Canadian Food Inspection Agency | Agence canadienne d'inspection des aliments

Canadian Food Inspection Agency




Our vision:
To excel as a science-based regulator, trusted and respected by Canadians and the international community.

Our mission:
Dedicated to safeguarding food, animals and plants, which enhances the health and well-being of Canada's people, environment and economy.

International Twinning of Reference Labs and Harmonization of Diagnostics

John Pasick

National Centre for Foreign Animal Disease

Canada

Harmonization/Twinning Activities at NCFAD

- **North American Animal Health Laboratory Network**
  - Security and Prosperity Partnership of North America
  - Foot-and-mouth disease and Avian Influenza
  - Newcastle disease and Classical swine fever
- **Inter-laboratory Comparison Tests/Ring Trials**
  - African and classical swine fevers
  - Bluetongue
  - Foot-and-mouth disease
  - Avian influenza
  - Newcastle disease
- **International Training Initiatives**
  - Procinorte sponsored workshop on molecular diagnostic methods for influenza A viruses
  - OIE Twinning Project to provide technical support to the LNDV-ICA-Colombia for the diagnosis and control of avian influenza and Newcastle disease

North American Animal Health Laboratory Network

- Efforts to harmonize diagnostic tests for avian influenza, foot-and-mouth disease and tuberculus began in 2007
- Classical swine fever and Newcastle disease were added in 2011
- Initial meetings were held in Mexico City to:

1. Review each country's diagnostic testing capabilities
2. Decide which diagnostic tests to be harmonized
3. ID training and reagent requirements (Mexico)
4. Design of panels to assess harmonization

Avian Influenza

- **Serology**
  - **Group specific antibody – AGID**
  - **Subtype specific antibody – Hemagglutination-inhibition test**
- **Virus Isolation/Characterization**
  - **Matrix real-time RT-PCR**
  - **Virus isolation**
  - **HA subtyping by HI**
  - **NA subtyping by NI**
  - **Molecular pathotyping**
  - **IVPI**

North American Animal Health Laboratory Network Avian Influenza Characterization Panel

Panel #	Matrix RRT-PCR (Ct)	H5 RRT-PCR (Ct)	H7 RRT-PCR (Ct)	HI	NI	HA_0 Cleavage Site	IVPI	Final Diagnosis
1	22.4	21.41	No Ct	H5	N2	PQRETR*GLF	0	North American lineage LPAI H5N2
2	22.39	No Ct	No Ct	H2	N2		ND	H2N2
3	25.35	No Ct	No Ct	H6	N8		ND	H6N8
4	24.10	No Ct	23.22	H7	N9	PENPKTR*GLF	0	North American lineage LPAI H7N9
5	21.50	No Ct	No Ct	H10	N7		ND	H10N7
6	23.10	20.45	No Ct	H5	N1	PQRETR*GLF	0	North American lineage LPAI H5N1

FMDV harmonized tests: Canada, USA and Mexico

- Virus isolation in cell culture
- Virus Neutralization test

} Canada & USA

- DAS ELISA
- Real-Time RT-PCR
- 3ABC competitive ELISA (non-structural protein ELISA)

} All 3 countries

North American Animal Health Laboratory Network Newcastle Disease Virus Characterization Panel

Sample ID	Original Sample		Following Amplification in Chicken Embryos					
	APMV-1 Matrix Ct	APMV-1 Fusion Ct	APMV-1 HI Titer	APMV-1 Matrix Ct	APMV-1 Fusion Ct	F_0 Cleavage Site	ICPI	Interpretation
F69	20.7	29.11	256	15.35	18.33	PGGRRQKR*FIG	1.69	Velogenic
F73	29.17	31.62	512	15.8	18.0	PGGRRQKR*FIG	1.75	Velogenic
F87	24.35	Neg	2048	12.22	Neg	SGGGRQGR*LIG	0	Lentogenic
F91	15.96	34.82	16	17.15	30.72	SGGRRQKR*FIG	1.22	Mesogenic
FAS	Neg	Neg	Neg	Neg	Neg	Neg		Neg

Classical Swine Fever Serology Panel

- Assembled at NCFAD
- 12 Sera originating from experimentally infected pigs
- CSFV, BVDV and BDV antisera
- Each lab will screen sera by ELISA
- Confirm by NPLA
- Where necessary perform differential NPLA

Inter-laboratory Comparison Tests/Ring Trials

African and Classical Swine Fevers

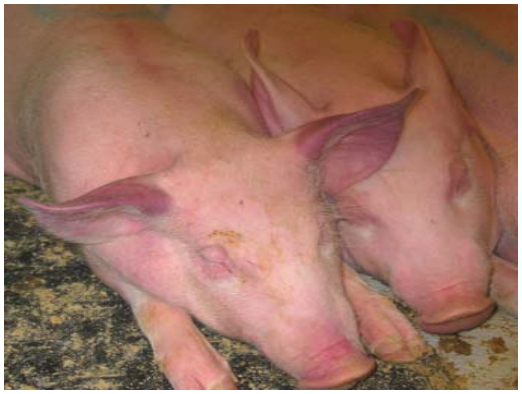
- ASF ILCT is administered by the European Union Reference Laboratory for African Swine Fever (Centro de Investigación en Sanidad Animal, Instituto Nacional de Investigación y Technolgía Agraria y Alimentaria) Madrid, Spain
- CSF ILTC is administered by the Community Reference Laboratory for Classical Swine Fever (Institute for Virology, University of Veterinary Medicine) Hannover, Germany
- Results of both are reviewed at an ASF/CSF Workshop that is held annually in Europe

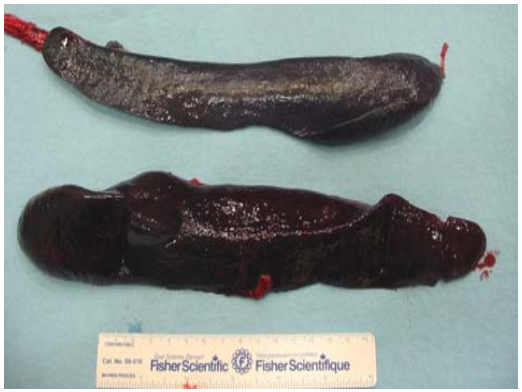
2010-2011 ASF ILCT

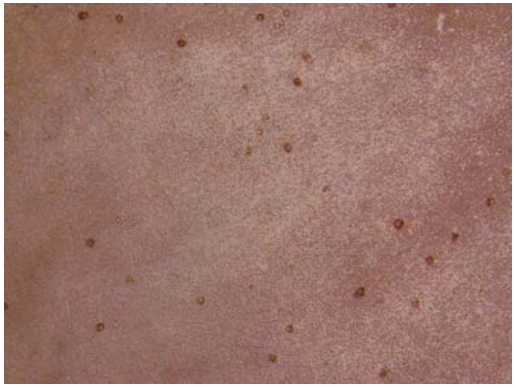
- 24 NRLs from EU Member States
  - Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Lativia, Lithuania, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden and UK
- 4 non-reference laboratories from EU Member States
  - France, Italy (3)
- 7 RLs from European non-EU Member States
  - Russia, Serbia, Belarus, Croatia, Norway, Ukraine and Switzerland
- 4 RLs from non-European countries
  - Canada, South Africa, USA (2)

ASF ILTC

- 2011/2012 Panel consisted of 9 coded serum samples (S1 to S9) and 7 coded tissue samples (T1 to T7)
- Virus isolation/PCR was carried out on all 16 samples while testing for ASFV specific antibodies was carried out on the serum samples
- Results for both were designated as correct and ‘fit for purpose’ for detection of ASF virus and antibodies to ASF in field samples







ASF ILTC-2012

SAMPLE ID	CLINICAL FORM	ASFV VIRULENCE	ASFV ISOLATE	GENOTYPE	ORIGIN OF SAMPLES	
					DPI	DESCRIPTION
S1			Naïve pig (ASFV free)			
S2	CHRONIC	ATTENUATED	NH/P68	I	D48PI (D20 post challenge	NHV i.m. + L60 i.m. at 29 dpi. Serum obtained at 20 dpc
S3	ACUTE	VIRULENT	L60	I	D5PI	L60 i.m. Serum obtained at 5 dpi
S4	ACUTE	VIRULENT	Az08D	II	D18	Pig in contact with Az08D infected animals. Serum obtained at 18 dpi
S5			Naïve pig (ASFV free)			
S6	CHRONIC	MODERATE	Ken05/Tk1	X	D70PI	Ken05/Tk (10HAU) i.m. + 10^4 HAU i.m. 46 dpi
S7	CHRONIC	ATTENUATED	E75 CV1-4	I	D15PI	E75 CV-1 i.m.
S8	ACUTE	VIRULENT	Ug03.H1	IX	D12PI	Pigs kept in contact with infected animals. Serum obtained at 12 dpi
S9	CHRONIC	ATTENUATED	NHV/P68	I	1:16 Dil of S2	
T1			Naïve pig (ASFV free)			
T2	SUBACUTE	MODERATE	Ken05/K2	X	D18PI	Spleen
T3	ACUTE	VIRULENT	Arm07	II	D9PI	Spleen
T4			Naïve pig (ASFV free)			
T5	ACUTE	VIRULENT	L60	I	D5PI	Kidney
T6	ACUTE	VIRULENT	Ken10/Kis027	IX		Field sample
T7	ACUTE	VIRULENT	Ni08/LaOK.1	I	D8PI	Liver

Classical Swine Fever ILTC

- 20 porcine serum samples
- Pigs inoculated with CSFV, BDV or BVDV
- All sera tested for virus nucleic acid, virus antigen and virus specific antibodies
- 2010 ILTC included 50 laboratories from 42 countries
- Non-EU countries included Switzerland, Norway, Canada, Chile, China, Colombia, Cuba, Dominican Republic, Mexico, Russia and USA

Decoding and characterization list of sera CSF ILCT 2011					
Code ILCT 2010	**Code ILCT 2011**	**EURL Serum-ID**	**Inoculum**	**dpi**	**Characterization**
7-2010	*1-2011*	**2009/01/0253/063**	BDV-1 137/4	63	porcine serum positive for BDV serology, cross neutralization with BVDV and CSFV, virology negative
8.1-2010	*2-2011*	**2009/01/0258/063**	BVDV-1 Viro1764/4 2008	63	porcine serum positive for BVDV serology, cross neutralization with BDV and CSFV, virology negative
-	*3-2011*	***1:100*** **2007/04/0152/006**	CSFV Koslov gt. 1.1	6	dilution series of a CSF virus positive serum, serology negative
-	*4-2011*	***1:10***			
-	*5-2011*	***undiluted***			
-	*6-2011*	**2001/01/0075/000**	no inoculum	-	native pig serum
-	*7-2011*	**2008/02/0191/000**	no inoculum	-	native pig serum
4-2010	*8-2011*	***1:100*** **2008/03/0242/020**	CSF0864 Bulgaria 2007 gt. 2.3	20	dilution series of a CSF virus positive serum, serology negative
5-2010	*9-2011*	***1:10***			
6-2010	*10-2011*	***undiluted***			
-	*11- & 12-2011*	**2010/02/0335/029**	CSF0306 Malaysia 1986 gt. 1.3	29	medium antibody titre against homologue virus and lower titre against CSFV Alfort and gt. 2.3 viruses, low cross reactivity with BDV. CSFV virology negative.
-	*13- & 14-2011*	**2010/02/0339/020**	CSF0822 France 2003 gt. 2.3	20	medium antibody titre against homologue virus and other gt. 2.3 viruses and lower titre against CSFV Alfort, low cross reactivity with BDV. CSF virology negative, but weak positive results in RT-PCR observed.
12- & 19-2010	*15- & 16-2011*	***1:16*** **2006/07/0061/064**	CSF0902 Alfort187 gt. 1.1	64	1:16 dilution of serum with high antibody titre against CSFV Alfort187, medium titre against gt 2.3 strains, no cross reactivity with BDV or BVDV. The dilution is expected to be found positive in Ab ELISA and CSF VNT (see also ILCT evaluation 2009 and 2010).
13- & 20-2010	*17- & 18-2011*	**2009/02/0265/067**	CSF1048 Lithuania 2009 gt. 2.1	67	High antibody titre against gt 2.3 strain and homologue virus, medium antibody titre against CSFV Alfort187, low cross reactivity with BDV and BVDV. CSF virology negative.
-	*19- & 20-2011*	**2008/03/0224/032**	CSF0940 C-strain (vaccine) gt.1.1	32	medium antibody titre against homologue virus and other gt. 1.1 viruses and low titre against gt. 2.3 viruses, no cross reactivity with BDV or BVDV. CSF virology negative.
	21- & 22-2011	**2010/02/0338/018**	CSF0822 France 2003 gt. 2.3	18	medium antibody titre against homologue virus and other gt. 2.3 viruses and lower titre against CSFV Alfort, low cross reactivity with BDV. CSF virology positive (Ag ELISA and PCR), isolation of infectious virus not observed.

NCFAD Results

ILCT 2011 - VIROLOGY & SEROLOGY

Serum Code	OVERALL INTERPRETATION, regarding all tests routinely used for CSF diagnosis	Remarks
1-2011	CSF virology and CSF serology negative, other pestivirus serology positive	BDV serology positive
2-2011	CSF virology and CSF serology negative, other pestivirus serology positive	BVDV serology positive
3-2011	CSF virology positive, pestivirus serology negative	live virus + genome
4-2011	CSF virology positive, pestivirus serology negative	live virus + genome
5-2011	CSF virology positive, pestivirus serology negative	live virus + genome
6-2011	CSF virology and pestivirus serology negative	
7-2011	CSF virology and pestivirus serology negative	
8-2011	CSF virology positive, pestivirus serology negative	genome only
9-2011	**CSF virology and CSF serology positive**	live virus + genome, weak serology
10-2011	**CSF virology and CSF serology positive**	live virus + genome, weak serology
11-2011	CSF virology negative, CSF serology positive	
12-2011	CSF virology negative, CSF serology positive	
13-2011	CSF virology and CSF serology positive	weak genome only
14-2011	CSF virology and CSF serology positive	weak genome only
15-2011	CSF virology negative, CSF serology positive	
16-2011	CSF virology negative, CSF serology positive	
17-2011	CSF virology negative, CSF serology positive	
18-2011	CSF virology negative, CSF serology positive	
19-2011	CSF virology negative, CSF serology positive	
20-2011	CSF virology negative, CSF serology positive	
21-2011	CSF virology and CSF serology positive	genome only
22-2011	CSF virology and CSF serology positive	genome only

Wrong interpretation of test results are marked in blue.
Correct interpretations of test results but not meeting the sample characteristics are marked in green.

FMD and SVD combined proficiency test scheme

Aims: To assist National FMD Laboratories in improving and maintaining accurate and reproducible FMD and SVD diagnostic tests for virology and serology

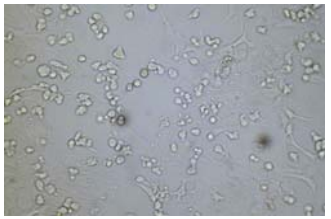
Organiser :FAO/OIE World Reference Laboratory for foot-and-mouth disease , Pirbright laboratory, Institute for Animal Health, UK

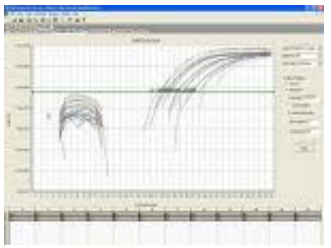
Frequency: Annual

Consist of: 4 panels

FMD Virology
two different panels
FMD Serology
one panels
SVD Serology
one panel

NCFA: participate annually





Results
FMD and SVD combined proficiency test scheme 2010

4 different panels from FAO/OIE World Reference Laboratory for foot-and-mouth disease, Pirbright laboratory, Institute for Animal Health, UK

Panel 1 contains live virus in suspension

- virus isolation on both a primary lamb kidney cell line and IBRS-2 continuous cell line.
- Both DAS ELISA (7 FMDV serotypes plus SVD) and real-time RT-PCR were run on positive cell culture
- **Overall result** : No action required

Panel 2 non-infectious for virus genome/antigen detection

- We run both a DAS ELISA (7 FMDV serotypes plus SVD) and real-time RT-PCR
- **Overall result** : No action required

Panel 3 non-infectious for FMD serology

- 3ABC ELISA, FMD-VNT test and FMD SPCE (only for A and O)
- **Overall result**: FMD SPCE test
  - Improve serotype specificity, and establish Asia1 SPCE test
  - FMD-VNT - improve type A test sensitivity
  - 3ABC ELISA - No action required

Panel 4 non-infectious for SVD serology

- SVD cELISA and SVD-VNT test
- **Overall result:** No action required

Overall Results

FMDV virology: Live FMDV and SVD in suspension

Panel 1		Results for each test		Overall interpretation for each sample
		VI/Antigen ELISA	RT-PCR	
1a	P1a -77	SVDV	SVDV POS	SVDV
	P1a -115	negative	negative	negative
	P1a -183	negative	negative	negative
1b	P1b-110	negative	negative	negative
	P1b-161	negative	negative	negative
	P1b-203	FMDV Type A	FMDV POS	FMDV

No action required

FMDV virology : Non-infectious for virus genome /antigen detection of FMDV and SVD

Panel 2	Antigen Detection ELISA Results								Real time RT-PCR		Overall interpretation for each sample
	OD value for FMDV serotypes and SVDV								FMDV	SVD	
	O	A	C	SAT1	SAT2	SAT3	Asia1	SVDV	Ct values	Ct values	
P2 - 122	0.22	0.21	0.07	2.10	0.30	0.37	0.17	0.03	27.31	0	FMDV Type SAT1
P2 - 136	0.06	0.00	0.00	0.04	0.04	0.04	0.01	0.48	0	26.65	SVDV
P2 - 149	0.07	0.01	0.00	0.03	0.02	0.05	0.00	0.01	0	0	Negative
P2 - 239	0.08	0.00	0.00	0.02	0.18	0.04	0.00	0.01	0	0	Negative
P2 - 330	1.06	1.05	1.11	0.28	0.29	1.16	3.12	0.02	23.76	0	FMDV Type ASIA
P2 - 340	0.05	0.01	0.00	0.05	0.05	0.04	0.00	0.49	0	29.23	SVDV
P2 - 405	2.59	0.36	0.50	0.23	0.24	0.51	0.60	0.03	31.21	0	FMDV Type O

No action required

FMDV serology

Panel 3	Results for each test			Overall interpretation for FMDV Ab* status for each sample	Overall interpretation for infection status for each sample
	NSP ELISA	SPCE only O and A tested	VNT		
Panel 3 - 12	Negative	Negative for Type O and A	Negative	Negative	non-infected and/or non-vaccinated implies not protected
Panel 3 - 22	Negative	Negative for Type O and A	Negative	Negative	non-infected and/or non-vaccinated implies not protected
Panel 3 - 41	Negative	FMDV type A	FMD A22	FMDV type A Ab	Vaccinatedand non-infected
Panel 3 - 140	Negative	FMDV type A	Suspicious	FMDV type A Ab	Inconclusive. May be early stages of either infection or vaccination. There is an antibody response but it is weak.
Panel 3 - 212	Positive	FMDV type A	FMD Asia	FMDV type Asia and A Ab	Vaccinated and Infected
Panel 3 - 344	Negative	Negative for Type O and A	FMD Asia	FMDV type Asia Ab	Vaccinated and non-infected

Improve serotype specificity, and Establish Asia1 SPCE test

FMD-VNT – Improve type A test sensitivity

3ABC ELISA – No Action required

SVD serology

Panel 4	Results for each test		Overall	Comments
	5BC MAC ELISA/titre	VNT/titre		
P4-4	POS/7.5	POS/2048	SVD Ab Pos	SVDV Positive
P4-71	NEG	0	SVD Ab Neg	Negative
P4-153	NEG	0	SVD Ab Neg	Negative
P4-182	NEG	0	SVD Ab Neg	Negative
P4-264	POS/7.5	POS/1024	SVD Ab Pos	SVDV Positive
P4-298	POS/7.5	POS/512	SVD Ab Pos	SVDV Positive

No action required

BTV Proficiency Test 2011

- Administered by the Institute for Animal Health, Pirbright Laboratory, UK
- 8 serum samples for serology
- 10 whole blood samples for PCR
- 42 laboratories participated in the 2011 ILTC

BTV Ringtrial 2011 – Antisera samples

Sample number	Serotype	Sample identity
1	BTV 8	BTV8 VP77 cattle serum Day 10 15/09/06
2	BTV 26	BTV26 B1 day28
3	EHDV	EHDV ME4 day37
4	BTV 6	German sera R8 28dpi BTV6 *[Sera supplied by Bernt Hofmann, FLI, Germany]*
5	BTV 1	6559&6601 BTV1
6	Negative	Adult bovine serum
7	EHDV	EHDV TE1 day37
8	BTV26	BTV26 B5 day28

BTV Ringtrial 2011 – EDTA blood samples

Sample number	Serotype	Sample identity
9	EHDV 6	Negative blood spiked with TUR2007/01 KC2
10	Negative	Negative Blood sample
11	BTV 8	Negative blood spiked with FRA2009/01 BHK1 (duplicate of #18)
12	BTV 26	Negative blood spiked with A9/10 22 E1/BHK1/V2
13	BTV 4	Negative blood spiked with MOR2009/09 KC1
14	BTV 1	Blood from animal experiment at IAH [ALG2006/04] (duplicate of #15)
15	BTV 1	Blood from animal experiment at IAH [ALG2006/04] (duplicate of #14)
16	BTV16	Negative blood spiked with CYP2010/03 KC1
17	Negative	Negative Blood sample
18	BTV 8	Negative blood spiked with FRA2009/01 BHK1 (duplicate of #11)

Positive samples were created by spiking negative bovine blood with tissue cultured virus

OFFLU Proficiency Test for Avian Influenza

Objective: To standardize diagnostic testing for avian influenza virus through participation in a world-wide proficiency testing system for international reference laboratories “cross hemispheres” detection of notifiable AIV.

Molecular and serologic diagnostic assays were evaluated in the 2011 OFFLU proficiency test.

Participating Laboratories:

1. CFIA-NCFAD
2. USDA-NVSL
3. IZSV, Italy
4. CSIRO, Australia
5. USDA-SEPRL
6. IVRI, India
7. VLA, UK
8. Graduate School of Veterinary Medicine, Japan

Goals:

1. To determine if primers/probes designed for AI from North/South America can detect AI from Europe
2. To determine if primers/probes designed to detect AI from the Eastern Hemisphere can detect AI from North America and Europe
3. To determine if North American serological subtyping reagents are antigenically similar enough to subtype AI from North America and Europe
4. To determine if serological subtyping reagents from various countries in the Eastern Hemisphere are antigenically similar enough to subtype AI from North America and Germany

OFFLU Molecular Panel

Panel ID	Antigen ID	Expected AIV Matrix (Ct)	Expected AIV H7 (Ct)	Expected AIV H5 (Ct)	Expected Sequence of HA_0
1	A/Gs/MB/428/2006 (H5N2) LPAI	23.6	Neg	25.42	NVPQRETR*GLF
2	A/Ck/BC/CN-07/2004 (H7N3) HPAI	27.55	26.47	Neg	NPKQAYRKRMTR*GLF
3	A/Tk/Ger/R2379/2008 (H5N3) LPAI	25.24	Neg	24.01	NVPQRETR*GLF
4	A/Tk/Ger/R655-5/2009 (H7N7) LPAI	20.11	19.63	Neg	PEIPKGR*GLF
5	A/EC/Ger/R411/2010 (H10N8)	22.8	Neg	Neg	NA
6	APMV-1 LaSota	Neg	Neg	Neg	NA
7	A/Ck/BC/CN-07/2004 (H7N3) HPAI	31.33	30.8	Neg	NPKQAYRKRMTR*GLF
8	A/Tk/Ger/R2379/2008 (H5N3) LPAI	29.72	Neg	28.04	NVPQRETR*GLF
9	A/Tk/Ger/R655-5/2009 (H7N7) LPAI	27.72	25.58	Neg	PEIPKGR*GLF
10	SPF Allantoic Fluid	Neg	Neg	Neg	NA

OFFLU Molecular Panel

Panel ID	Antigen ID	Expected AIV Matrix (Ct)	Expected AIV H7 (Ct)	Expected AIV H5 (Ct)	Expected Sequence of HA_0
1	A/Gs/MB/428/2006 (H5N2) LPAI	23.6	Neg	25.42	NVPQRETR*GLF
2	A/Ck/BC/CN-07/2004 (H7N3) HPAI	27.55	26.47	Neg	NPKQAYRKRMTR*GLF
3	A/Tk/Ger/R2379/2008 (H5N3) LPAI	25.24	Neg	24.01	NVPQRETR*GLF
4	A/Tk/Ger/R655-5/2009 (H7N7) LPAI	20.11	19.63	Neg	PEIPKGR*GLF
5	A/EC/Ger/R411/2010 (H10N8)	22.8	Neg	Neg	NA
6	APMV-1 LaSota	Neg	Neg	Neg	NA
7	A/Ck/BC/CN-07/2004 (H7N3) HPAI	31.33	30.8	Neg	NPKQAYRKRMTR*GLF
8	A/Tk/Ger/R2379/2008 (H5N3) LPAI	29.72	Neg	28.04	NVPQRETR*GLF
9	A/Tk/Ger/R655-5/2009 (H7N7) LPAI	27.72	25.58	Neg	PEIPKGR*GLF
10	SPF Allantoic Fluid	Neg	Neg	Neg	NA

EPIZONE

- European Union funded Network of Excellence for Epizootic Disease Diagnosis and Control
- Initiated in 2006
- Goal is to develop a network of scientists to improve research on preparedness, prevention, detection, and control of epizootic diseases within Europe
- NDV real-time RT-PCR ring trial conducted in 2009
- 26 samples
- 12 participating laboratories

Newcastle Disease Real-Time RT-PCR Ring Trial

CONSENSUS DETECTION RESULTS				Institute number:											
			approx. Titer (EID50/ml)	1	2	3	4	5	6	7	8	9	10	11	12	13
sample	identification	Classification	before RNA extraction													
1	PMV1/dk/UK/7800/2006	Class I (genotype 6)	3 log 10	n	n	n	?	p	n	n	n	p		n	n	n
2	PMV1/ck/Romania/001267/2008	Class II -5d	3 log 10	p	p	p	p	p	p	p	p	p		p	p	p
3	PMV1/pi/Austria/2250/2007	Class II -4a	3 log 10	p	p	p	p	p	p	p	p	p		p	p	p
4	PMV1/Sweden/8195/2006	ClassII -5b	3 log 10	n	p	p	p	p	p	p	p	p		n	n	p
5	PMV1/Estonia/7655/2006	classII -4a	3 log 10	p	p	p	p	p	p	p	p	p		p	p	p
6	PMV1/Germany/R2919/06	Class I (genotype 6)	3 log 10	n	n	n	n	p	n	n	n	p		n	n	n
7	PMV1/Germany/R2919/06	Class I (genotype 6)	6 log 10	n	p	n	n	p	n	n	n	p		n	n	n
8	PMV1/Germany/R49/99	Class I (genotype 6)	3 log 10	n	n	n	n	p	n	n	n	p		p	n	n
9	PMV1/Germany/R49/99	Class I (genotype 6)	6 log 10	n	p	n	n	p	n	n	n	p		n	n	n
10	B1/ck/46	Class II -2	3 log 10	n	p	p	p	p	p	p	p	p		p	n	p
11	Hertz/ck/33	Class II -3	3 log 10	p	p	p	p	p	p	p	p	p		p	p	p
12	Ulster/ck/64	Class II -1	3 log 10	p	p	p	p	p	p	p	p	p		p	p	p
13	APMV3/pkt/Netherlands/449/75	APMV-3	5 log 10	n	n	n	n	n	n	n	n	n		n	n	n
14	A/mallard/Belgium/12827/07	LPAI	5 log 10	n	n	n	n	n	n	n	n	n		n	n	n
15	PMV1/pi/Belgium/1.3	Class II - 4 or 5	1	n	n	n	n	n	n	n	n	n		n	n	n
16	PMV1/pi/Belgium/1.3	Class II - 4 or 5	0.1	n	n	n	n	n	n	n	n	n		n	n	n
17	PMV1/pi/Belgium/1.3	Class II - 4 or 5	1 log 10	n	p	n	?	n	n	n	n	p		n	n	n
18	PMV1/pi/Belgium/1.3	Class II - 4 or 5	2 log 10	n	p	p	p	p	n	p	p	p		n	n	n
19	PMV1/pi/Belgium/1.3	Class II - 4 or 5	3 log 10	n	p	p	p	p	p	p	p	p		p	n	p
20	PMV1/pi/Belgium/1.3	Class II - 4 or 5	4 log 10	p	p	p	p	p	p	p	p	p		p	p	p
21	PMV1/LaSota/ck/46	Class II -2	1	n	n	n	n	n	n	n	n	n		n	n	n
22	PMV1/LaSota/ck/46	Class II -2	0.1	n	n	n	n	n	n	n	n	n		n	n	n
23	PMV1/LaSota/ck/46	Class II -2	1 log 10	n	p	p	?	n	n	?	n	p		p	n	n
24	PMV1/LaSota/ck/46	Class II -2	2 log 10	n	p	p	p	p	p	?	n	p		n	p	p
25	PMV1/LaSota/ck/46	Class II -2	3 log 10	n	p	p	p	p	p	p	p	p		n	p	p
26	PMV1/LaSota/ck/46	Class II -2	4 log 10	n	p	p	p	p	p	p	p	p		n	p	p

Institute result over all tests. If several tests were done, detection with one of those tests was interpreted as correct result

color coding of results: green=correct, red=wrong, yellow=dilution not detected

reported results: n=negative p=positive ?=doubtfull

Newcastle Disease Real-Time RT-PCR Ring Trial

CONSENSUS DETECTION RESULTS				Institute number:											
			approx. Titer (EID50/ml)	1	2	3	4	5	6	7	8	9	10	11	12	13
sample	identification	Classification	before RNA extraction													
1	PMV1/dk/UK/7800/2006	Class I (genotype 6)	3 log 10	n	n	n	?	p	n	n	n	p		n	n	n
2	PMV1/ck/Romania/001267/2008	Class II -5d	3 log 10	p	p	p	p	p	p	p	p	p		p	p	p
3	PMV1/pi/Austria/2250/2007	Class II -4a	3 log 10	p	p	p	p	p	p	p	p	p		p	p	p
4	PMV1/Sweden/8195/2006	ClassII -5b	3 log 10	n	p	p	p	p	p	p	p	p		n	n	p
5	PMV1/Estonia/7655/2006	classII -4a	3 log 10	p	p	p	p	p	p	p	p	p		p	p	p
6	PMV1/Germany/R2919/06	Class I (genotype 6)	3 log 10	n	n	n	n	p	n	n	n	p		n	n	n
7	PMV1/Germany/R2919/06	Class I (genotype 6)	6 log 10	n	p	n	n	p	n	n	n	p		n	n	n
8	PMV1/Germany/R49/99	Class I (genotype 6)	3 log 10	n	n	n	n	p	n	n	n	p		p	n	n
9	PMV1/Germany/R49/99	Class I (genotype 6)	6 log 10	n	p	n	n	p	n	n	n	p		n	n	n
10	B1/ck/46	Class II -2	3 log 10	n	p	p	p	p	p	p	p	p		p	n	p
11	Hertz/ck/33	Class II -3	3 log 10	p	p	p	p	p	p	p	p	p		p	p	p
12	Ulster/ck/64	Class II -1	3 log 10	p	p	p	p	p	p	p	p	p		p	p	p
13	APMV3/pkt/Netherlands/449/75	APMV-3	5 log 10	n	n	n	n	n	n	n	n	n		n	n	n
14	A/mallard/Belgium/12827/07	LPAI	5 log 10	n	n	n	n	n	n	n	n	n		n	n	n
15	PMV1/pi/Belgium/1.3	Class II - 4 or 5	1	n	n	n	n	n	n	n	n	n		n	n	n
16	PMV1/pi/Belgium/1.3	Class II - 4 or 5	0.1	n	n	n	n	n	n	n	n	n		n	n	n
17	PMV1/pi/Belgium/1.3	Class II - 4 or 5	1 log 10	n	p	n	?	n	n	n	n	p		n	n	n
18	PMV1/pi/Belgium/1.3	Class II - 4 or 5	2 log 10	n	p	p	p	p	n	p	p	p		n	n	n
19	PMV1/pi/Belgium/1.3	Class II - 4 or 5	3 log 10	n	p	p	p	p	p	p	p	p		p	n	p
20	PMV1/pi/Belgium/1.3	Class II - 4 or 5	4 log 10	p	p	p	p	p	p	p	p	p		p	p	p
21	PMV1/LaSota/ck/46	Class II -2	1	n	n	n	n	n	n	n	n	n		n	n	n
22	PMV1/LaSota/ck/46	Class II -2	0.1	n	n	n	n	n	n	n	n	n		n	n	n
23	PMV1/LaSota/ck/46	Class II -2	1 log 10	n	p	p	?	n	n	?	n	p		p	n	n
24	PMV1/LaSota/ck/46	Class II -2	2 log 10	n	p	p	p	p	p	?	n	p		n	p	p
25	PMV1/LaSota/ck/46	Class II -2	3 log 10	n	p	p	p	p	p	p	p	p		n	p	p
26	PMV1/LaSota/ck/46	Class II -2	4 log 10	n	p	p	p	p	p	p	p	p		n	p	p

Institute result over all tests. If several tests were done, detection with one of those tests was interpreted as correct result

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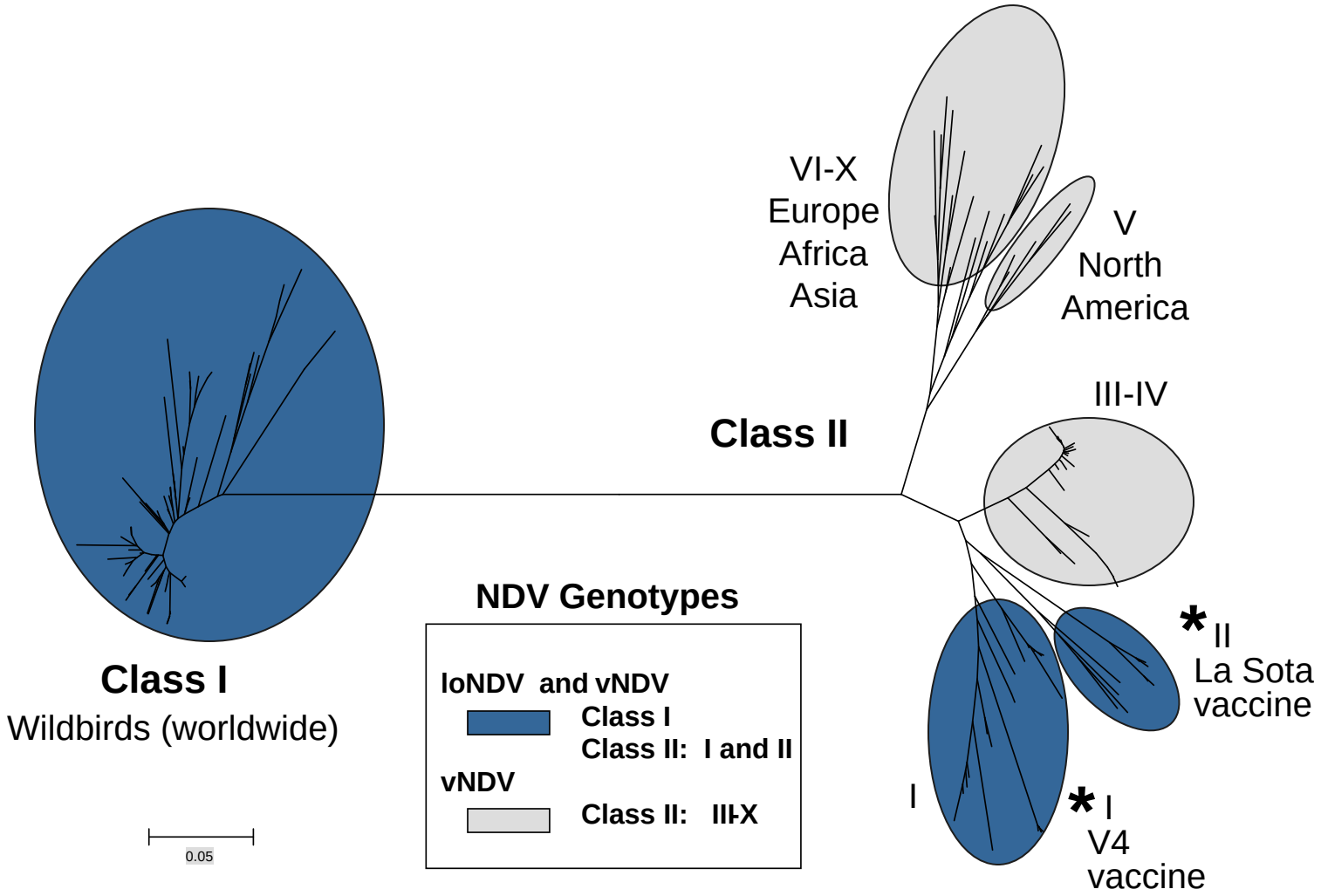

Courtesy: Claudio Afonso, SEPRL

2011 Procinorte Workshop on Influenza A Virus Molecular Diagnostic Techniques

- Procinorte is a Cooperative Program in Agricultural Research and Technology that facilitates cooperation among the 3 countries of IICA's Northern Region (Canada, USA and Mexico)
- The Workshop's objectives were:

1. To share experiences, information and hands-on approaches used in the molecular diagnosis and characterization of influenza A virus infections.
2. To identify the best practices used in the molecular diagnosis of influenza A virus infections with the focus on sequencing
3. To discuss methods used in virus isolation and characterization
4. To identify and discuss ways in which the 3 countries could harmonize influenza A virus molecular diagnostic methods

OIE Twinning Project

Laboratorio National de Diagnóstico Veterinario
Instituto Colombiano Agropecuario I.C.A.
Bogotá-Colombia

Goals

Implement laboratory diagnostic methods at the National Veterinary Diagnostic Laboratory (NVDL-ICA) for surveillance, identification and characterization of AIV and NDV

- Based upon OIE standards
- Accomplished with support of parent lab (NCFAD)
- Direct interactions through workshops, hands-on training, test result evaluation, trouble-shooting and quality assurance

Inter-laboratory comparison testing through exchange of proficiency panels and reagent preparation

Short-Term Objectives

- Implement and review diagnostic tools to identify and characterize AIV/NDV (molecular and biological)
- Develop continuous education program
  - ensure lab personnel are capable of performing relevant tests
- Establish monitoring and auditing programs at NVDL
- Establish support network to confirm/verify test results
- Establish continuous supply of reagents
- Improve diagnostic capabilities and acquire necessary modern technology
- Render NVDL capable to provide diagnostic and technical support to other countries (Andes)

Accomplishments to Date

- Assessed Colombian laboratory infrastructure, technical capabilities and quality system
- Provided training in basic molecular diagnostic techniques
- Reference antisera and antigens required for serodiagnostics
- Reagents for molecular diagnostics + initial proficiency panel
- Detailed characterization of Colombian NDV isolates

Colombian NDV Isolates

ID	Sample Type	Cleavage Site Sequen
14402-17	Cecal Tonsil	SGGRRQKR*FIGA
1326-13207	Brain	SGGRRQKR*FIGA
2063-25	Trachea/Lung/Brain	SGGRRQKR*FIGA
1326-13285	Trachea/Lung/Brain	SGGRRQKR*FIGA
2077-26	Trachea/Lung/Brain	SGGRRQKR*FIGA
2541-05	Trachea/Lung	SGGGRQGR*LIGA
2985-08	Trachea/Lung/Brain	SGGRRQKR*FIGA
1326-13563	Cloacal Swab 3	SGGGKQGR*LIGA
8080-18	Trachea/Lung/Brain	SGGGRQGR*LIGA
1326-13409	Cloacal Swab 2	SGGGKQGR*LIGA
1326-13286	Tracheal Swab	SGGRRQKR*FIGA
5728-17	Trachea/Lung/Brain	SGGRRQKR*FIGA
1326-13433	Cloacal Swab	SGGGRQGR*LIGA
1326-14301	Cloacal Swab 2	SGGRRQKR*FIGA
12129-14	Cecal Tonsil	SGGRRQKR*FIGA
1326-14475	Cloacal Swab 1	SGGGKQGR*LIGA
1326-13919	Cloacal Swab 2	SGGRRQKR*FIGA
12196-07	Trachea/Lung	SGGGRQGR*LIGA
1326-14322	Cloacal Swab 1	SGGRRQKR*FIGA
1326-14493	Cloacal Swab 1	SGGGKQGR*LIGA
1326-14553	Cloacal Swab 1-Cloacal Swab 2-Cloacal Swab 3	SGGGKQGR*LIGA
2420-06	Trachea/Lung/Brain	SGGRRQKR*FIGA
4707-00	Brain	SGGGRQGR*LIGA
4061-00	Litter	SGGGRQGR*LIGA
4677-23	Cloacal Swab 1-Cloacal Swab 2-Cloacal Swab 3	SGGGKQGR*LIGA
3951-10	Trachea/Lung	SGGGRQGR*LIGA
2389-17	Brain	SRGRRQKR*FIGA

Next Steps

- Workshop on classical and molecular diagnostic assays to be given at NVDL-ICA in 2012
- Transfer reference virus strains to NVDL-ICA once BSL3 laboratory is certified
- Assess laboratory biosafety/biosecurity
- Administer serology and virus characterization panels