Extended validation data of ELISAs & qPCR for the diagnosis of African Swine Fever

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Introduction

African Swine Fever Virus (ASFV) is a highly virulent swine disease characterized by fever, hemorrhages and high mortality rates. ASF control and eradication programs require accurate and reliable diagnostic tests. IDvet offers solutions for **antibody detection** : **indirect (i)** and **competitive** (c) **ELISAs** in **serum** (i/c), **blood filter paper** (i) and **meat juice samples** (i), as well as **a real-time qPCR.**

As wild boar play a major role in the dissemination of the disease, it is important to determine ELISAs performances for that type of samples, as they are difficult samples that could be the source of incorrect ASFV serological status.

This poster shows **complementary data** for IDvet's ELISAs performance on wild boar samples, but also **qPCR validation data and preliminary results on an ELISA prototype for oral fluids.**

ID Screen® ELISAs performances on wild boar

▶ Context

Wild boar samples can be problematic for ASF diagnosis ; previous studies (Dixon, L. et al.) reported specificity issues with wild board samples (initially selected for their reactivity on test A) tested with different ELISAs.

Origin of wild boar sera	Number of sera (total : 243)	Test A Commercial cELISA, P72 based (Sp %)	Test B Commercial iELISA, P30 based (Sp %)	OIE ELISA Indirect ELISA, whole antigen based (Sp %)	ID Screen® indirect ELISA P32, P62, P72 based (Sp %)
Europe – Poland	145	52.4%	80.7%	95.2%	100%
Europe – Belgium	13	46.2%	61.5%	76.9%	100%
Europe – Spain	85	84.7%	91.8%	95.3%	100%
SPECIFICITY %		63%	84%	94%	100%

From Dixon *et al.*

The initial performances of the ID Screen® ASF competitive ELISA were determined as follows : Sp: 100% [99,2 – 100] on domestic pigs n=452 ; 96,4% [87,7 – 99,0] on wild boar n=55 ; Se: 95.8% [92.2 - 97.8] n=213.
Aim : better assess the specificity on wild boar samples for the ID Screen® ASF cELISA and iELISA by testing a larger number of wild boar samples. Kits were performed as per manufacturer's instructions. Results are presented in the table below :

▶ Results

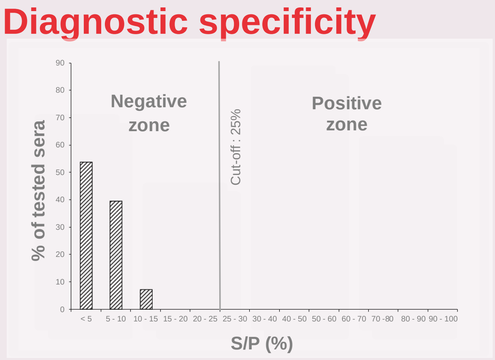
Wild boar sera origin	Sample status definition	Tested at	ID Screen® indirect ELISA negative / total tested	ID Screen® competitive ELISA negative / total tested
Europe – Poland (2014-2015)	IPT	EURL / CISIA-INIA, kit validation study	165 / 165	ND
Europe – Spain (2014-2015)	IPT	EURL / CISIA-INIA, kit validation study	85 / 85	ND
Europe – Lithuania (2014-2015)	IPT	EURL / CISIA-INIA, kit validation study	ND	2 / 3 (false positive : haemolysed)
Europe – Latvia (2014-2015)	IPT	EURL / CISIA-INIA, kit validation study	ND	2 / 2
Europe – France (2013)	Non-endemic aera	IDvet	40 / 40	49 / 50
Europe – Spain (2016-2017)	Non-endemic aera	IDvet	200 / 200	198 / 200
		TOTAL	**490 / 490**	**251 / 255**
		Specificity on wild boar samples	**100 %**	**98.4 %**
		[$CI_{95\%}$]	[99.2 - 100]	[96.3 – 99.4]

▶Both **ID Screen®** kits show very **high specificity** with **wild boar samples**

An ELISA prototype for ASFV antibody detection in oral fluids

Plates are coated with P32, P62 and P72 ASFV recombinant proteins. Oral fluids are added on both coated and non-coated adjacent well (bi-well ELISA) and incubated for 1h30 at 37° C ; the HRP conjugate (recognizing both IgG and IgA) is then added for 30 min at room temperature. After final washes, the TMB substrate is added (15 min at RT), OD450 nm are read after addition of the stop solution, Results are expressed as S/P% (cut-off 25%)

▶ Diagnostic specificity



56 individual oral fluids collected with a cotton rope-based oral fluid collection kit (IDvet) from conventional farm pigs (aged 40-150 days) in a non-endemic area (France) were tested with the OF ELISA prototype. The graph shows the S/P distribution obtained :

▶Measured specificity : 100% $IC_{95\%}$ (93,6 – 100), *n*=56

▶ Preliminary evaluation (EURL reference lab – 2014) :

Oral fluids collected at 0, 4, 8, 11, 14, 18, 21, 30, 37, 44, 52, 58 and 65 dpi (according Mur et al., 2013) from experimental infections were tested on the ELISA prototype. Samples were also tested with the ImmunoPeroxydase Test (IPT) and a modified OIE ELISA for OF:

		IPT		
		Positive	Negative	TOTAL
IDvet ELISA	Positive	44	0	**44**
	Negative	5	36	**41**
	TOTAL	**49**	**36**	**85**

		OIE indirect ELISA			
		Positive	Doubtful	Negative	TOTAL
IDvet ELISA	Positive	38	6	0	**44**
	Negative	1	1	39	**41**
	TOTAL	**39**	**7**	**39**	**85**

Samples were collected in two studies involving experimentally-infected pigs: i) four Landrace Large White pigs inoculated (IM) with 105 TCID50/ml - ASFV isolate NH/P68 (NHV), challenged at 30 dpi with 10 HAD50/ml of Armenia ASFV Arm07 isolate; and ii) four Landrace Large White pigs inoculated (IM) with 103 TCID50/ml of attenuated Portugal ASFV isolate NH/P68 (NHV).

▶**Excellent correlation of the ASF OF ELISA prototype(κ ; [CI95])**
with the **OIE modified ELISA*** (κ=0.953 ; [0,888-1]) and **with the IPT** (κ=0,882 ; [0,782-0,982])
*** Considering doubtful results as positive .**

ID Gene® ASF Duplex Real-Time PCR

The **ID Gene® ASF Duplex** is a **ready-to-use** qPCR kit assay detecting simultaneously ASFV and an endogenous internal positive control, on blood, serum, plasma, swabs and tissues. A freeze-dried calibrated positive control containing inactivated virus, can be used as a sentinel. Results may be obtained in less than 1,5 hour (extraction in 20 minutes, fully automatable with the IDeal™ robot, and amplification in 50 minutes).

MAGFAST™ universal for automated DNA/RNA extraction

qPCR kit with calibrated sentinel/positive control

The IDeal™ extraction robot

▶ Diagnostic sensitivity

The sensitivity of the IDASF PCR kit was evaluated by the EURL on 279 positives samples

▶The IDASF perfectly detects field samples from current outbreaks in Eastern European countries (genotype II) and endemic African countries

▶Measured sensitivity : 98,6%
(CI_{95}%: 96.4% - 99.4 %), *n*=279.

Location	Host	N° sample	IDASF PCR kit
			% POS
Estonia	European wild boar	38	89 %
Latvia	European wild boar	48	98 %
Lithuania	European wild boar	103	85 %
Poland	European wild boar	32	78 %
Estonia	Domestic pig	11	100 %
Poland	Domestic pig	20	100 %
Moldova	Domestic pig	11	100 %
Endemic African countries	Homogenates soft ticks and domestic pig	40	98 %
Total		279	91 %

▶ Comparison with reference tests

162 positives samples were tested in parallel with the UPL-PCR test, the OIE-PCR test and the IDVET PCR kit. Results were obtained by EURL reference lab.

Total samples	UPL-PCR	OIE-PCR	ID Gene™ ASF Duplex
	% POS	% POS	% POS
112	98 %	76 %	97 %
18	100 %	56 %	100 %
32	63 %	41 %	84 %
162	91 %	67 %	95 %

▶ On this panel, the IDASF kit was able to detect 95% of infected or exposed animals versus 67 and 91% for the 2 other kits, indicating improved sensitivity compared to other methods tested. Cq values were at least 2 Cq earlier on the panels versus UPL-PCR and OIE-PCR

▶ Inclusivity and exclusivity

A DNA panel (14 DNA) and two sample reference panels (experimental and clinical field samples), obtained from the EURL, were tested.
DNA extraction was performed by magnetic beads (MAGFAST384) as per manufacturer's instructions.

▶ The IDASF kit correctly identified all ASF panels form the CISA-INIA and did not show any cross-reactions with the 31 other pathogens tested

	Strain	ID Gene™ ASF Duplex
Inclusivity	14 URL-ASF reference DNAs (from CISA-INIA)	Detected
	16 URL-ASF reference samples (from CISA-INIA)	Detected
	23 URL-ASF references samples (in CISA-INIA)	Detected
Exlusivity	Influenza (H1N1, H5N2, H7N1)	Not detected
	Classical Swine Fever	Not detected
	Mycoplasma hyopneumoniae	Not detected
	PRRSV	Not detected
	25 other virus and bacteria from different species	Not detected

▶ PCR characteristics

Characteristics	ID Gene™ ASF Duplex
Analytical specificity	100 %
Efficiency	~ 100 %
Limit of detection	12 copies per PCR
Repeatability	CV < 2 %
Intermediate precision	CV < 3 %
Robustness	Unaffected by all parameters tested (± 1°C / ±10% RNA volume)
Experimental LOD	5.10^4 TDICT50 / mL on positive lung homogenate

Rigorous system of controls:
▶ Endogenous internal control to confirm sample presence and to validate both extraction and amplification steps.
▶ Freeze-dried calibrated Positive Control supplied in the kit : a sentinel to check run to run reproducibility over time

Conclusion

In this study, new validation data on the **ID Screen® African Swine Fever ELISA** range were obtained on wild boar samples. Both the competitive and indirect kits offer high specificity on these difficult samples (98,4 and 100%).

Preliminary data obtained on **an oral fluid ELISA** look encouraging.

The **ID Gene® ASF Duplex** allows the accurate detection of all genotypes of ASFV, including those currently circulating in Eastern Europe. It has an excellent limit of detection and diagnostic sensitivity (98,6%).
The kit was evaluated and validated by the EURL (CISA-INIA, Spain), is registered in Poland (Piwet-Pulawy) and Germany (FLI : Friedrich-Loeffler-Institut) and under registration progress in France.

IDvet offers a full range of performant tools for accurate and rapid diagnosis of African Swine Fever

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References :
Dixon L. et al ***ASFV Challenges for Diagnosis : preventing and controlling viral disease,***
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