

INTERNAL VALIDATION REPORT



ID SCREEN® PPR COMPETITION

COMPETITIVE ELISA FOR THE DETECTION OF ANTIBODIES AGAINST PPRV NUCLEOPROTEIN ANTIBODIES IN SERUM AND PLASMA FROM SHEEP, GOAT, CAMELID, SWINE AND OTHER SUSCEPTIBLE SPECIES

METHOD	Competitive ELISA
TARGET	Antibodies directed against PPRV nucleoprotein
SAMPLE TYPES	• Serum • Plasma
VALIDATED SPECIES	• Sheep • Goat • Camelid • Swine • Other susceptible species
PRODUCT CODE	PPRC

With you at every step

Table of contents

INTRODUCTION.....	3
DESCRIPTION AND PRINCIPLE OF THE TEST.....	3
SPECIFICITY	4
OVINE SERA	4
SWINE SERA	5
CAMELID SERA	6
<i>Innovative Diagnostics Internal study.....</i>	<i>6</i>
<i>External study.....</i>	<i>7</i>
<i>Global specificity on camelid sera.....</i>	<i>8</i>
ANALYTICAL SENSITIVITY	9
INNOVATIVE DIAGNOSTICS INTERNAL POSITIVE REFERENCE SERUM.....	9
SENSITIVITY: SEROCONVERSION KINETICS.....	10
VIRULENT FIELD STRAIN	10
VACCINE STRAIN	11
CAMELID SEROCONVERSION KINETICS	12
RELATIVE SENSITIVITY AND SPECIFICITY: COMPARISON WITH THE CIRAD IN-HOUSE ELISA	14
REPEATABILITY	15
REPRODUCIBILITY.....	16
ROBUSTNESS.....	17
STABILITY	18
CONCLUSION.....	19
RELATED PRODUCTS	19
REFERENCE.....	19
HISTORY OF REVISIONS.....	20

INTRODUCTION

Ovine rinderpest, also commonly known as Peste des Petits ruminants (PPR), is a contagious disease affecting primarily goats and sheep especially in Africa, the Middle-East and the Indian subcontinent. It is caused by a species of the Morbillivirus genus of viruses. The disease is highly contagious, with approximately 80 percent of mortality in acute cases.

In June 2008, the disease invaded Morocco, crossing the natural barrier of Sahara and causing concern that the disease could spread across Europe.

Serology may be used to identify and control outbreaks. The ID Screen® PPR Competition ELISA efficiently detects antibodies directed against the virus nucleoprotein.

The test uses technology (antigen and monoclonal antibody) developed by the FAO /OIE reference laboratory for PPR (CIRAD-EMVT, Montpellier, France) [1]. All components are ready-to-use, including coated plates, and each sample is deposited only once.

This report summarizes validation data for this test.

DESCRIPTION AND PRINCIPLE

Microwells are coated with purified recombinant PPR nucleoprotein (NP). Samples to be tested and controls are added to the microwells. Anti-NP antibodies, if present, form an antigen-antibody complex which masks the NP epitopes. After washing, an anti-NP peroxidase (HRP) conjugate is added to the wells. It fixes to the remaining free NP epitopes, forming an antigen-conjugate-HRP complex. After elimination of the excess conjugate by washing, the substrate solution (TMB) is added.

The resulting coloration is proportional to the quantity of specific antibodies present in the sample. 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: $S/N \% = \frac{OD_{\text{sample}}}{OD_{\text{NC}}} \times 100$

RESULT FOR SHEEP, GOAT AND CAMELID SAMPLES	STATUS
$S/N \% \leq 50\%$	Positive
$50\% < S/N \% \leq 60\%$	Doubtful
$S/N \% > 60\%$	Negative

RESULT FOR SWINE SAMPLES	STATUS
$S/N \% \leq 30\%$	Positive
$30\% < S/N \% \leq 40\%$	Doubtful
$S/N \% > 40\%$	Negative

SPECIFICITY

OVINE SERA

Ovine sera (n = 405) from a disease-free region (Hérault, France) were tested.

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

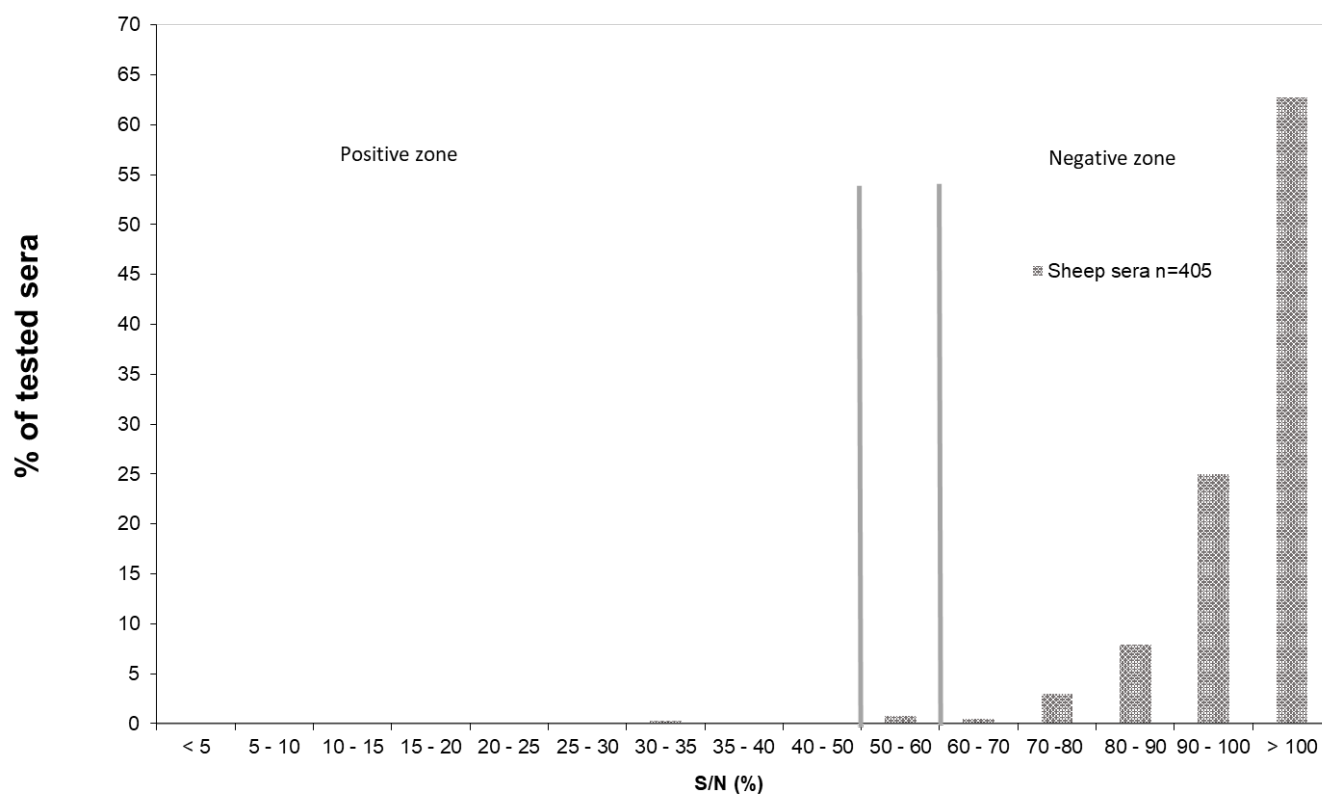


Figure 1: Specificity of the ID Screen® ELISA on ovine sera from disease-free regions in France, n=405

RESULTS (Figure 1) :

- 401/405 sera were found negative. Three sera were found doubtful.
- **Measured specificity = 99.0 % (95% CI [97.5, 99.6], n=405).**
- **When doubtful sera are considered negative, measured specificity = 99.8 % (95% CI [98.6, 99.9], n=405).**

SWINE SERA

Experimental data, first presented during the joint ESVV & Epizone congress (2015, Montpellier), then published in 2018 (Schulz and al., 2018; [3]) by the Friedrich loeffler Institute (FLI, Germany) suggested that suids should be considered as potential sources of PPRV reservoir hosts.

Consequently, the specificity of the ID Screen® kit was assessed on swine samples to evaluate the kit's cut-off relevance for this species.

478 pigs from disease-free areas were tested :

- 176 Iberian pigs,
- 302 French pigs (Rennes, Acseclate).

Results obtained are presented in Figure 2.

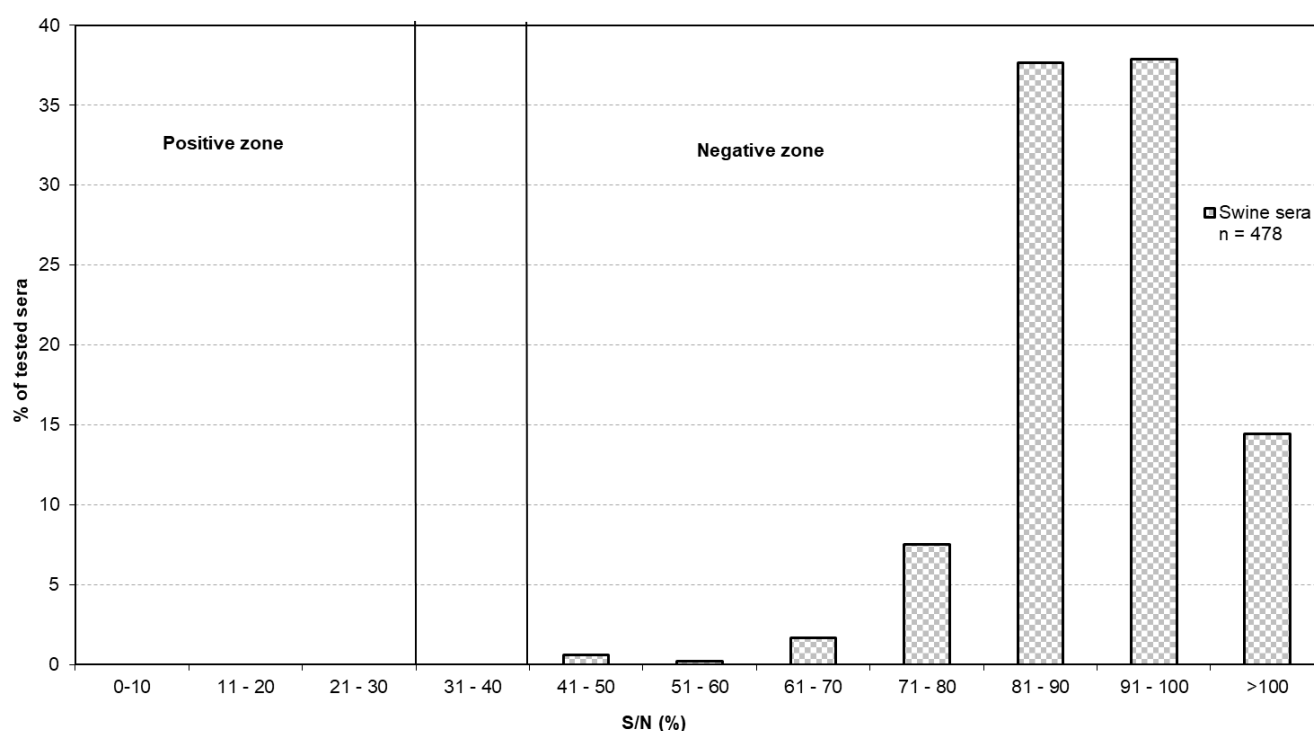


Figure 2: Specificity of the ID Screen® ELISA on swine sera from disease-free areas (Spain, France), n=478

RESULTS (Figure 2) :

- All sera were found negative.
- **Measured specificity = 100% (95% CI [99.2, 100], n=478).**

CAMELID SERA

Innovative Diagnostics Internal study

The epidemiological role of camelids in PPRV transmission has been investigated and are assumed to be dead-end hosts for the virus (Schulz C. and al., 2019; [4]).

To evaluate the specificity, as well as the cut-off's relevance on this species, 696 camelids from disease-free areas were tested :

- 204 Ethiopian camelids,
- 492 Moroccan camelids.

Results obtained are presented in Figure 3.

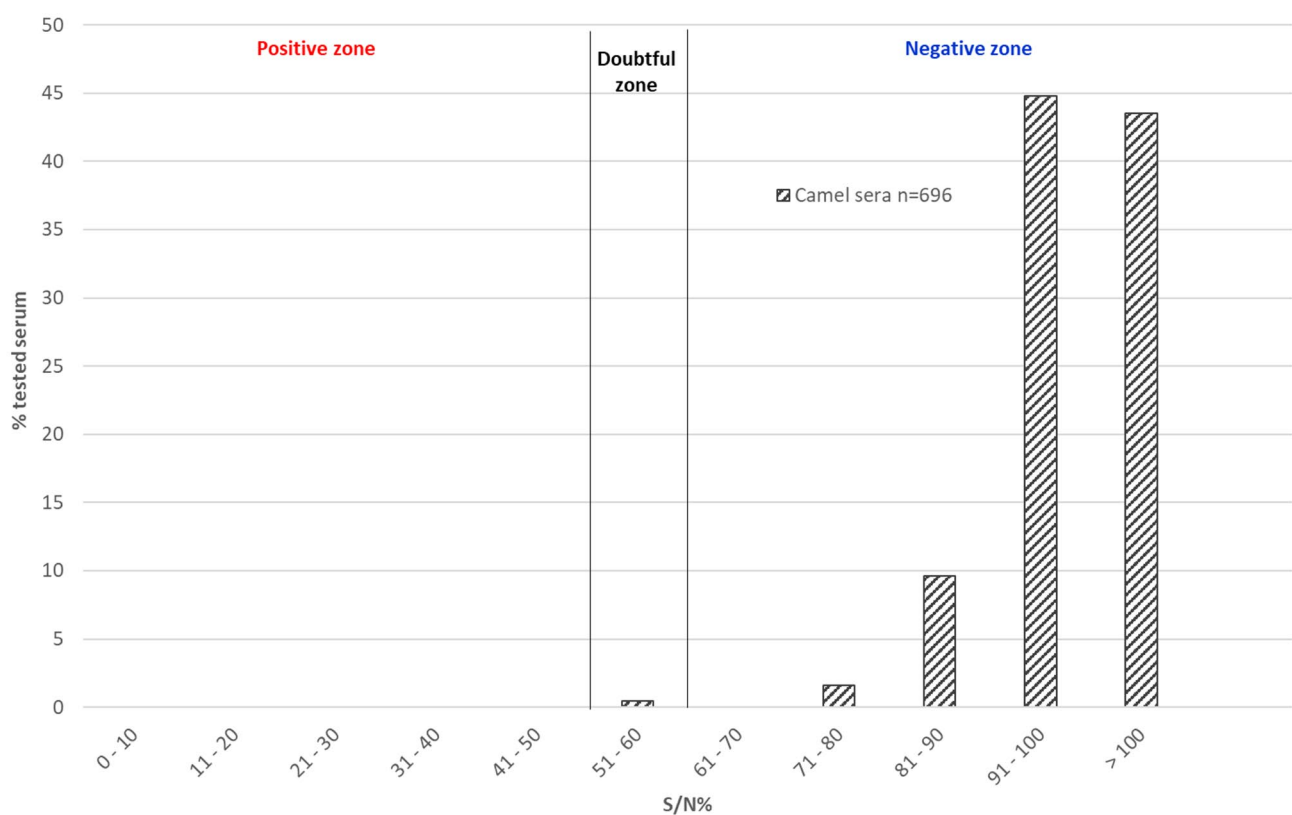


Figure 3: S/N % distribution for camelid negative samples, n = 696

RESULTS (Figure 3) :

- 695/696 sera were found negative. One sera was found doubtful.
- **Measured specificity = 99.86 % (95% CI [99.2, 99.9], n=696).**
- **When doubtful sera are considered negative, measured specificity = 100 % (95% CI [99.5, 100], n=696).**

External study

Specificity was evaluated on 200 PPR-negative sera from Algerian camel in a study carried out by Dr. Satya Parida (Laboratory and Vaccine Specialist at Food and Agriculture Organization of United Nations).

The data was presented at the annual PPR Global Research and Expertise Network (PPR-GREN), December 6-8, 2021 [5].

Results obtained are presented in Figure 4.

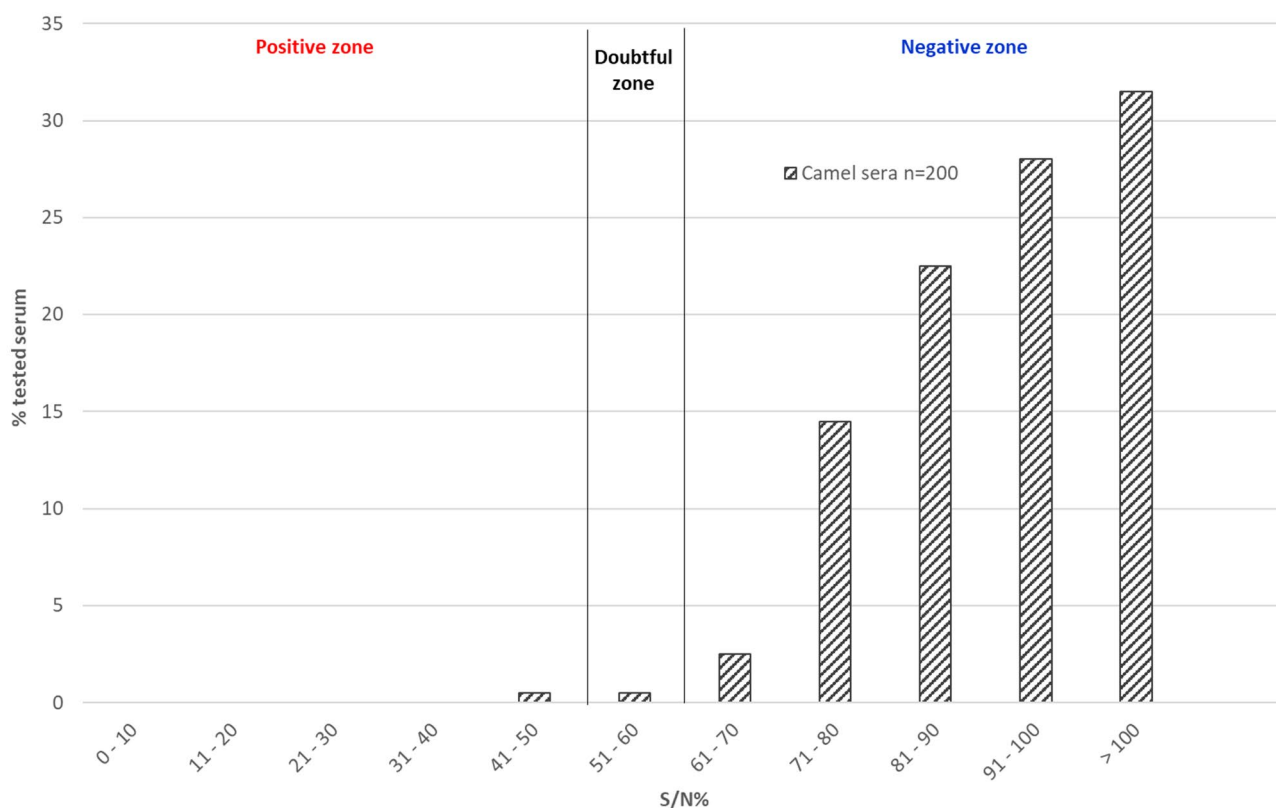


Figure 4: S/N % distribution for camelid negative samples, n = 200

RESULTS (Figure 4) :

- 198/200 sera were found negative. 1 sera was found doubtful and 1 sera positive.
- **Measured specificity = 99.0% (95% CI [96.4, 99.7], n=200).**
- **If doubtful sera are considered negative, measured specificity = 99.5 % (95% CI [97.2, 99.9], n=200).**

Global specificity on camelid sera

The overall specificity was evaluated on a total of 896 camelids serum samples from 3 countries, the table below summarizes these data.

COUNTRY	SPECIFICITY (%) (number of samples)	95 % CI
Ethiopia	99.5 (n = 204)	[97.3, 99.9]
Morocco	100 (n = 492)	[99.2, 100]
Algeria	99.0 (n = 200)	[96.4, 99.7]
TOTAL	99.67 (n = 896)	[99.0, 99.9]

Table 1: Global Specificity of the ID Screen® ELISA on camelid samples, n=896

RESULTS (Table 1) :

- 893/896 sera were found negative. 2 sera were found doubtful and 1 sera positive.
- **Measured specificity = 99.67% (95% CI [99.0, 99.9], n=896).**

ANALYTICAL SENSITIVITY

INNOVATIVE DIAGNOSTICS INTERNAL POSITIVE REFERENCE SERUM

As no international standard exists for PPR serodiagnosis, Innovative Diagnostics developed a caprine positive PPRC freeze-dried serum standard (containing anti-PPRV NP antibodies) 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-PPR.

Analytical sensitivity was tested using serial dilutions of the MRI-PPR on the ID Screen® and CIRAD in-house ELISA.

DILUTION	ID SCREEN® ELISA CUT-OFF: 50-60%		CIRAD IN-HOUSE ELISA CUT-OFF: 50-60%	
	S/N%	STATUS	S/N%	STATUS
1 : 2	16	(+)	25	(+)
1 : 4	28	(+)	44	(+)
1 : 8	51	(+/-)	62	(-)
1 : 16	65	(-)	86	(-)

Table 2 : Titration of Innovative Diagnostics' PPR freeze-dried serum standard with the ID Screen® ELISA and CIRAD in-house ELISA

RESULTS (Table 2) :

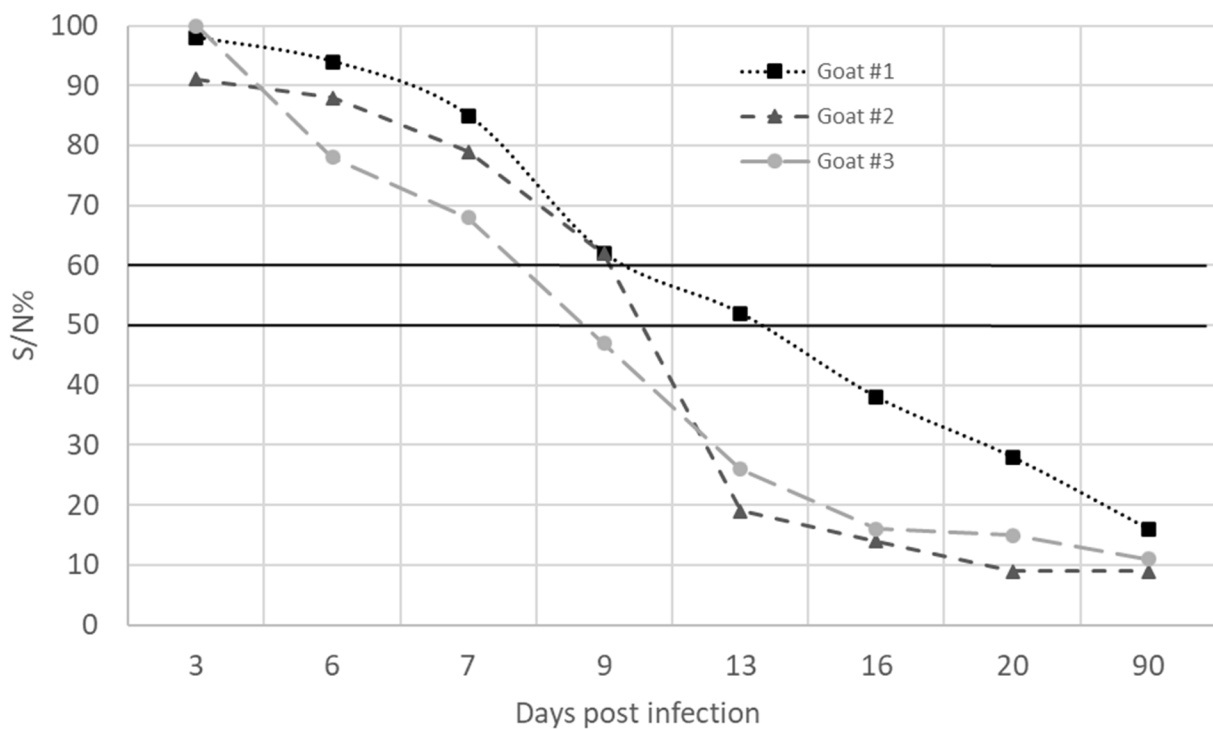
- The MRI-PPR was detected as positive when diluted up to 1:4 on both kits.

SENSITIVITY: SEROCONVERSION KINETICS

VIRULENT FIELD STRAIN

Detection of seroconversion was determined by studying three goats experimentally-infected by a virulent strain of PPRV (Ivory Coast strain 1989 [CI89]).

Animals were bled and tested at 3, 6, 7, 9, 13, 16, 20 and 90 days post-infection. Results obtained are presented in Figure 5.



RESULTS (Figure 5) :

- Seroconversion began at least 9 days post-infection, and all sera were found positive at 16 days post-infection. (Seroconversion for PPR is generally detected between the first and second week by virus neutralization (VNT)).

VACCINE STRAIN

To determine the ID Screen® test's suitability for vaccination monitoring, 3 goats were vaccinated with a dose ($10^{2.5}$ TCID₅₀) of the PPRV vaccine strain Nigeria 75-1.

Animals were bled and tested with the ID Screen® ELISA at 3, 6, 7, 9, 13, 16, 20 and 90 days post-vaccination (dpv). Results obtained are presented in Figure 6.

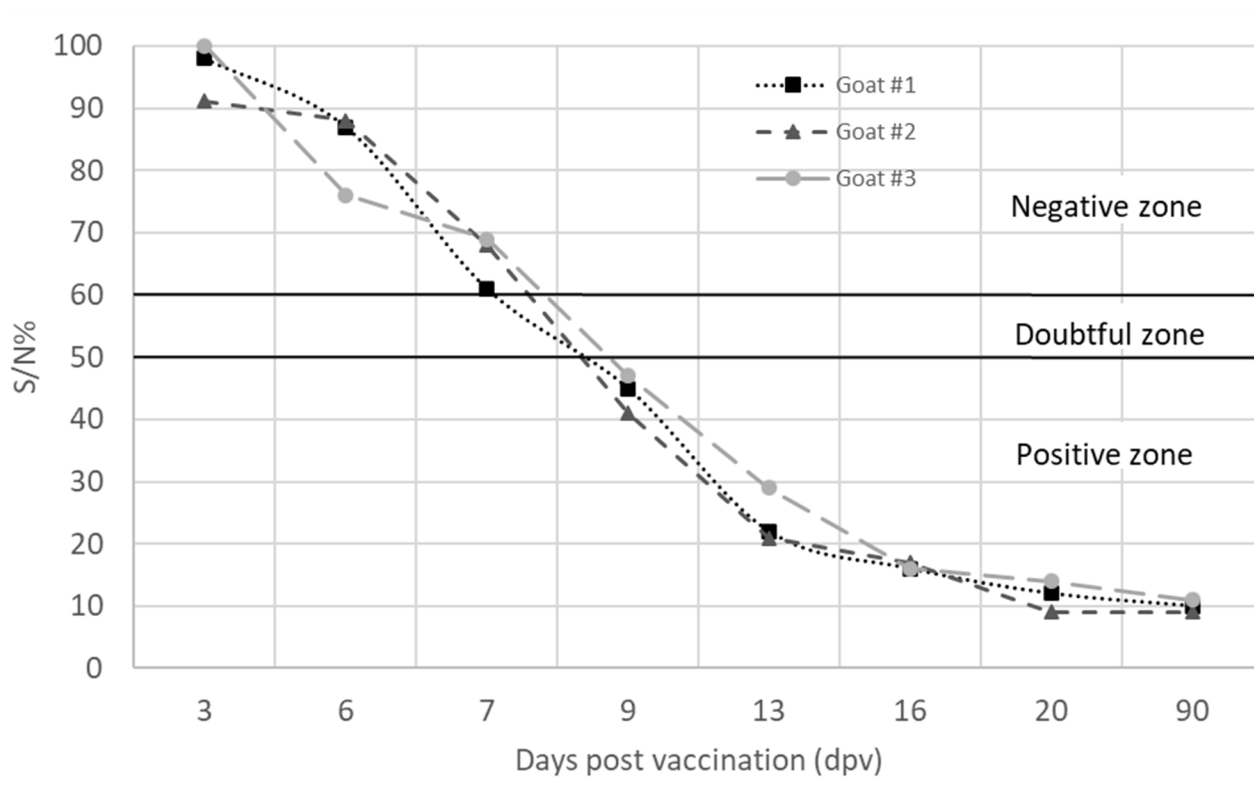


Figure 6 : Goat seroconversion kinetics on vaccine strain with the ID Screen® ELISA

RESULTS (Figure 6) :

- Sera were found positive between the first and second weeks post-vaccination, with seroconversion beginning at least 7 days post-vaccination. All sera were found positive at 9 days post-vaccination.

CAMELID SEROCONVERSION KINETICS

Sensitivity was evaluated in a study carried out by Dr. Satya Parida (Laboratory and Vaccine Specialist at Food and Agriculture Organization of United Nations). Data were presented at the annual PPR-GREN meeting, December 6-8, 2021 [5].

Detection of seroconversion was determined by studying 8 Sudanese camels (sample A to H) vaccinated by a PPRV strain Nigeria 75-1. Animals were bled and tested at 0, 1, 2, 3, 4, 5, 7, 9, 11, 13, 15, 21 and 28 days post-immunisation (dpi).

The samples between D9 and D28 are first tested by Virus Neutralisation Test (VNT), to determine a neutralising antibody titer (Nab). Results obtained are presented in Figure 7.

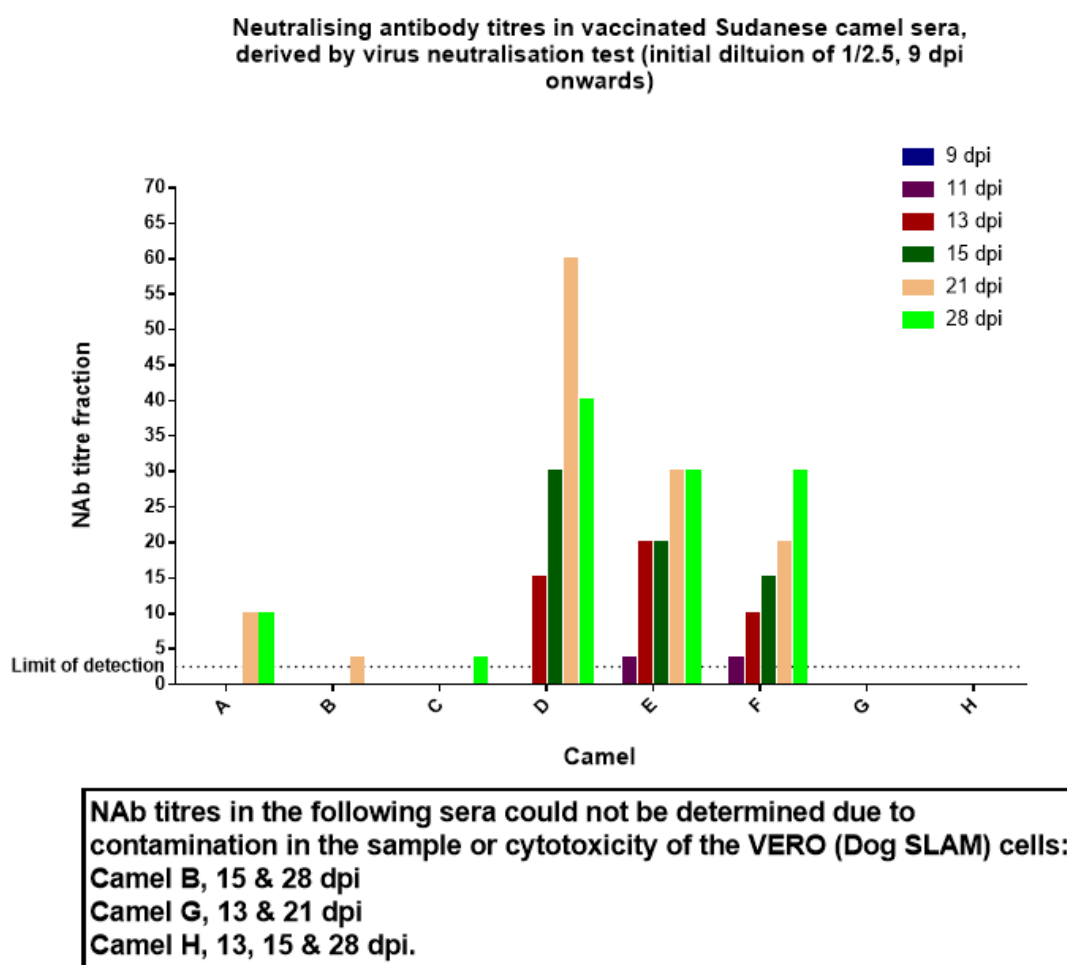


Figure 7 : Neutralizing antibody titre determination in camel sera by Virus neutralization test

RESULTS (Figure 7):

- Seroconversion is detected as early as day 11 days post-immunisation, with an average of samples seroconverted between 13 to 28 dpi.
- Samples D, E and F are strongly seroconverted.
- Samples A, B and C are weakly seroconverted.
- Samples G and H did not seroconvert.

All samples were tested at 0, 1, 2, 3, 4, 5, 7, 9, 11, 13, 15, 21 and 28 days post-immunisation with the ID Screen® ELISA (n=104 sample). Results obtained are presented in Figure 8.

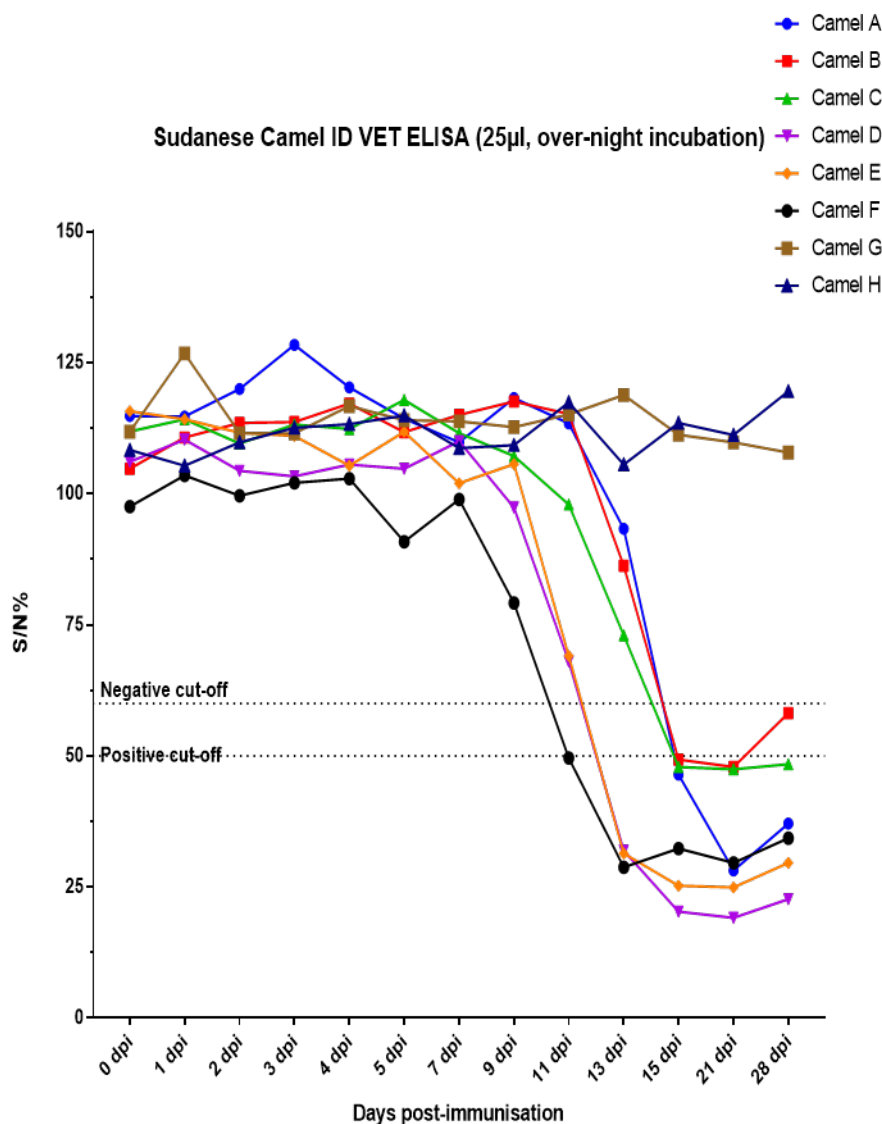


Figure 8 : Camelid seroconversion kinetics with the ID Screen® ELISA

RESULTS (Figure 8) :

- Seroconversion began at least 11 days post-immunisation, and all sera were found positive at 15 dpi (except for samples G and H which did not seroconvert following vaccination).
- Excellent correlation between Virus Neutralisation Test and cELISA.

RELATIVE SENSITIVITY AND SPECIFICITY: COMPARISON WITH THE CIRAD IN-HOUSE ELISA

118 goat sera from experimental infections and from suspected field cases in Mayotte were tested in parallel with the ID Screen® and the CIRAD ELISAs.

Results obtained with both the ID Screen® ELISA and Kit A are summarized in Table 3 below.

		ID SCREEN® ELISA		
		Positive	Negative	TOTAL
CIRAD ELISA	Positive	28	0	28
	Negative	0	90	90
TOTAL		28	90	118

Table 3 : Relative specificity and sensitivity of the CIRAD and ID Screen ELISAs

Results (Table 3) :

- Identical results were obtained with both ELISAs. Test agreement = 100%.
- Relative measured specificity of the ID Screen / CIRAD kits = 100% (95% CI [96, 100]).
- Relative measured sensitivity of the ID Screen / CIRAD kits = 100% (95% CI [88, 100]).

REPEATABILITY

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

Results are considered compliant if the CV% is less than 15%. OD results are shown on Table 4 below.

OD AT 450NM											
0.692	0.704	0.694	0.694	0.685	1.323	1.336	0.690	0.697	0.674	0.681	0.632
0.705	0.682	0.683	0.675	0.680	1.312	1.337	0.690	0.665	0.671	0.650	0.660
0.689	0.671	0.679	0.688	0.673	1.302	1.322	0.671	0.695	0.655	0.644	0.652
1.335	1.308	1.295	1.310	1.286	1.296	1.296	1.320	1.310	1.281	1.294	1.340
1.339	1.318	1.337	1.332	1.300	1.327	1.331	1.327	1.354	1.310	1.293	1.312
0.696	0.679	0.683	0.701	0.684	1.298	1.324	0.703	0.687	0.672	0.669	0.662
0.708	0.672	0.677	0.681	0.682	1.310	1.300	0.677	0.679	0.671	0.680	0.676
0.706	0.707	0.705	0.701	0.702	1.320	1.341	0.688	0.703	0.660	0.638	0.667

	AVERAGE OD	STANDARD DEVIATION	MINIMUM	MAXIMUM	CV%
WEAK POSITIVE SAMPLE	0.681	0.018	0.629	0.698	3
NEGATIVE POOL SERUM	1.316	0.018	1.281	1.354	1

Table 4 : Repeatability study for the ID Screen® ELISA (results expressed as OD values)

RESULTS (Table 4) :

- The CV% obtained were 3% for the weak positive sample and 1% for the negative pool serum, demonstrating **excellent repeatability test**.

REPRODUCIBILITY

A positive serum was diluted in a negative serum pool in order to generate a threshold sample. This threshold dilution was tested in 25 independent runs by different operators and on different days. Results are shown in Figure 9.

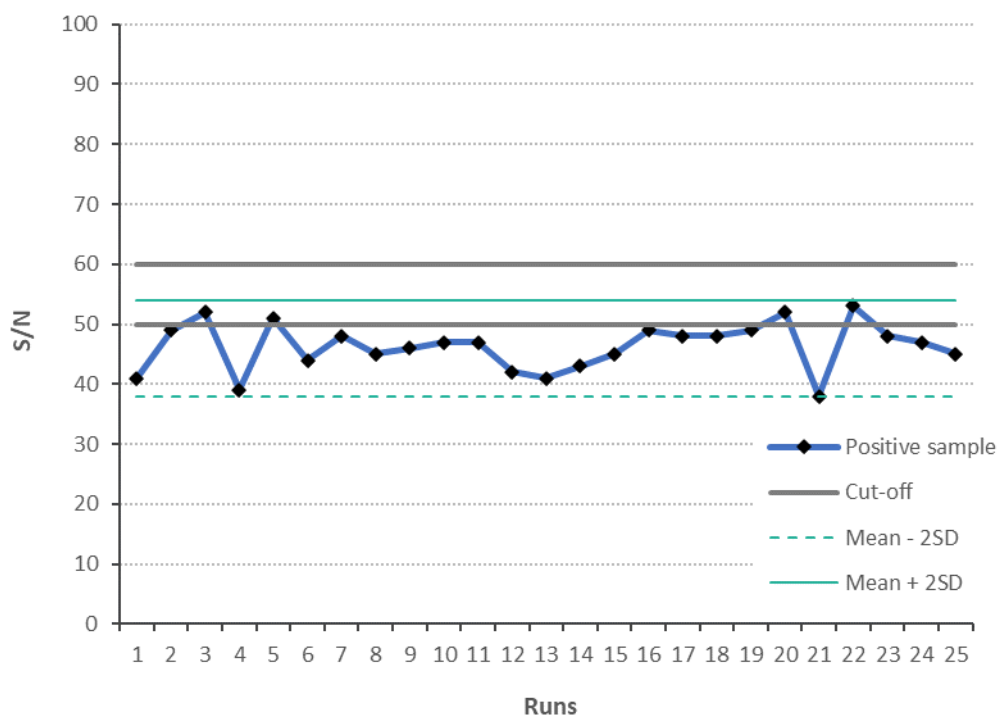


Figure 9: S/N values for a threshold dilution of a positive serum sample tested in 25 independent runs

RESULTS (Figure 9) :

- All values are within a range of 2 standard deviations around the mean, with a CV% of 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:

- Samples incubation: 45 minutes $\pm$ 4 minutes at 37°C ($\pm$ 3°C);
- Conjugate incubation: 30 minutes $\pm$ 3 minutes at 21°C ($\pm$ 5°C);
- Substrate Solution incubation: 15 minutes $\pm$ 2 minutes at 21°C ($\pm$ 5°C).

For each condition, the test is validated if:

- The mean value of the negative control OD (OD_{NC}) is greater than 0.7 ($OD_{NC} > 0.700$).
- The ratio of the mean values of the Positive and Negative Controls (OD_{PC} and OD_{NC}) is less than 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 Figure 10 below. Three dilutions of the MRI-PPRC 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			41 MIN / 27 MIN / 13 MIN	49 MIN / 33 MIN / 17 MIN	
TEMPERATURE OF INCUBATION	34°C/16°C/16°C	37°C/21°C/21°C	40°C/26°C/26°C	34°C/16°C/16°C	40°C/26°C/26°C	
Negative control	1.371	1.620	1.970	1.098	2.369	OD 450 NM
	1.365	1.692	1.930	1.097	2.333	
Positive control	0.149	0.195	0.208	0.130	0.228	
	0.159	0.196	0.216	0.129	0.231	
$OD_{NC} > 0.700$	✓	✓	✓	✓	✓	
$OD_{PC} / OD_{NC} < 0.30$	✓	✓	✓	✓	✓	
MRI-PPRC diluted 1:2	16	18	17	19	15	S/N %
MRI-PPRC diluted 1:4	28	29	27	30	21	
MRI-PPRC diluted 1:8	44	48	46	51	41	
Negative sample 1	95	98	97	96	95	
Negative sample 2	89	90	91	87	88	

Figure 10: Robustness study for the ID Screen® ELISA

RESULTS (Figure 10):

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**.

STABILITY

The product's shelf-life is evaluated by the technique of accelerated ageing.

The stability of the plates, the positive control and the conjugate was tested by evaluating the residual activity of individual components after storage at $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$, with respect to storage at $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$. The measured residual activity at $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$ should be greater than 75% after two months.

Results are shown in Figure 11 below.

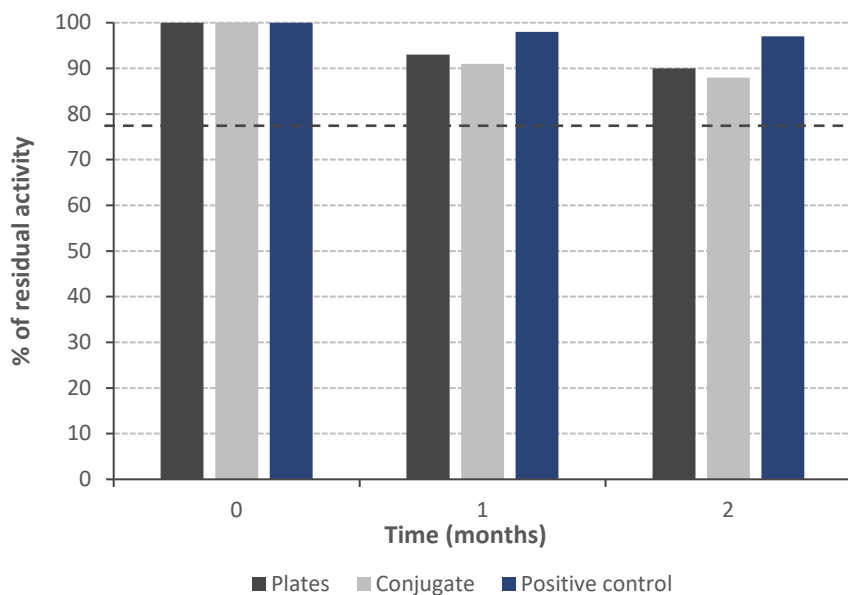


Figure 11: Percentage of residual activity of the plates, positive control and conjugate after stability testing at 37°C

RESULTS (Figure 11):

- After 2 months at 37°C , the plates, the conjugate and the positive control showed residual activity of 88%, 87% and 95% respectively, thus indicating **high component stability**.

CONCLUSION

The ID Screen® PPR Competition ELISA:

- is the **only commercial ELISA based on biologicals developed by a reference laboratory** (CIRAD, France) [2].
- is also the only commercial ELISA supplying **ready-to-use coated plates** and **all other components in liquid form**.
- is particularly easy-to-use, with results in 90 minutes. Thanks to its **screening format**, each sample is only deposited once.
- shows **high agreement (100%) with the CIRAD's in-house ELISA**.
- shows **high specificity** in disease-free animals. It **detects seroconversion to virulent and vaccinal strains one to two weeks post-infection / vaccination**.
- is a reliable tool for the detection of antibodies against the PPRV with **high reproducibility, repeatability, robustness and stability**.

Related products

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

Reference

- [1] **Development of a competitive ELISA for detecting antibodies to the Peste des Petits Ruminants virus using a recombinant nucleoprotein**. Libeau G, Préhaud C, Lancelot R, Colas F, Guerre L, Bishop DH, Diallo A., Res Vet Sci. 1995 Jan;58(1):50-5.
- [2] **Validation report of the ID Screen® PPR Competition ELISA**. CIRAD-EMVT, Montpellier, France. March 2011.
- [3] Schulz, Claudia & Fast, Christine & Schlottau, Kore & Hoffmann, Bernd & Beer, Martin. (2018). **Neglected Hosts of Small Ruminant Morbillivirus**. Emerging infectious diseases. 24. 2334-2337. 10.3201/eid2412.180507.
- [4] Schulz C., Fast C. et al. (2019) **Camelids and Cattle Are Dead-End Hosts for Peste des Petits-Ruminants Virus**. Viruses, 11, 1133
- [5] **Annual PPR-GREN meeting**. Validation of cELISAs for the Detection of Antibodies Against PPR in Camels, presented by Dr. Satya Parida, December 6-8, 2021.

History of revisions

VERSION	EDIT DATE	REFERENCE	TYPE OF REVISION	REVISION MADE
0814	09/2020	doc927	Not applicable (first version)	N/A
	11/2020	doc971	Update : Addition/Edition of validation data	Update of data / test performance (specificity) Correction of typos.
0821	08/2021	doc1037	Technical modification : Update of the document following technical modification of the kit	Addition of a new cut-off dedicated to swine sample testing. Chapters mentioning swine samples have been updated accordingly.
	05/2023	doc1332	Technical modification : Update of the document following technical modification of the kit	Addition of a protocol dedicated to camelid sample testing (with an overnight sample incubation). Chapters mentioning camelid samples have been updated accordingly.