INTERNAL VALIDATION REPORT

ID SCREEN®RIFT VALLEY FEVER COMPETITION MULTI-SPECIES

COMPETITIVE ELISA KIT FOR THE DETECTION OF ANTIBODIES AGAINST THE RIFTV NUCLEOPROTEIN IN SERA OR PLASMA

METHOD	**Competition ELISA**
TARGET	• Antibodies directed against Rift Valley Fever virus nucleoprotein (RIFTV-NP)
SAMPLE TYPES	• Serum • Plasma
VALIDATED SPECIES	• Multiple species including ruminants, horses, cats, and dogs. • Humans (for research use only)
PRODUCT CODE	RIFTC

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INTRODUCTION

Rift Valley Fever (RVF) is a vector-borne viral zoonosis transmitted by mosquitoes that infects a wide range of vertebrate hosts, including cattle, sheep and goats. In livestock, infection is characterised by high rates of abortion and neonatal mortality.

The RVF virus, which causes in humans flu-like symptoms and death in the most severe cases, is endemic in many countries of sub-Saharan Africa and in Egypt. In 2000, outbreaks occurred for the first time outside of Africa in Yemen and Saudi Arabia, raising fears that the virus will emerge in new areas.

RVF diagnosis may be obtained through tissue culture, PCR, histopathology and serology. Seroneutralisation test is the prescribed serological test for international trade, but it may only be performed with live virus, raising biosecurity questions in non-endemic areas. In contrast, the ELISA method does not pose such risks, and is therefore particularly suited to antibody surveillance in disease-free countries.

Innovative Diagnostics offers a full range of diagnostic kits to detect antibodies directed against the nucleoprotein (NP) of the RVF virus (RVFV): a blocking ELISA, the ID Screen® Rift Valley Fever Competition Multi-Species ELISA, and an IgM Antibody Capture (IMAC) ELISA to detect IgM antibodies, the ID Screen® Rift Valley Fever IgM Capture ELISA. The detection of anti-nucleoprotein antibodies by ELISA method indicates exposure to the virus by natural infection or by vaccination.

The ID Screen® Rift Competition Multi-Species ELISA is easy-to-use and detects anti-RVFV-NP antibodies in multiple species, including ruminants, horses, cats, dogs. It can be used for research use only on human samples.

This report summarizes validation data for this test.

DESCRIPTION AND PRINCIPLE OF THE TEST

Microwells are coated with a recombinant Rift Valley Fever Virus nucleoprotein. Samples to be tested and controls are added to the microwells. Anti-nucleoprotein antibodies, if present, form an antigen-antibody complex which masks the nucleoprotein epitopes.

After washing, an anti-nucleoprotein-peroxidase (HRP) conjugate is added to the wells. It fixes the remaining free nucleoprotein 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 presence of antibodies, no coloration appears. In the absence of antibodies, a blue coloration appears which becomes yellow after addition of the stop solution.

The microplate is read at 450 nm.

For each sample, the S/N ratio is calculated as follows: $\mathrm{S/N\%} = \frac{\mathrm{OD_{sample}}}{\mathrm{OD_{NC}}} \mathrm{x100}$

RESULT	STATUS
S/N% ≤ 40%	**Positive**
40% < S/N% ≤ 50%	**Doubtful**
S/N%> 50%	**Negative**

SPECIFICITY

STUDY 1: FIELD SAMPLES

A panel of 920 sera from disease-free European populations were tested using the ID Screen® Rift Competition Multi-Species ELISA kit [(1)]:

- 370 bovine sera.
- 68 horse sera.
- 280 ovine sera.
- 70 caprine sera.
- 16 feline sera.
- 35 canine sera.
- 81 human sera.

All samples were found negative when tested by the French Agricultural Research Centre for International Development (CIRAD, Montpellier, France) using the Virus Neutralisation Test (VNT).

Results are summarized in Figure 1 and Table 1 below.

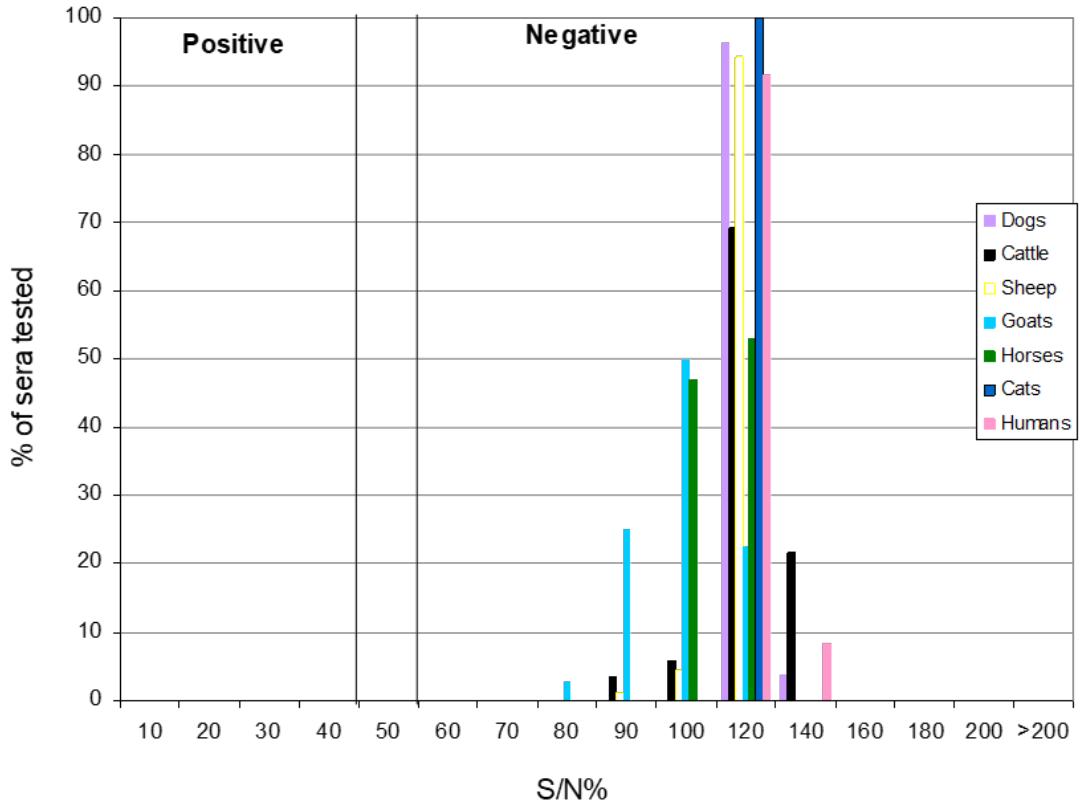

Figure 1: S/N% distribution for disease-free animals, n=920

SPECIES	NUMBER OF SAMPLES TESTED	MEASURED SPECIFICITY (%)	95% CI
Bovine	370	100	[99.0, 100]
Horse	68	100	[94.7, 100]
Ovine	280	100	[98.7, 100]
Caprine	70	100	[94.0, 100]
Feline	16	100	[80.6, 100]
Canine	35	100	[90.1, 100]
Human	81	100	[95.5, 100]
TOTAL	**920**	**100**	**[99.58, 100]**

Table 1: Measured specificities according to the species, n=920

RESULTS (Figure 1 and Table 1) :

- All sera tested were found negative.
- **Measured specificity = 100% (95% CI [99.58, 100], n=920).**

STUDY 2: ALPINE WILDLIFE SAMPLES

333 sera of alpine wildlife (chamois, deer, ibex and bighorn sheep) from a disease -free area (Hautes Alpes, France) were tested using the ID Screen® Rift Competition Multi-Species kit.
The results shown in Figure 2 are expressed as percentages of sample to negative control ratios (S/N%).

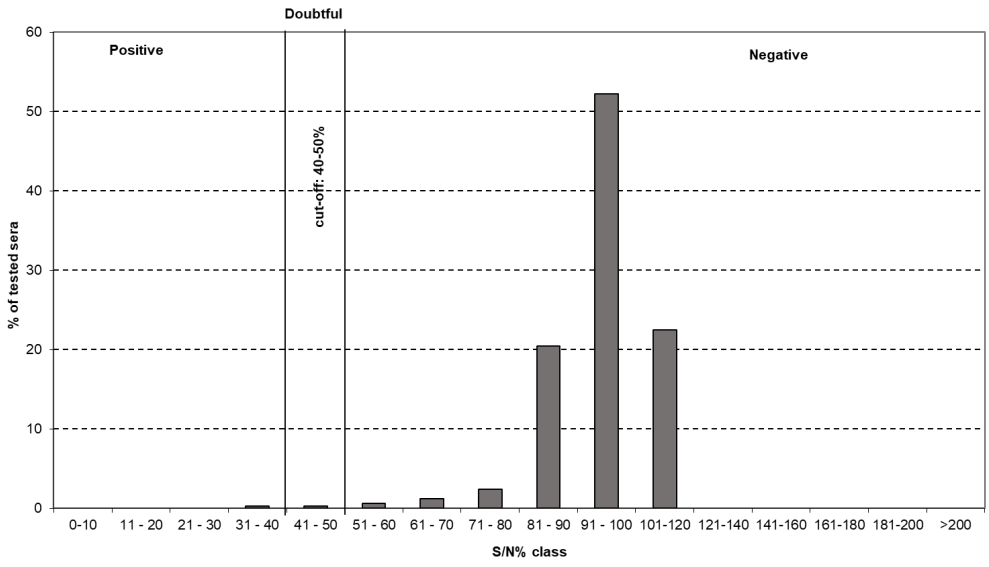

Figure 2: S/N% distribution for alpine wildlife animals, n=333

RESULTS (Figure 2) :

- 331/333 sera tested were found negative.
- **Measured specificity = 99.4% (95% CI [97.8, 99.8], n=333).**

GLOBAL RESULTS

Results from studies 1 and 2, indicating the measured specificity for each species tested, are summarized in the Table 2 below.

SPECIES	NUMBER OF SAMPLES TESTED	MEASURED SPECIFICITY (%)	95% CI
Bovine	370	100	[99.0, 100]
Horse	68	100	[94.7, 100]
Ovine	280	100	[98.7, 100]
Caprine	70	100	[94.0, 100]
Feline	16	100	[80.6, 100]
Canine	35	100	[90.1, 100]
Human	81	100	[95.5, 100]
Alpine wildlife	333	99.4	[97.8, 100]
TOTAL	**1253**	**99.8**	**[99.4, 100]**

Table 2: Measured specificity for each species tested

RESULTS (Table 2) :

- 1251/1253 sera tested were found negative with the D Screen® Rift Competition Multi-Species ELISA kit.
- **Measured specificity = 99.8% (95%CI [99.4 , 100], n=1253).**

ANALYTICAL SENSITIVITY

As no international standard exists for RVF serodiagnosis, Innovative diagnostics produces a freeze-died bovine serum containing anti-Rift Valley Fever virus (RVFV) specific IgG antibodies, which may be used to check that the test analytical sensitivity does not vary between runs, operators, and batches (available for purchase, product code MRI-RIFT).

The MRI-RIFT batch 002 was titrated in a negative serum and tested using the ID Screen® Rift Valley Fever Competition Multi-species.

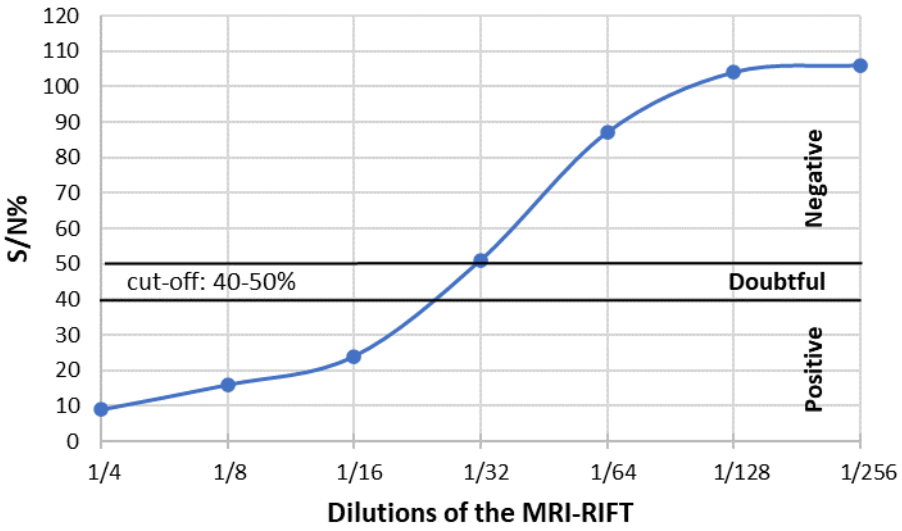

Figure 3: Titration of the IDvet freeze-dried positive serum

RESULTS (Figure 3):

- The MRI-RIFT was detected as positive when diluted up to 1:16.

SENSITIVITY

INTERNAL STUDY

40 sera, collected in 2008 , from infected bovine from Djibouti and Mayotte were tested using the ID Screen® Rift Competition Multi-Species ELISA, of which 18 were tested positive by Virus Neutralisation Test (VNT) [1].

Results are summarized in Table 3 and Figure 4 below.

SAMPLE ID	VNT Cut-off: >10 TITER VALUE (STATUS)	ID SCREEN® ELISA Cut-off: 40-50% S/N% (STATUS)
Mayotte 276	120 (+)	11 (+)
Mayotte 277	640 (+)	8 (+)
Mayotte 278	10 (+)	29 (+)
Mayotte 280	640 (+)	7 (+)
Mayotte 281	640 (+)	9 (+)
Mayotte 282	160 (+)	12 (+)
Mayotte 283	160 (+)	9 (+)
Mayotte 284	320 (+)	16 (+)
Mayotte 285	160 (+)	8 (+)
Mayotte 286	40 (+)	23 (+)
Mayotte 287	120 (+)	25 (+)
Mayotte 288	240 (+)	8 (+)
Mayotte 289	80 (+)	11 (+)
Mayotte 290	80 (+)	9 (+)
Mayotte 494	320 (+)	22 (+)
Mayotte 495	320 (+)	9 (+)
Mayotte 496	320 (+)	8 (+)
Mayotte 497	160 (+)	14 (+)

Table 3: Comparison between the ID Screen® ELISA and VNT

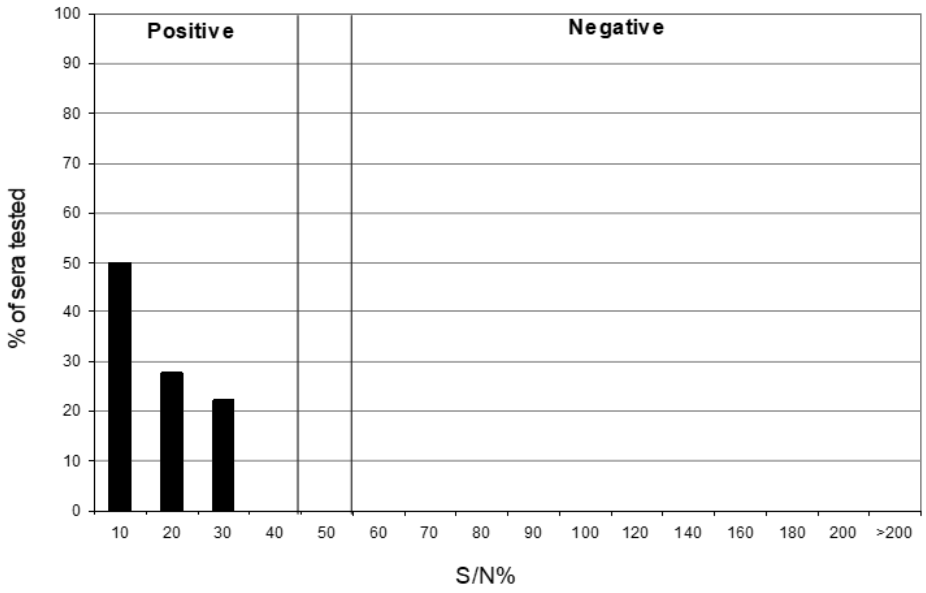

Figure 4: S/N% distribution for sera from infected cattle, n=40

RESULTS (Table 3 and Figure 4):

- All 40 sera were found positive by the ID Screen® ELISA**.**
- **Measured sensitivity = 100% (95%CI [91.24, 100], n=40).**
- **The measured correlation between VNT and the ID Screen® ELISA was 100%** (n=18).
- The ID Screen® Rift Competition Multi-Species ELISA shows an **excellent sensitivity.**

EXTERNAL STUDY

500 small ruminant samples from sacrificed livestock and 100 samples of their close human contacts collected in 2011, in Makkah, Saudi Arabia were tested using the ID Screen® Rift Competition Multi-Species ELISA [(2)].

SPECIES	INVESTIGATED SAMPLES	POSITIVE SAMPLES	SEROPREVALENCE(%)
Small ruminants	500	84	16.8
Humans	100	9	9

Table 4: Seroprevalence in small ruminants (n=500) and humans (n=100) [(2)]

RESULTS (Table 4):

- The ID Screen® Rift Competition Multi-Species ELISA was able to detect anti-RVFV antibodies in **small ruminants** and **in a human population in close contact with this RVFV-infected livestock.**

COMPARISON WITH ANOTHER COMMERCIAL BLOCKING ELISA

A comparison study was performed by Innovative Diagnostics between the ID Screen® Rift Competition Multi-Species ELISA and another commercial blocking ELISA (Kit A) following the manufacturer's instructions.

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

ANALYTICAL SENSITIVITY

Analytical sensitivity was compared between the ID Screen® ELISA and Kit A using the IDvet freeze-dried RVF IgG-positive serum (product code: MRI-RIFT).

To determine the last positive dilution, the serum was serially diluted in a pool of negative samples and then tested in parallel with both ELISAs.

Results obtained are shown in Table 5.

ID SAMPLE	DILUTION	ID SCREEN® ELISA Cut-off: 40-50%		KIT A Cut-off: 40-45%	
		S/N%	STATUS	S/N%	STATUS
MRI-RIFT	1 :4	8	(+)	67	(-)
	1 :8	12	(+)	74	(-)
	1 :16	23	(+)	83	(-)
	1 :32	43	(-)	94	(-)

Table 5: Analytical sensitivity obtained with the ID Screen® ELISA and Kit A

RESULTS (Table 5):

- The ID Screen® ELISA was abe to detect the sample as positive up to dilution 1:16, while Kit A did not detect any of the dilutions tested.
- The ID Screen® Rift Competition Multi-Species ELISA shows **a better analytical sensitivity** than Kit A.

DISTRIBUTION OF NEGATIVE AND POSITIVE SERA

The following sera were tested in parallel with ID Screen® ELISA and the Kit A:

- 13 bovine sera from an infected herd in Mayotte (IgG detection),
- 7 ovine samples from an experimental infection (IgM detection),
- 63 negative bovine sera from disease-free areas.

Results are shown in Figure 5.

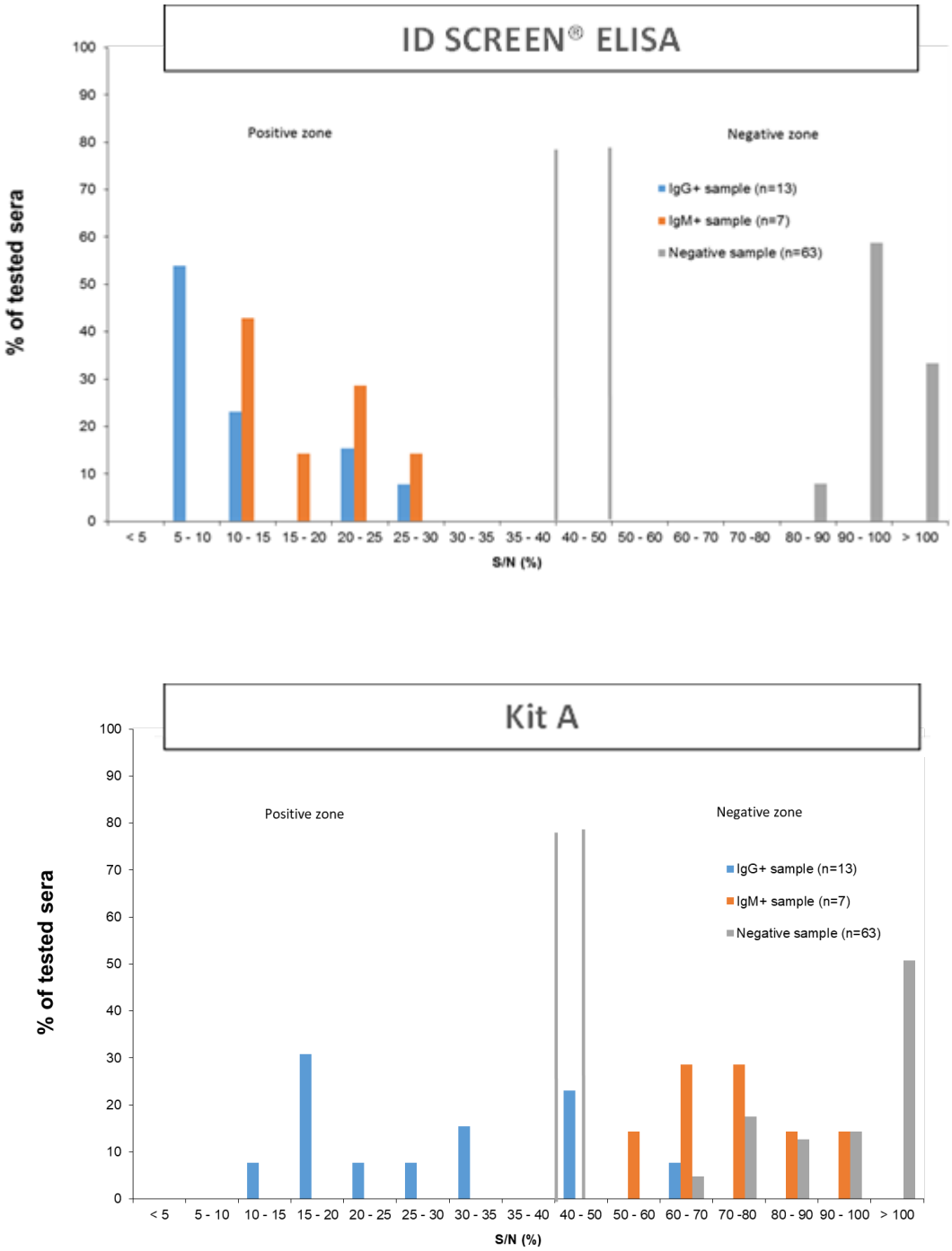

Figure 5: S/N% distribution for anti-RVFV IgG positive, IgM positive, and negative sera obtained with the ID Screen® ELISA and Kit A

RESULTS (Figure 5):

- The ID Screen® test showed **better discrimination between negative and positive sera** than Kit A (with thus lower risks of false negative and false positive results).
- **The ID Screen® Rift Competition Multi-Species ELISA is able to detect IgM antibodies, while Kit A cannot.**

REPEATABILITY

Intra-plate repeatability was evaluated by measuring the coefficient of variation (CV%) for 96 repetitions of a negative sample and 96 repetitions of a weakly positive sample.

The OD values obtained are shown in Table 6 below. Results are considered compliant if the CV% is less than 10%.

OD AT 450 NM											
NEGATIVE SAMPLE											
1.424	1.399	1.343	1.432	1.346	1.426	1.346	1.379	1.381	1.322	1.248	1.260
1.168	1.200	1.358	1.316	1.340	1.337	1.348	1.368	1.394	1.286	1.323	1.378
1.304	1.405	1.402	1.430	1.390	1.432	1.399	1.368	1.400	1.344	1.351	1.233
1.343	1.292	1.310	1.390	1.352	1.427	1.327	1.337	1.322	1.302	1.293	1.248
1.381	1.388	1.370	1.414	1.336	1.417	1.387	1.356	1.398	1.308	1.355	1.330
1.423	1.396	1.370	1.430	1.384	1.391	1.311	1.391	1.356	1.274	1.315	1.337
1.340	1.334	1.308	1.378	1.393	1.320	1.306	1.329	1.306	1.274	1.283	1.305
1.409	1.395	1.401	1.441	1.355	1.443	1.395	1.408	1.375	1.368	1.386	1.392
WEAK POSITIVE SAMPLE											
0.844	0.855	0.892	0.918	0.890	0.900	0.801	0.829	0.834	0.833	0.781	0.844
0.808	0.877	0.885	0.891	0.870	0.888	0.873	0.884	0.872	0.845	0.773	0.850
0.878	0.950	0.910	0.910	0.918	0.919	0.852	0.850	0.839	0.843	0.848	0.822
0.876	0.948	0.945	0.910	0.931	0.948	0.891	0.860	0.857	0.860	0.812	0.819
0.903	0.946	0.953	0.950	0.959	0.949	0.904	0.897	0.891	0.893	0.884	0.836
0.913	0.904	0.887	0.913	0.913	0.928	0.870	0.868	0.842	0.854	0.837	0.869
0.861	0.872	0.895	0.786	0.865	0.860	0.836	0.830	0.828	0.804	0.802	0.788
0.878	0.850	0.866	0.869	0.872	0.838	0.842	0.836	0.846	0.748	0.804	0.836

	AVERAGE OD	STANDARD DEVIATION	MINIMUM	MAXIMUM	CV%
NEGATIVE SAMPLE	1.354	0.054	1.168	1.443	**4%**
WEAK POSITIVE SAMPLE	0.869	0.045	0.748	0.959	**5%**

Table 6: Repeatability study for the ID Screen® ELISA

RESULTS (Table 6):

- The CV% obtained were 4% for the negative sample and 5% for the weak positive serum, demonstrating **excellent repeatability of the ID Screen® Rift Competition Multi-Species ELISA.**

REPRODUCIBILITY

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

This threshold dilution was tested in 16 independent runs by different operators and on different days.

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

Results are shown in Figure 6.

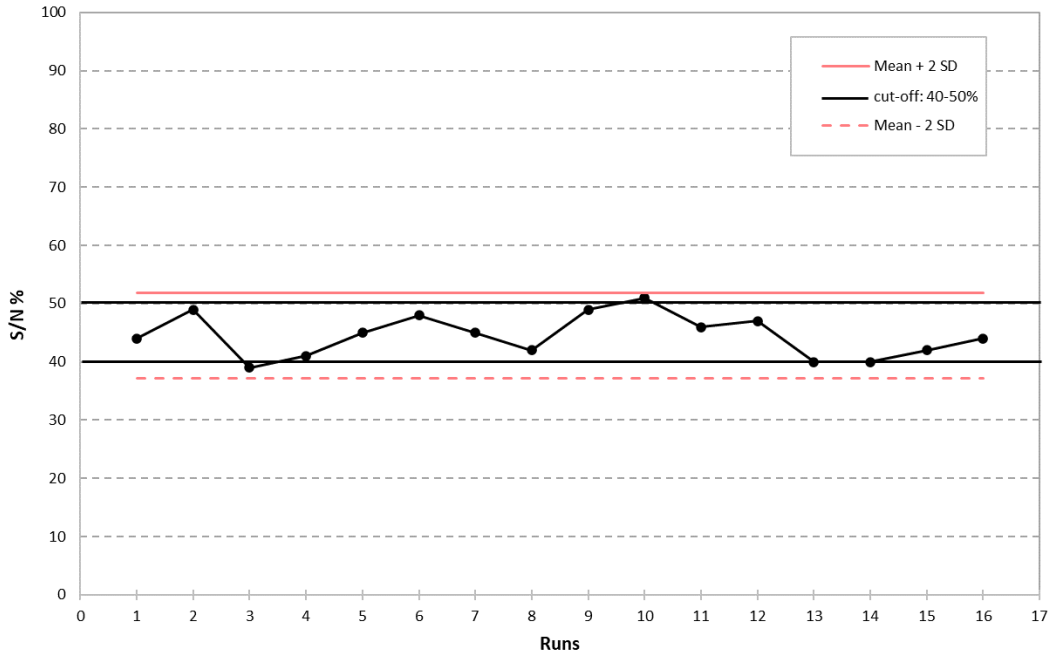

Figure 6: S/N% values for a threshold dilution of a positive serum sample tested in 16 independent runs

RESULTS (Figure 6):

- All values were within a range of 2 standard deviations around the mean, with a CV% of 8.2%.
- These results illustrate the **high reproducibility of the ID Screen® Rift Competition Multi-Species ELISA.**

ROBUSTNESS

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: 1 hour ± 6 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 O.D. value of the Negative Control (OD_{NC}) is greater than 0.7.
- the mean O.D. value of the Positive Control (OD_{PC}) is less than 30 % of the OD_{NC}.

Optical densities at 450nm obtained, in each condition, for both negative and positive controls, and the S/N% values obtained for 4 dilutions of the MRI-RIFT (batch 002) and 2 negative samples are detailed in Table 7 below.

SAMPLES/CONJUGATE/SUBSTRATE INCUBATION TIME		60 MIN / 30 MIN / 15 MIN			54 MIN / 27 MIN / 13 MIN	66MIN / 33 MIN / 17 MIN	
TEMPERATURE OF INCUBATION		16°C	21°C	26°C	16°C	26°C	
Positive control		0.074	0.064	0.055	0.052	0.055	OD 450 NM
		0.056	0.064	0.073	0.062	0.073	
Negative Control		1.601	1.802	2.123	1.488	2.123	
		1.647	1.775	2.063	1.431	2.063	
OD_{NC} > 0.7		√	√	√	√	√	
OD_{PC} /OD_{NC} < 0.3		√	√	√	√	√	
MRI-RIFT	diluted 1 :8	13	11	11	16	11	S/N%
	diluted 1 :16	27	26	22	32	22	
	diluted 1 :32	45	44	41	50	38	
	diluted 1 :64	61	62	54	66	51	
Negative sample	1	89	89	94	90	98	
	2	93	97	101	93	102	

Table 7: Robustness study for the ID Screen® ELISA

RESULTS (Table 7):

- **For each run and for each time and temperature condition tested**, the **test validation criteria** for both positive and negative controls **were obtained**, the **S/N% values obtained were similar,** and **analytical sensitivity was constant**, thereby demonstrating the **good robustness** of the **ID Screen® Rift Competition Multi-Species ELISA test.**

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 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 at 37°C ± 2°C should be greater than 75% after two months.

Results are shown in Figure 7 below.

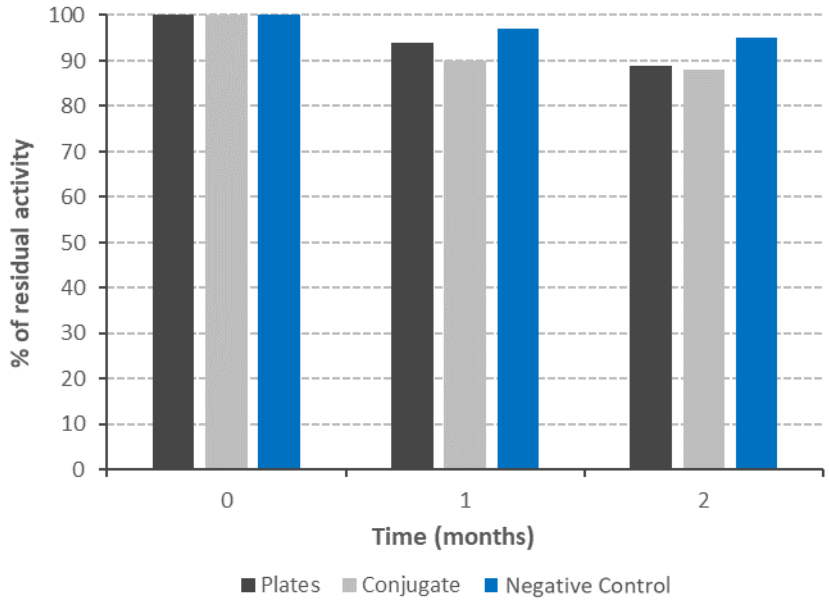

Figure 7: Percentage of residual activity of the plates, negative control and conjugate after stability testing at 37°C

RESULTS (Figure 7):

- After 2 months at 37°C, the plates, the conjugate and the negative control showed residual activity of 89%, 88% and 95% respectively, thus indicating **the high component stability** the **ID Screen® Rift Competition Multi-Species kit.**

CONCLUSION

The **ID Screen® Rift Competition Multi-Species** ELISA:

- demonstrates **excellent specificity on sera from multiple species, including humans.**
- demonstrates **excellent sensitivity on cattle sera.**
- was able to detect RVFV antibodies in RVFV-infected small ruminants and in **a human population in close contact with this livestock**.
- shows **better performances than another commercial blocking ELISA (Kit A).**
- is **a reliable tool** for detecting antibodies against the RVFV with **high reproducibility**, **repeatability and robustness.**

REFERENCES

(1) Comtet, L. et Pourquier, P. (Idvet),. Marié,. J-L. & Davoust, B. (French Defence Medical Service, Working group of animal epidemiology, Marseille, France), Cêtre-Sossah, C. (CIRAD, Montpellier, France). **Preliminary validation of the ID Screen® Rift Valley Fever Competition Multi-species ELISA.** Poster presented at the 2010 EAVLD meeting, Lelystad, The Netherlands.

(2) Mohamed A. M., Ashshi, A.M. et al. **Seroepidemiological survey on Rift Valley fever among small ruminants and their close human contacts in Makkah, Saudi Arabia,in 2011**. Rev Sci Tech2014 Dec; 33(3): 903–915

Related products

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
1114	12/2023	doc1243	Update: Addition/Edition of validation data	• Addition of several new chapters: - Specificity data on alpine wildlife (study 2) - External sensitivity data on small ruminants and their close human contacts. - Study comparison with other commercial ELISA - Repeatability, reproducibility, robustness, and stability data - Related products • Update of the general conclusion and references. • Innovative Diagnostics now mentioned as the kit's manufacturer.

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