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INTERNAL VALIDATION REPORT

ID SCREEN® AFRICAN HORSE SICKNESS INDIRECT

INDIRECT ELISA FOR THE DETECTION OF ANTIBODIES DIRECTED AGAINST THE VP7 PROTEIN OF THE AFRICAN HORSE SICKNESS VIRUS IN SERUM OR PLASMA FROM HORSES AND DONKEYS

METHOD	**Indirect ELISA**
TARGET	Antibodies directed against the VP7 of the African Horse Sickness Virus (AHSV)
SAMPLE TYPES	• Serum • Plasma
VALIDATED SPECIES	• Horse • Donkey
PRODUCT CODE	AHSS

With you at every step

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INTRODUCTION

African horse sickness (AHS) is an infectious disease caused by an Orbivirus of the family *Reoviridae*, which is transmitted by hematophagous midges of *Culicoïdes* spp.

9 serotypes of African horse sickness virus (AHSV) have been identified, causing respiratory and circulatory disorders. Horses are the most susceptible, with mortality rates of up to 90%. Donkeys and mules are also susceptible, but generally develop a milder form of the disease, while Zebras are highly resistant.

AHS is considered endemic in sub-Saharan Africa but its repercussions extend far beyond geographical borders. Major outbreaks have occurred in the Middle East, Asia and Southern Europe, mainly due to importation of infected animals.

Given the severity of the disease, and the potential for transmission through international horse movements, AHS is listed by the World Organisation for Animal Health (WOAH). It is the only equine disease for which the WOAH has introduced guidelines for member states to apply for official recognition of disease-free status.

The ID Screen® African Horse Sickness Indirect ELISA kit has been designed to detect antibodies directed against the VP7 protein, which is conserved among all 9 AHSV serotypes . It is a useful tool for managing international movements of equids, monitoring disease-free areas and implementing sanitary measures.

This report summarizes validation data obtained for this test.

DESCRIPTION AND PRINCIPLE OF THE TEST

Microwells are coated with AHSV VP7 recombinant protein. Samples to be tested and controls are added to the microwells. Anti-AHSV antibodies, if present, form an antigen-antibody complex.

After washing , an anti-equine IgG horseradish peroxidase (HRP) conjugate is added to the wells. It fixes to the anti-AHSV antibodies, forming an antigen-antibody-conjugate-HRP complex. The excess conjugate is then removed by washing, and the Substrate Solution (TMB) is added.

The resulting coloration depends on the quantity of specific antibodies present in the sample to be tested. In the presence of antibodies, a blue coloration appears which 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 sample to positive control ratio is calculated and interpreted as follows:

$$\mathbf{S/P\%} = \frac{\mathbf{OD_{sample} - OD_{NC}}}{\mathbf{OD_{PC} - OD_{NC}}} \times \mathbf{100}$$

RESULT	STATUS
S/P % ≤ 60 %	**Negative**
60% < S/P % ≤ 70%	**Doubtful**
S/P % > 70 %	**Positive**

SPECIFICITY

The following panel of 1015 samples from AHS-free countries (France, Brazil, Argentina and Iceland) was tested using the ID Screen® African Horse Sickness Indirect ELISA kit:

- 946 sera from horses,
- 27 sera from mules,
- 42 sera from donkeys.

The distribution of the results (expressed as sample to positive control ratios, S/P%) is shown in Figure 1.

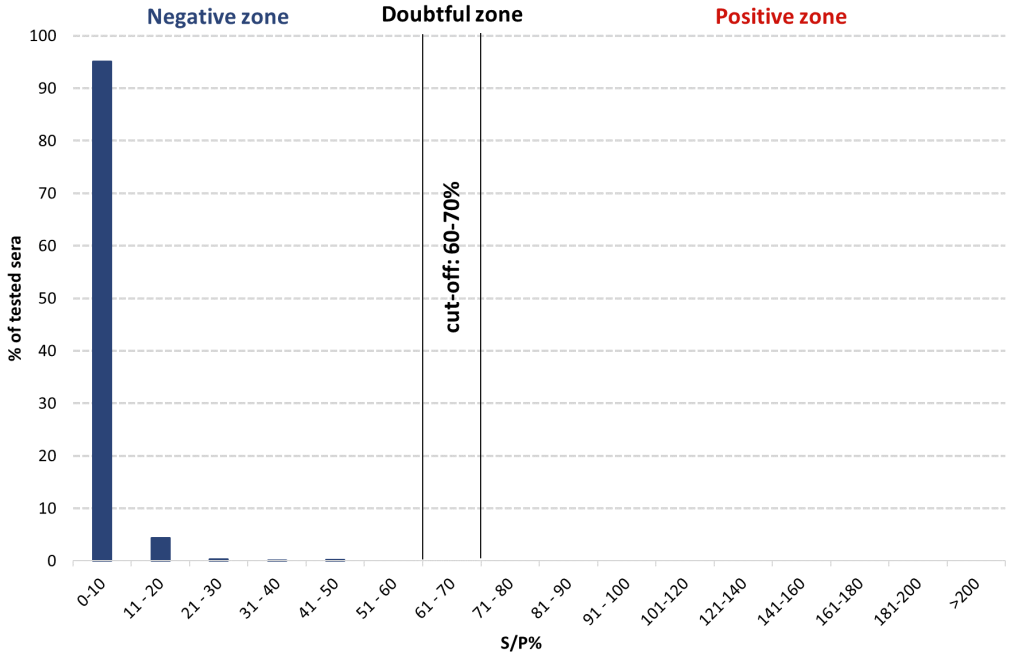


Figure 1: S/P% distribution for negative sera, n=1015

RESULTS (Figure 1) :

- 1015/1015 sera were found negative.
- **Measured specificity = 100% (95% CI [99.6, 100.0], n=1015).**

ANALYTICAL SENSITIVITY

The following 3 positive samples from the European Union (EURL) and WOAH Reference Laboratory for African Horse Sickness (Algete, Madrid, Spain) were tested:

- 2 serums collected from horses vaccinated with a mutated live vaccine(noted LCV 256 and LCV457),
- 1 sample composed of a pool of sera from naturally infected horses (noted LCV 565).

These sera were serially diluted and tested by Innovative Diagnostics using the ID Screen® ELISA and another commercially available ELISA kit called "Kit A".

Kit A is a competitive ELISA in which sera are diluted 1:5 and incubated 1 hour at 37°C, the conjugate is added for 30 minutes at 37°C, and the substrate incubated for 10 minutes at room temperature. The obtained results are expressed as Blocking Percentage (BP), as per the manufacturer's recommendations.

Results are presented in Table 1.

SAMPLE DILUTION	VACCINATED HORSES								INFECTED HORSES			
	Serum LCV 256				Serum LCV 457 (diluted 1/30)				Sample LCV 565			
	ID SCREEN® ELISA Cut-off: 60-70%		KIT A Cut-off: 45-50%		ID SCREEN® ELISA Cut-off: 60-70%		KIT A Cut-off: 45-50%		ID SCREEN® ELISA Cut-off: 60-70%		KIT A Cut-off: 45-50%	
	S/P%	STATUS	BP	STATUS	S/P%	STATUS	BP	STATUS	S/P%	STATUS	BP	STATUS
1:1	277	(+)	111	(+)	192	(+)	58	(+)	239	(+)	94	(+)
1:4	279	(+)	106	(+)	126	(+)	39	(-)	197	(+)	57	(+)
1:8	266	(+)	100	(+)	82	(+)	32	(-)	154	(+)	31	(-)
1:16	260	(+)	84	(+)	54	(-)	22	(-)	104	(+)	17	(-)
1:32	217	(+)	58	(+)	29	(-)	16	(-)	63	(+/-)	15	(-)
1:64	170	(+)	29	(-)	18	(-)	10	(-)	37	(-)	9	(-)
1:128	108	(+)	16	(-)	11	(-)	8	(-)	24	(-)	6	(-)
1:256	76	(+)	5	(-)	6	(-)	4	(-)	11	(-)	1	(-)

Table 1: Analytical sensitivity results obtained for 3 positive samples from AHS EU/WOAH-RL with the ID Screen® ELISA and Kit A

RESULTS (Table 1):

- The 3 positive sera were respectively identified as positive :
  up to the 1:256 dilution ; 1:8 dilution and 1:16 dilution with the ID Screen ELISA®.
  up to the 1:32 dilution ; 1:1 dilution; and 1:4 dilution with Kit A
- **On this serum panel, the analytical sensitivity of the ID Screen ELISA® is higher than that of Kit A.**

SENSITIVITY

Thirteen sera were tested in parallel using the ID Screen® ELISA and Kit A. Four of them were also tested on another commercially available ELISA kit, called "Kit B".

Kit B is an indirect ELISA in which sera are diluted 1:20 and incubated 30 minutes at room temperature. The conjugate is added for 30 minutes at room temperature, and substrate incubated for 15 minutes at room temperature. The obtained results are expressed as Positivity Percentage (PP), as per the manufacturer's recommendations.

Results are shown in Table 2.

SAMPLE DESCRIPTION				ID SCREEN® ELISA Cut-off: 60-70%		KIT A Cut-off: 45-50%		KIT B Cut-off: 60%	
ORIGIN	HORSE	AHSV SEROTYPE	SAMPLE ID	S/P%	STATUS	BP	STATUS	PP	STATUS
Sera from the Pirbright Institute (UK)	Experimentally infected	1 (20 dpi)	#NVRL-AHS-01	222	(+)	75	(+)	Not tested	
		2 (6 dpi)	#NVRL-AHS-02	228	(+)	78	(+)	Not tested	
		3 (46 dpi)	#NVRL-AHS-03	241	(+)	102	(+)	Not tested	
		4 (48 dpi)	#NVRL-AHS-04	238	(+)	91	(+)	Not tested	
		5 (16 dpi)	#NVRL-AHS-05	184	(+)	87	(+)	Not tested	
		6 (39 dpi)	#NVRL-AHS-06	250	(+)	88	(+)	Not tested	
		7 (80 dpi)	#NVRL-AHS-07	154	(+)	63	(+)	Not tested	
		8 (35 dpi)	#NVRL-AHS-08	204	(+)	102	(+)	Not tested	
		9 (39 dpi)	#NVRL-AHS-09	210	(+)	94	(+)	Not tested	
Reference sera from the EU/WOAH-RL (Spain)	Vaccinated	4 (Spain 2001)	LCV 457 (1:30)	192	(+)	86	(+)	31	(-)
	Vaccinated	4 (Spain 2001)	LCV 457 (1:60)	139	(+)	51	(+)	24	(-)
	Naturally infected and convalescent	4 (Spain 1989)	LCV 565 (1:15)	239	(+)	94	(+)	48	(-)
	Vaccinated	4 (Morocco 2008)	LCV 256	277	(+)	111	(+)	136	(+)

Table 2: Sensitivity results obtained for 13 positive samples with the ID Screen® ELISA, Kit A and Kit B

RESULTS (Table 2):

- **The ID Screen® ELISA was able to detect as highly positive** all sera against the **9 AHSV serotypes.**
- The **ID Screen® ELISA** shows **similar results** to those obtained with the **commercial competitive ELISA Kit A.**
- On the tested panel, the **ID Screen® ELISA presents better sensitivity** than that observed for the **commercial indirect ELISA Kit B.**

CUT-OFF VALUE DETERMINATION

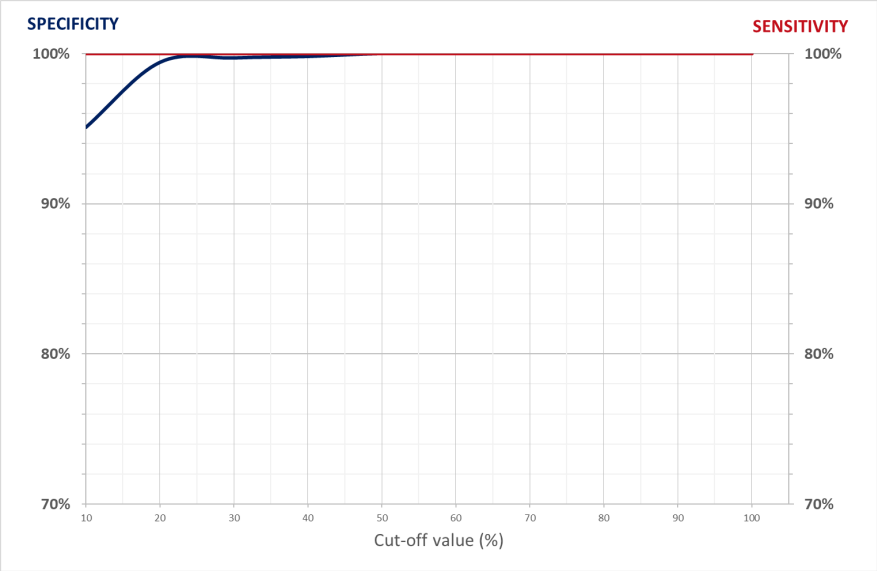
IMPACT OF THE CUT-OFF VALUE

The performances of the ID Screen® African Horse Sickness Indirect ELISA were evaluated, for different cut-off values, based on the previously described data acquired on 1015 negative and 13 positive samples.

Diagnostic specificity and sensitivity were calculated, with the 95% Confidence Interval (lower and upper limits), for different threshold values.

Results are presented in the Figure 2 .

A



B

CUT-OFF VALUES	SPECIFICITY		SENSITIVITY	
	Sp (%)	95% CI	Se (%)	95% CI
10	95.1	[93.6-96.2]	100	[75.7-99.4]
20	99.4	[98.7-99.7]	100	[75.7-100]
30	99.7	[99.1-99.9]	100	[75.7-100]
40	99.8	[99.6-100]	100	[75.7-100]
50	100	[99.6-100]	100	[75.7-100]
60	**100**	**[99.6-100]**	**100**	**[75.7-100]**
70	**100**	**[99.6-100]**	**100**	**[75.7-100]**
80	100	[99.6-100]	100	[75.7-100]
90	100	[99.6-100]	100	[75.7-100]
100	100	[99.6-100]	100	[75.7-100]

Figure 2: Specificity and sensitivity for different cut-off values: graphical representation (A) and summary table (B)

ROC CURVE

The data acquired was also used to plot a Receiver Operating Curve (ROC), a graphical way to show the connection/trade-off between sensitivity and specificity for every possible cut-off value (Figure 3).

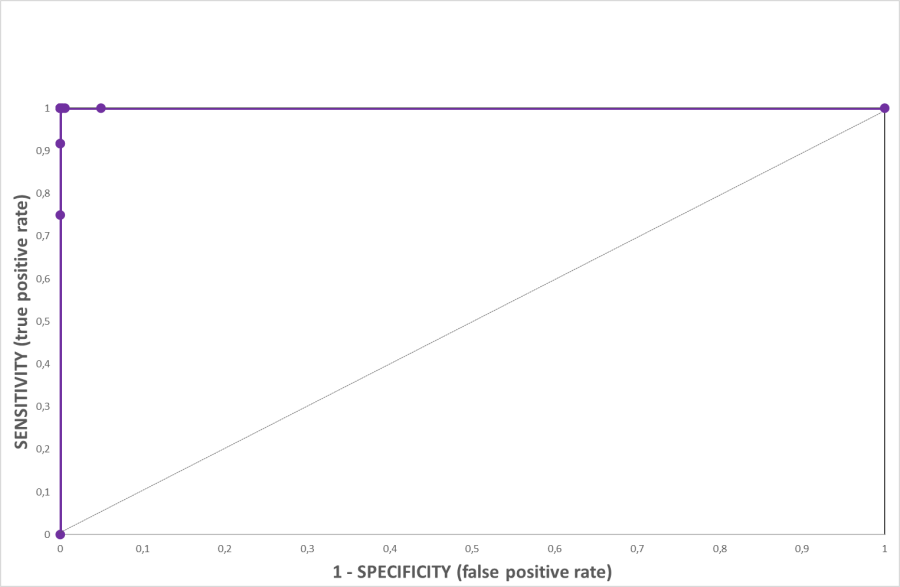


Figure 3: ROC curve obtained with the ID Screen® ELISA kit for 1015 negative and 13 positive horse sera (calculation of AUC)

RESULTS (Figures 2 and 3) :

- As illustrated by **an Area Under Curve (AUC) of 0.95** (95% CI [92.3, 97.9]) the obtained ROC curve confirms **the excellent diagnostic and discrimination capabilities of the ID Screen® African Horse Sickness Indirect ELISA.**
- The diagnostic sensitivity and specificity of the ID Screen® ELISA according to each threshold value confirms that **setting the S/P% cut-off value at 70%** provides **optimum sensitivity and specificity conditions**.
- Considering the economic and health impact of a misdiagnosis, **a doubtful zone is established for S/P% values between 60% and 70%.**

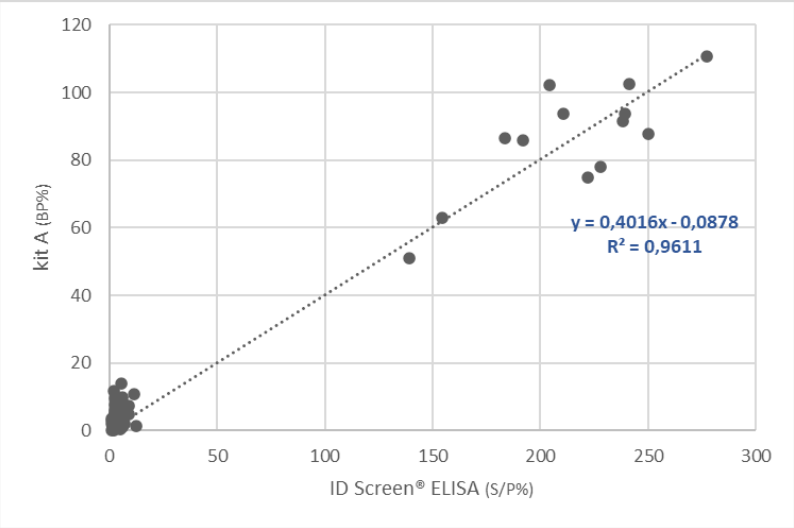
GLOBAL CORRELATION WITH KIT A

The following panel of 109 horse sera was tested in parallel with the ID Screen® ELISA and Kit A:

- 96 negative horse sera from AHS-free countries,
- 13 positive horse sera (described previously in the Sensitivity Chapter).

Correlation between the S/P% obtained with the ID Screen® ELISA and Blocking Percentage (BP%) obtained with Kit A is shown in Figure 4 A and the results obtained with both kits are summarized in Figure 4 B.

A



B

		ID SCREEN® ELISA		
		POSITIVE	NEGATIVE	TOTAL
KIT A	POSITIVE	13	0	13
	NEGATIVE	0	96	96
	TOTAL	13	96	109

Figure 4: Comparison of results obtained with the ID Screen® ELISA and Kit A: graphical representation (A) and summary table (B)

RESULTS (Figure 4):

- **109/109 samples** gave identical results on both ELISA tests.
- The **measured percentage of correlation was of 100%.**
- **Test agreement** : **k = 1** (95% CI [1.00, 1.00]). Please refer to note 1 for further explanation of kappa correlation coefficient (k).
- **The ID Screen® ELISA shows excellent agreement with Kit A.**

NOTE 1:

The inter-rater agreement statistic, Kappa correlation coefficient can be interpreted as follows:

κ value	Strength of agreement
< 0.20	**Very weak**
0.21 - 0.40	**Weak**
0.41 – 0.60	**Medium**
0.61 – 0.80	**Statisfactory**
0.81 – 1.00	**Excellent**

References:
- Altman DG (1991) Practical statistics for medical research. London: Chapman and Hall.
- Cohen J (1960) A coefficient of agreement for nominal scales. Educational and Psychological Measurement 20:37-46.
- Fleiss JL, Levin B, Paik MC (2003) Statistical methods for rates and proportions, 3rd ed. Hoboken: John Wiley & Sons.

EXTERNAL STUDIES

EVALUATION BY THE EU/WOAH REFERENCE LABORATORY

The European Union Reference Laboratory (EURL) for African horse sickness, which is also WOAH Reference Laboratory (Laboratorio Central de Veterinaria, Algete, Madrid, Spain), evaluated the performances of the ID Screen® African Horse Sickness Indirect ELISA and made, in its report on January 2024, the conclusion cited below:

CITATION FROM THE AHS EU/WOAH-RL REPORT:

"this kit is suitable for the detection of specific antibodies against African horse sickness virus, and when used according to the established protocol, complies with data from validation provided by the manufacturer".

EVALUATION BY THE GERMAN REFERENCE LABORATORY FOR AHS

The ID Screen® African Horse Sickness Indirect ELISA was tested by the German Reference Laboratory for AHS (Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, FLI Insel Riems) on 54 horse sera, including :

- 32 negative sera collected from Germany,
- 8 monospecific sera from experimentally infected horses obtained by the Pirbright Institute, UK (noted #NVRL-AHS).
- 5 other positive sera,
- 9 monospecific sera from horses immunized against each AHSV serotype with inactivated vaccines during a study carried out by CVRL in Dubai (Wernery U et al. 2021).

The samples were also tested in parallel with Kit A.

SAMPLE	ID SCREEN® ELISA Cut-off: 60-70%		KIT A Cut-off : 45-50%		SAMPLE	ID SCREEN® ELISA Cut-off: 60-70%		KITA Cut-off: 45-50%	
	S/P%	STATUS	BP	STATUS		S/P%	STATUS	BP	STATUS
HS 14/16-4038	3	(-)	12	(-)	HS 16/16-4219	2	(-)	13	(-)
HS 14/16-4083	4	(-)	21	(-)	HS 16/16-4221	1	(-)	18	(-)
HS 14/16-4085	2	(-)	18	(-)	HS 16/16-4234	2	(-)	19	(-)
HS 14/16-4086	2	(-)	15	(-)	HS 16/16-4235	4	(-)	14	(-)
HS 14/16-4087	2	(-)	20	(-)	HS 16/16-4254	2	(-)	23	(-)
HS 14/16-4088	2	(-)	16	(-)	HS 16/16-4255	1	(-)	15	(-)
HS 14/16-4095	2	(-)	12	(-)	HS 16/16-4257	1	(-)	17	(-)
HS 14/16-4096	3	(-)	14	(-)	HS 16/16-4262	1	(-)	10	(-)
HS 14/16-4097	1	(-)	14	(-)	HS 17/16-353	1	(-)	13	(-)
HS 14/16-4098	1	(-)	14	(-)	HS 17/16-621	1	(-)	9	(-)
HS 14/16-4099	1	(-)	20	(-)	HS 19/16-815	2	(-)	11	(-)
HS 14/16-4115	1	(-)	17	(-)	HS 19/16-816	1	(-)	7	(-)
HS 14/16-4196	4	(-)	19	(-)	HS 19/16-817	2	(-)	7	(-)
HS 14/16-4117	1	(-)	16	(-)	HS 20/16-110	1	(-)	10	(-)
HS 14/16-4118	2	(-)	20	(-)	HS 23/16-311	3	(-)	10	(-)
HS 14/16-4119	2	(-)	15	(-)	HS 23/16-312	2	(-)	11	(-)

Table 3: Comparison of results obtained by the German reference laboratory for AHS on 32 negative samples with the ID Screen® ELISA and Kit A

SAMPLE		ID SCREEN® ELISA Cut-off : 60-70%		KIT A Cut-off : 45-50%	
DESCRIPTION	DILUTION	S/P%	STATUS	BP	STATUS
#NVRL-AHS-01	1:10	116	(+)	59	(+)
	1:20	112	(+)	28	(-)
#NVRL-AHS-02	1:10	160	(+)	71	(+)
	1:20	147	(+)	55	(+)
#NVRL-AHS-03	1:10	132	(+)	78	(+)
	1:20	139	(+)	66	(+)
#NVRL-AHS-04	1:10	191	(+)	76	(+)
	1:20	139	(+)	67	(+)
#NVRL-AHS-05	1:10	112	(+)	65	(+)
	1:20	103	(+)	47	(+/-)
#NVRL-AHS-06	1:10	179	(+)	72	(+)
	1:20	127	(+)	60	(+)
#NVRL-AHS-08	1:10	228	(+)	93	(+)
	1:20	205	(+)	90	(+)
#NVRL-AHS-09	1:10	157	(+)	76	(+)
	1:20	150	(+)	70	(+)
Freeze dried positive serum	1:10	243	(+)	96	(+)
	1:20	237	(+)	94	(+)
118/98 Nr. 1	1:1	97	(+)	63	(+)
MB110/3 Nadra 4.9.98	1:1	154	(+)	79	(+)
MB 092/2 19.1.98 54/98	1:1	211	(+)	94	(+)
MB 134/99 2907	1:1	164	(+)	87	(+)

Table 4: Comparison of results obtained by the German reference laboratory for AHS on 13 positive samples with the ID Screen® ELISA and Kit A

SAMPLE	TITER (LAST POSITIVE DILUTION)	
	ID SCREEN® ELISA Cut-off: 60-70%	KIT A Cut-off: 45-50%
CVRL Dubai AHSV-1	1:32	1:4
CVRL Dubai AHSV-2	1:128	1:16
CVRL Dubai AHSV-3	1:128	1:8
CVRL Dubai AHSV-4	1:32	1:1
CVRL Dubai AHSV-5	1:64	1:4
CVRL Dubai AHSV-6	1:16	1:1
CVRL Dubai AHSV-7	1:64	1:4
CVRL Dubai AHSV-8	1:128	1:8
CVRL Dubai AHSV-9	1:128	1:8
#NVRL-AHS-08	1:320	1:320

Table 5: Comparison of results obtained by the German reference laboratory for AHS on 10 positive monospecific horse sera with the ID Screen® ELISA and Kit A

Log2 dilution series were prepared in PBS, tested with the kits and the last positive dilution was defined as the titer.

RESULTS FROM THE GERMAN REFERENCE LABORATORY FOR AHS

(Tables 3, 4 and 5):

- 54/54 samples gave **identical results on both ELISA tests**.
- All sera directed against **each of the 9 AHSV serotypes were found highly positive** on the ID Screen® ELISA**.**
- When comparing the last positive dilution, 10/10 positive monospecific sera gave **higher ELISA titer on the the ID Screen® ELISA than on Kit A.**
- **This study confirms the excellent status correlation between both kits and further suggests that the ID Screen® ELISA has a higher analytical sensitivity than Kit A.**

REPEATABILITY

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

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

OD AT 450 NM											
POSITIVE SAMPLE											
0.886	0.931	0.917	0.918	0.927	0.944	0.908	0.9	0.888	0.975	0.898	0.942
0.904	0.944	0.944	0.945	0.965	0.94	0.946	0.912	0.939	0.917	0.931	0.94
0.879	0.863	0.89	0.939	0.887	0.91	0.934	0.937	0.889	0.924	0.883	0.924
0.883	0.896	0.896	0.894	0.933	0.921	0.941	0.905	0.907	0.859	0.923	0.937
0.919	0.918	0.93	0.954	0.915	0.947	0.897	0.925	0.908	0.917	0.933	0.955
0.882	0.891	0.908	0.883	0.919	0.877	0.949	0.913	0.946	0.923	0.953	0.93
0.92	0.87	0.945	0.789	0.942	0.799	0.97	0.835	0.943	0.865	0.982	0.886
0.935	0.876	0.963	0.901	0.947	0.837	1.002	0.947	0.972	0.995	1.019	0.995
THRESHOLD SAMPLE											
0.531	0.585	0.591	0.578	0.568	0.589	0.556	0.586	0.567	0.604	0.576	0.618
0.59	0.578	0.584	0.598	0.607	0.586	0.599	0.565	0.595	0.593	0.594	0.608
0.563	0.517	0.539	0.598	0.545	0.545	0.558	0.548	0.571	0.578	0.559	0.584
0.558	0.563	0.558	0.56	0.579	0.547	0.547	0.578	0.589	0.546	0.573	0.593
0.555	0.526	0.562	0.579	0.544	0.562	0.556	0.564	0.576	0.578	0.577	0.621
0.565	0.557	0.566	0.566	0.562	0.53	0.581	0.562	0.592	0.57	0.582	0.578
0.574	0.565	0.588	0.549	0.581	0.538	0.585	0.492	0.586	0.579	0.598	0.589
0.587	0.564	0.591	0.563	0.563	0.571	0.579	0.595	0.616	0.608	0.605	0.612

	AVERAGE OD	STANDARD DEVIATION	MINIMUM	MAXIMUM	CV%
POSITIVE SAMPLE	0.919	0.039	0.789	1.019	**4.2%**
THRESHOLD SAMPLE	0.573	0.023	0.492	0.621	**4.0%**

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

RESULTS (Table 6):

- The CV% obtained were 4.2% for the positive sample and 4.0% for the threshold sample, demonstrating the **excellent repeatability of the ID Screen® African Horse Sickness Indirect ELISA test**.

REPRODUCIBILITY

A positive serum was diluted in a negative serum 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 considered compliant if the values are within ± 2 standard deviations around the mean value and the CV% is less than 15%.

Results are shown in Figure 5.

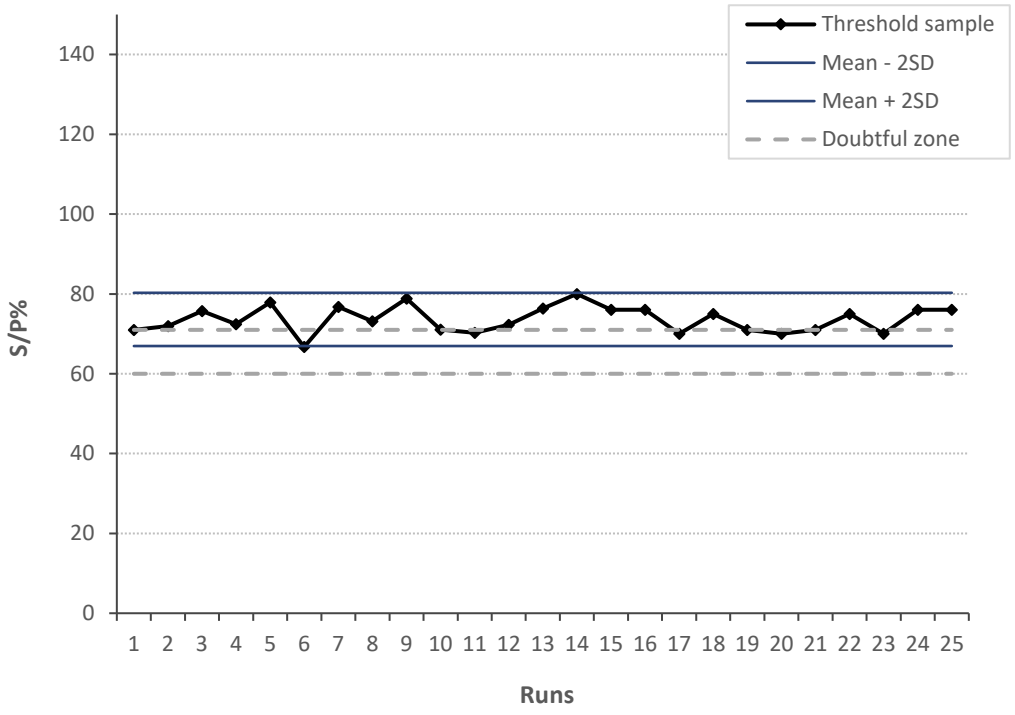


Figure 5: S/P% values obtained for a threshold dilution of a positive serum sample tested in 25 independent runs

RESULTS (Figure 5):

- All values are within a range of 2 standard deviations around the mean value, with a **CV% of 4.5%.**
- These results illustrate the **high reproducibility of the ID Screen® African Horse Sickness Indirect ELISA test.**

ROBUSTNESS

Test robustness was evaluated by 3 operators in 3 independent runs 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 ($OD_{PC} \geq 0.350$).
- The ratio of the mean value of the Positive Control and Negative Control (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 are detailed in the Table 7. Three dilutions of a strong positive sample and 2 negative samples were also tested and the S/P% values obtained are detailed below.

SAMPLES/CONJUGATE/SUBSTRATE INCUBATION TIME		45 MIN / 30 MIN / 15 MIN			40 MIN / 27 MIN / 13 MIN	50 MIN / 33 MIN / 17 MIN	
TEMPERATURE OF INCUBATION		16°C	21°C	26°C	16°C	26°C	
Negative Control		0.045	0.121	0.047	0.055	0.045	OD 450 NM
		0.054	0.054	0.047	0.046	0.039	
Positive Control		0.696	0.857	1.032	0.52	1.246	
		0.737	0.897	1.067	0.522	1.25	
$OD_{PC} \geq 0.350$		√	√	√	√	√	
$OD_{PC}/OD_{NC} > 3$		√	√	√	√	√	
Positive sample	diluted 1:64	169	159	158	172	148	S/P%
	diluted 1:128	109	107	112	110	115	
	diluted 1:256	62	67	79	66	77	
Negative sample	1	7	1	5	6	5	
	2	7	0	3	5	4	

Table 7: Robustness study for the ID Screen® ELISA

RESULTS (Table 7):

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

STABILITY

The shelf-life of the products 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°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 6 below.

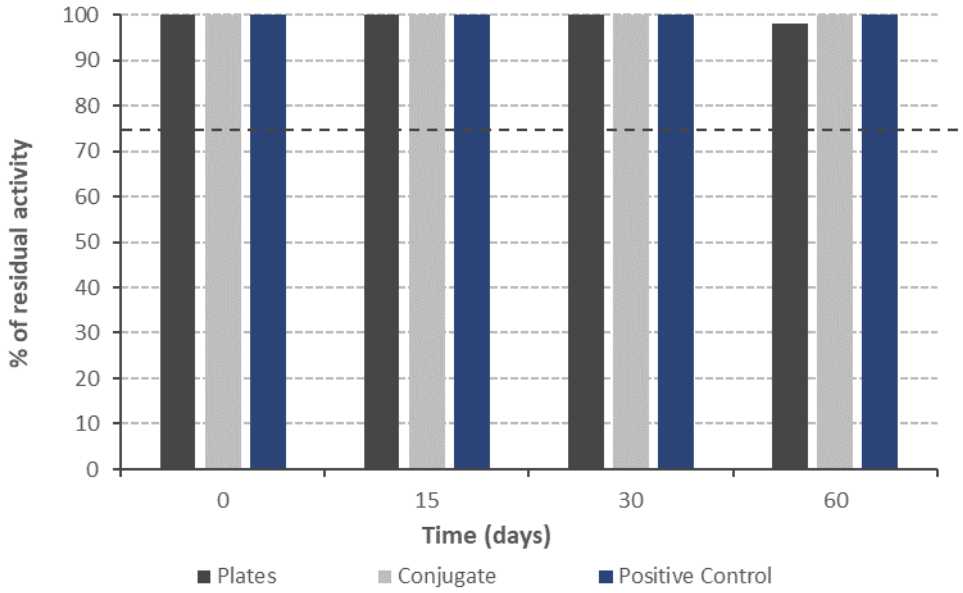


Figure 6: Percentage of residual activity of the plates, positive control and conjugate after stability testing at 37°C

RESULTS (Figure 6):

- After 2 months at 37°C, the plates, the conjugate and the positive control showed residual activity of 98%, 100% and 100% respectively, thus indicating **high component stability for the ID Screen® African Horse Sickness Indirect ELISA kit.**

CONCLUSION

The **ID Screen® African Horse Sickness Indirect** ELISA:

- is a **reliable tool for** the detection of antibodies against **AHSV VP7 protein**, allowing efficient **detection of all the 9 serotypes** of the virus.
- shows a **very high specificity.**
- shows **excellent diagnostic correlation** with another commercial ELISA (Kit A) with a **higher analytical sensitivity.**
- gave **excellent results** during **external evaluations** by the **AHS EURL/WOAH reference laboratory** (Algete, Madrid, Spain) a**nd the German reference laboratory for AHS (**FLI, Insel Riems)
- is easy-to-use with results in just 90 minutes.
- harbours **good repeatability, reproducibility and robustness**.

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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
0224	02/2024	doc1272	Not applicable (first version)	N/A

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