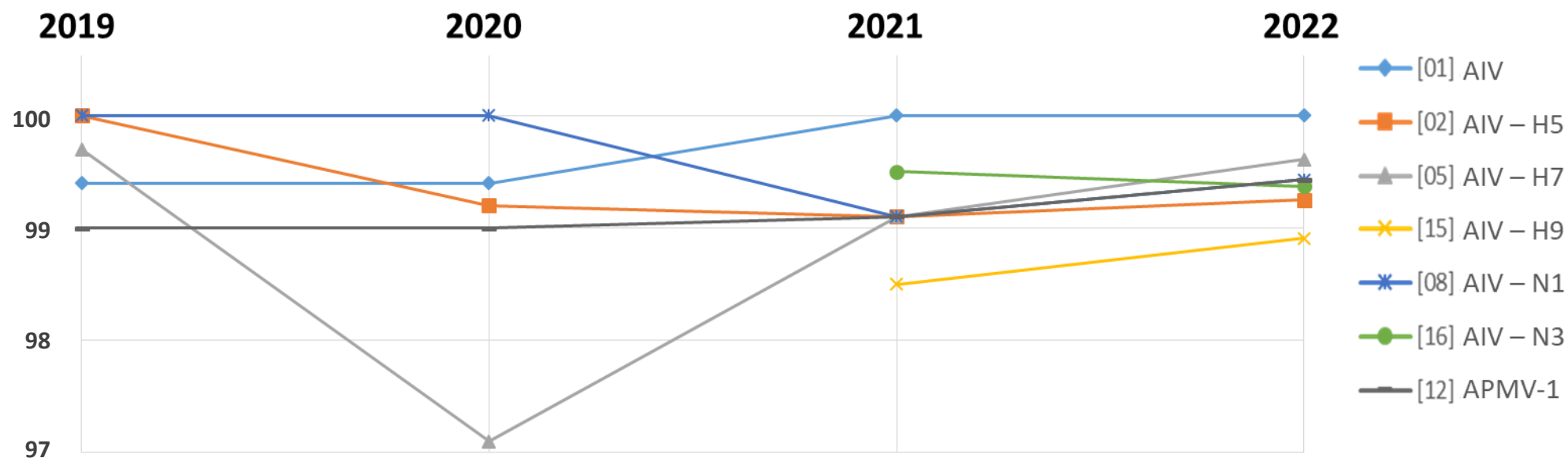
() Number of correct results/Number of total results

INTRA-METHODS RESULTS



PERFORMANCE MONITORING

Parameter	Success rate (%)			
	2022	2021	2020	2019
[01] AIV	100	100	99.4	99.4
[02] AIV-H5	99.3	99.1	99.2	100
[05] AIV-H7	99.6	99.1	97.1	99.7
[15] AIV-H9	98.9	98.5	-	-
[08] AIV-N1	99.4	99.1	100	100
[16] AIV-N3	99.4	99.5	-	-
[18] AIV-N4	100			
[12] APMV-1	99.4	99.1	99	99



VIROLOGICAL PANEL COMPOSITION

- **12 lyophilized samples** including:
- 10 containing haemagglutinin antigens
 - 2 Negatives

	Virus	Type	Pathotype	HA titer
AIV-H5	A/Anas platyrhynchos/Belgium/10811_6_19VIR8799-53/2019	H5N6	LPAI	1:128
	A/yellow legged gull/Switzerland/15_2021_0039_21VIR3035-9/2021	H5N4	HPAI	1:64
	A/knot wader/Ireland/PV21-000472_21VIR2956-11/2021	H5N3	HPAI	1:128
AIV-H7	A/turkey/Italy/20VIR1969-10/2020	H7N1	LPAI	1:128
	A/chicken hen/Italy/13VIR4527-11/2013	H7N7	HPAI	1:256
AIV-H9	A/poultry/Niger/ET1_21VIR2131-45/2021	H9N2	LPAI	1:256
	A/swan/Italy/17VIR1205/2017	H9N2	LPAI	1:128
APMV-1	PPMV1/collared dove/Cyprus/20VIR3543-12/2020	PPMV-1	Virulent	1:128
	NDV/chicken/Rus/Krasnodar/9.1_20VIR6516/19	APMV-1	Virulent	1:128
Other	APMV6/mallard/Italy/18VIR6847-297/2018	APMV-6	-	1:128

RESULTS

Sample	Strain	Type	Pathotype	Success rate and False results (No.)				
				HAV	H5	H7	H9	APMV-1
V01	A/turkey/Italy/20VIR1969-10/2020	H7N1	LPAI					
V02	PPMV1/collared dove/Cyprus/20VIR3543-12/2020	APMV-1	Virulent					
V03	A/Anas platyrhynchos/Belgium/10811_6_19VIR8799-53/2019	H5N6	LPAI					
V04	Negative	-	-					
V05	APMV6/mallard/Italy/18VIR6847-297/2018	APMV-6	-		97 (1)		97 (1)	94 (2)
V06	A/chicken hen/Italy/13VIR4527-11/2013	H7N7	HPAI					
V07	A/swan/Italy/17VIR1205/2017	H9N2	-					
V08	A/yellow legged gull/Switzerland/15_2021_0039_21VIR3035-9/2021	H5N4	HPAI	97 (1)	97 (1)			
V09	Negative	-	-					
V10	NDV/chicken/Rus/Krasnodar/9.1_20VIR6516/19	APMV-1	Virulent					97 (1)
V11	A/poultry/Niger/ET1_21VIR2131-45/2021	H9N2	-					
V12	A/knot wader/Ireland/PV21-000472_21VIR2956-11/2021	H5N3	HPAI					

 100% success rate
 () Number of false results
 HAV = Haemagglutinating virus

CROSS REACTIONS RESULTS

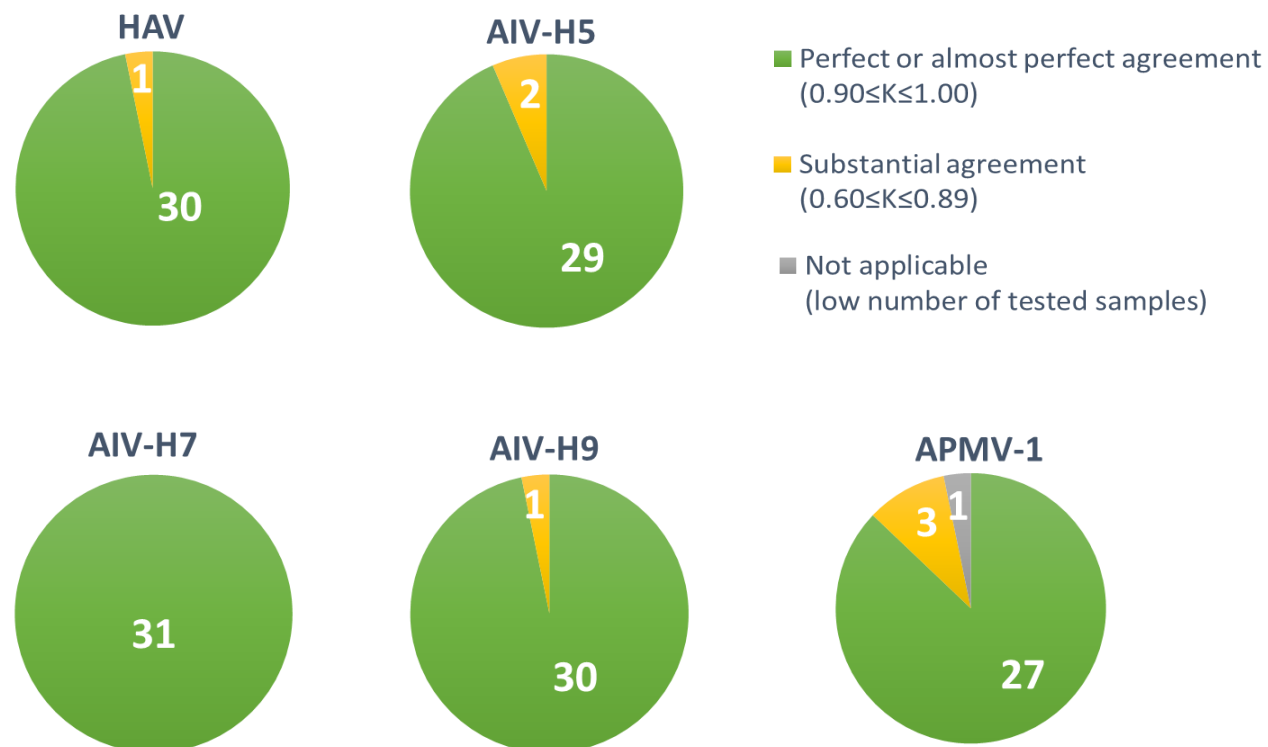
SERUM				HI Titer to test Sample
Immunizing antigen	Type	Pathotype	HI Titer to homologous antigen	V05 APMV-6
A/turkey/Italy/7898/2014	H5N8	HPAI	1:256	N
A/chicken/Scotland/1/59	H5N1	HPAI	1:512	N
A/teal/England/7394-2805/06	H5N3	LPAI	1:512	N
A/africa starling/England/983/79	H7N1	LPAI	1:512	N
A/turkey/England/647/77	H7N7	LPAI	1:512	N
A/turkey/Wisconsin/66	H9N2	LPAI	1:512	N
A/mallard/Italy/3817-34/05	H9N2	LPAI	1:256	N
Ulster 2C	NDV	Avirulent	1:256	1:4
PPMV-1/dove/Italy/4400/2000	APMV-1	Virulent	1:512	1:4
APMV-6/duck/HongKong/199/77	APMV-6	-	1:512	1:256

Significant difference between the titer of the reference serum to the unknown sample and its known titer suggests only partial antigenic identity

ASSESSMENT

- 31 Participating Labs
- Success rate = 99.6%

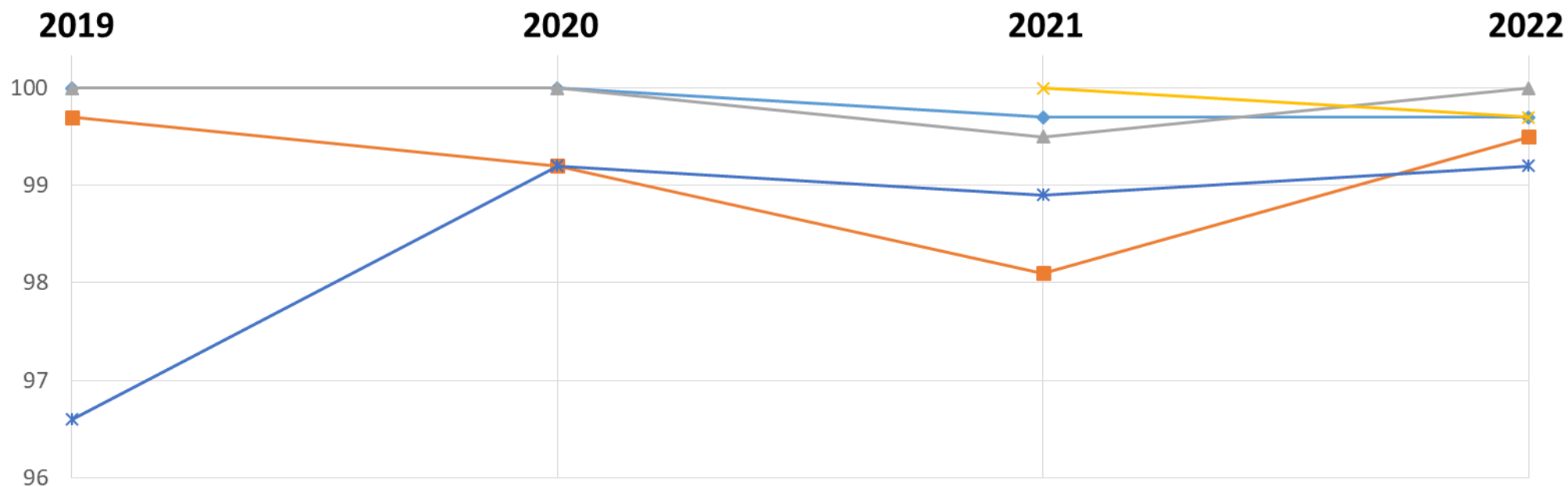
- 26 LABS submitted results in **Perfect agreement** with the EURL for **ALL PARAMETERS**
- 4 LABS submitted results in **Partial agreement** with the EURL only for **ONE PARAMETER**
- 1 LAB submitted results in **Partial agreement** with EURL for **THREE out of FOUR PARAMETERS**



PERFORMANCE MONITORING

Parameter	Success rate (%)			
	2022	2021	2020	2019
— [20] Haemagglutinating viruses (HAVs)	99.7	99.7	100	100
— [21] Avian influenza viruses of H5 subtype	99.5	98.1	99.2	99.7
— [22] Avian influenza viruses of H7 subtype	100	99.5	100	100
— [24] Avian influenza viruses of H9 subtype	99.7	100	-	-
— [23] Avian paramyxoviruses type 1	99.2	98.9	99.2	96.6

> 1%



SEROLOGICAL PANEL COMPOSITION AND RESULTS

- **34 Participating Labs**
- **Success rate = 99.7%**

- **12 lyophilized sera including:**
10 AI/APMV-1 positive sera obtained from **RECENT STRAINS**
2 Negatives

Sample	Immunizing antigen	Type	HI titer [^]	Success rate and False results (No.)				
				AIV	H5	H7	H9	APMV-1
S01	A/duck/Italy/15VIR6558/2015	H5N2	1:512					
S02	Negative	Negative	-					
S03§	A/mallard/Italy/20VIR4911-52/2020	H5N3	1:512					
S04	A/turkey/Italy/16VIR8643-60/2016	H9N2	1:512					
S05	A/turkey/Italy/20VIR1969-10/2020	H7N1	1:512					
S06	NDV Ulster	APMV-1	1:512					
S07§	A/chicken/Italy/19VIR5895-21/2019	H7N3	1:512					
S08§	A/mallard/Italy/20VIR4911-52/2020	H5N3	1:64					
S09	Negative	Negative						
S10§	A/chicken/Italy/19VIR5895-21/2019	H7N3	1:64	97 (1)				
S11	A/Anas platyrhynchos/Belgium/10811_6_19VIR8799-53/2019	H5N6	1:512					
S12	PPMV-1/pigeon/Cyprus/20VIR3543-9/2020	APMV-1	1:512					

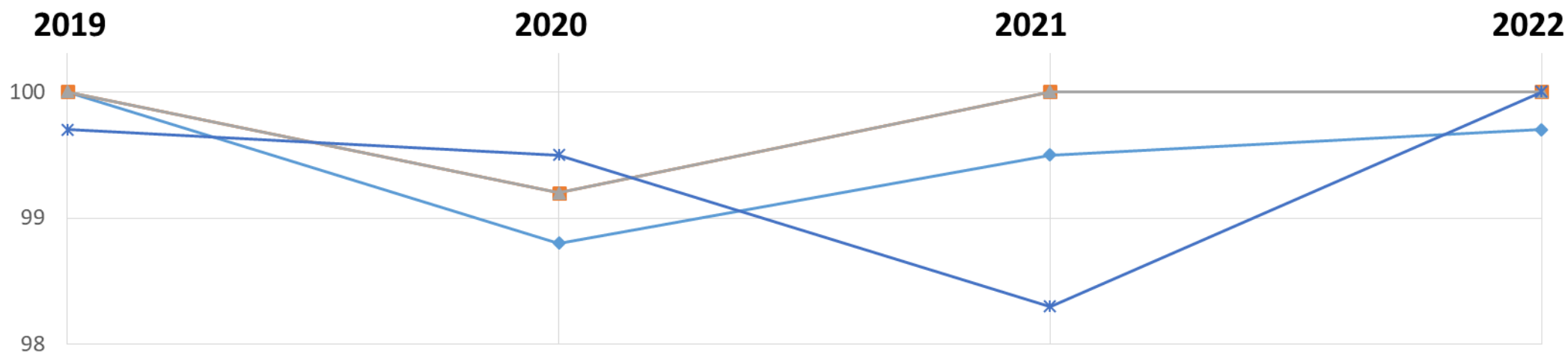
 100% success rate

() Number of false results; § Sample pairs S03 and S08 as well as S07 and S10 represent different dilution steps performed with the same stock serum; [^] Against homologous antigen

PERFORMANCE MONITORING

Parameter	Success rate (%)			
	2022	2021	2020	2019
—◆ [30] Antibodies to type A avian influenza viruses	99.7	99.5	98.8	100
—■ [31] Antibodies to H5 subtype of avian influenza viruses	100	100	99.2	100
—▲ [32] Antibodies to H7 subtype of avian influenza viruses	100	100	99.2	100
—✕ [34] Antibodies to H9 subtype of avian influenza viruses	100	100	-	-
—* [33] Antibodies to avian paramyxoviruses type 1	100	98.3	99.5	99.7

~ 2 %





HIGHLIGHTS

- NRLs demonstrated an excellent proficiency testing level in the three schemes (>99%)
- Recommended molecular methods were recognized fit for purpose and failure was mainly strain dependant
- To improve the identification of the H9 strains it is recommended the adoption of the newly developed method by Panzarin et al., 2022
- As for the virological scheme, significant difference between the known titer of the reference serum and the titer to the unknown sample might be due to only partial antigenic identity
- The use of a panel which includes sera to the most common APMV types might help in avoiding interpretative errors and false positive reporting for APMV-1 target



ACKNOWLEDGMENT

- Laura Boscarato
- Lorenza Boscolo
- Valeria D'Amico
- Francesca Ellero
- Alessandra D'Anna
- Alessandra Drago
- Silvia Maniero
- Sabrina Marciano
- Valentina Panzarin
- Crispina Veggiato



**THANK YOU!
SEE YOU IN
APRIL FOR
PT 2023**