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INTERNAL VALIDATION REPORT

ID SCREEN® CLASSICAL SWINE FEVER E2 COMPETITION

COMPETITIVE ELISA FOR THE DETECTION OF ANTIBODIES AGAINST THE CSFV E2 GLYCOPROTEIN IN SERUM AND PLASMA FROM SWINE OR WILD BOAR

METHOD	**Competitive ELISA**
TARGET	• Antibodies directed against the E2 glycoprotein of the Classical Swine Fever virus (CSFV)
SAMPLE TYPES	• Serum • Plasma
VALIDATED SPECIES	• Swine
PRODUCT CODE	CSFE2C

With you at every step

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INTRODUCTION

Classical swine fever (CSF) or hog cholera is a highly contagious disease affecting pigs and wild boars that occurs in an acute, subacute, chronic, or persistent form.

The disease is caused by the CSF virus (CSFV, genus *Pestivirus* family *Flaviviridae*). CSFV is closely related to the ruminant *Pestivirus* which cause Bovine Viral Diarrhoea (BVD) and Border Disease (BD).

In its acute form, the disease is characterized by high fever, severe depression, multiple superficial and internal haemorrhages, as well as high morbidity and mortality. In its chronic form, the signs of depression, anorexia, and fever are less severe than in the acute form, and recovery is occasionally seen in mature animals. Transplacental infection with viral strains of low virulence often results in persistently infected piglets, which constitute a major cause of virus dissemination to non-infected farms.

Serology may be used for disease diagnosis and control.

The ID Screen® Classical Swine Fever E2 Competition kit detects antibodies directed against the E2 glycoprotein in serum or plasma from swine and wild boar. The method indicates exposure to the virus by natural infection or by vaccination.

This report summarizes the validation data for this assay.

DESCRIPTION AND PRINCIPLE OF THE TEST

Wells are coated with the recombinant E2 glycoprotein. Samples to be tested and controls are added to the microwells. Anti-E2 antibodies, if present, form an antibody-antigen complex which masks the E2 epitopes.

An anti-E2 horseradish -peroxidase (HRP) conjugate is added to the microwells. It binds the remaining free E2 epitopes, forming an antigen-conjugate-HRP complex. After washing in order to eliminate the excess conjugate, the substrate solution (TMB) is added.

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

For each sample, the S/N% ratio is calculated and interpreted as follows : $\mathrm{S/N\%} = \frac{\mathrm{OD_{Sample}}}{\mathrm{OD_{NC}}} \mathrm{x}\ 100$

RESULT	STATUS
S/N% ≤ 50%	**Positive**
50% < S/N% ≤ 60%	**Doubtful**
S/N% > 60%	**Negative**

SPECIFICITY

Specificity of the ID Screen® CSFE2C ELISA kit was evaluated by testing :

- with the short incubation protocol (n=466):
  - 350 swine sera from CSF-free herds from Switzerland,
  - 116 swine sera from French CSF-free herds, that were provided by the Laboratoire des Pyrénées et des Landes (France).
- with the long incubation protocol (n=668):
  - 527 swine sera from CSF-free herds from Switzerland,
  - 141 swine sera from French CSF-free herds, that were provided by the Laboratoire des Pyrénées et des Landes (France).

The results with the short and long incubation protocols are respectively shown in Figure 1 and Figure 2.

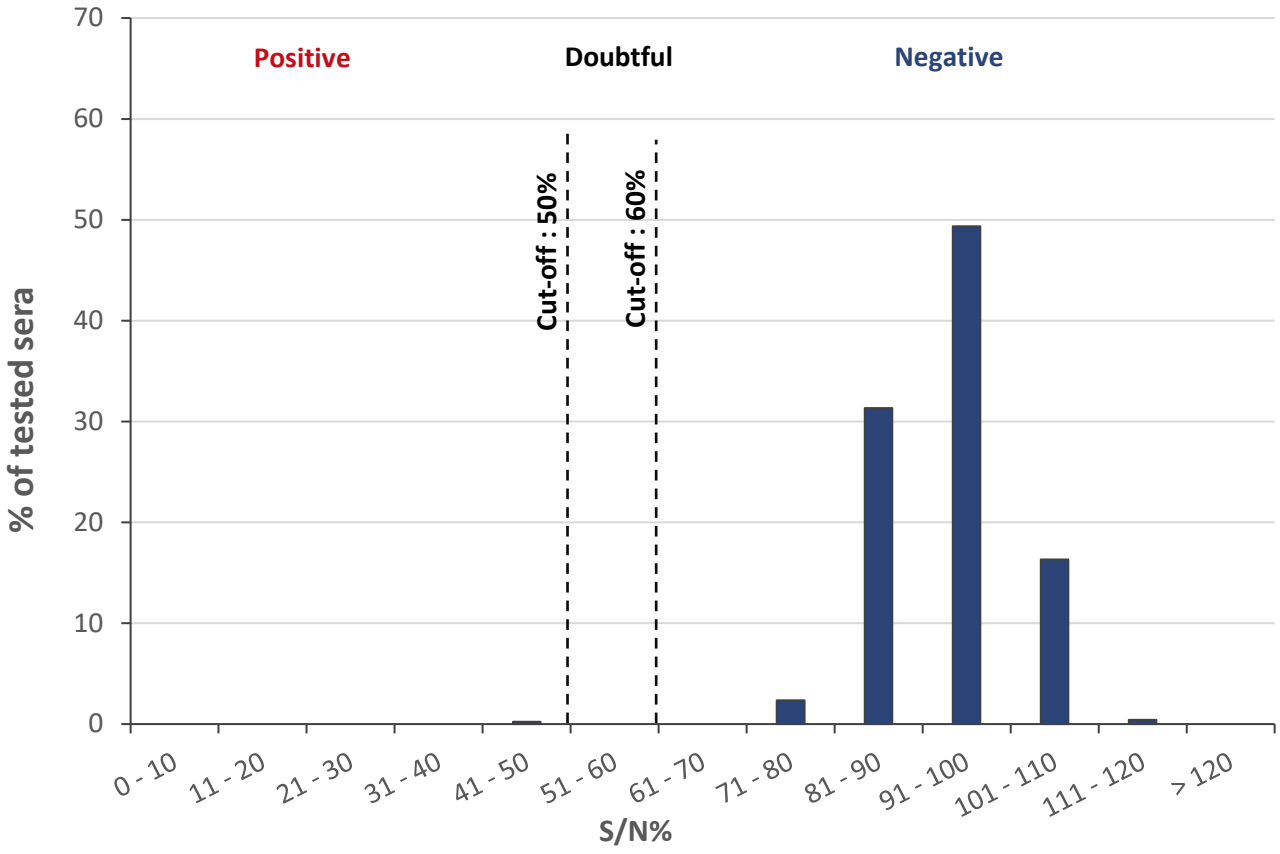


Figure 1 : S/N% distribution for negative sera obtained with the short incubation protocol, n=466

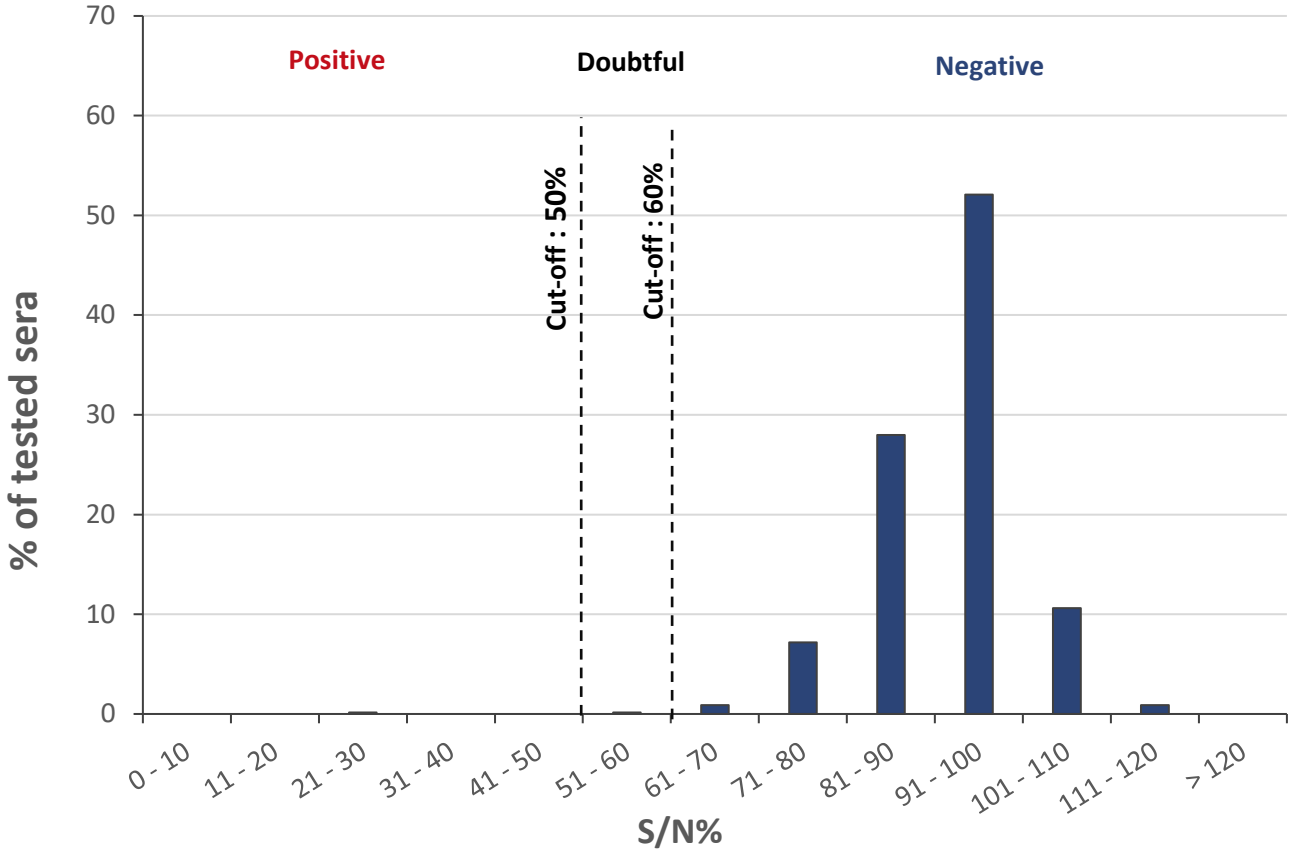


Figure 2 : S/N% distribution for negative sera obtained with the long incubation protocol, n=668

RESULTS (Figures 1 and 2) :

- 465/466 sera are found negative with the short incubation protocol.
- 666/668 sera are found negative with the long incubation protocol.
- **Measured specificity :**
    - **Short incubation : 99.9 %,** 95% CI [98.8, 100.0], n=466
    - **Long incubation : 99.7%,** 95% CI [98.9, 99.9], n=668

ANALYTICAL SENSITIVITY

Innovative Diagnostics produces a positive freeze-dried swine serum containing specific antibodies against the CSFV E2 glycoprotein which may be used to check that the test analytical sensitivity does not vary between runs, operators and batches. This serum standard is available for purchase, product code MRI-CSFE2.

To evaluate the analytical sensitivity of the ID Screen® CSFE2C ELISA kit :

- a batch of the MRI-CSFE2 was titrated and tested by Innovative Diagnostics (Table 1)
- positive sera (identified as #1, #2, #3, #4, #5, #6) from vaccinated animals from Asian countries were titrated and tested in parallel with the ID Screen® CSFE2C kit and another commercially available kit (kit A) (Table 2).

Serum standard (MRI-CSFE2)

		ID SCREEN® CSFE2C			
		SHORT INCUBATION		LONG INCUBATION	
	DILUTION	S/N%	STATUS	S/N%	STATUS
MRI-CSFE2	1:8	26	POSITIVE	28	POSITIVE
	1:16	44	POSITIVE	44	POSITIVE
	1:32	59	DOUBTFUL	71	NEGATIVE
	1 :64	72	NEGATIVE	85	NEGATIVE

Table 1 **:** *Titration of a batch of Innovative Diagnostics' freeze-dried serum standard (MRI-CSFE2) with both protocols of the ID Screen®CSFE2C ELISA*

RESULTS (Table 1):

- With doubtful results considered positive, **the MRI-CFSE2 was detected positive** :
  - diluted 1:32 with the short incubation protocol.
  - diluted 1:16 with the long incubation protocol.

Positive sera (vaccinated animals from Asian countries)

ID SAMPLE	DILUTION	ID SCREEN® CSFE2C			
		CUT-OFF: 50% - 60%			
		SHORT INCUBATION		LONG INCUBATION	
		S/N%	STATUS	S/N%	STATUS
#1	1:2	6	POSITIVE	4	POSITIVE
	1:8	17	POSITIVE	5	POSITIVE
	1:32	53	DOUBTFUL	11	POSITIVE
	1:128	73	NEGATIVE	47	POSITIVE
#2	1:2	8	POSITIVE	5	POSITIVE
	1:8	27	POSITIVE	5	POSITIVE
	1:32	66	NEGATIVE	21	POSITIVE
	1:128	91	NEGATIVE	69	NEGATIVE
#3	1:2	7	POSITIVE	4	POSITIVE
	1:8	23	POSITIVE	4	POSITIVE
	1:32	65	NEGATIVE	13	POSITIVE
	1:128	80	NEGATIVE	50	POSITIVE
#4	1:2	6	POSITIVE	6	POSITIVE
	1:8	16	POSITIVE	4	POSITIVE
	1:32	35	POSITIVE	6	POSITIVE
	1:128	70	NEGATIVE	23	POSITIVE
#5	1:2	26	POSITIVE	5	POSITIVE
	1:8	43	POSITIVE	7	POSITIVE
	1:32	79	NEGATIVE	34	POSITIVE
	1:128	83	NEGATIVE	67	NEGATIF
#6	1:2	18	POSITIVE	6	POSITIVE
	1:8	54	DOUBTFUL	14	POSITIVE
	1:32	81	NEGATIVE	52	DOUBTFUL
	1:128	91	NEGATIVE	76	NEGATIVE

Table 2 : Titration of the sera from vaccinated swine tested with both protocols of the ID Screen® CSFE2C ELISA and kit A, n=6

RESULTS (Table 2):

- With doubtful results considered positive, **the ID Screen® CSFE2C ELISA kit detected positive:**
  - for short incubation protocol :
    - samples #2, #3, #5 and #6 diluted 1:16,
    - samples #1 and #4 diluted 1:32.
  - for long incubation protocol :
    - samples #2, #5 and #6 diluted 1:32,
    - samples #1, #3 and #4 diluted 1:128.

SENSITIVITY

Sensitivity of the ID Screen® CSFE2C ELISA kit was evaluated by testing the following sample :

- With the short protocol (n=103) :
  - 15 CSF-positive sera (genogroup 1.1, 1.2, 1.3, 2.1, 2.2, 2.3) from a panel of swine sera from the European reference laboratory for CSF (Virology laboratory, Hannover University),
  - 88 CSF-positive swine sera taken from vaccinated pigs in Asian countries.
- With the long protocol (n=118) :
  - 15 CSF-positive sera (genogroup 1.1, 1.2, 1.3, 2.1, 2.2, 2.3) from a panel of swine sera from the European reference laboratory for CSF (Virology laboratory, Hannover University),
  - 83 CSF-positive swine sera taken from vaccinated pigs in Asian countries,
  - a panel of 20 CSF-positive sera provided by the French national reference laboratory (Anses, Ploufragan- Plouzané-Niort).

Results for both protocols are presented respectively in Figures 3 and 4.

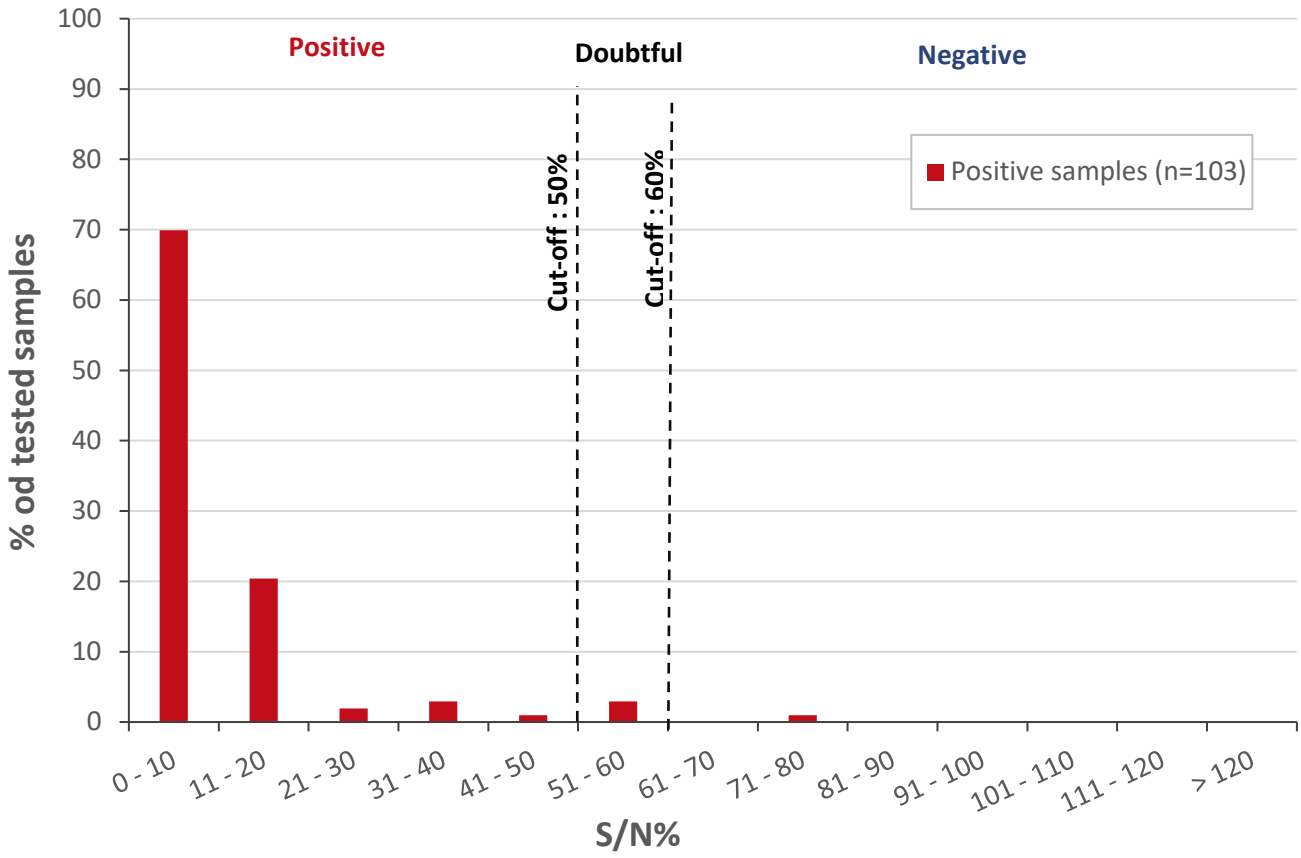


Figure 3 : S/N% distribution for positive sera tested with the ID Screen® CSFE2C ELISA kit for the short incubation protocol, n=103

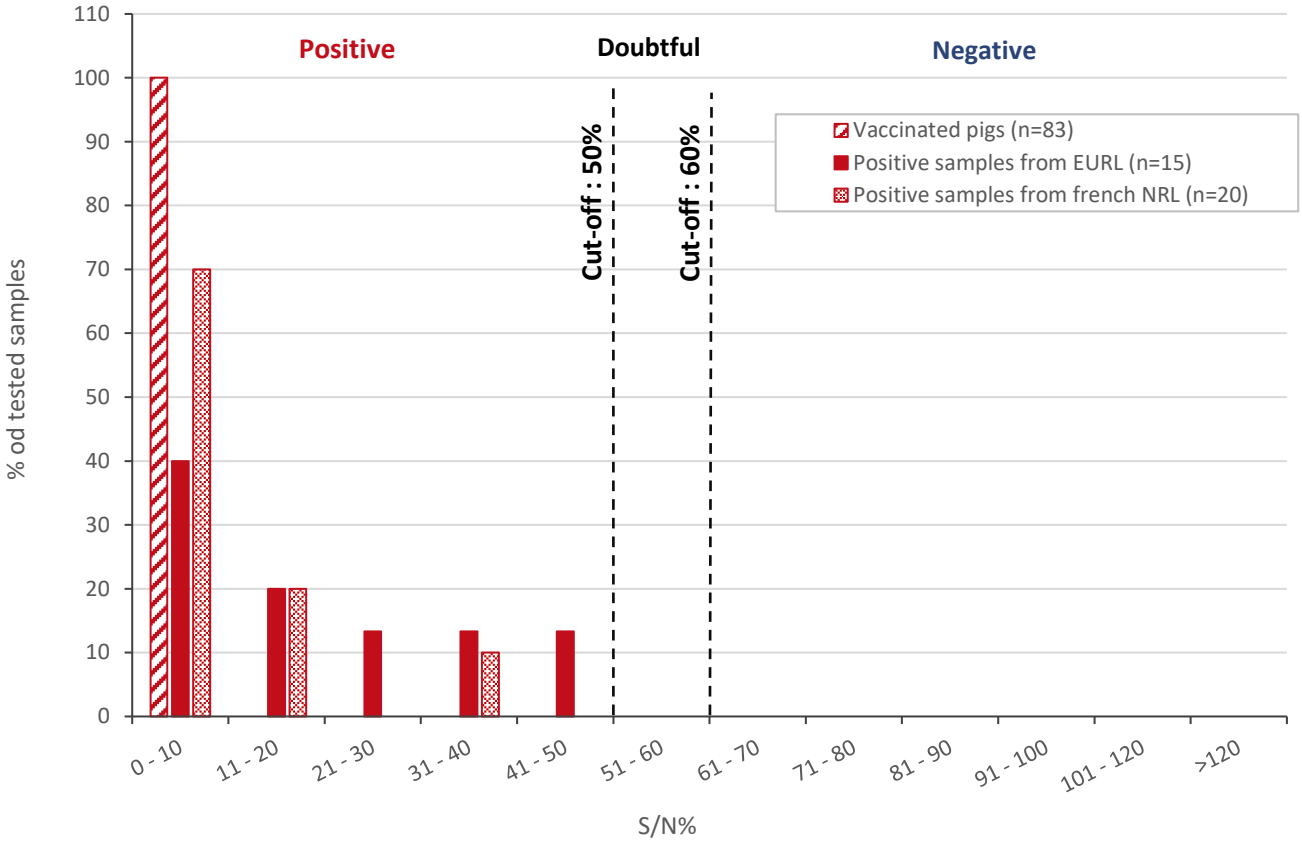


Figure 4 : S/N% distribution for positive sera tested with the ID Screen® CSFE2C ELISA kit for the long incubation protocol, n=118

RESULTS (Figures 3 and 4) :

- 99 / 103 sera were found positive with the short incubation protocol
- 118 / 118 sera were found positive with the long incubation protocol.
- **Measured sensitivity:**
  - **Short incubation protocol : 96.1%**, 95% CI [90.4, 98.5].
  - **Long incubation protocol : 100%**, 95% CI [96, 100].

CUT-OFF VALUE DETERMINATION

To establish the cut-off value of the ID Screen® Classical Swine Fever E2 Competition ELISA kit, a large panel was tested using the short and long incubation protocols.

SHORT INCUBATION PROTOCOL

The panel tested includes :

- Negative samples (n=466):
  - 466 swine sera sampled in Swiss CSF-free herds in 2017.
- Positive samples (n=103):
  - 15 CSF-positive sera (genogroup 1.1, 1.2, 1.3, 2.1, 2.2, 2.3) from a panel of swine sera provided by the European reference laboratory for CSF (Virology laboratory, Hannover University),
  - 88 CSF-positive swine sera taken from vaccinated pigs in Asian countries.

The results, shown in Figure 5, are expressed as S/N% ratios :

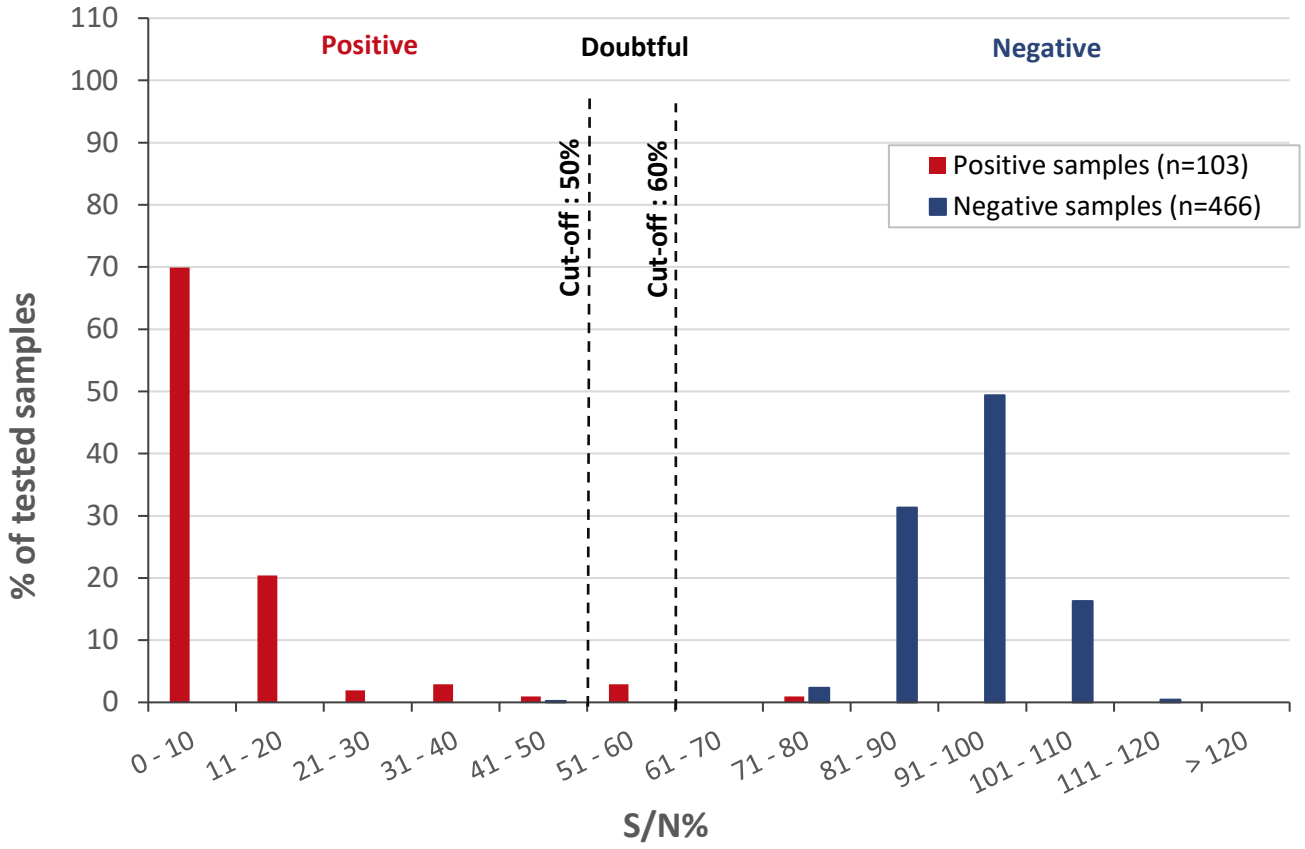


Figure 5 : S/N% distribution for negative (n=466) and positive (n=103) samples tested with the short incubation protocol

LONG INCUBATION PROTOCOL

The panel tested includes :

- Negative samples (n=668):
  - 527 swine sera sampled in Swiss CSF-free herds in 2017,
  - 141 swine sera from French CSF-free herds, that were provided by the Laboratoire des Pyrénées et des Landes, France.
- Positive samples (n=118):
  - 15 CSF-positive sera (genogroup 1.1, 1.2, 1.3, 2.1, 2.2, 2.3) from a panel of swine sera from the European reference laboratory for CSF (Virology laboratory, Hannover University),
  - 83 CSF-positive swine sera taken from vaccinated pigs in Asian countries,
  - a panel of 20 CSF-positive sera provided by the French national reference laboratory (Anses, Ploufragan- Plouzané-Niort).

The results, shown in Figure 6, are expressed as S/N% ratios :

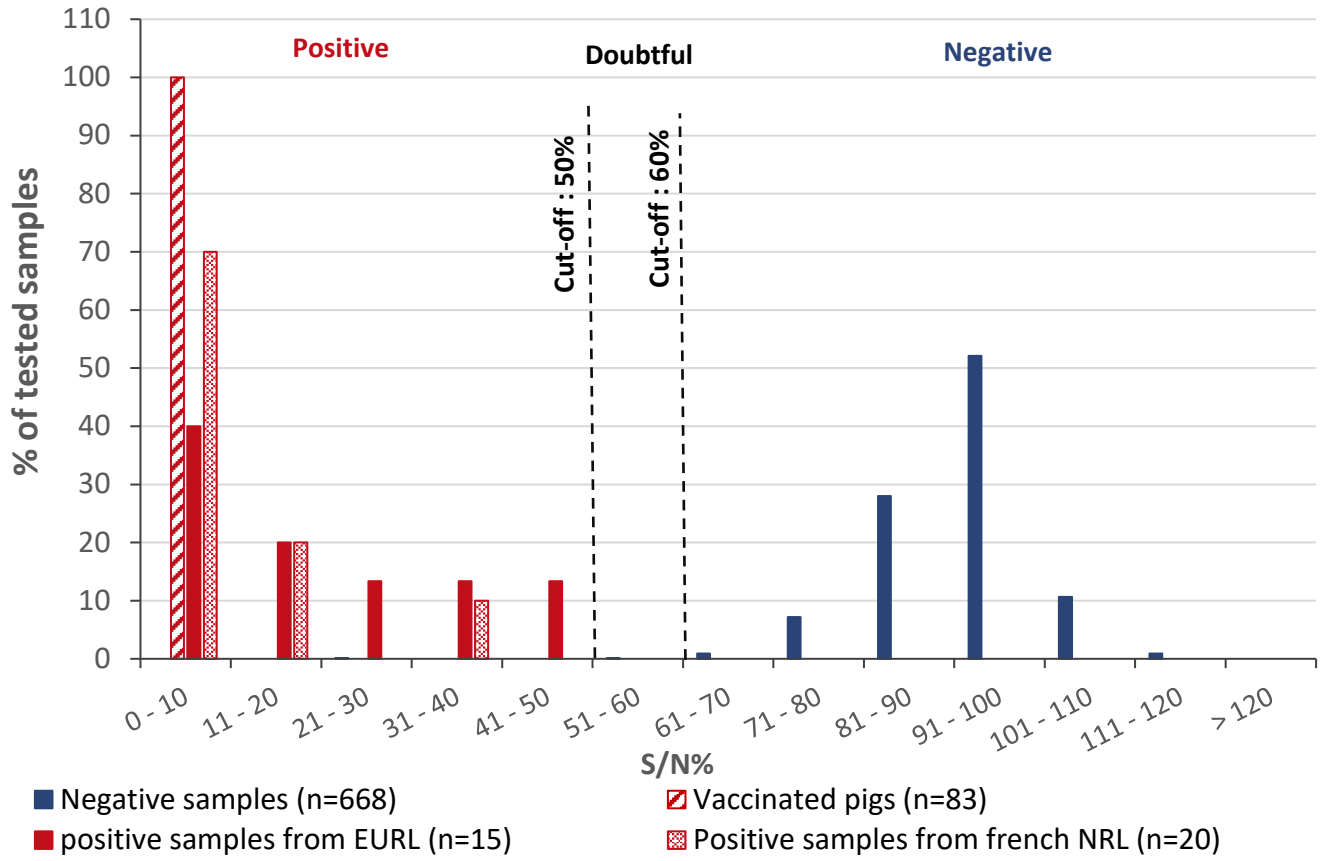


Figure 6 : S/N% distribution for negative (n=668) and positive (n=118) samples tested with the long incubation protocol

Considering the total amount of samples included in this validation, Table 3 below presents, for each threshold value, the diagnostic sensitivity and specificity data, with the 95% confidence interval (lower and upper limits) for the short and the long incubation protocols.

PROTOCOL	THRESHOLD VALUE (S/N%)	SPECIFICITY			SENSITIVITY		
		SPECIFICITY	LOWER LIMIT	UPPER LIMIT	SENSITIVITY	LOWER LIMIT	UPPER LIMIT
SHORT INCUBATION	10	100%	99.4	100	69.9%	61.0	78.8
	20	100%	99.4	100	90.3%	84.6	96.0
	30	100%	99.4	100	92.2%	87.1	97.4
	40	100%	99.4	100	95.1%	91.0	99.3
	50	**99.8%**	**99.4**	**100**	**96.1%**	**92.4**	**99.8**
	60	**99.8%**	**99.4**	**100**	**99.0%**	**97.1**	**100**
	70	99.8%	99.4	100	99.0%	**97.1**	100
	80	97.4%	96.0	98.9	100%	**97.1**	100
	90	66.1%	61.8	70.4	100%	**97.1**	100
	100	16.7%	13.3	20.1	100%	**97.1**	100
LONG INCUBATION	10	100%	99.6	100	87.3%	81.3	93.3
	20	100%	99.6	100	93.2%	88.7	97.8
	30	99.9%	99.6	100	94.9%	91	98.9
	40	99.9%	99.6	100	98.3%	96	100
	50	**99.9%**	**99.6**	**100**	**100%**	**97.5**	**100**
	60	**99.7%**	**99.3**	**100**	**100%**	**97.5**	**100**
	70	98.8%	98.0	99.6	100%	97.5	100
	80	91.6%	89.5	93.7	100%	97.5	100
	90	63.6%	60.0	67.3	100%	97.5	100
	100	11.5%	9.1	13.9	100%	97.5	100

Table 3 : Specificity and sensitivity values obtained for different threshold values for the short and the long incubation protocols

RESULTS (Figures 5, 6 and Table 3):

- Figures 5 and 6 confirm **the very high diagnostic and discrimination capacities** for the short and the long incubation protocols.
- For each incubation protocol, Table 3 showing the diagnostic sensitivity and specificity obtained for the ID Screen® CSFE2C ELISA kit at different cut-offs, confirms that **established threshold** at S/N% values of 60%, with a doubtful zone between 50% and 60%, provides **optimum sensitivity and specificity conditions.**

INCLUSIVITY

Inclusivity of the ID Screen® CSFE2C ELISA kit was tested using 15 samples from a panel of sera taken from pigs infected with 11 different CSF strains. This panel was provided by the European reference laboratory for CSF (Virology laboratory, Hannover University).

Results obtained with both protocols (short and long) are presented in Table 4 below :

					SHORT INCUBATION		LONG INCUBATION	
SAMPLE ID	STRAIN		DAY POST-INFECTION	GENOGROUP	ID Screen® CSFE2C		ID Screen® CSFE2C	
					S/N%	STATUS	S/N%	STATUS
#1	CSF0695	759/Ru	21	1.1	32	POSITIVE	18	POSITIVE
#2	CSF0902	Alfort/187	20	1.1	7	POSITIVE	4	POSITIVE
#3	CSF0375	3795/96	19	1.2	30	POSITIVE	15	POSITIVE
#4	CSF0650	Guatemala HC	42	1.3	27	POSITIVE	16	POSITIVE
#5	CSF0277	Paderborn, V1240/97	44	2.1	12	POSITIVE	8	POSITIVE
#6	CSF0277	Paderborn, V1240/97	65	2.1	40	POSITIVE	26	POSITIVE
#7	CSF0277	Paderborn, V1240/97	30	2.1	57	DOUBTFUL	35	POSITIVE
#8	CSF0573	Parma	69	2.2	20	POSITIVE	9	POSITIVE
#9	CSF0634	VI 3837/38	29	2.3	15	POSITIVE	6	POSITIVE
#10	CSF0104	Diepholz	21	2.3	13	POSITIVE	7	POSITIVE
#11	CSF0104	Diepholz	58	2.3	15	POSITIVE	6	POSITIVE
#12	CSF0864	BG/Jambul	18	2.3	34	POSITIVE	26	POSITIVE
#13	CSF0864	BG/Jambul	27	2.3	58	DOUBTFUL	40	POSITIVE
#14	CSF1019	Romania	22	2.3	74	NEGATIVE	48	POSITIVE
#15	CSF0822	030657; SS 00024 (France 2003, Bas-Rhin)	21	2.3	54	DOUBTFUL	34	POSITIVE

Table 4 : Results obtained with 15 samples from a panel provided by the EURL for CSF tested with the short and the long incubation protocols (n=15)

RESULTS (Table 4):

- With doubtful resuts considered positive, **14/15 samples** were found positive with the short incubation protocol , 1 is found negative with a S/N% value near the cut-off.
- All samples were found positive with the long incubation protocol.
- **The ID Screen® CSFE2C ELISA kit has an excellent inclusivity of the tested strains.**

EXCLUSIVITY

As CSFV is closely related to viruses inducing BVD and BD, the exclusivity of the kit was evaluated by testing the following samples with both the short and the long incubation protocols :

- 4 swine sera from a panel provided by the European reference laboratory for CSF (Virology laboratory, Hannover University) described as follows :
  - 2 BVDV-positive sera (strain NADL and 22146),
  - 2 BDV-positive sera (strain Frijters and Moredun).
- 5 samples provided by the French national reference laboratory for CSF (ANSES, Ploufagran) :
  - 2 swine sera hyperimmunized with BVDV,
  - 3 swine sera hyperimmunized with BDV.

Results are presented in the following Table 5:

VIRUS	ORIGINE	STRAIN	DAY POST-INFECTION	SHORT INCUBATION		LONG INCUBATION	
				S/N%	STATUS	S/N%	STATUS
BVDV-POSITIVE SAMPLES	Germany	NADL	90	91	NEGATIVE	86	NEGATIVE
	Germany	22146	69	101	NEGATIVE	91	NEGATIVE
	France	SHI BVD 261 1987	-	83	NEGATIVE	72	NEGATIVE
	France	SHI BVD 263 1987	-	87	NEGATIVE	79	NEGATIVE
BDV-POSITIVE SAMPLES	Germany	Frijters	41	29	POSITIVE	16	POSITIVE
	Germany	Moredun	58	90	NEGATIVE	92	NEGATIVE
	France	SHI V08 S BD01 2008	-	85	NEGATIVE	82	NEGATIVE
	France	SHI V98 S BD 01 1998	-	69	NEGATIVE	59	DOUBTFUL
	France	SHI BD 791 1987	-	67	NEGATIVE	43	POSITIVE

Table 5 : Exclusivity study of the ID Screen® CSFE2C ELISA kit with both incubation protocols

RESULTS (Table 5) :

- **All BVDV-positive** samples were found **negative**.
- 1/5 BDV-positive samples was found positive with the short incubation protocol and 2/5 with the long incubation protocol (1/5 doubtful), indicating that the kit may cross-react with antibodies directed against Border Disease Virus.
- The ID Screen® kit shows a excellent exclusivity **regarding the BVD Virus**.

REPEATABILITY

SHORT INCUBATION PROTOCOL

Intra-plate repeatability with the short incubation protocol was evaluated by measuring the coefficient of variation (CV%) for 96 repetitions of a weak positive sample.

Results are considered compliant if the CV% is less than 10%. O.D. results are shown in Table 6 below.

OD AT 450NM											
0.855	0.852	0.815	0.828	0.851	0.820	0.827	0.871	0.800	0.835	0.844	0.821
0.838	0.848	0.839	0.795	0.779	0.823	0.807	0.792	0.782	0.792	0.807	0.797
0.825	0.792	0.778	0.771	0.743	0.754	0.754	0.780	0.757	0.776	0.819	0.812
0.819	0.782	0.779	0.760	0.751	0.744	0.722	0.712	0.733	0.756	0.790	0.810
0.791	0.761	0.746	0.738	0.692	0.698	0.740	0.731	0.721	0.728	0.766	0.774
0.811	0.830	0.789	0.772	0.741	0.731	0.726	0.775	0.772	0.818	0.814	0.826
0.847	0.817	0.797	0.756	0.746	0.839	0.789	0.836	0.820	0.835	0.843	0.852
0.844	0.821	0.826	0.814	0.789	0.844	0.857	0.843	0.856	0.834	0.850	0.849

	AVERAGE OD	STANDARD DEVIATION	MINIMUM	MAXIMUM	CV%
Weak positive sample	0.795	0.042	0.692	0.871	**5.3**

Table 6 : Repeatability study for the ID Screen® CSFE2C ELISA (results expressed as OD values)

RESULTS (Table 6) :

- The CV% was determined at 5.3% for the weak positive sample tested, demonstrating **excellent test repeatability**.

LONG INCUBATION PROTOCOL

Intra-plate repeatability with the long incubation protocol was evaluated by measuring the coefficient of variation (CV%) for 288 repetitions of a weak positive sample.

Results are considered compliant if the CV% is less than 10%. O.D. results are shown in Table 7 below.

OD AT 450NM											
0.541	0.547	0.544	0.524	0.568	0.563	0.509	0.520	0.514	0.535	0.505	0.534
0.570	0.535	0.543	0.516	0.534	0.547	0.534	0.546	0.538	0.543	0.535	0.539
0.547	0.524	0.518	0.509	0.533	0.558	0.549	0.574	0.562	0.569	0.540	0.534
0.542	0.533	0.497	0.513	0.519	0.525	0.521	0.533	0.537	0.559	0.546	0.507
0.537	0.556	0.511	0.502	0.521	0.513	0.515	0.524	0.550	0.546	0.542	0.517
0.565	0.563	0.535	0.521	0.536	0.533	0.548	0.557	0.565	0.551	0.553	0.503
0.547	0.561	0.536	0.536	0.565	0.532	0.538	0.558	0.563	0.554	0.616	0.513
0.532	0.535	0.534	0.539	0.536	0.545	0.540	0.531	0.551	0.543	0.499	0.477

	AVERAGE OD	STANDARD DEVIATION	MINIMUM	MAXIMUM	CV%
Weak positive sample	0.537	0.021	0.477	0.616	**3.8**

Table 7 : Repeatability study for the ID Screen® ELISA (results expressed as OD values)

RESULTS (Table 7) :

- The CV% was determined at 3.8% for the weak positive sample tested, demonstrating **excellent test repeatability**.

REPRODUCIBILITY

SHORT INCUBATION PROTOCOL

A positive serum was diluted in a negative serum pool in order to generate a threshold sample.

This threshold dilution was tested in 13 independent runs by different operators and on different days. Results are considered compliant if the CV% is less than 15% and the values are within ± 2 standard deviations around the mean.

Results are shown in Figure 7.

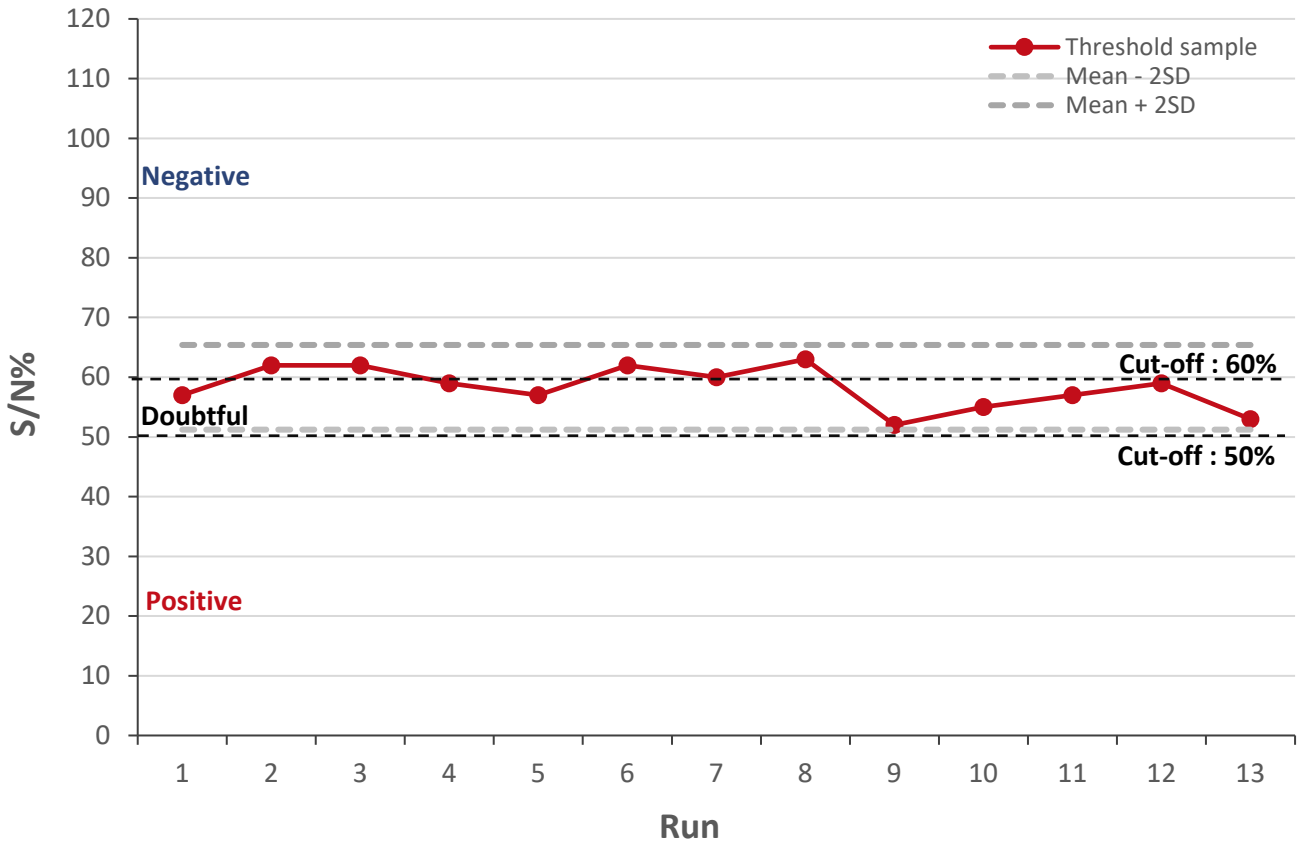


Figure 7 : S/N% values for a threshold dilution of a positive serum sample tested in 13 independent run

LONG INCUBATION PROTOCOL

A positive serum was diluted in a negative serum pool in order to generate a threshold sample.

This threshold dilution was tested in 6 independent runs by different operators and on different days with the long incubation protocol. Results are considered compliant if the CV% is less than 15% and the values are within ± 2 standard deviations around the mean.

Results are shown in Figure 8.

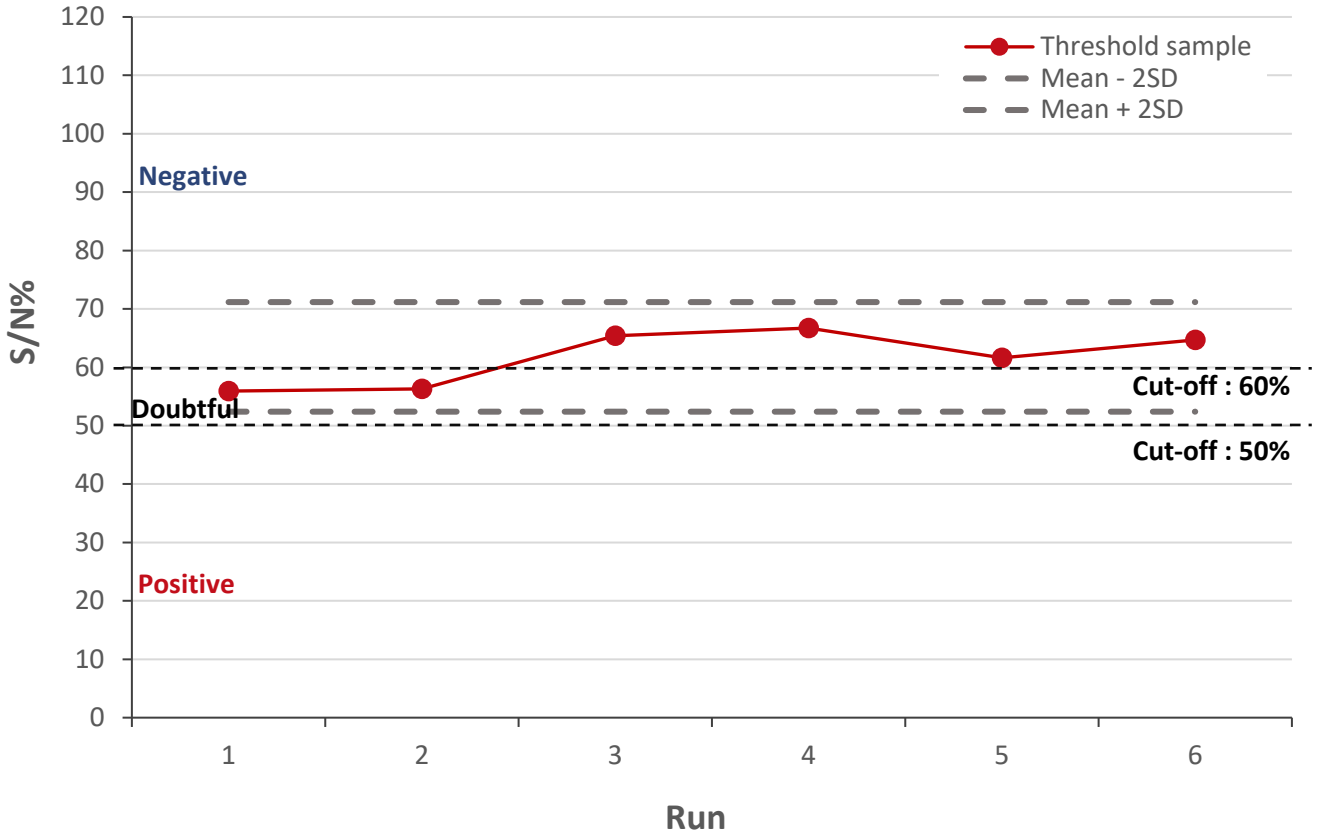


Figure 8 : S/N% values for a threshold dilution of a positive serum sample tested in 6 independent runs

RESULTS (Figures 7 and 8)**:**

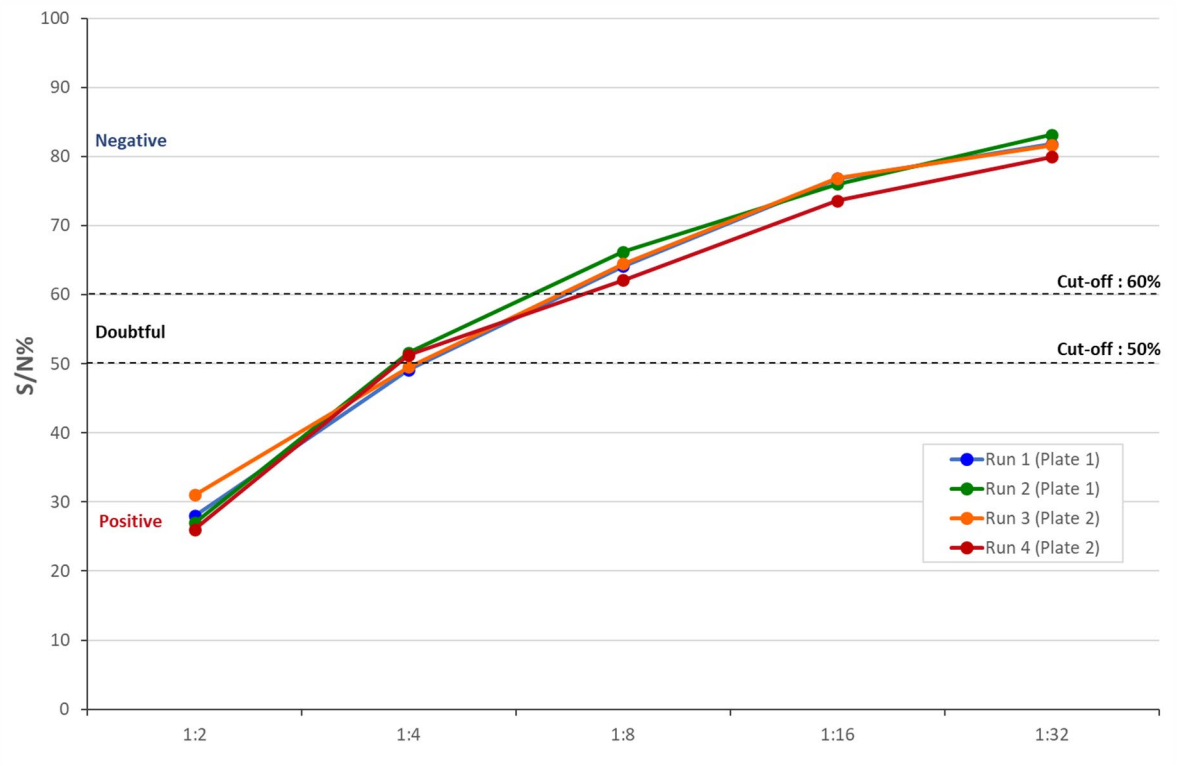
- For each protocols, the described criterias (CV% inferior to 15% and values within ± 2 standard deviations around the mean) are met, with respectively for the short and long incubation protocols CV values of 7.6% and 12.3%.
- These results demonstrate **the excellent reproducibility** of the ID Screen® CSFE2C ELISA kit.

LINEARITY

A positive serum sample was titrated and tested in duplicates on 2 different plates with the ID Screen® CSFE2C ELISA kit with the short and the long incubation protocols.

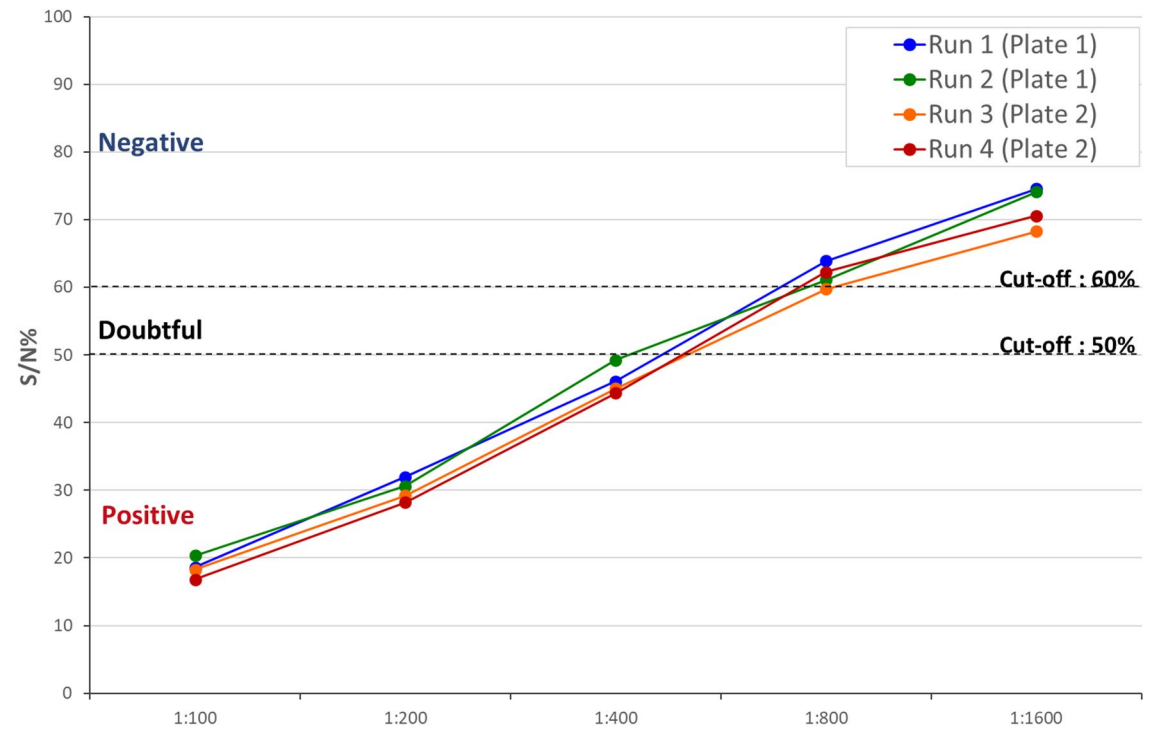
The results, shown in Figure 9, are expressed as S/N% ratios :

A



SAMPLE DILUTION	MEAN	STANDARD DEVIATION	CV%	MIN	MAX
1:2	28 (+)	2.2	**7.7**	26	31
1:4	50 (+)	1.2	**2.4**	49	52
1:8	64 (-)	1.7	**2.6**	62	66
1:16	76 (-)	1.5	**2.0**	74	77
1:32	82 (-)	1.3	**1.6**	80	83

B



SAMPLE DILUTION	MEAN	STANDARD DEVIATION	CV%	MIN	MAX
1:100	19 (+)	1.4	**7.8**	17	20
1:200	30 (+)	1.7	**5.5**	28	32
1:400	46 (+)	2.2	**4.7**	44	49
1:800	62 (-)	1.8	**2.9**	60	64
1:1600	72 (-)	3.0	**4.2**	68	75

Figure 9 : Linearity for the short (A) and the long (B) incubation protocols

RESULTS (Figure 9):

- **The ID Screen® CSFE2C ELISA offers a consitent linearity.**
- For each dilution and eah protocol, the **CV% obtained were less than 10%**, indicating high reproducibility.

ROBUSTNESS

SHORT INCUBATION PROTOCOL

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

RESULTS :

For each run :

- **The validation criteria** described in the insert for both **Positive and Negative Controls were met.**
- **S/N% values for Negative Control, Positive Control and threshold samples were equivalent, regardless of the test conditions.**

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 37°C (± 2°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 Negative Control O.D. (OD_{NC}) is strictly superior to 0.7 ($OD_{NC} > 0.7$).
- The ratio of the mean values of the Positive and Negative Controls (OD_{PC} and OD_{NC}) is strictly inferior to 0.3 ($OD_{PC} / OD_{NC} < 0.3$)

Optical densities at 450nm obtained in each condition for both negative and positive controls are detailed in the Table 7 below. 3 dilutions of a positive sample and 2 negative samples were also tested and the S/N% values obtained are detailed below.

SAMPLES / CONJUGATE / SUBSTRATE INCUBATION TIME	45 MIN / 30 MIN / 15 MIN			40MIN / 27 MIN / 13 MIN	50MIN / 33 MIN / 17 MIN	
SAMPLES INCUBATION TEMPERATURE	35°C	37°C	39°C	35°C	39°C	
CONJUGATE / SUBSTRATE INCUBATION TEMPERATURE	16°C	21°C	26°C	16°C	26°C	
POSITIVE CONTROL	0.088 0.102	0.087 0.097	0.098 0.108	0.119 0.117	0.106 0.104	OD 450 NM
NEGATIVE CONTROL	0.888 0.868	0.849 0.884	1.222 1.203	1.715 1.724	1.483 1.423	
$OD_{NC} > 0,7$	√	√	√	√	√	
$OD_{PC} / OD_{NC} < 0,3$	√	√	√	√	√	
POSITIVE SAMPLE 1:4	49 (+)	49 (+)	50 (+)	46 (+)	44 (+)	S/N%
POSITIVE SAMPLE 1:8	64 (-)	62 (-)	64 (-)	58 (+/-)	61 (-)	
POSITIVE SAMPLE 1:16	74 (-)	74 (-)	75 (-)	69 (-)	77 (-)	
NEGATIVE SAMPLE #1	94 (-)	100 (-)	99 (-)	96 (-)	101 (-)	
NEGATIVE SAMPLE #2	96 (-)	102 (-)	101 (-)	100 (-)	107 (-)	

Table 8 : Robustness study for the short incubation protocol of the ID Screen® CSFE2C ELISA

LONG INCUBATION PROTOCOL

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

RESULTS :

For each run :

- **The validation criteria** described in the insert for both **Positive and Negative Controls were obtained.**
- **S/N% values for Negative Control, Positive Control and threshold samples were equivalent, regardless of the test conditions.**

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: 18 hours ± 2 hours 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 Negative Control O.D. (OD_{NC}) is strictly superior to 0.7 ($OD_{NC} > 0.7$).
- The ratio of the mean values of the Positive and Negative controls (OD_{PC} and OD_{NC}) is strictly inferior to 0.3 ($OD_{PC} / OD_{NC} < 0.3$)

Optical densities at 450nm obtained in each condition for both negative and positive controls are detailed in the Table 8 below. 3 dilutions of a positive sample and 2 negative samples were each tested twice and the S/N% ratios values obtained are detailed below.

SAMPLES / CONJUGATE / SUBSTRATE INCUBATION TIME	18H / 30 MIN / 15 MIN			16H / 27 MIN / 13 MIN	20H / 33 MIN / 17 MIN	
TEMPERATURE OF INCUBATION	16°C	21°C	26°C	16°C	26°C	
POSITIVE CONTROL	0.061 0.063	0.052 0.059	0.069 0.071	0.062 0.059	0.086 0.077	OD 450 NM
NEGATIVE CONTROL	1.078 1.035	1.41 1.388	1.697 1.736	0.891 0.926	1.741 1.715	
$OD_{NC} > 0,7$	√	√	√	√	√	
$OD_{PC} / OD_{NC} < 0,3$	√	√	√	√	√	
POSITIVE SAMPLE 1:4	18 (+) 17 (+)	18 (+) 21 (+)	14 (+) 16 (+)	22 (+) 18 (+)	12 (+) 13 (+)	S/N%
POSITIVE SAMPLE 1:8	42 (+) 39 (+)	44 (+) 39 (+)	36 (+) 38 (+)	40 (+) 37 (+)	38 (+) 40 (+)	
POSITIVE SAMPLE 1:16	62 (-) 59 (+/-)	61 (-) 60 (-)	61 (-) 64 (-)	63 (-) 62 (-)	61 (-) 57 (+/-)	
NEGATIVE SAMPLE #1	100 (-) 99 (-)	98 (-) 96 (-)	96 (-) 98 (-)	105 (-) 101 (-)	97 (-) 100 (-)	
NEGATIVE SAMPLE #2	101 (-) 97 (-)	101 (-) 97 (-)	98 (-) 102 (-)	96 (-) 102 (-)	106 (-) 104 (-)	

Table 9 : Robustness study for the long incubation protocol of the ID Screen® CSFE2C ELISA

RESULTS (Tables 8 and 9):

- For each protocol, the test validation criteria for both positive and negative controls were obtained.
- For protocol, the S/N% ratios values obtained were similar, and analytical sensitivity was constant, thereby demonstrating the **excellent robustness** of the ID Screen® CSFE2C ELISA.

STABILITY

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

The stability of the plates, the Negative Control and the conjugate were 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 at 37°C ± 2°C should be greater than 75% after two months.

Results are shown in Figure 10 below.

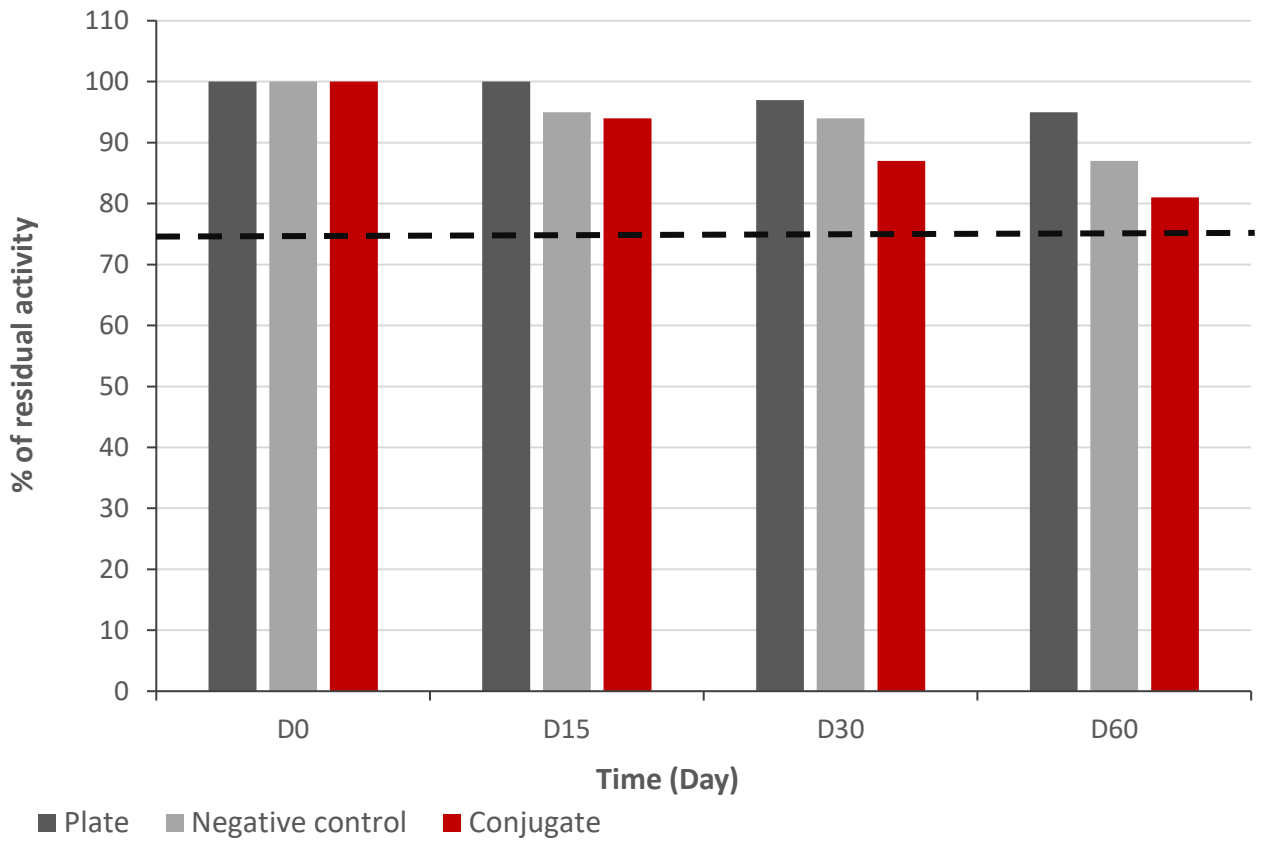


Figure 10 : Percentage of residual activity of the plates, Negative Control and conjugate after stability testing at 37°C

RESULTS (Figure 10):

- After 2 months at 37°C, the plates, the Conjugate and the Negative Control showed residual activity of 95%, 81% and 87% respectively, thus indicating **high component stability**.

CONCLUSION

The **ID Screen® Classical Swine Fever E2 Competition** ELISA:

- demonstrates **excellent specificity and sensitivity** on field samples as well as on reference sera.
- **efficiently detects** all CSF virus strains without cross-reacting with related pestiviruses such as Bovine Viral Diarrhea virus (BVDV).
- is easy-to-use with results in **just 90 minutes**.
- offers **convenient technical features** such as very high reproducibility, repeatability and robustness as well as component stability.

RELATED PRODUCTS

- **Freeze-dried CSF reference serum** (product code : MRI-CSFE2) : Freeze-dried swine serum containing anti-CSFV E2 glycoprotein specific antibodies.

For associated products, please consult the Innovative Diagnostics website: www.innovative-diagnostics.com.

History of revisions

VERSION	EDIT DATE	REFERENCE	TYPE OF REVISION	REVISION MADE
0516	06/2019	doc782	Not applicable (first version)	N/A
	07/2024	doc1377	Update: Addition/Edition of validation data	Addition of: Analytical sensitivity, Sensitivity, Specificity, Repeatability data. Addition of: Cut-off value determination, Inclusivity, Exclusivity, Reproducibility, Linearity, Robustness, Stability chapters.

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