INTERNAL VALIDATION REPORT

ID SCREEN® AFRICAN SWINE FEVER INDIRECT

INDIRECT ELISA FOR THE DETECTION OF ANTIBODIES AGAINST ASFV IN SERUM, PLASMA, MEAT JUICE OR BLOOD FILTER PAPER SAMPLES FROM PORCINE

METHOD	**Indirect ELISA**
TARGET	Antibodies directed against African Swine Fever Virus
SAMPLE TYPES	• Serum • Plasma • Meat juice • Blood filter paper
VALIDATED SPECIES	Porcine (pigs, wild boars, warthogs)
PRODUCT CODE	ASFS

With you at every step

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INTRODUCTION

African Swine Fever (ASF) is a serious viral disease of pigs caused by the African Swine Fever virus (ASFV). It is highly contagious and can spread very rapidly in pig populations by direct contact with infected animals, indirect contact on fomites, and tick vectors[1].

The ID Screen® African Swine Fever Indirect ELISA effectively detects anti-ASFV antibodies in serum, plasma and blood filter paper samples. It can be used on domestic pigs, wild boars and warthogs. It is the only commercial ELISA test which uses three recombinant antigens: p32, p62 and p72.

This report summarizes validation data for this test.

DESCRIPTION AND PRINCIPLE OF THE TEST

Samples to be tested and controls are added to microwells coated with p32, p62 and p72 ASFV recombinant proteins. Anti-ASFV antibodies, if present, form an antigen-antibody complex.

After washing, an anti-multi-species horseradish peroxidase (HRP) conjugated secondary antibody is added to the wells. It binds to the antibodies, forming an antigen-antibody-conjugate-HRP complex. After elimination of the excessive amount of conjugate by washing, the substrate solution (TMB) is added.

The resulting coloration depends on the quantity of specific antibodies present in the specimen to be tested. In the presence of antibodies, a blue coloration appears, and it becomes yellow after addition of the stop solution. In the absence of antibodies, no coloration appears. The microplate is read at 450 nm.

For each sample, the S/P% is calculated. Samples are then classified as positive, negative or doubtful depending on their S/P% result, as indicated below:

RESULT	STATUS
S/P % ≤ 30%	**Negative**
30% < S/P % < 40%	**Doubtful**
S/P % ≥ 40%	**Positive**

SERUM SAMPLES

INNOVATIVE DIAGNOSTICS VALIDATION

ANALYTICAL SENSITIVITY

Analytical sensitivity was evaluated through the titration of the two positive sera below:

IDvet internal positive reference serum:

Innovative Diagnostics produces, for its IDvet range, a positive ASF freeze-dried serum reference material which may be used to check that the test's analytical sensitivity does not vary between runs, operators and batches. This serum standard is available for purchase under the product code MRI-ASF.

The MRI-ASF was titrated and tested by Innovative Diagnostics.

	MRI-ASF	
DILUTION	**S/P%**	**STATUS**
1:16	79	(+)
1:64	59	(+)
1:256	38	(+/-)
1:1024	19	**(-)**

Table 1: Titration of the IDvet freeze-dried serum standard on the ID Screen® ELISA

RESULTS (Table 1):

- **The MRI-ASF was detected as positive up to dilution 1:64 and doubtful at dilution 1:256.**

ASF-CP C+93 reference serum from the ASF-EURL, CISA-INIA:

The European Union Reference Laboratory for African Swine Fever in Madrid, Spain (ASF-EURL, CISA-INIA) produces a strong positive reference serum for ASF antibody detection called ASF-CP. ASF-CP Batch C+93 was tested. When using the ASF-EURL in-house ELISA, the last dilution of this serum found positive is 1:5000 (final dilution).

	ASF-EURL, CISA-INIA, ASF positive reference serum: ASF-CP Batch C+93		
PRE-DILUTION	**FINAL DILUTION IN THE PLATE**	**S/P%**	**STATUS**
pure	1:20	122	(+)
1:100	1:2000	61	(+)
1:200	1:4000	42	(+)

Table 2: Titration of the ASF positive reference serum on the ID Screen® ELISA

RESULTS (Table 2) :

- **This serum was detected positive diluted 1:4000**

SENSITIVITY

Sensitivity was evaluated on the serum panels below:

Panel from the ASF-EURL, CISA-INIA:

A reference panel from the ASF-EURL, CISA-INIA, composed of 8 sera from experimentally infected pigs were tested using the ID Screen® ELISA and reference techniques such as the EURL Western Blot (WB), the EURL Indirect Immunoperoxidase Test (IPT) and the EURL ELISA.

DESCRIPTION				EURL WB	EURL IPT	EURL ELISA	ID Screen® ELISA	
SAMPLE ID	STATUS	ORIGIN	ASFV STRAIN				S/P%	STATUS
Serum 1	Negative serum	Disease-free pig	-	-	-	-	**0**	**Negative**
Serum 2	Positive serum	Serum obtained at 50 dpi	E75/E70 (Spain)	**+++**	**+++**	**+++**	**130**	**Positive**
Serum 3	Antibody positive serum	Serum obtained at 36 dpi	E75/E70 (Spain)	**++**	**++**	**++**	**98**	**Positive**
Serum 4	Antibody positive serum	Serum obtained at 24 dpi	Ken05.Tk 1 (Kenya)	**++**	**+++**	**++**	**115**	**Positive**
Serum 6	Antibody weak positive serum	Serum obtained at 50 dpi	E75/E70	**++**	**++**	**+**	**95**	**Positive**
Serum 9	Negative serum	Disease-free pig	-	-	-	-	**3**	**Negative**
Serum 10	Antibody positive serum	Serum obtained at 67 dpi	E75/E70	**++**	**++**	**++**	**95**	**Positive**
Serum 11	Antibody positive serum	Serum 2 diluted 1:8 in S2	E75/E70	**++**	**++**	**++**	**112**	**Positive**

***+++**: strong positive; **++**: positive; **+**: weak positive, **-**: negative*
dpi: days post-infection

Table 3: Reference sera from the ASF-EURL, CISA-INIA, tested using the ID Screen® ELISA and other techniques.

RESULTS (Table 3):

- **For each tested serum,** the ID Screen® African Swine Fever Indirect ELISA **gave the expected result.**
- The results obtained with the ID Screen® ELISA are **completely correlated** with the ones obtained with **the reference techniques used by the ASF-EURL.**

Panel from ANSES, Ploufragan, France:

Sera were obtained from three pigs vaccinated at day 0 and day 24 with the ASFV Ourt88/3 strain and challenged at day 42 using a mild strain (ANSES, Ploufragan, France).

Pigs were sampled at day 62 and day 63, and sera were tested using the ID Screen® African Swine Fever Indirect ELISA.

RESULTS (data not shown):

- **All sera were found positive.**

SPECIFICITY

Pig serum samples

723 pig serum samples from disease-free areas in France, Spain and Norway were tested using the ID Screen® African Swine Fever Indirect ELISA.

The results shown in Figure 1 are expressed as sample to positive control ratios (S/P%).

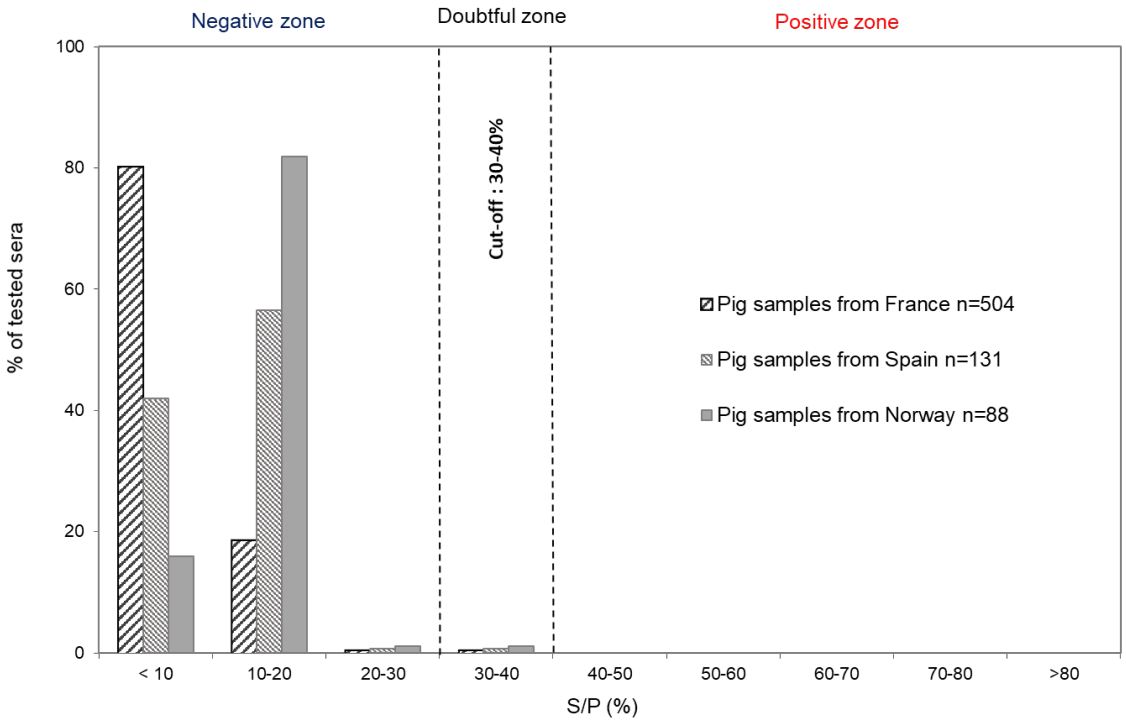


Figure 1: S/P distribution for negative pig sera, n=723

RESULTS (Figure 1):

- 719/723 pig sera were found negative and 8 sera gave doubtful results with the ID Screen® ELISA.
- **Measured specificity is:**
  - **99.5% (CI_{95}%: 98.6% - 99.8%), n = 723, doubtful results are considered as positive.**
  - **100% (CI_{95}%: 99.5% - 100%), n = 723, doubtful results are considered as negative.**

Wild boar serum samples

240 wild boar serum samples from disease-free areas in Spain (2013-2017) were tested using the ID Screen® African Swine Fever Indirect ELISA.

The results shown in Figure 2 are expressed as sample to positive control ratios (S/P%).

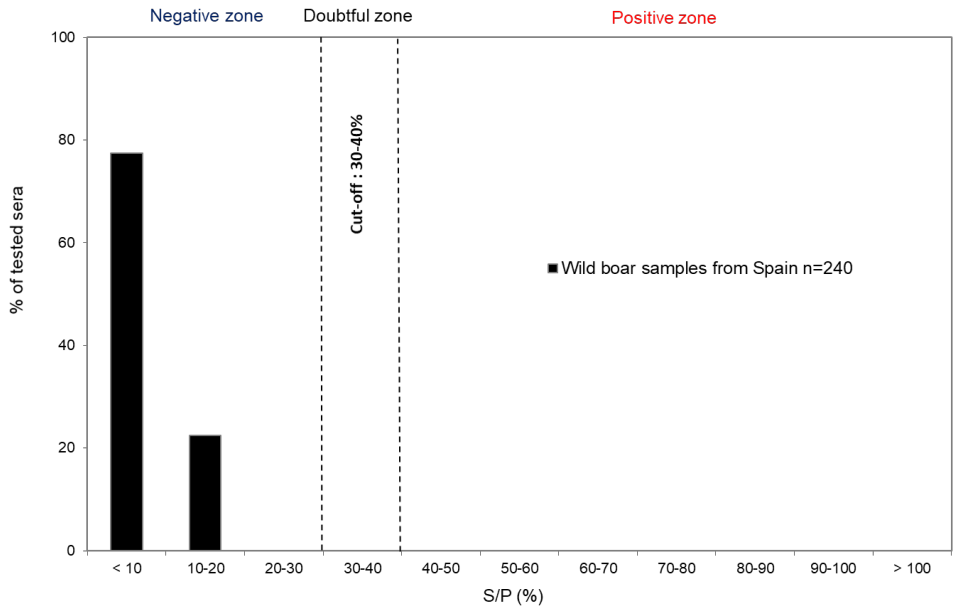


Figure 2: S/P distribution for negative wild boar sera, n=240

RESULTS (Figure 2):

- 240/240 wild boar sera were found negative with the ID Screen®ELISA.
- **Measured specificity = 100% (CI_{95}%: 99.5% - 100%), n = 240.**

Specificity summary: pigs and wild boar samples

SPECIES	SPECIFICITY	$CI_{95\%}$
Pigs	100%	99.5% - 100%, n = 723*
Wild boars	100%	99.5% - 100%, n = 240
TOTAL	**100%**	**99.6%-100%, n=963**

**doubtful results are considered as negative*

Table 4: Specificity for negative pigs and wild boars, n=250

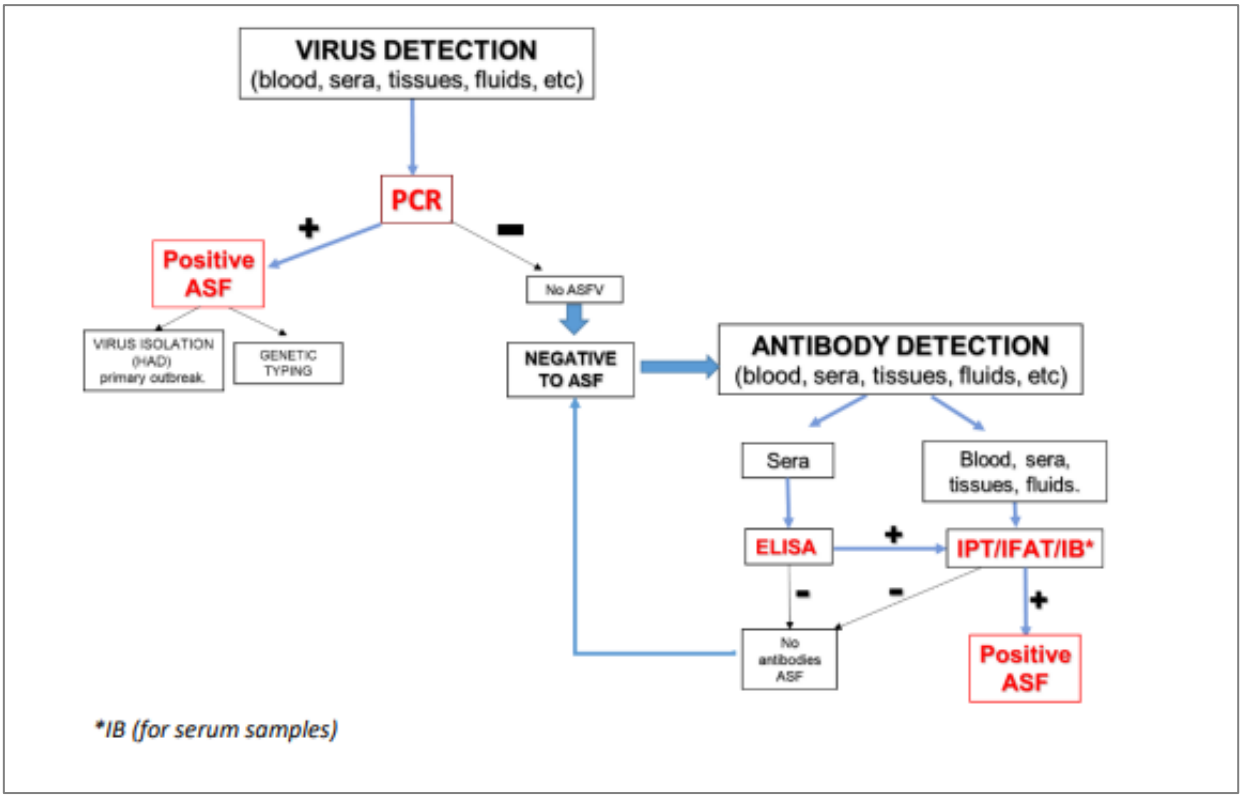
RESULTS (Table 4):

- **Measured specificity is 100 % in pigs as well as wild boars.**
- The ID Screen® ELISA shows **an excellent specificity**.

EUROPEAN REFERENCE LABORATORY VALIDATION (2)

PRELEMINARY NOTE

The ASF-EURL communicated a recommended scheme for ASF Diagnostics in case of ASF suspicion, available on-line at the following address:
https://asfreferencelab.info/asf/images/GUIDELINES_DIAGNOSIS/Link_2_workflow_MA.pdf



SPECIFICITY

In April 2014, the ASF-EURL, CISA-INIA, (Spain) validated the ID Screen® African Swine Fever Indirect ELISA by analysing a total of 375 negative samples from domestic pigs as well as from European wild boars and African warthogs. The obtained results are transcribed in Table 5.

SPECIES	NUMBER OF NEGATIVE SERA TESTED	NUMBER OF SERA FOUND NEGATIVE BY ID SCREEN® ELISA	MEASURED SPECIFICITY	$CI_{95\%}$
Domestic pigs	141	141	100 %	97.35-100 %
European wild boars	230	229	99.57 %	97.59-99.92 %
African warthogs	4	4	100 %	51.01 -100%
TOTAL SAMPLES	**375**	**374**	**99.73 %**	**98.5-99.95 %**

Table 5: Specificity as measured by the ASF-EURL, CISA-INIA

SENSITIVITY

To complete the ID Screen® validation, 217 positive samples from domestic pigs and African warthogs were tested by the ASF-EURL, CISA-INIA. The obtained results are transcribed in Table 6.

SPECIES	NUMBER OF POSITIVE SERA TESTED	NUMBER OF SERA FOUND POSITIVE BY ID SCREEN® ELISA	MEASURED SENSITIVITY	$CI_{95\%}$
Domestic pigs	89	84	94.38%	87.51-97.58%
African warthogs	128	125	97.66%	93.34-99.2%
TOTAL SAMPLES	**217**	**209**	**96.31%**	**92.89-98.12%**

Table 6: Sensitivity as measured by the ASF-EURL, CISA-INIA

POPULATION DISTRIBUTION

The obtained data were further analysed to determine the distribution of the S/P% values and are represented in Figure 3.

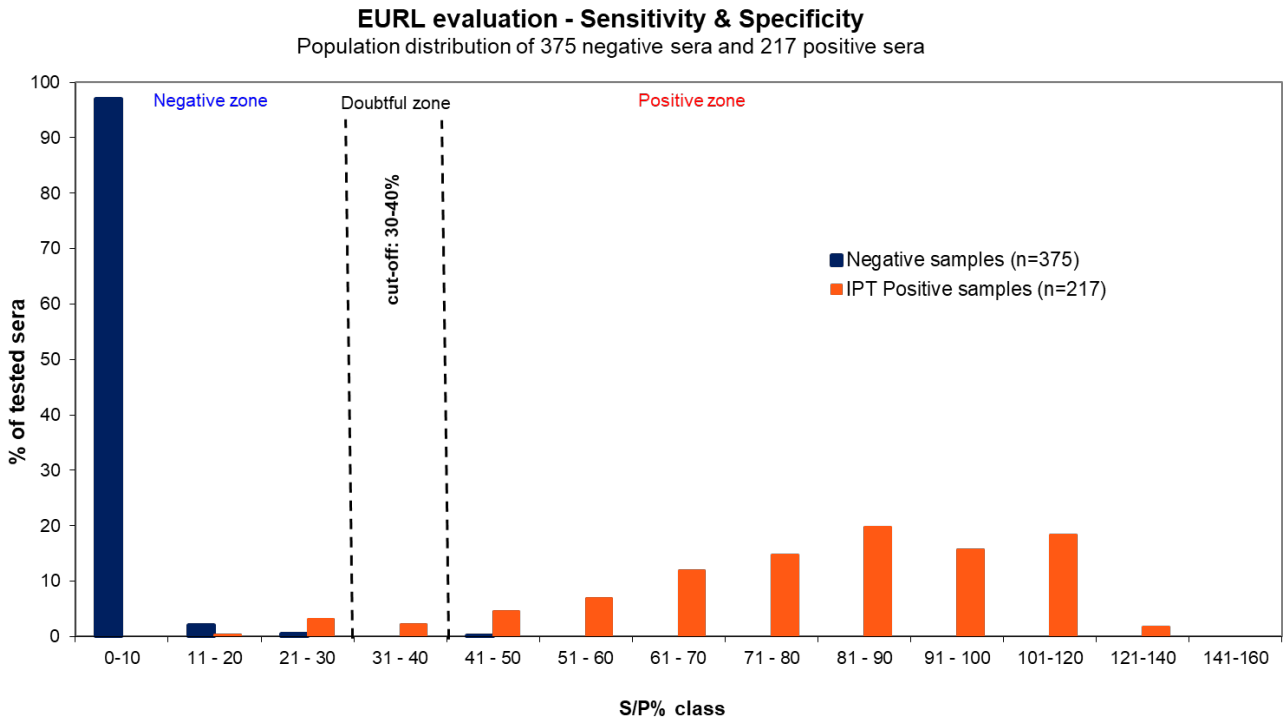


Figure 3: S/P% distribution for negative (n=375) and positive (n=217) sera

RESULTS (Table 5, Table 6 and Figure 3):

- **Measured sensitivity: 96.3% ($CI_{95\%}$: 92.9– 98.1%), n=217.**
- **Measured specificity: 99.7% ($CI_{95\%}$: 98.5– 100%), n=375.**

ASF-EURL, CISA-INIA, CONCLUSION

The ASF-EURL, CISA-INIA concluded in its report published on April 21, 2014:

> **Overall we conclude from the final validation of the test that the ID SCREEN indirect ELISA is appropriated for the detection of antibodies against ASF in serum samples with the CO range established in 30%.**

2021 ASF-EURL COMMUNICATION

Recently the ASF-EURL published a report listing international prescribed African swine fever diagnostics tests[3]. The Table below shows an extract from this publication, which gives an overview of validated African Swine fever virus and antibody detection tests.

Detection	Available tests	Type: in house/commercial	Recommended use
Virus	genome detection	PCR (OIE TaqMan probe[1], OIE UPL probe[1] or OIE conventional PCR[1], and commercial kits[2])	suspicion; surveillance; individual and herd testing
	virus isolation	VI/haemadsorption (HAD) test[1] (i.h.)	confirmation of primary outbreak
	antigen detection	Direct Immuno fluorescence (DIF)[1] (i.h.)	individual testing (acute forms)
		Antigen ELISA commercial kit INgezim PPA DAS, Double Ab Sandwich	surveillance; herd testing (acute forms)
	pen-side test	Lateral flow assay (LFA) commercial kit (INgezim ASF CROM Ag)	herd testing (acute forms)
Antibody	ELISA	ELISA (OIE, commercial kits[3])	surveillance; herd testing
	confirmatory test	Immunoblot (IB) test[1] (i.h.)	confirmatory; herd testing
		Immunofluorescence Antibody (IFAT) test[1] (i.h.)	confirmatory; herd testing
		Indirect Immunoperoxidase test[1] (IPT) (i.h.)	confirmatory; herd testing
	pen-side test	LFA commercial kit INgezim PPA CROM	herd testing

[1] Included in the OIE Terrestrial Manual for Diagnostic Test and Vaccines, 2019; i.h. = in house methods.

[2] PCR Commercial Kits currently validated: INgene q PPA, INGENASA. 11.PPA.K.5TX/Q; Tetracore TC-9017-064; Virotype ASFV PCR Kit, INDICAL BIOSCIENCE; LSI VetMAXTM Thermo Fisher Scientific; IDEXX RealPCR ASFV Mix, IDEXX; ID Gene® African Swine Fever Duplex – IDVet; ADIAVET ASFV REAL TIME 100R, BIO-X DIAGNOSTICS.

[3] Antibody ELISA Commercial Kits currently validated: INgezim PPA COMPAC competition-ELISA, INGENASA; IDScreen® ASF Indirect ELISA, IDVET; ID Screen® ASF Competition-ELISA, IDVET; SVANOVIR® ASFV Indirect-ELISA, SVANOVA.

- The ID Screen® African Swine Fever Indirect ELISA **is included in the ASF-EURL validated African Swine Virus antibody detection tests.**

SUMMARY OF PERFORMANCE ON SERUM SAMPLES

SPECIFICITY FOR WILD BOARS:

Specificity results on wild boar samples obtained at ASF-EURL, CISA-INIA, and Innovative Diagnostics are summarized in Table 7 below.

WILD BOAR SERUM DESCRIPTION			ID SCREEN®ELISA
ORIGIN	SAMPLE STATUS	TESTED BY	FOUND NEGATIVE/ NUMBER TESTED
Europe – Poland (2014/15)	IPT	ASF-EURL/CISA	165 / 165
Europe – Spain (2014/15)	IPT	ASF-EURL/CISA	85 / 85
Europe – France (2013)	Non-endemic area	Innovative Diagnostics	40/40
Europe – Spain (2016/17)		Innovative Diagnostics	200/200
TOTAL			**490/490**
Specificity on wild boar samples			**100 % ($CI_{95\%}$: 99.2-100%)**

Table 7: Summary of specificity on wild boar samples

GLOBAL SPECIFICITY AND SENSITIVITY

Performance evaluations at ASF-EURL, CISA-INIA and Innovative Diagnostics on total samples (pigs, wild boars and warthogs) are summarized in Figure 4 and Table 8.

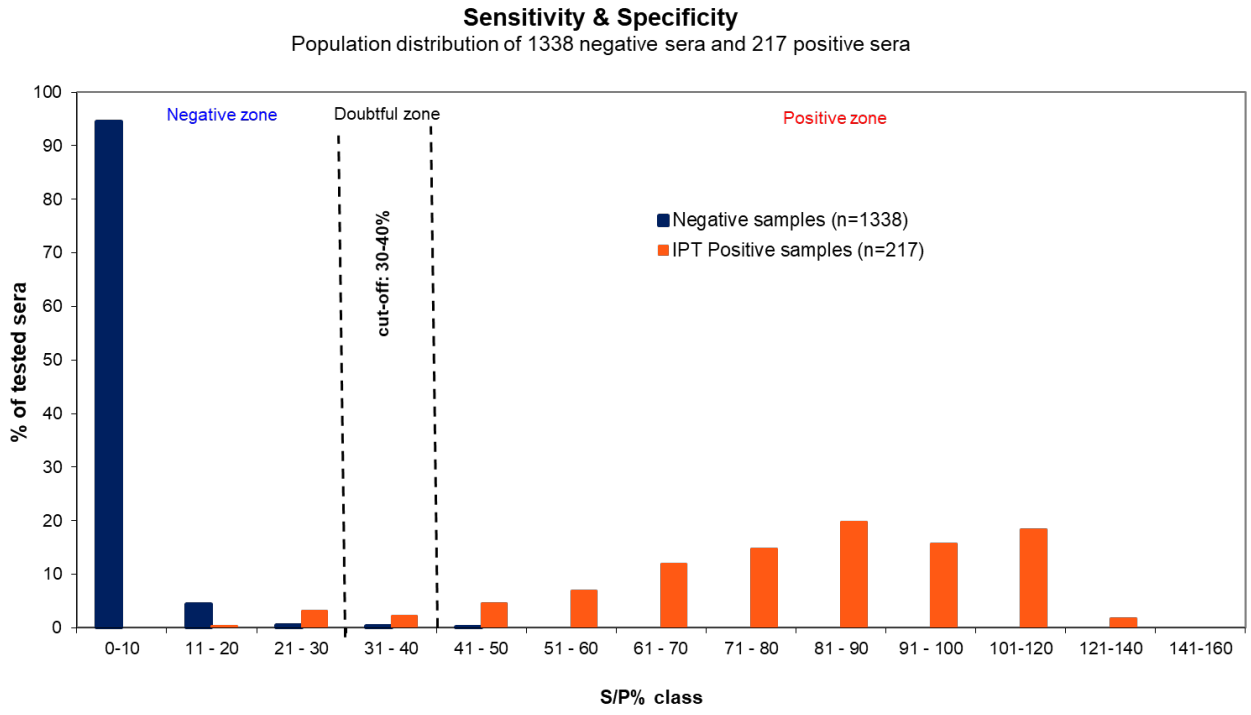


Figure 4: Global S/P% distribution for serum samples

	ID SCREEN® AFRICAN SWINE FEVER INDIRECT ELISA		
	ASF-EURL, CISA-INIA VALIDATION	**INNOVATIVE DIAGNOSTICS VALIDATION**	**TOTAL**
SPECIFICITY	**99.7% ($CI_{95\%}$: 98.5%-99.5%), n=375**	**100% ($CI_{95\%}$: 99.6%-100%), n=963**	**99.9% ($CI_{95\%}$: 99.5%-100%), n=1338**
SENSITIVITY	**96.3% ($CI_{95\%}$: 92.9%-98.1%), n=217**	*Sensitivity results were not statistically significant, due to a limited number of available samples*	**96.3% ($CI_{95\%}$: 92.9%-98.1%), n=217**

Table 8: Summary of the ID Screen® performance for serum samples

IMPACT ON TEST SPECIFICITY AND SENSITIVITY

Based on the validation data from the ASF-EURL, CISA-INIA (217 positive and 375 negative samples) and Innovative Diagnostics (963 negative samples), the test performance was evaluated for different cut-off values.

Specificity and sensitivity were calculated for different threshold values. The cut-off value was chosen to ensure the best sensitivity while preserving the best specificity. Results are presented in the Figure 5 and Table 9 below.

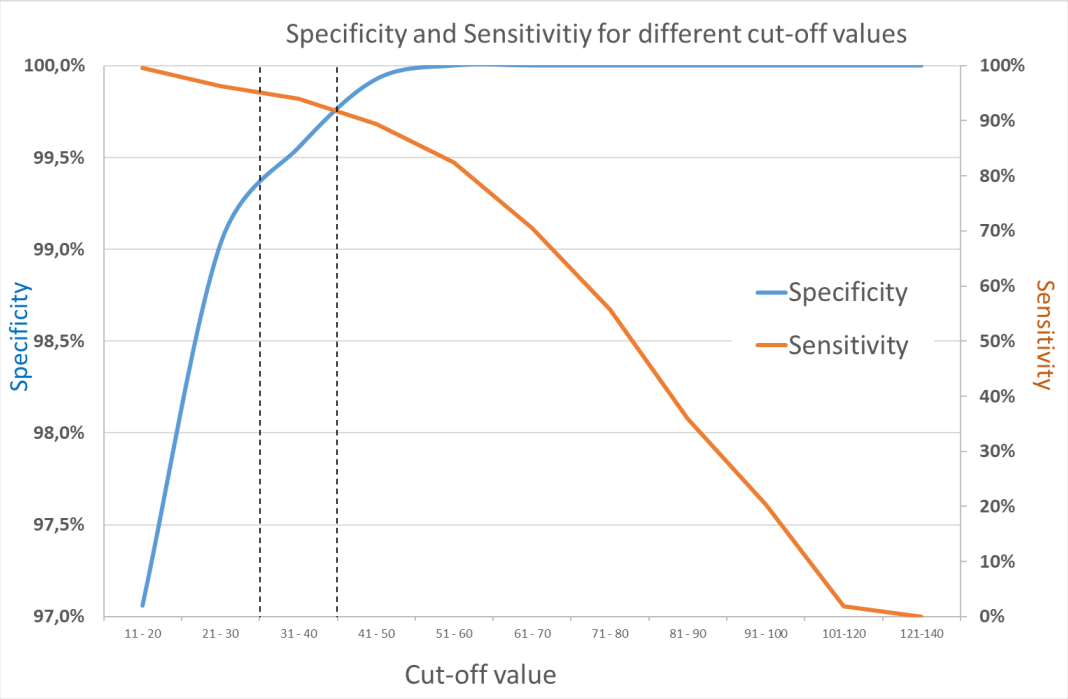


Figure 5: Graph of specificity and sensitivity for different cut-off values

CUT OFF VALUE (S/P%)	SPECIFICITY		SENSITIVITY	
	Sp (%)	$CI_{95\%}$ (%)	Se (%)	$CI_{95\%}$ (%)
10	97.1	96-97.8	100	93.9-98.6
20	99	98.6-99.6	99.5	96.9-99.8
30	**99.6**	99.3-99.9	**96.3**	97.8-100
40	**99.9**	99.3-99.9	**94**	97.8-100
50	100	99.7-100	89.4	98.3-100
60	100	99.7-100	82.5	98.3-100
70	100	99.7-100	70.5	98.3-100
80	100	99.7-100	55.8	98.3-100
90	100	99.7-100	35.9	98.3-100
100	100	99.7-100	20.3	98.3-100
120	100	99.7-100	1.8	98.3-100
140	100	99.7-100	0	98.3-100

Table 9: Specificity and sensitivity for different cut-off values

RESULTS (Figure 5 and Table 9):

- **A threshold at 30-40 % was chosen by Innovative Diagnostics to ensure the best sensitivity while keeping the highest specificity.**

ROC CURVE

A Receiver Operating Curve (ROC) curve was generated to make a graphical representation of the connection/trade-off between sensitivity and specificity, for every possible cut-off of a diagnostic test.

ROC analysis was performed from the specificity and sensitivity results obtained by the ASF-EURL, CISA-INIA, and Innovative Diagnostics, on the distribution of negative and positive suids sera (1338 negative samples and 217 positive samples).

The ROC curve plot for the ID Screen® ELISA kit is presented in Figure 6 below.

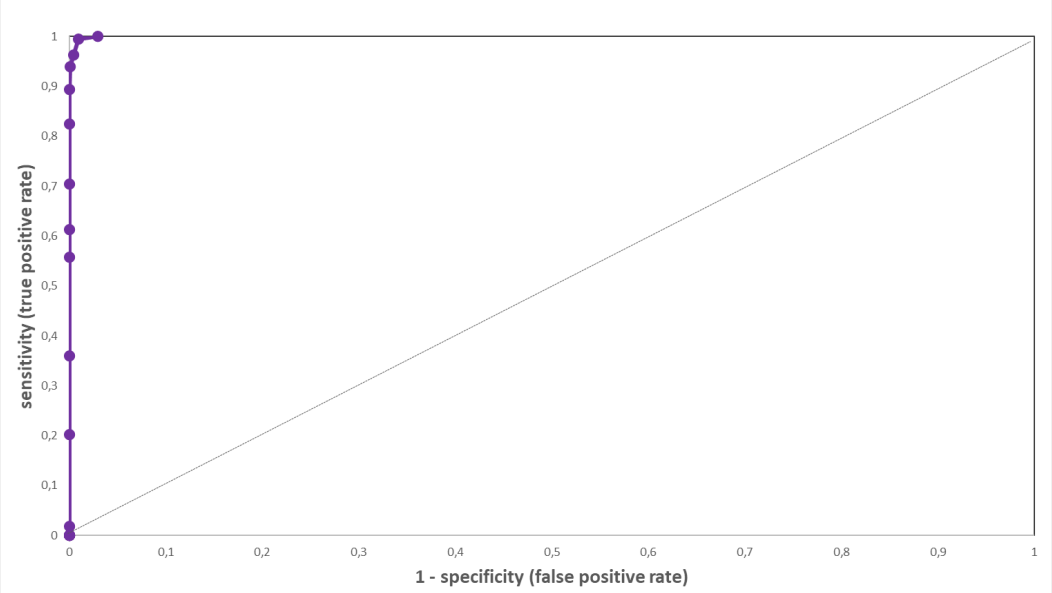


Figure 6: ROC curve, calculation of AUC

RESULTS (Figure 6):

- **Area Under Curve (AUC) = 0.992 ($CI_{95\%}$: 0.986– 0.999).**
- **The high AUC value demonstrates the excellent performance of the ID Screen® ELISA.**

PREDICTIVE VALUES

The Positive Predictive Value (PPV) and the Negative predictive value (NPV) depend on the measured sensitivity (Se) and specificity (Sp) of the method used for the diagnosis and of the known prevalence of the disease in the studied population.

Predictive values were calculated based on specificity and sensitivity measured previously:
Specificity: 99.9% ($CI_{95\%}$: 99.5%-100%), n=1338
Sensitivity: 96.3% ($CI_{95\%}$: 92.9%-98.1%), n=217

Calculations are shown in Table 10 below.

	PREVALENCE					
	1%	**5%**	**10%**	**20%**	**40%**	**60%**
PPV	90.7%	98.1%	99.1	99.6%	99.8%	99.9%
NPV	100.0%	99.8%	99.6%	99.1%	97.6%	94.7%

Table 10: Predictive values for different seroprevalences

RESULTS (Table 10):

- **Predictive positive value is excellent** while **predictive negative value is very high up to 20% prevalence.**
- This test is therefore **a reliable tool for ASF diagnosis**.

SEROCONVERSION STUDY

The study was performed by the French Reference Laboratory (ANSES, Ploufragan, France). 8 pigs were inoculated with the Ourt 88/3 strain and bled at 0,6,9,13,19 and 27 days post-infection (dpi). Sera were tested using the ID Screen® ELISA and time-course sera curves were generated (Figure 7).

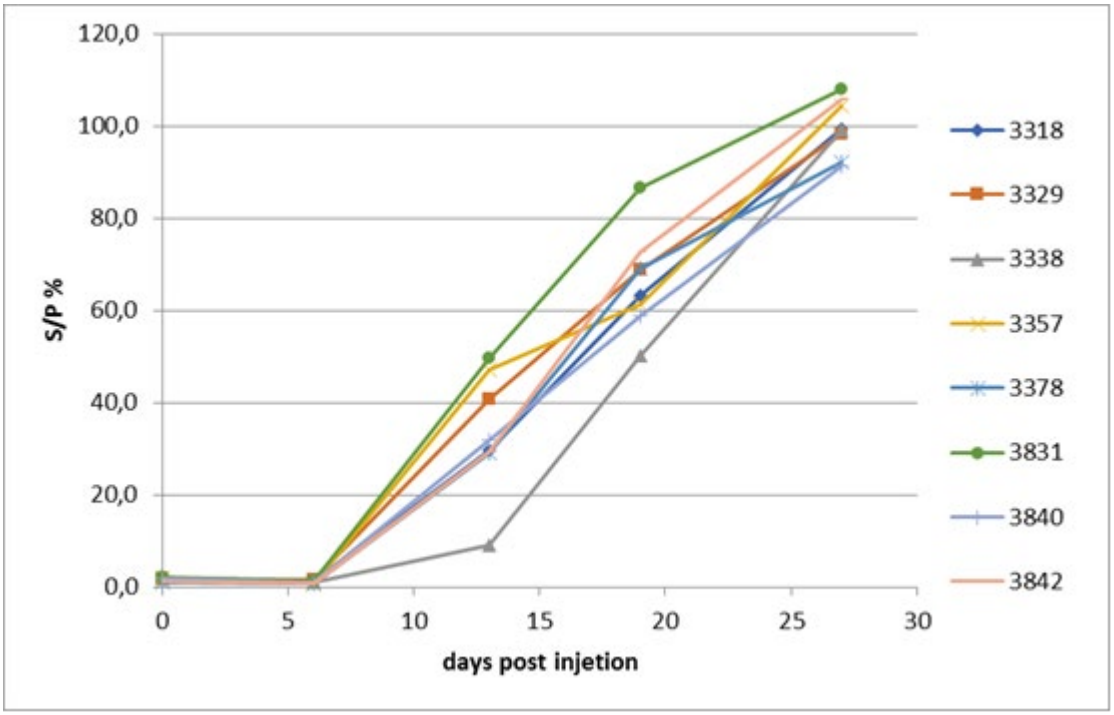


Figure 7: Seroconversion results (S/P% values)

RESULTS (Figure 7):

- **Seroconversion was detected by the ID Screen® African Swine Fever Indirect ELISA between 10 and 15 days post-infection.**

COMPARISON WITH OTHER COMMERCIAL ELISA TESTS

KIT DESCRIPTIONS

Analytical sensitivity, specificity and sensitivity were tested in parallel using the same qualified samples.

The comparison study was performed by Innovative Diagnostics using the 4 ELISA tests listed below and following the manufacturer's instructions:

- ID Screen ® African Swine Fever Indirect ELISA (product code: ASFS)
- ID Screen ® African Swine Fever Competition (product code: ASFC)
- Commercial competitive ELISA test based on p72 (Kit A competitive ELISA)
- Commercial indirect ELISA test based on cp312 and p30 (Kit A indirect ELISA) with a loss of analytical sensitivity of 2 steps of dilution described in their instructions for use compared to the competition ELISA test kit A
- Commercial competitive ELISA test (Kit B). The diagnostic target is not mentioned in the kit insert.

To standardize results expression, all the values were expressed as competition percentage (S/N%) by using the formula below:

$$\mathbf{S/N\%} = \frac{\mathbf{OD_{sample}}}{\mathbf{OD_{NC}}}\mathbf{x\ 100} \text{ with } \mathbf{S/N\%} = \mathbf{100 - S/P\%} \text{ or } \mathbf{100 - PI\%}$$

**** Negative – Doubtful – Positive***

ELISA TEST	FORMULA	CUT-OFF*	HARMONIZED CUT-OFF
ID Screen® indirect ELISA (ASFS)	$\mathbf{S/P\ \%} = \frac{\mathbf{OD_{sample} - OD_{NC}}}{\mathbf{OD_{PC} - OD_{NC}}}\mathbf{x\ 100}$	**30% < S/P % < 40%**	**60% < S/N % < 70%**
ID Screen® competitive ELISA (ASFC)	$\mathbf{S/N\%} = \frac{\mathbf{OD_{samples} - OD_{PC}}}{\mathbf{OD_{NC} - OD_{PC}}} \times \mathbf{100}$	**40% < S/N% < 50%**	**40% < S/N % < 50%**
Kit A competitive ELISA	$\mathbf{Blocking\%} = \frac{\mathbf{OD_{NC} - OD_{sample}}}{\mathbf{OD_{NC} - OD_{PC}}}\mathbf{x\ 100}$	**40 % <PI%<50%**	**50% < S/N % < 60%**
Kit A indirect ELISA	$\mathbf{S/P\%} = \frac{\mathbf{OD_{sample} - OD_{NC}}}{\mathbf{OD_{PC} - OD_{NC}}}\mathbf{x\ 100}$	**45% < S/P % < 50%**	**50% < S/N % < 55%**
Kit B ELISA	$\mathbf{Inhibition\%} = \mathbf{1} - \frac{\mathbf{OD_{sample}}}{\mathbf{OD_{NC}}}\mathbf{x\ 100}$	**25 % ≤ PI%**	**75% < S/N %**

ANALYTICAL SENSITIVITY

Analytical sensitivity was tested using serial dilutions of 11 samples positive for African Swine Fever:

- 8 positive sera from experimentally ASFV infected (vaccinated or not) pigs, sampled after recovery (Sample 1 to 8)
- 3 positive EU reference-pig serum, commercially available at ASF-EURL, CISA-INIA, for validation (Sample 9 to 11).

The ASF antibody status of these samples had been previously confirmed by other techniques (data not shown). The results are summarized in Table 11.

SAMPLE	DILUTION	ID SCREEN® iELISA (ASFS) Cut-off: 60-70%		ID SCREEN® cELISA (ASFC) Cut-off: 40-50%		Kit A competitive ELISA Cut-off: 50-60%		Kit A indirect ELISA Cut-off: 50-55%		Kit B Cut-off: 75%<	
		S/N%	STATUS	S/N%	STATUS	S/N%	STATUS	S/N%	STATUS	S/N%	STATUS
Sample 1	neat	0	(+)	2	(+)	1	(+)	25	(+)	6	(+)
	1: 16	31	(+)	36	(+)	-2	(+)	86	(-)	38	(+)
	1: 64	56	(+)	69	(-)	-1	(+)	96	(-)	70	(+)
	1: 256	81	(-)	96	(-)	8	(+)	98	(-)	92	(-)
	1: 1024	90	(-)	97	(-)	29	(+)	98	(-)	93	(-)
Sample 2	neat	0	(+)	1	(+)	2	(+)	0	(+)	5	(+)
	1: 16	2	(+)	12	(+)	-4	(+)	46	(+)	16	(+)
	1: 64	24	(+)	31	(+)	-3	(+)	78	(-)	56	(+)
	1: 256	57	(+)	64	(-)	1	(+)	93	(-)	81	(-)
	1: 1024	79	(-)	81	(-)	13	(+)	97	(-)	91	(-)
Sample 3	neat	0	(+)	-1	(+)	1	(+)	19	(+)	5	(+)
	1: 16	16	(+)	18	(+)	-1	(+)	81	(-)	49	(+)
	1: 64	48	(+)	56	(-)	7	(+)	94	(-)	76	(-)
	1: 256	75	(-)	93	(-)	26	(+)	97	(-)	88	(-)
	1: 1024	88	(-)	97	(-)	54	(+/-)	98	(-)	94	(-)
Sample 4	neat	2	(+)	-1	(+)	0	(+)	27	(+)	13	(+)
	1:16	17	(+)	21	(+)	-1	(+)	83	(-)	52	(+)
	1: 64	46	(+)	49	(+/-)	4	(+)	94	(-)	90	(-)
	1: 256	74	(-)	87	(-)	16	(+)	97	(-)	94	(-)
	1: 1024	89	(-)	93	(-)	43	(+)	98	(-)	104	(-)
Sample 5	neat	6	(+)	2	(+)	4	(+)	15	(+)	5	(+)
	1: 16	39	(+)	41	(+/-)	14	(+)	83	(-)	43	(+)
	1: 64	65	(+/-)	68	(-)	29	(+)	94	(-)	76	(-)
	1: 256	85	(-)	97	(-)	46	(+)	97	(-)	93	(-)
	1: 1024	92	(-)	107	(-)	71	(-)	98	(-)	94	(-)
Sample 6	neat	6	(+)	1	(+)	3	(+)	15	(+)	7	(+)
	1: 16	35	(+)	27	(+)	64	(-)	70	(-)	24	(+)
	1: 64	49	(+)	50	(+/-)	75	(-)	89	(-)	50	(+)
	1: 256	70	(-)	107	(-)	84	(-)	95	(-)	83	(-)
	1: 1024	86	(-)	87	(-)	89	(-)	98	(-)	88	(-)
Sample 7	neat	10	(+)	2	(+)	4	(+)	19	(+)	17	(+)
	1: 16	28	(+)	23	(+)	41	(+)	70	(-)	40	(+)
	1: 64	40	(+)	32	(+)	56	(+/-)	89	(-)	64	(+)
	1: 256	57	(+)	47	(+/-)	72	(-)	95	(-)	91	(-)
	1: 1024	77	(-)	63	(-)	80	(-)	98	(-)	90	(-)
Sample 8	neat	0	(+)	1	(+)	2	(+)	15	(+)	4	(+)
	1: 16	24	(+)	20	(+)	78	(-)	60	(-)	19	(+)
	1: 64	34	(+)	32	(+)	89	(-)	81	(-)	59	(+)
	1: 256	54	(+)	52	(-)	92	(-)	92	(-)	88	(-)
	1: 1024	75	(-)	79	(-)	99	(-)	97	(-)	91	(-)
Sample 9	neat	14	(+)	37	(+)	37	(+)	56	(-)	14	(+)
	1: 4	23	(+)	57	(-)	60	(-)	80	(-)	32	(+)
	1: 16	44	(+)	66	(-)	70	(-)	91	(-)	62	(+)
Sample 10	1: 4	-6	(+)	-2	(+)	1	(+)	0	(+)	14	(+)
	1: 16	3	(+)	-1	(+)	2	(+)	27	(+)	49	(+)
	1: 64	9	(+)	3	(+)	7	(+)	61	(-)	84	(-)
Sample 11	neat	47	(+)	12	(+)	25	(+)	71	(-)	90	(-)
	1: 4	52	(+)	40	(+)	54	(+/-)	89	(-)	93	(-)
	1: 16	74	(-)	72	(-)	83	(-)	95	(-)	96	(-)

** (-) Negative – (+/-) Doubtful – (+) Positive*

SAMPLE	LAST POSITIVE DILUTION	ID SCREEN® Ielisa (ASFS)	ID SCREEN® Celisa (ASFC)	Kit A competitive ELISA	Kit A indirect ELISA	Kit B
Experimentally infected pig sera, n=8 (Sample 1 to 8)	**neat**	8/8	8/8	8/8	8/8	8/8
	1: 16	8/8	8/8	**6/8****	**1/8****	8/8
	1: 64	5/8	3/8	1/8	0/8	5/8
	1: 256	3/8	1/8	1/8	0/8	0/8
	1: 1024	0/8	0/8	4/8	0/8	0/8
EU reference samples, n=3 (Sample 9 to 11)	**neat**	3/3	3/3	3/3	**1/3***	**2/3***
	1: 4	3/3	2/3	2/3	1/3	2/3
	1: 16	2/3	1/3	1/3	1/3	1/3
	1: 64	1/3	1/3	0/3	0/3	0/3

** Sample 9 and 11 were not detected by Kit A indirect and Sample 11 by Kit B*

Table 11: Comparison of the analytical sensitivity between the ID Screen ® African Swine Fever Indirect ELISA and other commercial tests

RESULTS (Table 11):

- **Most neat (undiluted) samples were correctly identified by all ELISA tests** (2 EU ASF antibody reference samples were not detected by Kit A indirect and 1 by Kit B *)
- **When testing the 8 sera from experimentally infected pigs** (Sample 1 to 8) **diluted:**
  - 8 were correctly identified by the ID Screen® indirect ELISA (ASFS), the ID Screen® competitive ELISA (ASFC) and Kit B.
  - 6/8 samples were found positive with the Kit A competitive ELISA**
  - 1/8 was found positive with the Kit A indirect ELISA**
- **When comparing analytical sensitivity for theses 8 sera:**
  - ID Screen® iELISA ASFS detected 5 samples up to 1:64 and 3 samples up to 1:256 dilution
  - ID Screen® cELISA ASFC detected 4 samples up to 1:16 ; 3 samples up to 1:64 and 1 sample up to 1:256 dilution
  - Kit A competitive ELISA detected 1 sample up to 1:64 ; 1 sample up to 1:256 ; 4 samples up to 1:1024 and 2 samples are not detected
  - Kit A indirect ELISA detected 1 sample up to 1:16 and 7 samples are not detected
  - Kit B detected 3 samples up to 1:16 and 5 samples up to 1:64
- **For the 3 positive pig EU reference serum**:
  - ID Screen® indirect ELISA: all samples were positive (up to at least 1:4 dilution)
  - ID Screen® competitive ELISA: all samples were positive
  - Kit A competitive ELISA: all samples were positive
  - Kit A indirect ELISA: only one sample (Sample 11) was positive
  - Kit B: 2/3 samples (Samples 9 and 11) were positive
- **The analytical sensitivity of the ID Screen® and other commercial ELISA tests is similar, except in the case of indirect ELISA Kit A, which is much lower, and the competitive ELISA Kit A, which failed to detect 2 diluted samples.**

SPECIFICITY AND SENSITIVITY

96 negative pig samples from ASF-free areas, as well as 11 positive pig samples were tested with the 5 tests.

The ASF antibody status of samples had been previously confirmed by other techniques (data not shown).

Population distributions of negative and positive sera with the 5 kits are shown in Figure 8.

Table 12 presents comparison of specificity and sensitivity for the ID Screen® ELISA and the four other kits.

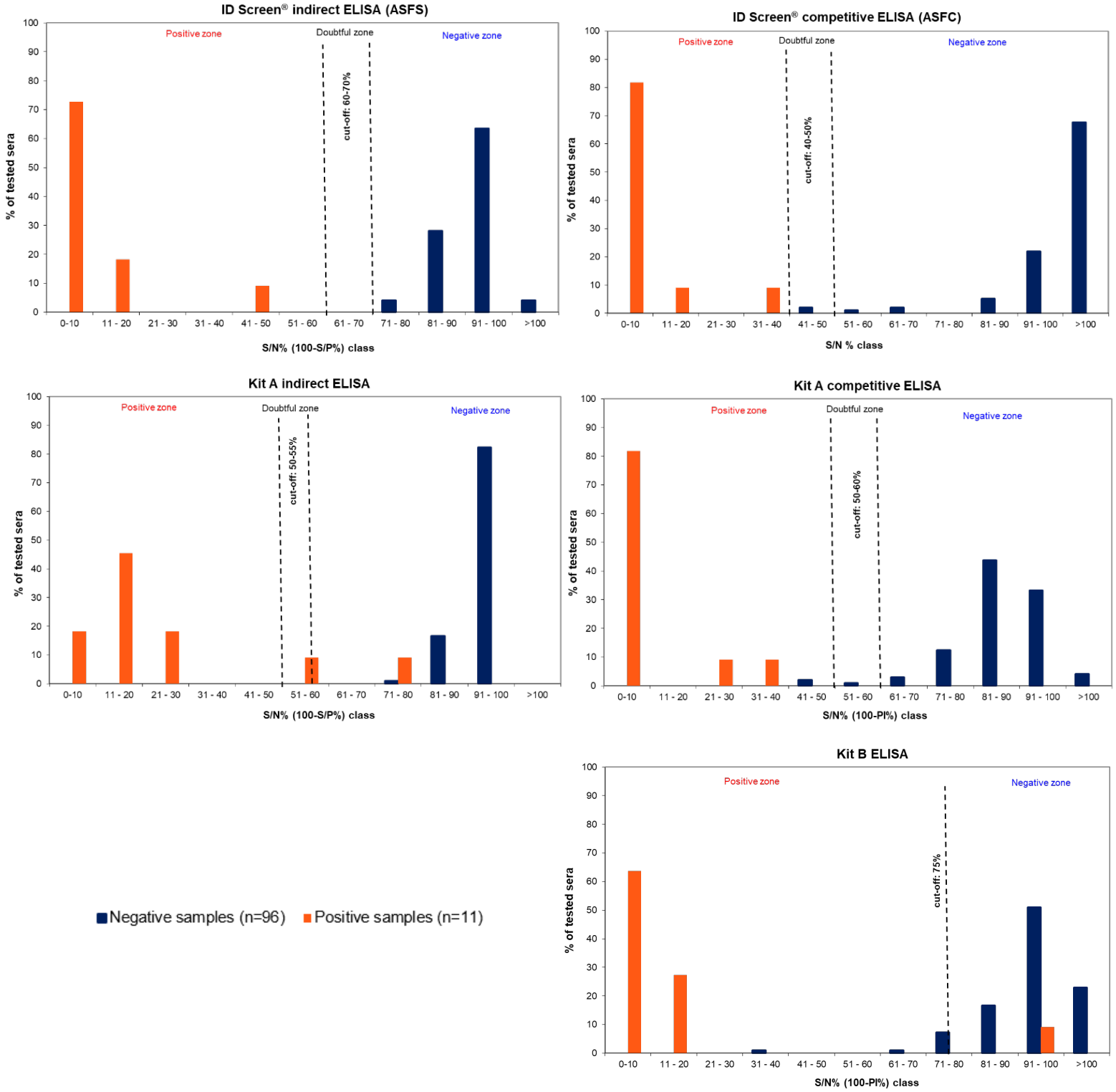


Figure 8: S/N% distributions obtained with the ID Screen® ELISAs and other commercial tests

ELISA TEST	SPECIFICITY $CI_{95\%}$	SENSITIVITY $CI_{95\%}$
ID Screen® indirect ELISA (ASFS)	**100 %** (n = 96) $CI_{95\%}$: 96.15 – 100 %	**100 %** (n = 11) $CI_{95\%}$: 74.12 – 100 %
ID Screen® competitive ELISA (ASFC)	100 % (n = 96) with doubtful sera considered as negative $CI_{95\%}$: 96.15 – 100 %	100 % (n = 11) $CI_{95\%}$: 74.12 – 100 %
Kit A competitive ELISA	97.9 % (n = 96) with doubtful sera considered as negative $CI_{95\%}$: 92.72 – 99.43 %	100 % (n = 11) $CI_{95\%}$: 74.12 – 100 %
Kit A indirect ELISA	100 % (n = 96) $CI_{95\%}$: 96.15 – 100 %	81.8 % (n = 11) $CI_{95\%}$: 52.30 – 94.86 %
Kit B ELISA	95.8 % (n = 96) $CI_{95\%}$: 89.77 – 98.37 %	90.91 % (n = 11) $CI_{95\%}$: 62.27 – 98.38 %

Table 12: Comparison of performance obtained with the ID Screen® ELISAs and other commercial tests

RESULTS (Figure 8 and Table 12):

- **In this study, the ID Screen® indirect ELISA,** as well as the ID Screen® competitive ELISA, **offer the best performance with measured specificity and sensitivity of 100%.**

INFLUENCE OF HEMOLYSED SAMPLES ON ELISA TEST PERFORMANCE [4]

The ASF NRLs from Poland, Belgium and Spain, reported deficiency in the specificity of Kit A and false positive results when hemolysed serum samples from wild boars were analysed.

A comparative study was conducted in which these panels of sera were analysed in parallel using Kit A, the OIE ELISA, another commercially available kit (Kit C) and the Innovative Diagnostics Ielisa.

The results obtained by Dr Dixon, L [4]. Are presented in Table 13.

	FALSE POSITIVE (FP) RESULTS FOR HEMOLYZED WILD BOAR SERA								
	TOTAL NUMBER OF SERA	Kit A		OIE ELISA		Kit C		Innovative Diagnostics	
		Number of FP	FP%	Number of FP	FP %	Number of FP	FP %	Number of FP	FP %
Poland	145	69	**47.6**	7	**4.8**	28	**19.3**	0	**0**
Belgium	13	7	**53.8**	3	**23.1**	5	**38.5**	0	**0**
Spain	85	13	**15.3**	4	**4.7**	7	**8.2**	0	**0**
Total	243	89	**37**	14	**6**	40	**16**	0	**0**
MEASURED SPECIFICITY		**63%**		**94%**		**84%**		**100%**	

Table 13: ELISA results for the analysis of 243 negative wild boar sample (2014)

RESULTS (Table 13):

- The Innovative Diagnostics ID Screen® African Swine Fever Indirect ELISA showed the **best specificity, when analysing hemolysed wild boar sera.**

EXTERNAL DATA ON WARTHOGS: SEROPREVALENCE STUDIES[5]

Serum samples collected from 19 warthogs from Saadani National Park in Tanzania were tested using the ID Screen® ELISA [5]

Distribution of warthog samples , by sex and age, n=19 is presented in Table 14. The S/P% values are summarized in Figure 9.

	ADULT WARTHOGS		JUVENILE WARTHOGS (<6MONTHS)		TOTAL
	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	
Male	2	1	2	0	5
Female	9	0	3	2	14
TOTAL	**11**	**1**	**5**	**2**	**19**

Table 14: Distribution of sex and age groups of warthogs sampled, n=19

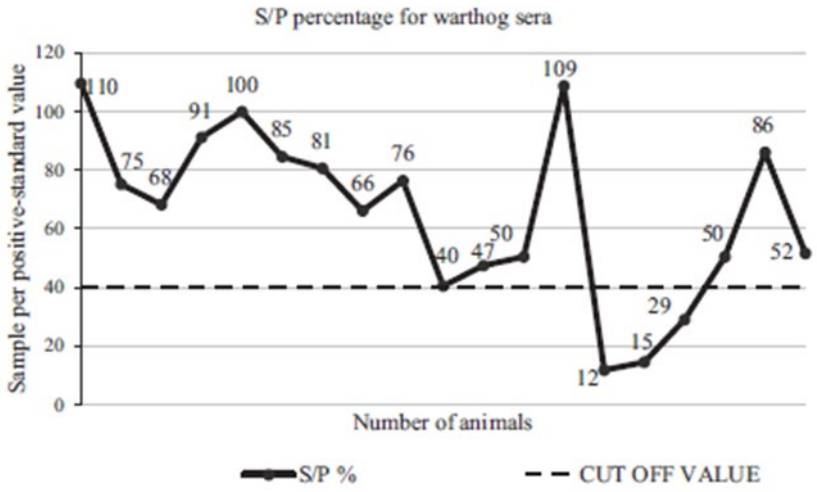


Figure 9: S/P % for warthog sera

RESULTS (Table 14 and Figure 9):

- **16 of the 19 warthog sera tested using the ID Screen® African Swine Fever Indirect ELISA were positive for antibodies against ASFV corresponding to a seroprevalence of 84%.**
- Of the 16 positive samples, 14 (87.5%) had an S/P% higher than 50, and two samples had an S/P% of above 100%, interpreted as intense exposure of warthogs to the virus.
- **the ID Screen® African Swine Fever Indirect ELISA can be used to detect antibodies against ASFV in warthogs.**

REPEATABILITY

Intra-plate repeatability was evaluated by measuring the coefficient of variation (CV%) for 36 repetitions of a strong positive sample, and 60 repetitions of a weak positive sample.

Results are considered conform if the CV% is less than 15%.

OD results are shown in the Table 15 below.

OD AT 450NM											
0.668	0.624	0.646	0.619	0.659	1.082	1.064	0.666	0.635	0.670	0.612	0.679
0.716	0.653	0.672	0.714	0.586	1.026	1.024	0.669	0.676	0.651	0.589	0.629
0.623	0.684	0.645	0.669	0.664	1.043	1.085	0.715	0.697	0.619	0.628	0.683
1.056	1.052	1.060	1.059	1.028	1.093	1.049	1.094	1.042	1.064	1.082	1.089
1.053	1.093	1.015	1.039	1.067	1.113	1.061	1.098	1.097	1.115	1.094	1.092
0.659	0.618	0.675	0.626	0.689	1.049	1.081	0.673	0.681	0.679	0.682	0.684
0.625	0.706	0.712	0.686	0.668	1.023	1.030	0.596	0.648	0.629	0.704	0.676
0.581	0.683	0.641	0.636	0.721	1.093	1.075	0.692	0.683	0.642	0.624	0.585

	AVERAGE OD	STANDARD DEVIATION	MINIMUM	MAXIMUM	CV%
Weak positive sample	0.658	0.036	0.581	0.721	**5**
Strong positive sample	1.066	0.027	1.015	1.115	**3**

Table 15: Intra-plate repeatability results for ID Screen® African Swine Fever Indirect ELISA.

RESULTS (Table 15):

- The CV% obtained were 5% for the weak positive sample and 3% for the strong positive sample, demonstrating **excellent test repeatability**.

REPRODUCIBILITY

A strong positive serum was diluted in a pool of negative sera in order to generate a positive, a doubtful and a negative sample.

Dilutions were tested in 10 independent runs by different operators and on different days.

Results are considered conform if the CV% is less than 15% and the values are within ± 2 standard deviations around the mean.

Results are expressed in Figure 10 and Table 16 below.

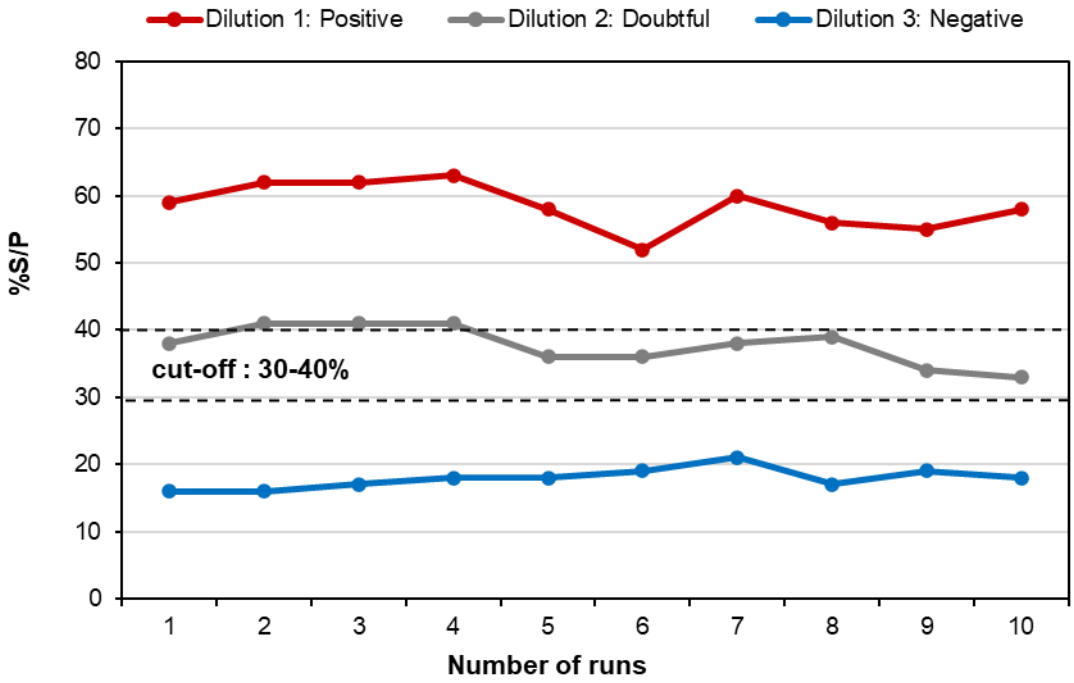


Figure 10: S/P% values for 3 diluted sera tested in 10 independent runs

	MEAN S/P%	STANDARD DEVIATION	CV%
Negative sample	17.9	2	**9**
Doubtful sample	37.7	2.9	**8**
Positive sample	58.5	3.1	**5**

Table 16: S/P% values for 3 sera diluted tested in 10 independent runs.

RESULTS (Figure 10 and Table 16):

- **All values are within a range of 2 standard deviations around the mean, with a CV% between 5% and 9%.**
- These results illustrate the **high reproducibility of the ID Screen® ELISA test.**

ROBUSTNESS

Test robustness was evaluated by 3 operators in 3 independent runs.

Robustness was evaluated by testing the maximum and minimum conditions of time and temperature of incubation as defined in the instructions for use:

- Sample incubation: 45 minutes ± 5 minutes at 21°C (± 5°C);
- Conjugate incubation: 30 minutes ± 3 minutes at 21°C (± 5°C);
- Substrate Solution incubation: 15 minutes ± 2 minutes at 21°C (± 5°C).

For each condition, the test is validated if:

- The mean value of the Positive Control OD (OD_{PC}) is greater than 0.350 ($OD_{PC} > 0.350$).
- The ratio of the mean values of the Positive and Negative Controls (OD_{PC} and OD_{NC}) is greater than 3 ($OD_{PC}/OD_{NC} > 3$).

Optical densities at 450nm obtained in each condition for both negative and positive controls and the S/P% values obtained for 3 dilutions of a positive sample and 2 negative samples are detailed in Table 17.

SAMPLES/CONJUGATE/SUBSTRATE INCUBATION TIME	45 MIN / 30 MIN / 15 MIN			41 MIN / 27 MIN / 13 MIN	49 MIN / 33 MIN / 17 MIN	
INCUBATION TEMPERATURE	16°C	21°C	26°C	16°C	26°C	
Positive control	0.832	1.013	1.023	0.722	1.14	OD 450 NM
	0.797	0.94	0.996	0.692	1.131	
Negative control	0.046	0.047	0.032	0.044	0.049	
	0.047	0.037	0.039	0.042	0.053	
$OD_{PC} > 0.350$	√	√	√	√	√	
$OD_{PC} / OD_{NC} > 3$	√	√	√	√	√	
MRI-ASF diluted 1:64	56	49	57	50	57	S/P%
MRI-ASF diluted 1:256	32	30	33	33	33	
MRI-ASF diluted 1:1024	18	18	20	17	20	
Negative sample 1	12	7	7	8	7	
Negative sample 2	7	6	7	6	7	

Table 17: Robustness study for the ID Screen® ELISA

RESULTS (Table 17):

- For each time and temperature condition, **the test validation criteria described for both positive and negative controls were obtained.**
- For each time and temperature condition, the S/P values obtained were similar, and analytical sensitivity was constant, thereby demonstrating the **excellent robustness** of the ID Screen® ELISA.

STABILITY

The shelf-life of the products is evaluated by the technique of accelerated ageing.

The stability of the plates and the positive control was tested by evaluating the residual activity of individual components after storage at 37°C ± 2°C, with respect to storage at 5°C ± 3°C.

The measured residual activity should be greater than 75% after 1 month of storage at 37°C ± 2°C.

Results are shown in Figure 11 below.

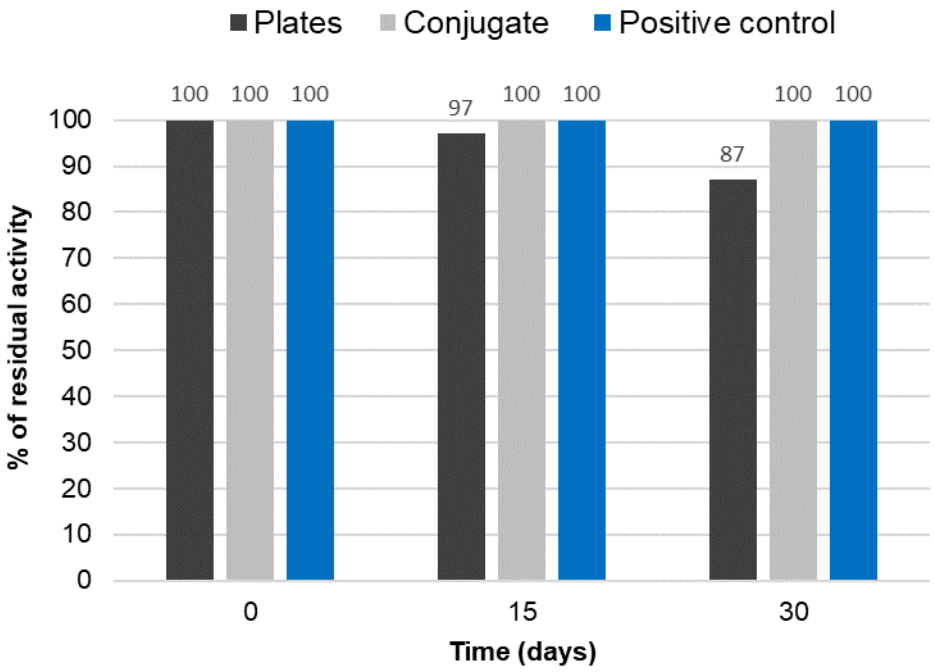


Figure 11: Percentage of residual activity of the plates. Positive control and conjugate after stability testing at 37°C.

RESULTS (Figure 11):

- After1 month at 37°C, the plates, the conjugate and the positive control showed residual activity of 87%, 100% and 100% respectively, thus indicating **high component stability**.

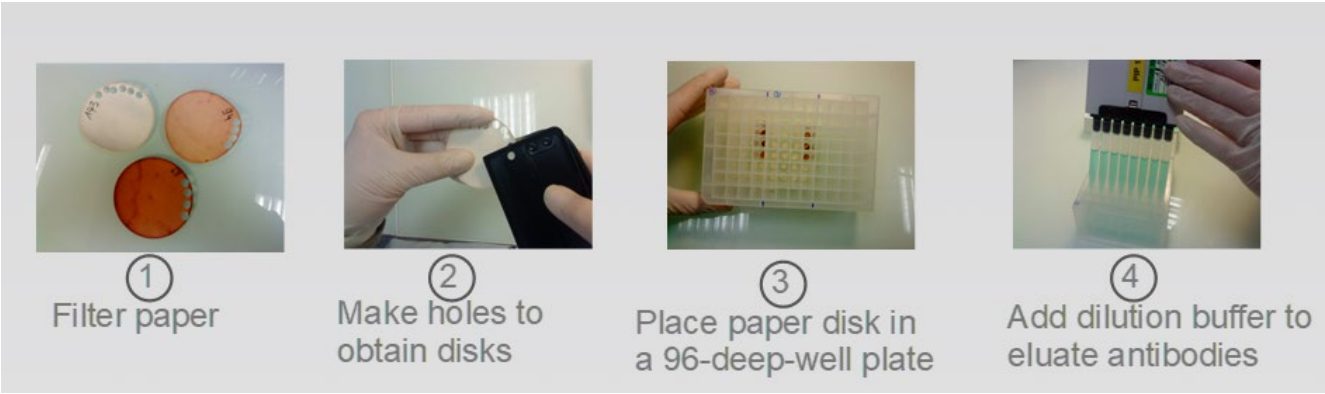
FILTER PAPER SAMPLES

SAMPLE COLLECTION

Filter paper samples are obtained by dipping Whatman paper (#1 or #3) in blood taken from animal's ears.

The use of this sample type facilitates sample processing: they are easily collected, and once dry, are easily transportable, cannot be contaminated, and are particularly adapted to collection in isolated regions.

The filter paper disk protocol is illustrated on the pictures below.



The deep-well format facilitates testing of large numbers of samples. In addition, this format allows the eluate to be easily re-tested if necessary.

SPECIFICITY

90 negative animals were tested in parallel using the serum protocol and the filter paper protocol.

These samples were obtained from disease-free animals in France (Cantal) within the framework of PRRS control.

Results are summarized in Figure 12.

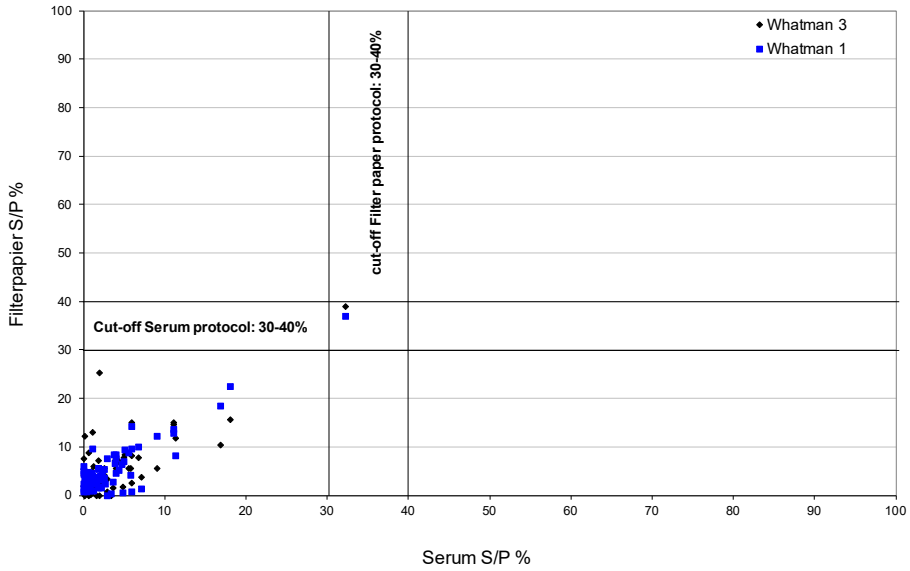


Figure 12: Correlation between S/P% values obtained by serum and paper disk protocols (n=90).

RESULTS (Figure 12):

- Excellent correlation between serum and filter paper sample was observed.
- **Measured test agreement = 100%.**

ANALYTICAL SENSITIVITY

3 positive sera from experimentally-infected pigs (ASFV strain Ourt 88/3) were tested:

- Each serum was serially-diluted in a negative serum
- Each dilution was then deposited on a filter paper before being tested, in parallel, using the serum protocol and the filter paper protocol.

SAMPLE	DILUTION	FILTER PAPER PROTOCOL		SERUM PROTOCOL
		Whatman #1 S/P%	Whatman# 3 S/P%	S/P%
Serum 01	Pure	204 (+)	198 (+)	201 (+)
	1:2	198 (+)	188 (+)	198 (+)
	1:4	182 (+)	174 (+)	192 (+)
	1:8	155 (+)	159 (+)	166 (+)
	1:16	123 (+)	113 (+)	130 (+)
	1:32	95 (+)	86 (+)	87 (+)
	1:64	53 (+)	51 (+)	56 (+)
Serum 02	Pure	196 (+)	191 (+)	183 (+)
	1:2	152 (+)	171 (+)	144 (+)
	1:4	125 (+)	133 (+)	103 (+)
	1:8	91 (+)	101 (+)	62 (+)
	1:16	56 (+)	59 (+)	35 (+ / -)
	1:32	39 (+ / -)	31 (+ / -)	19 (-)
	1:64	23 (-)	24 (-)	10 (-)
Serum 03	Pure	179 (+)	184 (+)	139 (+)
	1:2	113 (+)	133 (+)	91 (+)
	1:4	73 (+)	116 (+)	64 (+)
	1:8	51 (+)	74 (+)	37 (+ / -)
	1:16	31 (+ / -)	31 (+ / -)	22 (-)
	1:32	19 (-)	28 (-)	11 (-)
	1:64	9 (-)	21 (-)	6 (-)

Table 18: Comparison of the analytical sensitivity of serum and paper disk for three infected pigs.

RESULTS (Table 18):

- **Antibodies against ASFV strain Ourt 88/3 were detected using filter paper samples.**
- **Analytical sensitivity results obtained with the filter paper protocol are comparable to those obtained with the serum protocol.**

COMMUNICATION OF THE ASF-EURLS

Extract from ***Summary of EU Reference Laboratories meeting on African Swine Fever (ASF) and Classical Swine Fever (CSF)*** *Meeting organized by the European Commission, Brussels, 3 – 4 June 2013*

> *Whole blood collected on filter paper (DB samples) has been shown to be adequate sources of antibodies for laboratory testing using the "ID Screen® ASF Indirect ELISA KIT IDVET" and IPT aiming detection of antibodies to ASF.*

CONCLUSION

The ID Screen® ELISA test allows the use of filter papers, which makes animal sampling easier, especially with wild boars.

Moreover, using the elution protocol in deepwell tubes, with direct transfer to ELISA plates with a multi-channel pipette, is avoiding sample identification errors.

MEAT JUICE SAMPLES

SAMPLE COLLECTION

Meat juice samples are useful for testing carcasses at the slaughterhouse and sample collection is very easy.

The meat juice that exudes after thawing frozen meat is tested.

SPECIFICITY

97 negative samples were tested using the ID Screen® African Swine Fever Indirect ELISA. These samples were obtained from disease-free animals in France.

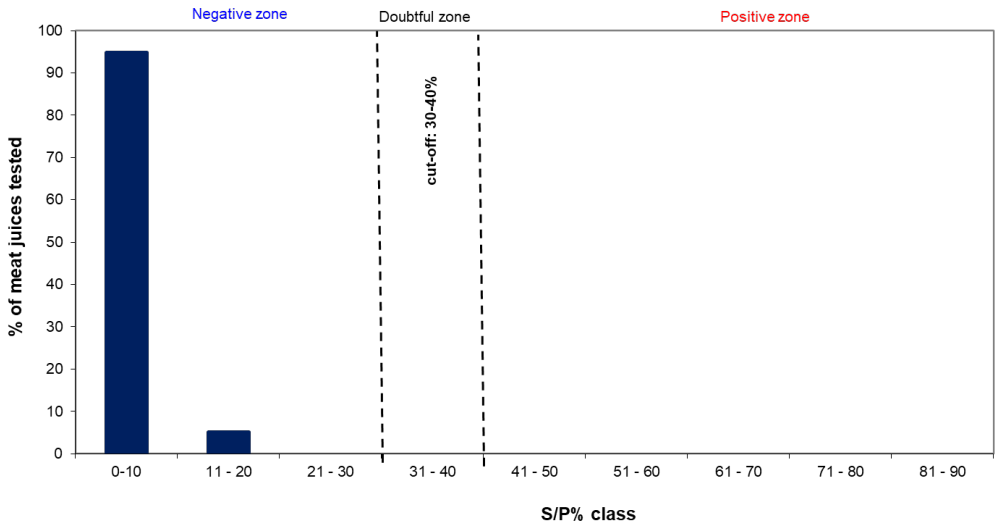


Figure 13: S/P% distribution obtained with the meat juice protocol (n = 97).

RESULTS (Figure 13):

- **All meat juice samples gave negative results**.
- **Measured specificity = 100%** ($CI_{95\%}$: 96.2% - 100%), n = 97.

SENSITIVITY

Innovative Diagnostics has not been able to test pig carcasses from infected animals.

Innovative Diagnostics has, however, developed meat juice protocols for other ID Screen® ELISA kits. Experience has shown that the concentration of antibodies in meat juice samples is 15 to 20 times inferior than in serum samples. Given this observation, test sensitivity was evaluated through the analysis of spiked samples: 5 positive sera were diluted at 1:30 in negative meat juice in order to obtain spiked samples.

RESULTS

- **All spiked samples were correctly identified as positive.**

GENERAL CONCLUSION

The **ID Screen® ASF Indirect** ELISA:

- Is **the only commercial ELISA based on the use of three different recombinant proteins: p32, p62 and p72**.
- **Has been validated by the European Union Reference Laboratory for ASF, in Spain (ASF-EURL, CISA)** (for the serum and filter paper applications)
- Shows an **excellent specificity** and **allows the correct identification of the ASF-EURL, CISA-INIA reference sera**. It also allows the detection of antibodies generated by an active ASFV circulation.
- Offers **the best sensitivity-specificity performance among the commercially-available serological tests**.
- Allows early **seroconversion detection.**
- **Is very suitable for wild boar testing: high specificity, even on haemolyzed samples**.
- **Allows the use of filter papers**, which makes animal sampling easier, especially with wild boars.
- Allows the analysis of **meat juice samples.**

Related products

- **ID Screen® African Swine Fever Competition** (product code: ASFC-2P; ASFC-5P): Competitive ELISA for the detection of anti-African Swine Fever antibodies in serum or plasma of swine, wild boar and other susceptible species.
- **ID Screen® ASF Oral Fluids-Biwell** (product code: ASFOFB-2P): Indirect ELISA for the detection of antibodies against the African Swine Fever virus in oral fluids from swine.
- **ASF positive Freeze-dried serum** (product code: MRI-ASF): Freeze-dried swine serum containing anti-ASF specific antibodies. To be used as internal reference material for quality control. This serum does not contain any infectious material.
- **ID Gene™ African Swine Fever Duplex** (product code: IDASF-50; IDASF-100): Real-time PCR kit that amplifies a target sequence in African Swine Fever (ASFV) viral genome.
- **ID Gene™ African Swine Fever Triplex** (product code: IDASFTRI-50; IDASFRI-100): Real-time PCR kit that amplifies a target sequence in the African Swine Fever virus (ASFV) genome, as well as endogenous and exogenous internal controls.
- **Freeze-dried ASF positive extraction control** (product code: PEC-ASF) For use with the liquid format of the ID Gene™ African Swine Fever Duplex and Triplex kits.

Since the ASF product range is evolving rapidly, please consult our website at www.innovative-diagnostics.com, to know the currently available associated products.

References

(1) OIE Terrestrial Manual, African Swine Fever (Infection with African Swine Fever Virus) , chapter 3.9.1, version adopted in May 2021.

(2) Final validation of the ASF diagnostic kit ID SCREEN® AFRICAN SWINE FEVER INDIRECT: *Assessment report performed by the Centro de Investigación en Sanidad Animal (CISA-INIA), European Union reference laboratory for ASF*, 21 April 2014 (available upon request).

(3) Gallardo, C et al. *5-Methods for African swine fever diagnosis in clinical and environmental samples.* Published Online: March 10, 2021. Pages 141-160*,* Understanding and combatting African Swine Fever: A European perspective. Lacolina, L. et al (eds.). https://doi.org/10.3920/978-90-8686-910-7_5

(4) Dixon, L. et al. ASFV Challenges for Diagnosis: preventing and controlling viral diseases. Presentation given at the OIE-CIC Joint international meeting on ASF, Paris, 30 June 1 July 2014.

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History of revisions

VERSION	EDIT DATE	REFERENCE	TYPE OF REVISION	REVISION MADE
0115	10/2018	DOC683	**Update:** Addition/Edition of validation data	Addition of the Repeatability, Reproducibility and Robustness data.
	03/2022	DOC1069	**Update:** Addition/Edition of validation data	• Specificity chapter: Additional data for wild boar samples, now provided in a separate graph • EURL validation chapter: Update to mention the most recent data and communication, with a new graphic representation population distribution • Addition of several new chapters: ○ Summary of performance on serum sample ○ Cut-off determination ○ Predictive values ○ Study comparison with other commercial ELISA ○ External data on warthogs ○ Stability • Filter paper samples chapter: Addition of a communication from the EURL validation report • Update of the general conclusion, related products, and references • Innovative Diagnostics now mentioned as the manufacturer of the IDvet product range.

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