Istituto Zooprofilattico Sperimentale delle Venezie

Aqua
Proficiency testing for quality assurance of results

EURL PROFICIENCY TEST 2020
AVIAN INFLUENZA AND NEWCASTLE DISEASE

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SCS5 – DSBIO IZSVe

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AI/ND EURL PT - Aim

- ✓ To assess the individual laboratories performance to detect and characterise AIV and APMV-1 or their specific antibodies
- ✓ To evaluate the performance of standard diagnostic methods/protocols
- ✓ PT organization, samples preparation and evaluation of lab performance according to ISO17043 and ISO13528

PT Circuit	**AQUA IN EU**		
PT Scheme	**AQUA IN EU MB 2020**	**AQUA IN EU S 2020**	**AQUA IN EU V 2020**
Sample	Virus isolates in PrimeStore	Freeze-dried sera-containing antibodies	Freeze-dried inactivated antigens
	RNA: AIV, AIV H5, AIV H7, AIV N1, AIV N2, AIV N6, AIV N8, APMV-1 Pathotype: AIV, APMV-1 CS Sequencing: AIV, APMV-1	Antibodies: AIV, AIV H5, AIV H7, APMV-1	Antigen: HAV, AIV H5, AIV H7, APMV-1
Testing	Real-time and conventional RT-PCR protocols	AGID/ELISA and HI test	HA and HI tests

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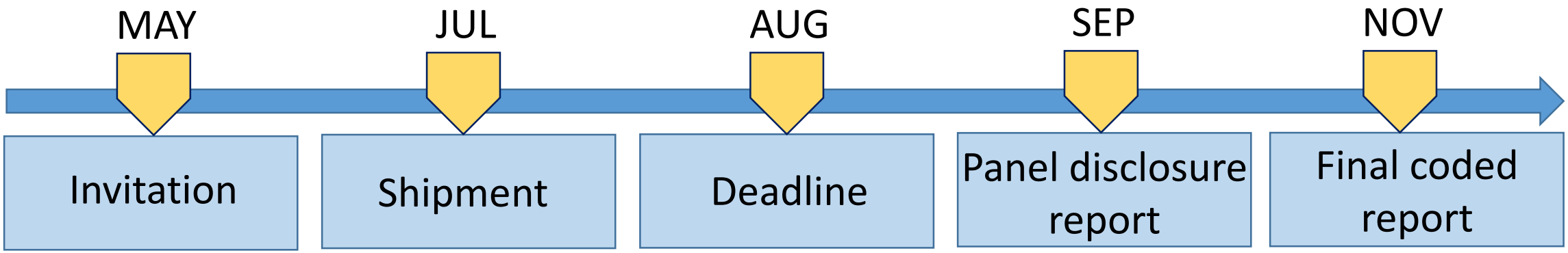
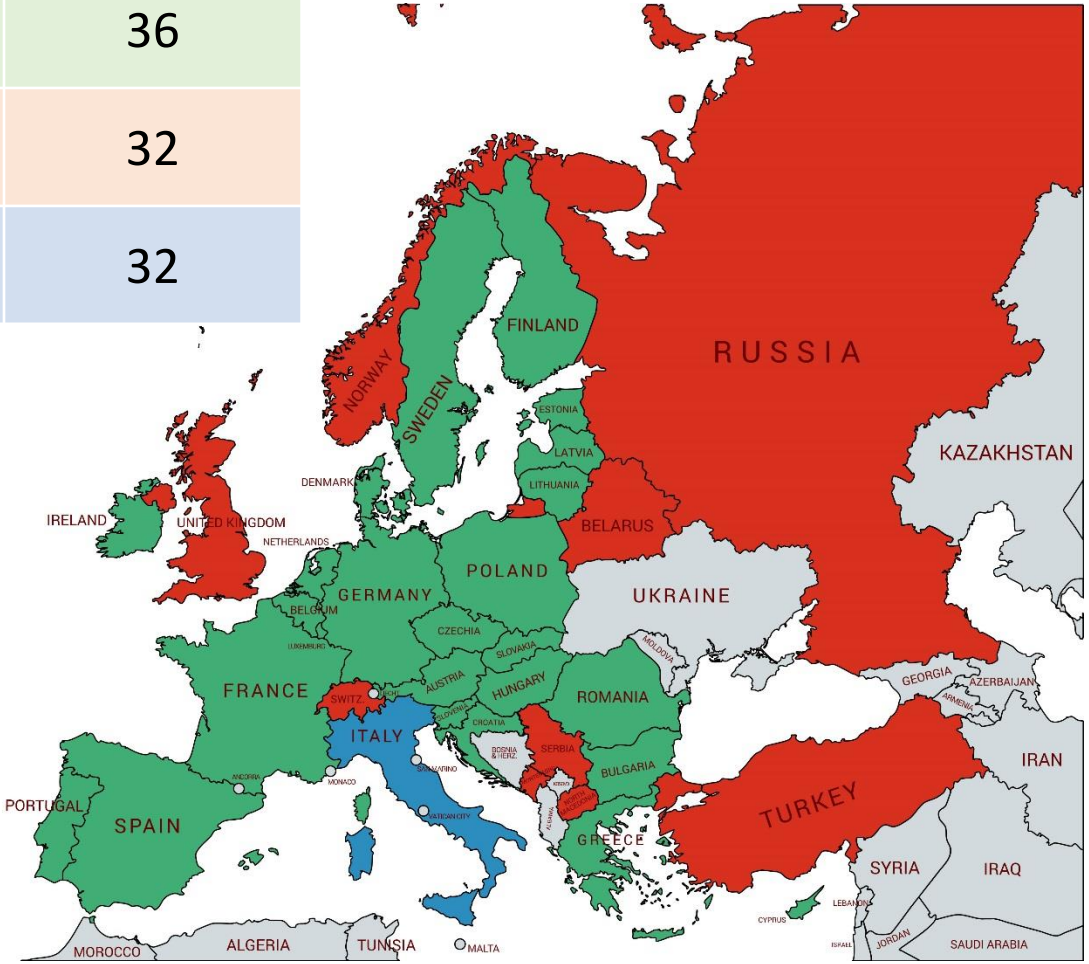
AI/ND EURL PT – Schedule/Timeline



AI/ND EURL PT – Participating Labs

PT Scheme	EU LABS	NON EU LABS	EFTA LABS	TOTAL
AQUA IN EU MB 2020	25	8	3	36
AQUA IN EU S 2020	24	7	1	32
AQUA IN EU V 2020	24	7	1	32



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AI/ND EURL PT – Samples preparation

- ✓ In conditions which should be as realistic as possible
- ✓ Choice of recent, relevant, circulating virus strains
- ✓ Virological and serological samples are lyophilised
- ✓ Molecular samples are prepared in a Molecular Transpot Medium (MTM)

AI/ND EURL PT – MTM fit for purpose

✓ To assess whether the MTM could be used for molecular PT samples

Stability test 8 weeks → No significant loss of Ct values

Inactivation test → No viable virus detected

Test of dilutions → Appropriate range of final Ct values to mimic routine conditions

AI/ND EURL PT – MB Scheme – Target

PT Circuit	AQUA IN EU
PT Scheme	**AQUA IN EU MB 2020**
Sample	Virus isolates in PrimeStore
Target	RNA: AIV, AIV H5, AIV H7, AIV N1, AIV N2, AIV N6, AIV N8, APMV-1 Pathotype: AIV, APMV-1 CS Sequencing: AIV, APMV-1
Testing	RT-PCR and real-time RT-PCR protocols

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AI/ND EURL PT – MB Scheme – Panel Composition

ID Sample	Isolate	Type	Clade/Lineage*	Pathotype	Cleavage site
M01	A/Anas platyrhynchos/Belgium/10811-6/2019	H5N6		LPAI	PQRETR/GLF
M02	A/chicken/Kostroma/2367/18	H5N2	2.3.4.4b	HPAI	PLREKRRKR/GLF
M03	APMV-1/chicken/California/18-016505-1/2018	APMV-1	V.1	Virulent	RRQKR*F
M04	A/duck/Italy/18Vir4932-2/2018	H7N7		LPAI	PEIPKGR/GLF
M05	Negative	-	-	-	-
M06	A/turkey/Poland/23/2019	H5N8	2.3.4.4b	HPAI	PLREKRRKR/GLF
M07	A/turkey/Italy/20VIR1969-10/2020	H7N1		LPAI	PEPPKGR/GLF
M08	APMV-1/pigeon/Italy/19Vir8321/2019	PPMV-1	VI	Virulent	RRQKR*F
M09	APMV-1/chicken/California/18-016505-1/2018	APMV-1	V.1	Virulent	RRQKR*FVGAII
M10	NDV La Sota L39/14	APMV-1	II	Avirulent	GRQGR*L
M11	A/chicken/Togo/18VIR3695-10/2018	H5N1	2.3.2.1c	HPAI	PQRERRRKR/GLF
M12	Negative	-	-	-	-
M13	A/chicken/Belgium/3497_0001/2019	H3N1		LPAI	
M14	A/duck/Japan/AQ-HE29-22/2017	H7N9		HPAI	PEVPKRKRTAR/GLF
M15	APMV1/Bulgaria/1262-2/19	APMV-1	VII.2	Virulent	RRQKR*F

AI/ND EURL PT – MB Scheme – Panel Composition

8 AIV Isolates

ID Sample	Isolate	Type	Clade/Lineage*	Pathotype	Cleavage site
M01	A/Anas platyrhynchos/Belgium/10811-6/2019	H5N6		LPAI	PQRETR/GLF
M02	A/chicken/Kostroma/2367/18	H5N2	2.3.4.4b	HPAI	PLREKRRKR/GLF
M04	A/duck/Italy/18Vir4932-2/2018	H7N7		LPAI	PEIPKGR/GLF
M06	A/turkey/Poland/23/2019	H5N8	2.3.4.4b	HPAI	PLREKRRKR/GLF
M07	A/turkey/Italy/20VIR1969-10/2020	H7N1		LPAI	PEPPKGR/GLF
M11	A/chicken/Togo/18VIR3695-10/2018	H5N1	2.3.2.1c	HPAI	PQRERRRKR/GLF
M13	A/chicken/Belgium/3497_0001/2019	H3N1		LPAI	
M14	A/duck/Japan/AQ-HE29-22/2017	H7N9		HPAI	PEVPKRKRTAR/GLF

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AI/ND EURL PT – MB Scheme – Panel Composition

4 AIV Isolates – H5 Subtype

ID Sample	Isolate	Type	Clade/Lineage*	Pathotype	Cleavage site
M01	**A/Anas platyrhynchos/Belgium/10811-6/2019**	**H5N6**		**LPAI**	**PQRETR/GLF**
M02	**A/chicken/Kostroma/2367/18**	**H5N2**	**2.3.4.4b**	**HPAI**	**PLREKRRKR/GLF**
M04	A/duck/Italy/18Vir4932-2/2018	H7N7		LPAI	PEIPKGR/GLF
M06	**A/turkey/Poland/23/2019**	**H5N8**	**2.3.4.4b**	**HPAI**	**PLREKRRKR/GLF**
M07	A/turkey/Italy/20VIR1969-10/2020	H7N1		LPAI	PEPPKGR/GLF
M11	**A/chicken/Togo/18VIR3695-10/2018**	**H5N1**	**2.3.2.1c**	**HPAI**	**PQRERRRKR/GLF**
M13	A/chicken/Belgium/3497_0001/2019	H3N1		LPAI	
M14	A/duck/Japan/AQ-HE29-22/2017	H7N9		HPAI	PEVPKRKRTAR/GLF

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AI/ND EURL PT – MB Scheme – Panel Composition

3 AIV Isolates – H7 Subtype

ID Sample	Isolate	Type	Clade/Lineage*	Pathotype	Cleavage site
M01	A/Anas platyrhynchos/Belgium/10811-6/2019	H5N6		LPAI	PQRETR/GLF
M02	A/chicken/Kostroma/2367/18	H5N2	2.3.4.4b	HPAI	PLREKRRKR/GLF
M04	**A/duck/Italy/18Vir4932-2/2018**	**H7N7**		**LPAI**	**PEIPKGR/GLF**
M06	A/turkey/Poland/23/2019	H5N8	2.3.4.4b	HPAI	PLREKRRKR/GLF
M07	**A/turkey/Italy/20VIR1969-10/2020**	**H7N1**		**LPAI**	**PEPPKGR/GLF**
M11	A/chicken/Togo/18VIR3695-10/2018	H5N1	2.3.2.1c	HPAI	PQRERRRKR/GLF
M13	A/chicken/Belgium/3497_0001/2019	H3N1		LPAI	
M14	**A/duck/Japan/AQ-HE29-22/2017**	**H7N9**		**HPAI**	**PEVPKRKRTAR/GLF**

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AI/ND EURL PT – MB Scheme – AIV Results (M gene)

2020

M Gene (8 samples)	Sensitivity (%)	Specificity (%)
25 EU Labs	99	100
9 other Labs	100	98.4
Overall	99.3	99.6

2019

M Gene (9 samples)	Sensitivity (%)	Specificity (%)
26 EU Labs	99.6	100
10 other Labs	98.9	98.3
Overall	99.4	99.5

AI/ND EURL PT – MB Scheme – AIV Protocols (M gene)

rRT-PCR/RT-PCR Protocol	No Labs	No False results statistically evaluated	Overall No False results
Heine et al., 2015	1	-	-
Hoffmann et al., 2016	1	-	-
Munster et al., 2009	1	-	-
Nagy et al., 2010	8	-	-
Spackman et al., 2002	24	3	4
Nagy et al., 2020	2	-	-
In House	2	-	-
Others	2	-	-

AI/ND EURL PT – MB Scheme – AIV H5 subtype Results

2020

H5 subtype (4 samples)	Sensitivity (%)	Specificity (%)
25 EU Labs	97	100
9 other Labs	100	98.9
Overall	99.3	99.6

2019

H5 subtype (5 samples)	Sensitivity (%)	Specificity (%)
26 EU Labs	100	100
10 other Labs	100	100
Overall	100	100

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AI/ND EURL PT – MB Scheme – AIV H5 subtype Protocols

rRT-PCR/RT-PCR Protocol	No Labs	No False results statistically evaluated	Overall No False results
Gall et al., 2008 (Pan HA)	1	-	-
Hoffmann et al., 2016	4	-	-
Monne et al., 2008	1	-	-
Naguib et al., 2017 (LP/HP)	1	-	-
Slomka et al., 2012 (CS)	3	1	3
Slomka et al., 2007a (Kha-1/Kha-3)	23	-	-
Slomka et al., 2007b (HA2)	24	1	1
Spackman et al., 2002	4	1	1
In house	3	-	-
Others	7	-	-

AI/ND EURL PT – MB Scheme – AIV H5 Pathotyping

2020	EU Labs	Other Labs	Overall
Achieved	20/23	6/8	26/31 (83.9%)
1 False Result	1/23	2/8	3/31 (9.7%)
> 1 False Result	2/23	-	2/31 (6.5%)

ID Sample	Isolate	Type	Pathotype	Cleavage site	No false results
M01	A/Anas platyrhynchos/Belgium/10811-6/2019	H5N6	LPAI	PQRETR/GLF	3
M02	A/chicken/Kostroma/2367/18	H5N2	HPAI	PLREKRRKR/GLF	1
M06	A/turkey/Poland/23/2019	H5N8	HPAI	PLREKRRKR/GLF	2
M11	A/chicken/Togo/18VIR3695-10/2018	H5N1	HPAI	PQRERRRKR/GLF	2

AI/ND EURL PT – MB Scheme – AIV H5 Pathotyping

2020

	EU Labs	Other Labs	Overall
Achieved	20/23	5/8	25/31 (80.6%)
1 False Result	1/23	3/8	4/31 (12.9%)
> 1 False Result	2/23	-	2/31 (6.5%)

2019

	EU Labs	Other Labs	Overall
Achieved	18/24	6/8	24/32 (75%)
1 False Result	4/24	-	4/32 (12.5%)
> 1 False Result	2/24	2/8	4/32 (12.5%)

AI/ND EURL PT – MB Scheme – AIV H7 subtype Results

2020

H7 subtype (3 samples)	Sensitivity (%)	Specificity (%)
25 EU Labs	84	100
9 other Labs	83.3	100
Overall	83.8	100

2019

H7 subtype (2 samples)	Sensitivity (%)	Specificity (%)
26 EU Labs	94.2	100
10 other Labs	95	95
Overall	94.4	99.8

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AI/ND EURL PT – MB Scheme – AIV H7 subtype Results

2020	H7 subtype (3 samples)	Sensitivity (%)	Specificity (%)
	25 EU Labs	84	100
	9 other Labs	83.3	100
	Overall	83.8	100

Sample M07 - H7N1 (A/turkey/Italy/20VIR1969-10/2020) responsible for 9 out of 11 false results

H7N1 LPAI Italy: Alert communication on IZSVe-EURL Platform on 23/04/20

→ Low detection sensitivity with Slomka et al., 2009 (probe mismatches)

→ Discrepancies in the results related to the real-time platforms used (RotorQ)

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AI/ND EURL PT – MB Scheme – AIV H7 subtype Results

rRT-PCR/RT-PCR Protocol	No Labs	No False results statistically evaluated	Overall No False results
Gall et al., 2008 (Pan HA)	1		
Graaf et al., 2017 (LP/HP)	1		
Hoffmann et al., 2016	4		
Monne et al., 2008	1		1
Slomka et al., 2009	29	7	10
Slomka et al., 2007a (GK7.3/GK7.4)	22		
Spackman et al., 2002	7	3	4
Van Borm et al., 2010	1		
In House	2		
Others	8	1	1

AI/ND EURL PT – MB Scheme – AIV H7 Pathotyping

2020	EU Labs	Other Labs	Overall
Achieved	18/23	6/8	24/31 (77.4%)
1 False Result	5/23	2/8	7/31 (22.6%)
> 1 False Result	-	-	-

ID Sample	Isolate	Type	Pathotype	Cleavage site	No false results
M04	A/duck/Italy/18Vir4932-2/2018	H7N7	LPAI	PEIPKGR/GLF	-
M07	A/turkey/Italy/20VIR1969-10/2020	H7N1	LPAI	PEPPKGR/GLF	5
M14	A/duck/Japan/AQ-HE29-22/2017	H7N9	HPAI	PEVPKRKRTAR/GLF	2

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AI/ND EURL PT – MB Scheme – AIV H7 Pathotyping

2020

	EU Labs	Other Labs	Overall
Achieved	16/23	7/8	23/31 (74.2%)
1 False Result	6/23	-	6/31 (19.4%)
> 1 False Result	1/23	1/8	2/31 (6.5%)

2019

	EU Labs	Other Labs	Overall
Achieved	22/24	6/8	28/32 (87.5%)
1 False Result	2/24	2/8	4/32 (12.5%)
> 1 False Result	-	-	0/32 (0%)

AI/ND EURL PT – MB Scheme – AIV N1 subtype Results

2020

N1 subtype (3 samples)	Sensitivity (%)	Specificity (%)
25 EU Labs	100	100
9 other Labs	100	100
Overall	100	100

2019

N1 subtype (0 sample)	Sensitivity (%)	Specificity (%)
25 EU Labs	100	100
9 other Labs	100	100
Overall	100	100

AI/ND EURL PT – MB Scheme – AIV N1 subtype Protocols

rRT-PCR/RT-PCR Protocol	No Labs
Agüero et al., 2007	1
Hoffmann et al., 2016	6
Payungporn et al., 2006	18
Slomka et al., 2012	6
Fereidouni et al., 2009	2
Others	2
In house	1

AI/ND EURL PT – MB Scheme – AIV N2 subtype Results

2020	N2 subtype (1 sample)	Sensitivity (%)	Specificity (%)	rRT-PCR/RT-PCR Protocol	No Labs
	20 EU Labs	100	100	Fereidouni et al., 2009	8
	7 other Labs	100	100	Hoffmann et al., 2016	17
	Overall	100	100	In house	1

AI/ND EURL PT – MB Scheme – AIV N6 subtype Results

2020

N6 subtype (1 sample)	Sensitivity (%)	Specificity (%)
23 EU Labs	100	100
9 other Labs	88.9	100
Overall	99.2	100

2019

N6 subtype (2 samples)	Sensitivity (%)	Specificity (%)
24 EU Labs	95.8	100
9 other Labs	77.8	100
Overall	93.9	100

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AI/ND EURL PT – MB Scheme – AIV N6 subtype Protocols

rRT-PCR/RT-PCR Protocol	No Labs	No False results statistically evaluated	Overall No False results
Fereidouni et al., 2009	4	-	-
Hoffmann et al., 2016	23	1	1
James et al., 2019	2	-	-
Others	2	-	-
In house	1	-	-

AI/ND EURL PT – MB Scheme – AIV N8 subtype Results

2020

N8 subtype (1 sample)	Sensitivity (%)	Specificity (%)
23 EU Labs	100	100
9 other Labs	100	99.2
Overall	100	99.7

2019

N8 subtype (1 sample)	Sensitivity (%)	Specificity (%)
25 EU Labs	88.8	97.6
9 other Labs	100	100
Overall	94.1	99.4

AI/ND EURL PT – MB Scheme – AIV N8 subtype Protocols

rRT-PCR/RT-PCR Protocol	No Labs	No False results statistically evaluated	Overall No False results
Fereidouni et al., 2009	3	-	-
Hoffmann et al., 2016	22	-	-
James et al., 2019	3	-	-
In house	4	1	1
Others	2	-	-

Conclusion

- High level of sensitivity and specificity (>99%) for the detection of AIV and subtype characterisation
- The low sensitivity in detection of H7 subtype (83.8%) was due to misidentification of sample M07 (H7N1) responsible for 9/11 FN results
- Correct pathotyping of H5 subtype was 5% higher in 2020 (80%) compared to PT EURL 2019
- Pathotyping of H7 subtype difficult to interprete
- Approved use of a MTM to prepare the molecular PT samples

AI/ND EURL PT – V Scheme - Target

PT Circuit	AQUA IN EU
PT Scheme	**AQUA IN EU V 2020**
Sample	Freeze-dried inactivated antigens
Target	Antigen HAV AIV H5 AIV H7 APMV-1
Testing	HA and HI tests

AI/ND EURL PT – V Scheme – Panel Composition

ID Sample	Isolate	Type	HI Titre
V01	A/mallard/Italy/3401/05	H5N1	1:256
V02	APMV1/dove/Italy/4400/2000	APMV-1	1:128
V03	A/chicken/Italy/22A/98	H5N9	1:128
V04	A/mallard/Italy/3817-34/05	H9N2	1:512
V05	Negative	-	-
V06	A/mute swan/England/AVP-18-1986/17	H5N6	1:128
V07	A/turkey/Italy/12VIR10208-2/2013	H9N2	1:128
V08	B1	APMV-1	1:256
V09	APMV4/Duck/Hong Kong D3/75	APMV-4	1:128
V10	A/turkey/Italy/9289/02	H7N3	1:512
V11	Negative	-	-
V12	NDV La sota	APMV-1	1:256

AI/ND EURL PT – V Scheme – Panel Composition

10 Haemagglutinating viruses (HAV)

ID Sample	Isolate	Type	HI Titre
V01	A/mallard/Italy/3401/05	H5N1	1:256
V02	APMV1/dove/Italy/4400/2000	APMV-1	1:128
V03	A/chicken/Italy/22A/98	H5N9	1:128
V04	A/mallard/Italy/3817-34/05	H9N2	1:512
V06	A/mute swan/England/AVP-18-1986/17	H5N6	1:128
V07	A/turkey/Italy/12VIR10208-2/2013	H9N2	1:128
V08	B1	APMV-1	1:256
V09	APMV4/Duck/Hong Kong D3/75	APMV-4	1:128
V10	A/turkey/Italy/9289/02	H7N3	1:512
V12	NDV La sota	APMV-1	1:256

AI/ND EURL PT – V Scheme – Panel Composition

3 AIV – H5 Subtype

ID Sample	Isolate	Type	HA Titre
V01	A/mallard/Italy/3401/05	H5N1	1:256
V03	A/chicken/Italy/22A/98	H5N9	1:128
V06	A/mute swan/England/AVP-18-1986/17	H5N6	1:128

AI/ND EURL PT – V Scheme – Panel Composition

1 AIV – H7 Subtype

ID Sample	Isolate	Type	HA Titre
V10	A/turkey/Italy/9289/02	H7N3	1:512

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AI/ND EURL PT – V Scheme – HAV Results

2020

HAV (10 samples)	Sensitivity (%)	Specificity (%)
24 EU Labs	100	100
8 other Labs	100	100
Overall	100	100

2019

HAV (8 samples)	Sensitivity (%)	Specificity (%)
25 EU Labs	100	100
7 other Labs	100	100
Overall	100	100

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AI/ND EURL PT – V Scheme – AIV H5 Results

2020

AIV H5 (3 samples)	Sensitivity (%)	Specificity (%)
24 EU Labs	100	99.7
8 other Labs	100	98.6
Overall	100	99

2019

AIV H5 (2 samples)	Sensitivity (%)	Specificity (%)
25 EU Labs	100	99.5
7 other Labs	100	100
Overall	100	99.7

AI/ND EURL PT – V Scheme – AIV H7 Results

2020	AIV H7 (1 sample)	Sensitivity (%)	Specificity (%)
	24 EU Labs	100	100
	8 other Labs	100	100
	Overall	100	100

2019	AIV H7 (3 samples)	Sensitivity (%)	Specificity (%)
	25 EU Labs	100	100
	7 other Labs	100	100
	Overall	100	100

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Conclusion

- High level of sensitivity and specificity (≥99%) for the detection of AIV and subtype characterisation
- Similar results compared to PT EURL 2019

AI/ND EURL PT – S Scheme - Target

PT Circuit	AQUA IN EU
PT Scheme	**AQUA IN EU S 2020**
Sample	Freeze-dried sera-containing antibodies
Target	Antibodies AIV AIV H5 AIV H7 APMV-1
Testing	AGID/ELISA and HI test

AI/ND EURL PT – S Scheme – Panel Composition

ID Sample	Isolate	Type	HI Titre
S01	Negative	-	-
S02	A/mallard/Italy/4810-79/04	H7N4	1:32
S03	A/turkey/Italy/1258/05	H5N2	1:512
S04	A/mallard/Italy/4810-79/04	H7N4	1:512
S05	A/turkey/England/n28/73	H5N2	1:32
S06	A/turkey/England/n28/73	H5N2	1:256
S07	Ulster 2 C	APMV-1	1:256
S08	A/chicken/Saudi Arabia/13VIR-3622-31/13	H9N2	1:512
S09	A/macaw/England/626/80	H7N7	1:512
S10	APMV1/Bulgaria/1262-1/19	APMV-1	1:256
S11	Negative	-	-
S12	A/mute swan/England/AVP-18-1986/17	H5N6	1:512

AI/ND EURL PT – S Scheme – Panel Composition

8 AIV (Abs)

ID Sample	Isolate	Type	HI Titre
S02	A/mallard/Italy/4810-79/04	H7N4	1:32
S03	A/turkey/Italy/1258/05	H5N2	1:512
S04	A/mallard/Italy/4810-79/04	H7N4	1:512
S05	A/turkey/England/n28/73	H5N2	1:32
S06	A/turkey/England/n28/73	H5N2	1:256
S08	A/chicken/Saudi Arabia/13VIR-3622-31/13	H9N2	1:512
S09	A/macaw/England/626/80	H7N7	1:512
S12	A/mute swan/England/AVP-18-1986/17	H5N6	1:512

AI/ND EURL PT – S Scheme – Panel Composition

4 AIV – H5 Subtype (Abs)

ID Sample	Isolate	Type	HI Titre
S03	A/turkey/Italy/1258/05	H5N2	1:512
S05	A/turkey/England/n28/73	H5N2	1:32
S06	A/turkey/England/n28/73	H5N2	1:256
S12	A/mute swan/England/AVP-18-1986/17	H5N6	1:512

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AI/ND EURL PT – S Scheme – Panel Composition

3 AIV – H7 Subtype (Abs)

ID Sample	Isolate	Type	HI Titre
S02	A/mallard/Italy/4810-79/04	H7N4	1:32
S04	A/mallard/Italy/4810-79/04	H7N4	1:512
S09	A/macaw/England/626/80	H7N7	1:512

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AI/ND EURL PT – S Scheme – AIV antibodies Results

2020

AIV Ab (8 samples)	Sensitivity (%)	Specificity (%)
20 EU Labs	100	98.8
7 other Labs	98.2	100
Overall	99.5	99.1

2019

AIV Ab (6 samples)	Sensitivity (%)	Specificity (%)
20 EU Labs	100	100
7 other Labs	100	100
Overall	100	100

AI/ND EURL PT – V Scheme – AIV H5 subtype antibodies Results

2020

AIV H5 (4 samples)	Sensitivity (%)	Specificity (%)
24 EU Labs	97.9	100
8 other Labs	100	98.4
Overall	98.4	99.6

2019

AIV H5 (3 samples)	Sensitivity (%)	Specificity (%)
25 EU Labs	100	100
7 other Labs	100	100
Overall	100	100

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AI/ND EURL PT – V Scheme – AIV H7 subtype antibodies Results

2020

AIV H7 (3 sample)	Sensitivity (%)	Specificity (%)
24 EU Labs	97.2	100
8 other Labs	95.8	100
Overall	99	100

2019

AIV H7 (2 samples)	Sensitivity (%)	Specificity (%)
25 EU Labs	100	100
7 other Labs	100	100
Overall	100	100

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Conclusion

- High level of sensitivity and specifcity in the detection and serotyping of AIV sera
- Similar results compared to PT EURL 2019

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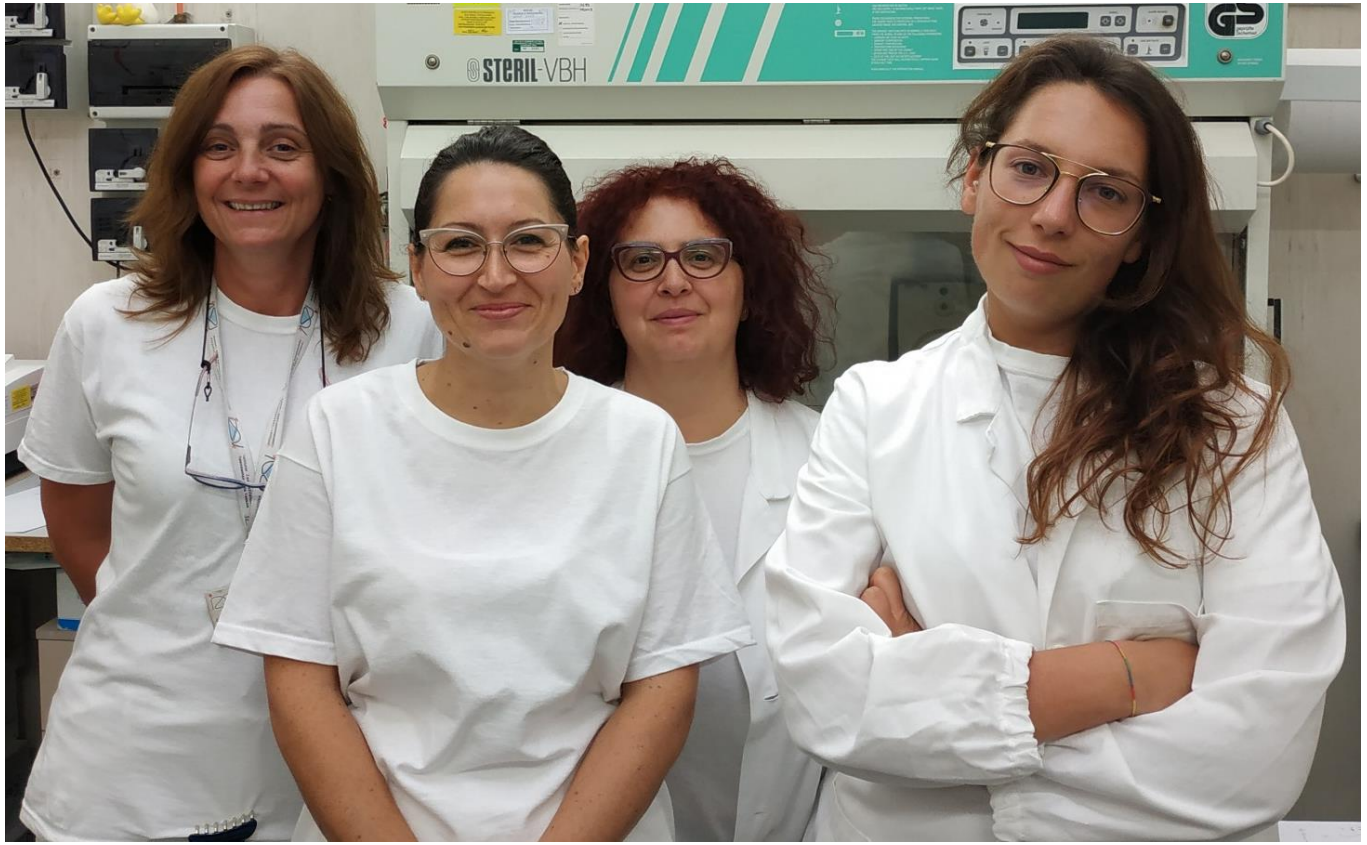

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