



**EURL PROFICIENCY TEST 2020
AVIAN INFLUENZA AND NEWCASTLE DISEASE**

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SCS5 – DS BIO IZSVe

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*FVGAI
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

5 APMV-1 Isolates

ID Sample	Isolate	Type	Clade/Lineage*	Pathotype	Cleavage site
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M03	APMV-1/chicken/California/18-016505-1/2018	APMV-1	V.1	Virulent	RRQKR*FVGAI
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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*FVGAI
M10	NDV La Sota L39/14	APMV-1	II	Avirulent	GRQGR*L

M15	APMV1/Bulgaria/1262-2/19	APMV-1	VII.2	Virulent	RRQKR*F
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AI/ND EURL PT – MB Scheme – AMPV-1 Results

2020	APMV-1 (5 samples)	Sensitivity (%)	Specificity (%)
	25 EU Labs	95.2	100
	9 other Labs	100	100
	Overall	96.7	100

2019	APMV-1 (3 samples)	Sensitivity (%)	Specificity (%)
	26 EU Labs	94.7	100
	10 other Labs	96.3	99.1
	Overall	95.37	99.77

AI/ND EURL PT – MB Scheme – APMV-1 Protocols

rRT-PCR/RT-PCR Protocol	Target	No Labs	No False results statistically evaluated	Overall No False results
Aldous et al., 2003	F Gene	2	-	-
APHA VI 448	F Gene	1	-	-
Collins et al., 1993	F Gene	1	-	-
Creelan et al., 2002	F Gene	6	-	-
Fuller et al., 2010	L Gene	20	4	9
Fuller et al., 2009	F Gene	13	-	-
Kant et al., 1997	F Gene	6	-	-
Kho et al., 2000	F Gene	1	-	-
Kim et al., 2008	Duplex L-M Genes	3	-	6
Oberdorfer et al., 1998	F Gene	2	-	-
Ramp et al., 2012	NP Gene	1	-	-

AI/ND EURL PT – MB Scheme – APMV-1 Protocols

rRT-PCR/RT-PCR Protocol	Target	No Labs	No False results statistically evaluated	Overall No False results
Sutton et al., 2019	L Gene	8	-	1
Wise et al., 2004	M Gene	14	-	3
	F Gene	5	-	5
	Duplex L-M Genes	1	-	-
Moharam et al., 2019	F Gene	2	-	3
Sutton et al., 2015	F Gene	1	-	-
Sealt et al., 1995	F Gene	2	-	-
Sabbre et al., 2017	F Gene	1	-	3
Werner et al., 1999	F Gene	1	-	-
Stäuber et al., 1995	F Gene	-	-	-
Others	F Gene	6	-	3
In house	F Gene	3	-	-

AI/ND EURL PT – MB Scheme – APMV-1 Pathotyping

2020			
	EU Labs	Other Labs	Overall
Achieved	21/24	6/9	27/33 (81.8%)
1 False Result	1/24	1/9	2/33 (6.1)
> 1 False Result	2/24	2/9	4/33 (12.1%)

ID Sample	Isolate	Type	Pathotype	Cleavage site	False results
M03	APMV-1/chicken/California/18-016505-1/2018	APMV-1	Virulent	RRQKR*FVGAI	4
M08	APMV-1/pigeon/Italy/19Vir8321/2019	PPMV-1	Virulent	RRQKR*F	2
M09	APMV-1/chicken/California/18-016505-1/2018	APMV-1	Virulent	RRQKR*FVGAI	1
M10	NDV La Sota L39/14	APMV-1	Avirulent	GRQGR*L	-
M15	APMV1/Bulgaria/1262-2/19	APMV-1	Virulent	RRQKR*F	3

AI/ND EURL PT – MB Scheme – APMV-1 Pathotyping

2020			
	EU Labs	Other Labs	Overall
Achieved	21/24	6/9	27/33 (81.8%)
1 False Result	1/24	1/9	2/33 (6.1%)
> 1 False Result	2/24	2/9	4/33 (12.1%)

2019			
	EU Labs	Other Labs	Overall
Achieved	20/25	6/8	26/33 (78.8%)
1 False Result	5/25	1/8	6/33 (18.2%)
> 1 False Result	-	1/8	1/33 (3%)

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	HA 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

3 APMV-1

ID Sample	Isolate	Type	HA Titre
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V02	APMV1/dove/Italy/4400/2000	APMV-1	1:128
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V08	B1	APMV-1	1:256
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V12	NDV La sota	APMV-1	1:256
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AI/ND EURL PT – V Scheme – APMV-1 Results

2020	APMV-1 (3 samples)	Sensitivity (%)	Specificity (%)
	24 EU Labs	100	99.6
	8 other Labs	100	97.2
	Overall	100	99

2019	APMV-1 (1 sample)	Sensitivity (%)	Specificity (%)
	25 EU Labs	100	96.9
	7 other Labs	100	93.7
	Overall	100	96.18

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

2 APMV-1 (Abs)

ID Sample	Isolate	Type	HI Titre
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S07	Ulster 2 C	APMV-1	1:256
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S10	APMV1/Bulgaria/1262-1/19	APMV-1	1:256
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AI/ND EURL PT – S Scheme – APMV-1 antibodies Results

2020	APMV-1 (2 samples)	Sensitivity (%)	Specificity (%)
	24 EU Labs	100	100
	7 other Labs	100	97.1
	Overall	100	99.7

2019	APMV-1 (2 samples)	Sensitivity (%)	Specificity (%)
	25 EU Labs	100	100
	6 other Labs	91.7	100
	Overall	98.4	100

● Conclusion

- High sensitivity and specificity in the molecular detection and pathotyping of APMV-1 positive samples
- Numerous molecular protocols used to analyse and characterise APMV-1 (23 without counting those modified)
- 100% sensitivity in the detection of APMV-1 antigens
- High sensitivity and specificity in the detection of APMV-1 positive sera

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