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INTERNAL VALIDATION REPORT

ID SCREEN® FLAVIVIRUS COMPETITION

COMPETITIVE ELISA FOR THE DETECTION OF ANTIBODIES AGAINST THE FLAVIVIRUS PR-E PROTEIN IN SERUM OR PLASMA FROM SUSCEPTIBLE SPECIES

METHOD	**Competitive ELISA**
TARGET	Antibodies directed against the Flavivirus prE protein
SAMPLE TYPES	• Serum • Plasma
VALIDATED SPECIES	• Equids • Birds • Humans (for Research Use Only) • Any other susceptible species *Please refer to our literature report referencing external publications and peer-reviewed references for more information.*
PRODUCT CODE	WNC

With you at every step

Table of contents

INTRODUCTION

West Nile virus (WNV) is a zoonotic mosquito-transmitted arbovirus belonging to the single genus in the family *Flaviviridae,* the *Flavivirus*.

Flaviviridae is a family of positive, single-stranded, enveloped RNA viruses that can occasionally infect humans. Members of this family are found in arthropods, in primarily mosquitoes and ticks. Mosquitoes are notably vectors of the Yellow Fever Virus, Dengue Fever Virus (DENV), Japanese Encephalitis Virus (JEV), Zika virus (ZIKAV), WNV and Usutu Virus (USUV), which causes widespread morbidity and mortality throughout the world. While other Flaviviruses, such as Tick-Borne Encephalitis Virus (TBEV), are transmitted by ticks and are responsible for encephalitis and haemorrhagic diseases.

The arboviruses are maintained in nature by cycling through birds and mosquitoes. Migratory birds are thought to be primarily responsible for virus dispersal (1).

For many avian species, WNV infection causes no overt signs, while other birds often succumb to fatal systemic illness. Among mammals, clinical disease is primarily exhibited in horses and humans, which are considered dead-end hosts. Clinical signs of WNV infection in horses arise from viral-induced encephalitis or encephalomyelitis (2).

Genetic analysis of WNV isolates separates strains into two clades. Lineage 1 isolates are found in Northern and Central Africa, Israel, Europe, India, Australia, and the American continent. Lineage 2 strains are endemic in Central and Southern Africa and Madagascar, with co-circulation of both virus lineages in Central Africa. In recent years, lineage 2 outbreaks have also occurred in Europe (3).

Serology, including the ELISA technique, may be used to diagnose and monitor disease.

Initially designed for the detection of anti- WNV antibodies in avian and horse sera (11, 12,13,14,15,16), the ID Screen® Flavivirus Competition ELISA has been proven to detect antibodies against a wide range of Flaviviruses in multiple species, including humans (4, 5, 6, 7, 8, 9, 10).

This report summarizes validation data obtained for this test for both equine and avian samples. Studies were performed either by Innovative Diagnostics directly or in collaboration with the French National Reference Laboratory for Flaviviruses (ANSES Maisons-Alfort), or by GD Animal Health (Deventer, The Netherlands).

DESCRIPTION AND PRINCIPLE OF THE TEST

Microwells are coated with a purified extract of the West Nile virus.

Samples to be tested and controls are added to the microwells. Anti-pr-E antibodies, if present, form an antigen- antibody complex.

An anti-pr-E antibody horseradish peroxidase (HRP) conjugate is added to the microwells. It binds to the remaining free pr-E epitopes, forming an antigen-conjugate-peroxidase complex.

After washing in order to eliminate the excess conjugate, the Substrate Solution (TMB) is added.

The resulting coloration depends on the quantity of specific antibodies present in the specimen to be tested. In the 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 sample to negative control ratio (S/N%) is calculated and interpreted as follows:

$$\mathbf{S/N\%} = \frac{\mathbf{OD_{sample}}}{\mathbf{OD_{NC}}} \times \mathbf{100}$$

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

SPECIFICITY

EQUINE SAMPLES

Innovative Diagnostics study

261 negative horse sera from Calvados, France (2008) were tested.

Results are shown in Figure 1 below.

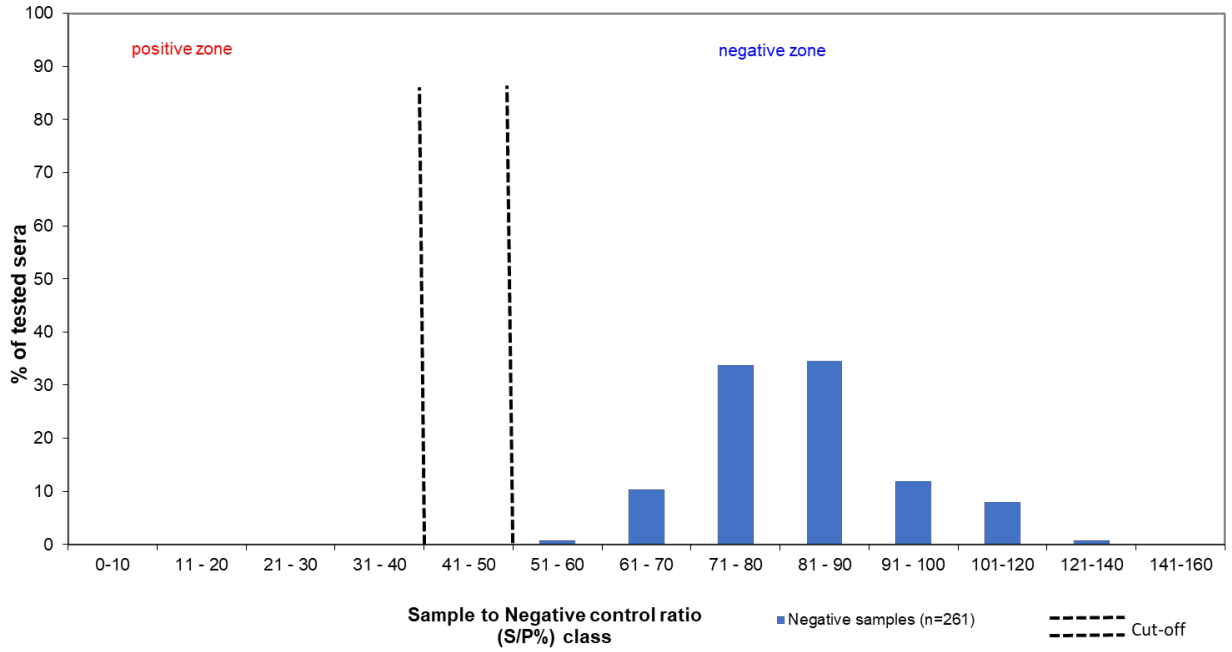


Figure 1 : S/N% distribution for disease-free horse sera, n=261

RESULTS (Figure 1) :

- **All sera gave negative results.**
- **Measured specificity = 100%** (95% CI [98.55, 100], n=261).

AVIAN SAMPLES

Innovative Diagnostics study on disease-free birds

Innovative Diagnostics tested 500 sera from disease-free ducks and chickens from the Loire-Atlantique region in France.

Results are shown in Figure 2 below.

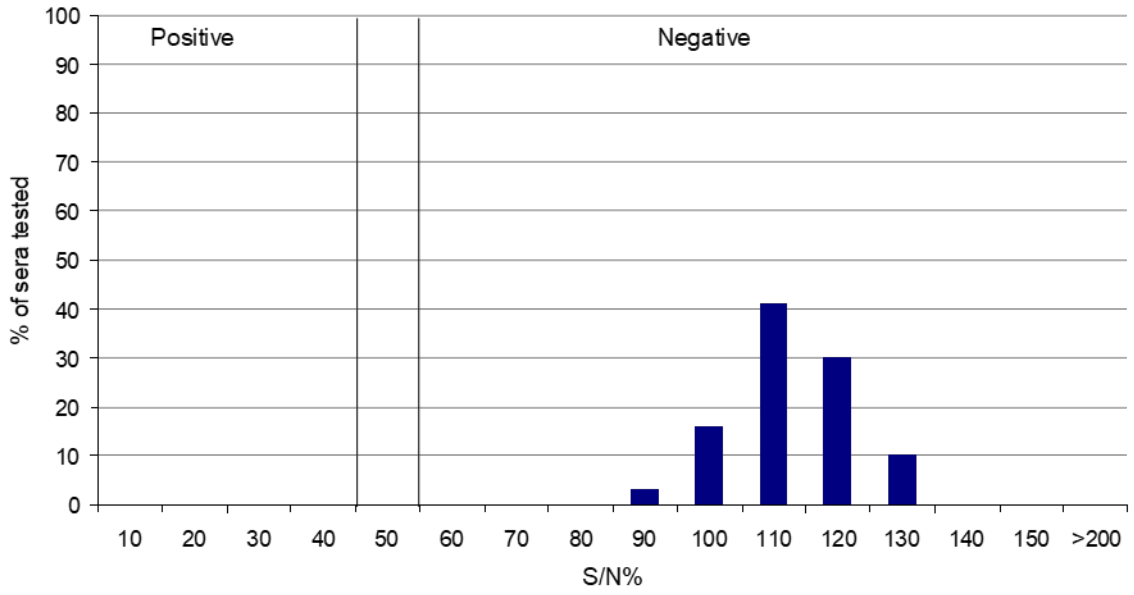


Figure 2 : S/N% distribution for disease-free avian sera, n=500

RESULTS (Figure 2):

- **All sera gave negative results.**
- **Measured specificity = 100%** (95% CI [99.24, 100], n = 500).

GD Animal Health study on disease-free chickens

45 chicken sera from The Netherlands were tested by GD Animal Health (Deventer, The Netherlands).

RESULTS

- **All sera gave negative results.**
- **Measured specificity = 100%** (95% CI [99.13, 100], n = 45).

GD Animal Health study on free-range layers

In a second study performed by GD Animal Health, 384 free-range layers from The Netherlands were tested.

Data obtained on old samples (2-3 weeks) and fresh samples (0-2 weeks) are shown in Figure 3 below.

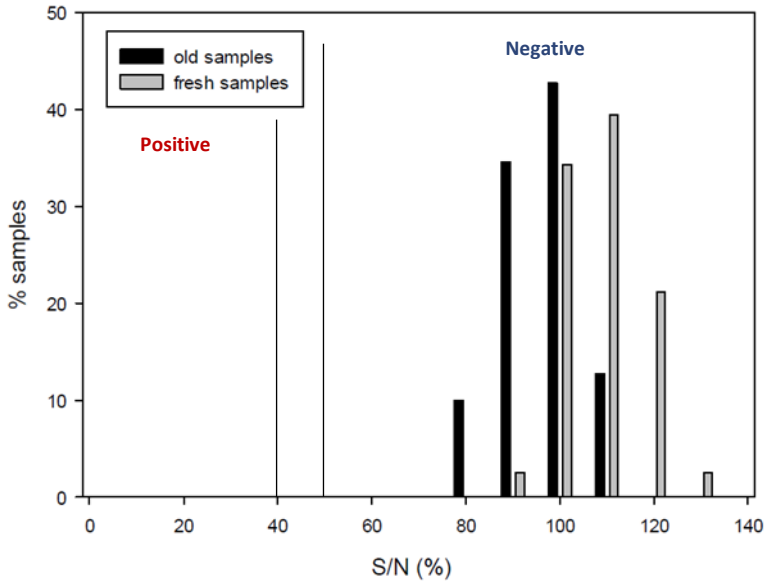


Figure 3 : Results obtained for free-range layers (n=384)
with comparison between old samples (2-3 weeks) and fresh samples (0-2 weeks)

RESULTS (Figure 3):

- **All sera gave negative results.**
- **Measured specificity = 100%** (95% CI [99.01, 100], n =384).
- Samples that were stored for more than 2 weeks at 4°C had a statistically significant (P<0.001) lower S/N% than samples that were stored up to 2 weeks at 4°C.

SPECIFICITY SUMMARY (EQUINE AND AVIAN SAMPLES)

Specificity results on equine and avian samples are summarized in Table 1 below.

SPECIES	SPECIFICITY [95% CI]
Equine	100% [98.65, 100], n=261
Avian	100% [99.59, 100], n=929
TOTAL	**100% [99.68, 100], n=1190**

Table 1: Measured specificity for negative horses and birds, n=1190

RESULTS (Table 1):

- **The measured specificity is 100 % for horses as well as birds.**
- The ID Screen® ELISA shows **an excellent specificity** for both equine and avian samples.
  Total measured specificity: 100% (95% CI [99.68, 100], n=1190).

SENSITIVITY

EQUINE SAMPLES

Innovative Diagnostics study

19 positive horse sera from the South of France were analysed. WNV outbreaks were observed in this region in 2003 (Camargue) and 2006 (Perpignan). The animals tested showed clinical signs and were confirmed positive by a Virus Neutralization Test (VNT) performed by the French National Reference Laboratory for Flaviviruses (ANSES Maisons-Alfort).

Results are shown in Figure 4 below.

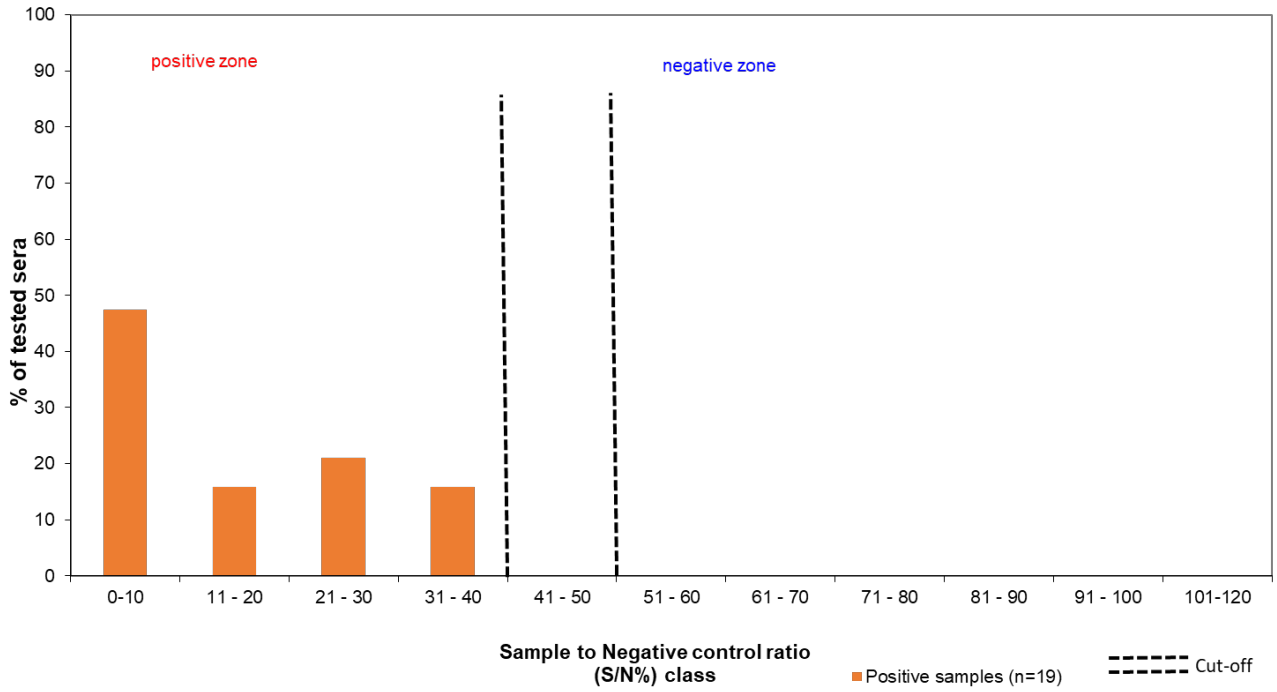


Figure 4 : S/N% distribution for positive horse sera, n=19

RESULTS (Figure 4) :

- **All 19 WNV-positive horse sera were correctly identified as positive by the ID Screen® ELISA.**
- **Measured sensitivity = 100%** (95% CI [83.18, 100], n=19).

AVIAN SAMPLES

GD Animal Health study on Romanian field sera

25 field sera from chickens tested positive using an in-house blocking ELISA for WNV (performed by G. Nicolescu in Bucharest, Romania) were diluted 1:16, instead of 1:2 due to limited volumes, and assayed with the ID Screen® ELISA.

RESULTS

- Of these sera tested at a 1:16 dilution:
  - 22/25 were found positive,
  - 1 serum gave a doubtful result,
  - 2 sera were found negative. When assayed again using a 1:2 dilution, as per the instructions for use, they were found positive (with S/N% < 20%).
- **Measured sensitivity** (with doubtful result compted as positive)**= 100%** (95% CI [86.68, 100], n= 25).

GD Animal Health study on experimental infections

3 chicken sera collected 4 months after infection (R. Bowen, Colorado, USA), and 2 pooled sera from an experimental infection with the WNV Goose/Israel/98 (L. Phipps, Veterinary Laboratories Agency, United Kingdom) were tested.

RESULTS

- **All sera were found positive by the ID Screen® ELISA.**

SENSITIVITY SUMMARY (EQUINE AND AVIAN SAMPLES)

Sensitivity results on equine and avian samples are summarized in Table 1 below.

SPECIES	SENSITIVITY [95% CI]
Equine	100% [83.2, 100], n=19
Avian	100% [86.7, 100], n=25
TOTAL	**100% [92.0, 100], n=44**

Table 2: Measured sensitivity for positive horses and birds, n=44

RESULTS (Table 2) :

- **The measured sensitivity is 100 % for horses as well as birds.**
- The ID Screen® ELISA shows **an excellent sensitivity** on both equine and avian samples.
  Total measured sensitivity: 100% (95% CI [92.0, 100], n=44).

CUT-OFF DETERMINATION AND PREDICTIVE VALUES

Test performances were evaluated for different cut-off values based on the previously described validation data.

EQUINE SAMPLES

Diagnostic specificity and sensitivity summary

As a reminder, the results used for this study were obtained for:

- 261 negative samples from Calvados, France (2008), which was an area free of Flaviviruses at that period.
- 19 positive samples from Camargue (2003) and Perpignan (2006), two area of France where WNV outbreaks were observed.

A compilation of the S/N% distributions obtained with the ID Screen® Flavivirus Competition ELISA kit for these populations of negative and positive samples is shown in Figure 5.

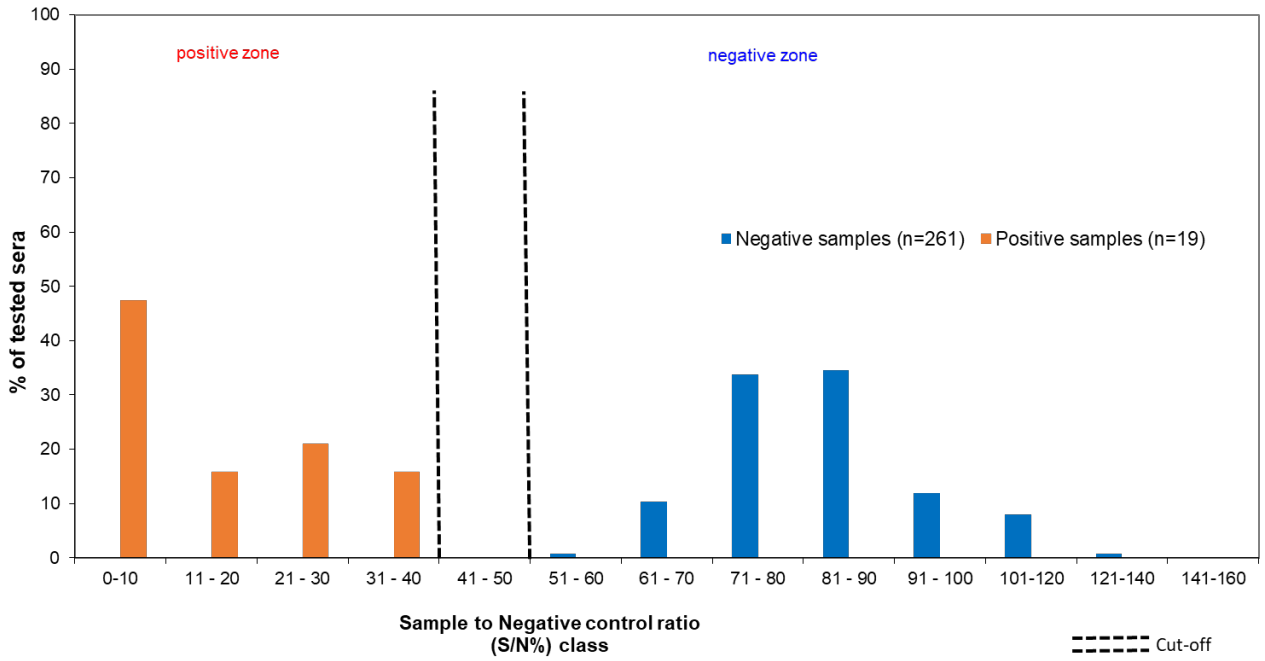


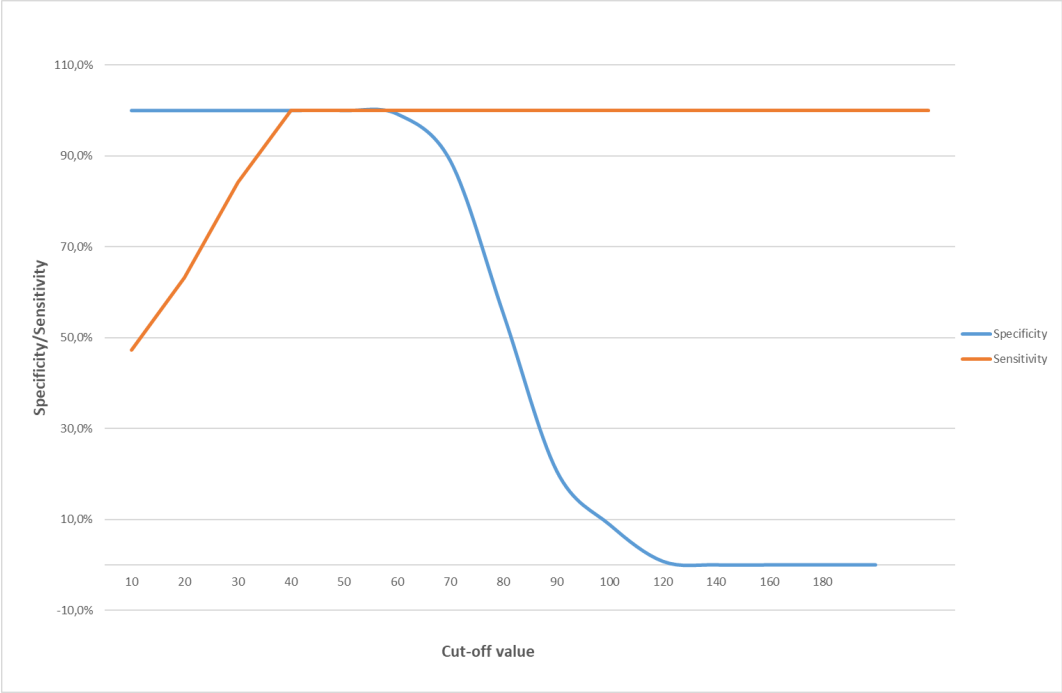
Figure 5 : S/N% distribution for negative and positive horse sera obtained with the ID Screen® Flavivirus Competition ELISA.

Impact of the cut-off values on test specificity and sensitivity

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 6 .

A



B

CUT-OFF VALUES	SPECIFICITY		SENSITIVITY	
	Sp (%)	95% CI	Se (%)	95% CI
10	100	[98.6-100]	47.4	[27.3-68.3]
20	100	[98.6-100]	63.2	[41-80.9]
30	100	[98.6-100]	84.2	[62.4-94.5]
40	**100**	**[98.6-100]**	**100**	**[83.2-100]**
50	**100**	**[98.6-100]**	**100**	**[83.2-100]**
60	99.2	[97.2-99.8]	100	[83.2-100]
70	88.9	[84.5-92.2]	100	[83.2-100]
80	55.2	[49.1-61.1]	100	[83.2-100]
90	20.7	[16.2-26.0]	100	[83.2-100]
100	8.8	[5.9-12.9]	100	[83.2-100]
120	0.8	[0.2-2.8]	100	[83.2-100]
140	0	[0-1.5]	100	[83.2-100]

Figure 6: Specificity and sensitivity for different cut-off values: graphical representation (A) and summary table (B)

RESULTS (Figure 6):

- The **cut-off values at 40% -50% S/N%** were chosen by Innovative Diagnostics ensures **optimum sensitivity and specificity conditions.**

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 7).

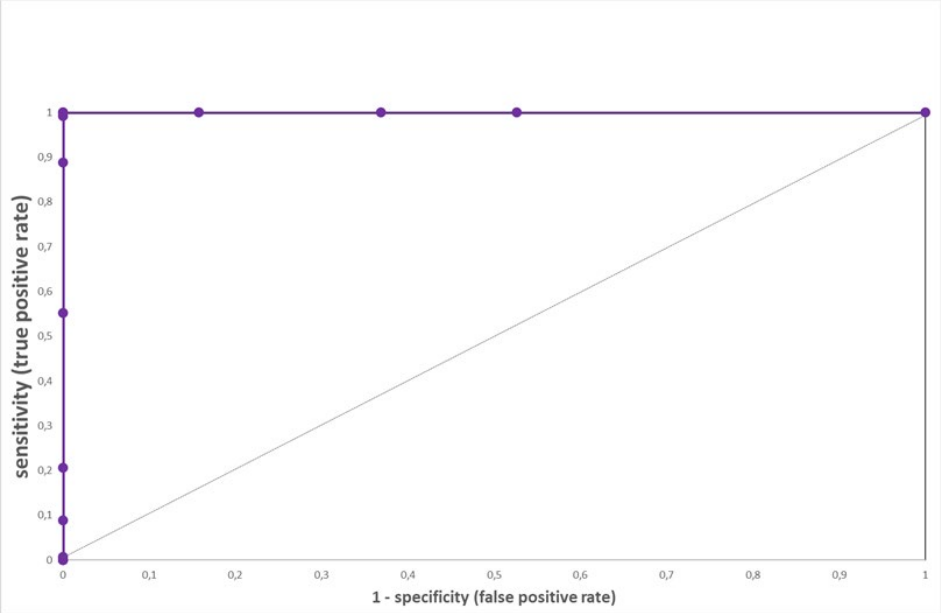


Figure 7: ROC curve obtained with the ID Screen® ELISA kit for 261 negative and 19 positive horse sera (calculation of AUC)

RESULTS (Figure 7) :

- As illustrated by **an Area Under Curve (AUC) of 1** (95% CI [98.7, 100]). the obtained ROC curve confirms **the excellent performance of the ID Screen® Flavivirus Competition ELISA.**

Predictive values

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

For the calculations shown in Table 3, based on the performance observed for horse serum samples, the lower limits of the 95% Confidence Interval summarised below were used :

- Specificity: 100% (95% CI [98.65, 100], n=261), and
- Sensitivity: 100% (95% CI [83.18, 100], n=19).

		PREVALENCE					
		1%	5%	10%	20%	40%	60%
PREDICTIVE VALUE	PPV	39%	77%	88%	94%	98%	99%
	NPV	100%	99%	98%	96%	90%	80%

Table 3: Predictive values for different seroprevalences obtained with ID Screen® Flavivirus Competition ELISA for horses exposed to the West Nile Virus

RESULTS (Table 3):

- The **Positive Predictive Value (PPV)** is **excellent** while the **Negative Predictive Value (NPV) is very high up to 20% prevalence (indicating a high reliability in low prevalence-areas).**
- The **ID Screen® Flavivirus Competition ELISA** is therefore **a reliable tool for West Nile diagnosis in horses**.

SEROCONVERSION KINETICS STUDIES

EQUINE SAMPLES

GD Animal Health study

Two horses were experimentally inoculated with WNV-virus lineage 2 and serum samples collected in time courses were tested using the ID Screen® ELISA .

Results are presented in Figure 8.

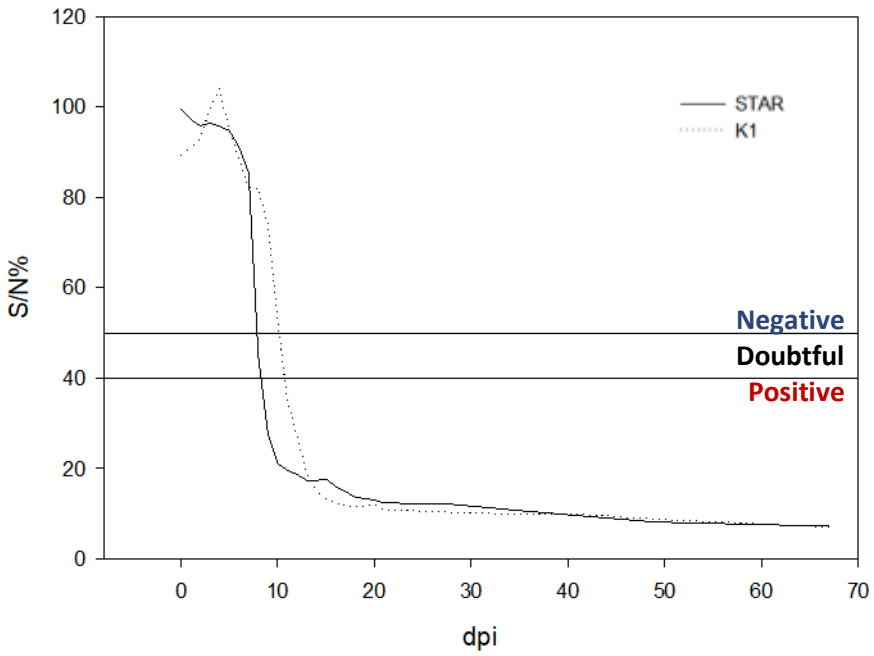


Figure 8: Seroconversion kinetics for two horses experimentally inoculated with WNV-virus lineage 2

RESULTS (Figure 8):

- **For the 2 WNV-infected horses, seroconversion was detected 10-15 days post-infection.**

ANSES study

An experimental infection was performed on horses using several Flaviviruses, in 2013, by the French National Reference Laboratory for Flaviviruses (ANSES Maisons-Alfort).

Five horses were infected subcutaneously with 10^7 pfu of either WNV lineage 1 (WNV1, IS-98-ST1 strain), lineage 2 (WNV2, Aus08), JEV (Nakayama strain), TBEV (Hypr strain), or USUV (SAAR1776 strain) and sera were sampled on day 8, day 20 and day 35.

Sera were tested by Innovative Diagnostics using in parallel the ID Screen® Flavivirus Competition and the ID Screen® West Nile IgM Capture ELISAs. To facilitate results comparison, results obtained with the ID Screen® Flavivirus Competition ELISA were expression as percentage of inhibition: PI%= 100- S/N%.

Results are presented in Figure 9.

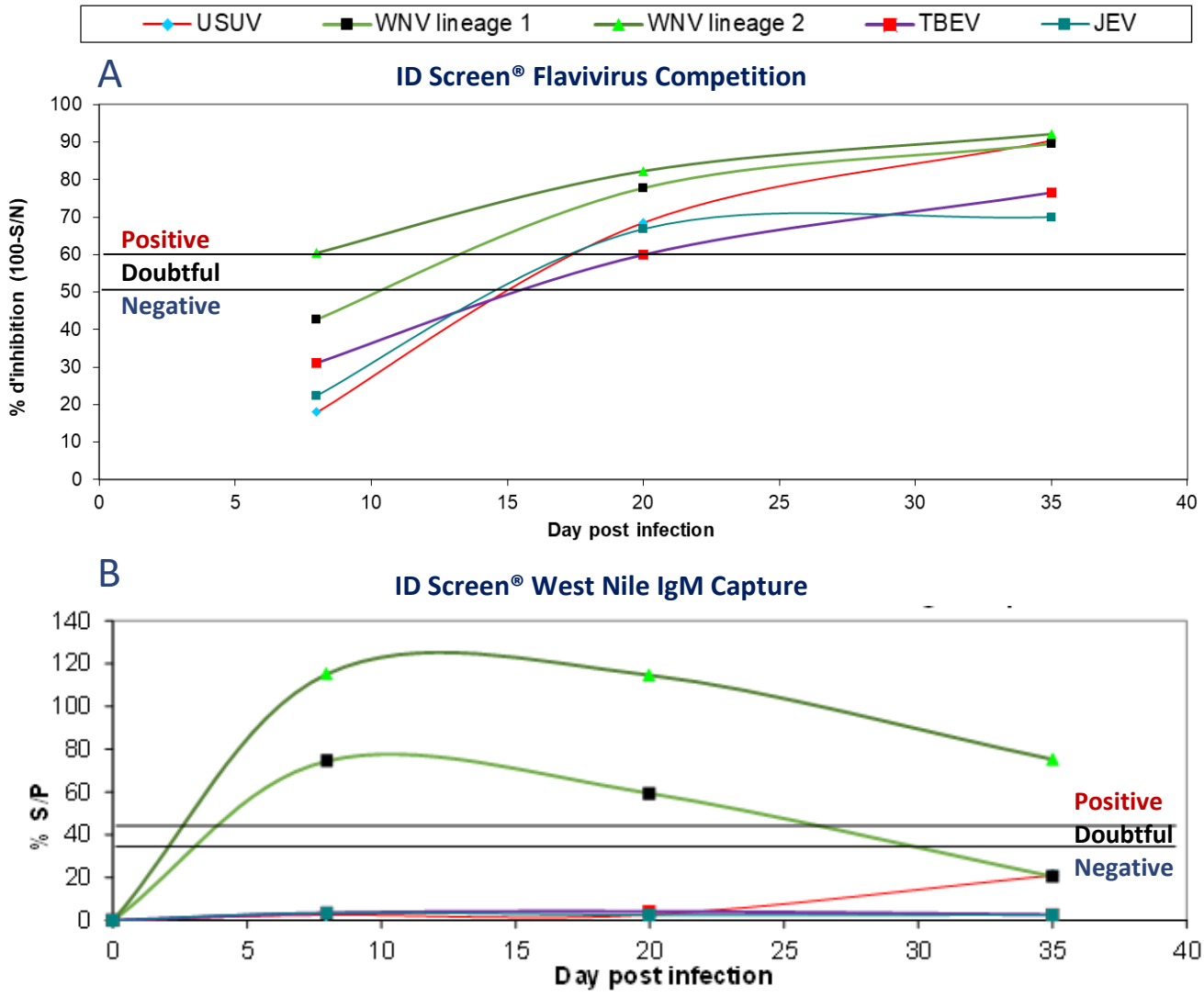


Figure 9: Seroconversion kinetics obtained for experimentally infected horses using the ID Screen® Flavivirus Competition (A) and the ID Screen® West Nile IgM Capture (B) ELISAs

RESULTS (Figure 9):

- With the **ID Screen® Flavivirus Competition** ELISA, **seroconversion in horses was detected:**
  - at **8 dpi** in animals infected with **WNV lineage 2**,
  - at **approximatly 13 dpi i**n animals infected with **WNV lineage 1**,
  - at **approximatly 17 dpi** in animals infected with **USUV or JEV**,
  - at **approximatly 20 dpi** in animals infected with **TBEV.**
- With the **ID Screen® West Nile IgM Capture** ELISA , **seroconversion in horses:**
  - **was not detected** in animals infected with **USUV, TBEV or JEV,**
  - **was detected before 5 dpi** in animals infected with **WNV lineage 1** , with an **IgM peak** at **approximatly 9 dpi**,
  - **was detected before 5 dpi** in animals infected with **WNV lineage 2**, with an **IgM peak** at **approximatly 10 dpi.**
- This study performed in horses shows that:
  - **the ID Screen® Flavivirus Competition ELISA is able to detect antibodies against various Flaviviruses: either of the 2 lineages of the WNV, USUV, TBEV, or JEV.**
  - **the ID Screen® West Nile IgM Capture ELISA is specifically detecting IgM antibodies against the WNV.**

AVIAN SAMPLES

GD Animal Health study

2 chickens, immunised intramuscularly with the Merial West Nile Virus vaccine (Live Canarypox vector), and 5 chickens, immunised intramuscularly with 1 dose of the Fort Dodge West Nile Virus vaccine (inactivated), were boosted 4 weeks later with the same vaccine and dose. Blood samples were collected on 1-, 14-, 28- and 42-days post-vaccination (dpv) and tested using the ID Screen® ELISA .

Results are presented in Figure 10.

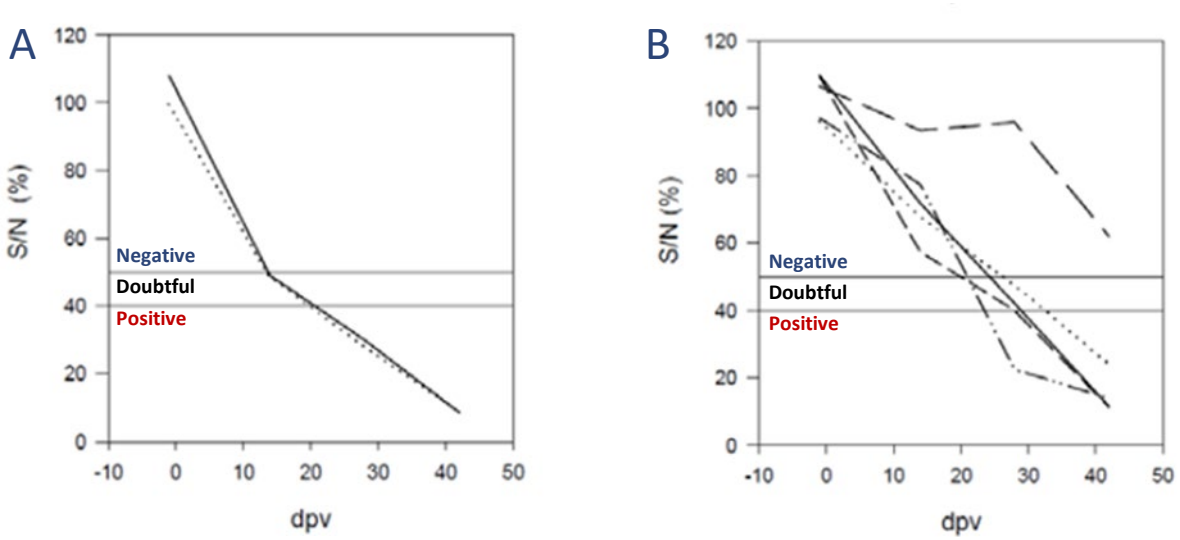


Figure 10: Seroconversion kinetics obtained for chickens vaccinated with either the Merial-WNV vaccine (A, n=2) or the Fort Dodge-WNV-vaccine (B, n=5) , and boosted at 28 dpv.

RESULTS (Figure 10) :

- These samples were analysed in duplicate and **repeatability was excellent.** The average difference in S/N% was -0.1% ± 2.6 (n = 28). The repeatability was around 4.9 S/N%.
- For one chicken, the S/N% values decreased over time, but remained above the cut-off values.
- **For 6/7 WNV-vaccinated chickens, seroconversion was clearly detected 40 days post-vaccination.**

ANALYTICAL SENSITIVITY

EQUINE SAMPLES

Innovative Diagnostics produces a freeze-dried WNV IgG positive horse serum, which may be used to check that the test analytical sensitivity does not vary between runs, operators, and batches. This positive serum is available for purchase (product code : MRI-WN).

The MRI-WN (batch 004) was diluted in a pool of negative sera, titrated, and tested.

Results are shown on Table 4 below.

SAMPLE	DILUTION TESTED	ID SCREEN® ELISA	
		S/N%	STATUS
MRI-WN (batch 004)	1/2	15	(+)
	1/4	39	(+)
	1/8	54	(-)
	1/16	83	(-)

Table 4: Titration of the IDvet freeze-dried WNV IgG positive serum

RESULTS (Table 4):

- The MRI-WN (batch 004) was detected as positive diluted up to 1:4.

REPEATABILITY

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

The OD values obtained are shown in the Table 5 below. Results are considered compliant if the CV% is less than 10%.

OD AT 450 NM											
0.630	0.568	0.634	0.630	0.611	1.731	1.737	0.599	0.620	0.604	0.643	0.611
0.600	0.626	0.620	0.613	0.600	1.627	1.488	0.528	0.534	0.483	0.505	0.499
0.638	0.608	0.563	0.543	0.511	1.493	1.432	0.501	0.516	0.458	0.524	0.553
1.703	1.561	1.398	1.385	1.324	1.427	1.431	1.415	1.395	1.421	1.506	1.557
1.627	1.538	1.411	1.452	1.400	1.481	1.353	1.296	1.335	1.337	1.397	1.581
0.604	0.635	0.605	0.586	0.589	1.528	1.420	0.532	0.518	0.485	0.525	0.543
0.554	0.583	0.576	0.585	0.575	1.462	1.477	0.534	0.545	0.541	0.549	0.537
0.600	0.617	0.594	0.608	0.604	1.702	1.662	0.544	0.565	0.520	0.576	0.553

	AVERAGE OD	STANDARD DEVIATION	MINIMUM	MAXIMUM	CV%
WEAK POSITIVE SAMPLE	0.569	0.046	0.458	0.643	**8%**
NEGATIVE SAMPLE	1.486	0.121	1.296	1.737	**8%**

Table 5: Repeatability study for the ID Screen® ELISA.

RESULTS (Table 5)

- The CV% obtained were 8% for the weak positive sample and 8% for the negative sample, demonstrating **good test repeatability of the ID Screen® ELISA test**.

REPRODUCIBILITY

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

This threshold dilution was tested in 11 independent runs by different operators and on different days.

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

Results are shown in Figure 11.

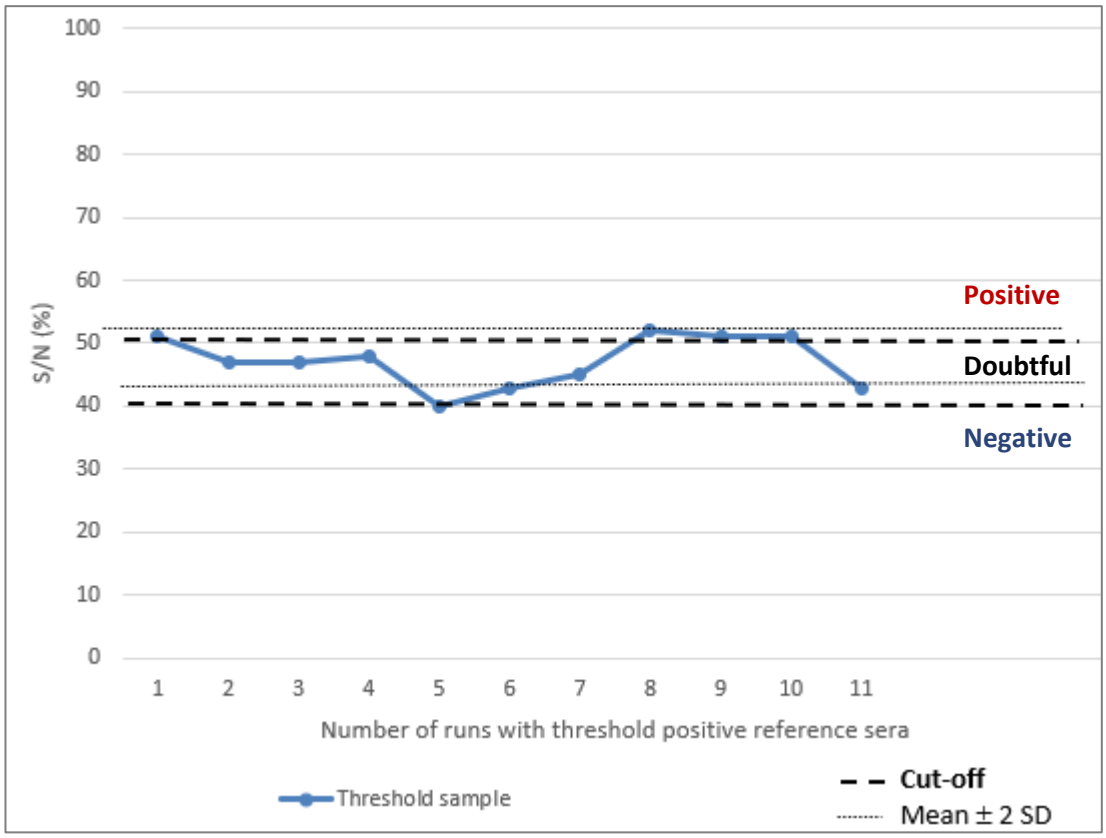


Figure 11: S/N% values obtained for a threshold dilution of a positive serum tested in 11 independent runs

RESULTS (Figure 11)

- **All values are within a range of 2 standard deviations around the mean, with a CV of 4%.**
- These results illustrate the **high reproducibility of the ID Screen® ELISA test.**

ROBUSTNESS

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: 90 minutes ± 6 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 Negative control OD (OD_{NC}) is greater than 0.700.
- The mean value of the Positive control OD (OD_{PC}) is less than 30% of the mean value of the Negative control OD (OD_{NC}).

Optical densities at 450 nm obtained, in each condition, for both Negative and Positive controls and the S/N% values obtained for 3 dilutions of a positive sample and 2 negative samples are detailed in Table 6 below.

SAMPLES/CONJUGATE/SUBSTRATE INCUBATION TIME		90 MIN / 30 MIN / 15 MIN			84 MIN / 27 MIN / 13 MIN	96 MIN / 33 MIN / 17 MIN	
INCUBATION TEMPERATURE		16°C	21°C	26°C	16°C	26°C	
Positive control		0.085	0.074	0.080	0.089	0.085	OD 450 NM
		0.080	0.075	0.070	0.089	0.090	
Negative control		0.933	1.333	1.312	0.846	1.354	
		1.104	1.358	1.173	0.933	1.518	
OD_{NC} > 0.700		✓	✓	✓	✓	✓	
OD_{PC} / OD_{NC} < 0.3		✓	✓	✓	✓	✓	
MRI-WN (batch 004)	pure	20	15	14	26	16	S/N%
	diluted 1 :2	31	20	26	35	28	
	diluted 1 :4	64	47	55	67	51	
Negative sample	1	114	115	111	128	108	
	2	107	98	113	112	103	

Table 6: Robustness study for the ID Screen® ELISA

RESULTS (Table 6) :

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

STABILITY

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

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

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

Results are shown in Figure 12 below.

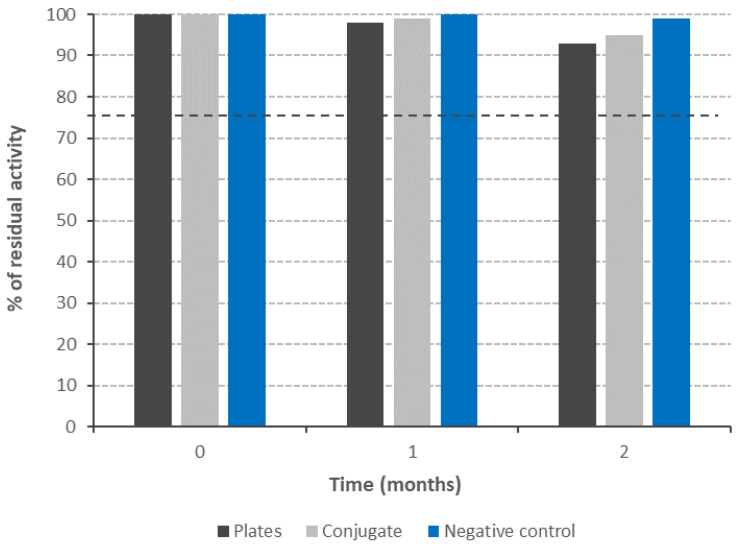


Figure 12: Percentage of residual activity of the plates, negative control and conjugate after stability testing at 37°C.

RESULTS (Figure 12) :

- After 2 months at 37°C, the plates, the conjugate and the negative control showed residual activity of 93%, 95% and 99% respectively, thus indicating the **high component stability** of the ID Screen® ELISA.

CONCLUSION

The **ID Screen® Flavivirus Competition** ELISA kit :

- enables the **detection of antibodies against the West Nile Virus lineages 1 and 2**: it shows **high specificity and sensitivity** on both chicken and horse sera.
- enables the **detection of pan-Flavivirus antibodies:** it has been proven to detect antibodies against a **broad spectrum of Flaviviruses** such as the WNV but also the **USUV, JEV, TBEV, ZIKAV and DENV** *(literature report referencing external publications and peer-reviewed references available upon request*).
- allows detection of **seroconversion:**
  - **in infected horses,** between **8 and 15 days post-infection** with the **WN1 or WN2,** at **approximatly 17 dpi** with the **USUV** or the **JEV,** and at **approximatly 20 dpi** with the **TBEV.**
  - **in vaccinated chicken** (boosted at 28 dpv)**, clearly at 40 days post-vaccination.**
- **may be used as a screening test.** Please note that positive results should be confirmed by assays such as the Virus Neutralization Test (VNT) or the Plaque Reduction Neutralization Test (PRNT).
- harbours **good repeatability, reproducibility, robustness** and **stability.**

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REFERENCES

(1) WOAH Terrestrial Manual, **West Nile fever, chapter 3.1.25**, version adopted in May 2018.

(2) Bahuon C. *et al*. (2016). **West Nile virus epizootics in the Camargue (France) in 2015 and reinforcement of surveillance and control networks**. Rev. Sci. Tech. Off. Int. Epiz., 35 (3), 811-824.

(3) Chaintoutis S. *et al*. (2015) **Evaluation of cross-protection of a lineage 1 West Nile virus inactivated vaccine against natural infections from a virulent lineage 2 strain in horses, under field conditions**. Clin Vaccine Immunol 22:1040 –1049.

(4) Cosseddu G.M. *et al*. (2021). **Sero-surveillance of emerging viral diseases in camels and cattle in Nouakchott, Mauritania: an abattoir study**. Tropical Animal Health and Production, 53(2), 1-6.

(5) Selim A. *et al*. (2020). **The first detection of anti-West Nile virus antibody in domestic ruminants in Egypt**. Tropical Animal Health and Production, 52(6), 3147-3151.

(6) Montagnaro S. *et al*. (2019). **Serological evidence of mosquito-borne flaviviruses circulation in hunting dogs in Campania Region, Italy**. Vector-Borne and Zoonotic Diseases, 19(2), 142-147.

(7) Tinto B. *et al*. (2022). S**creening of circulation of Usutu and West Nile Viruses: A one health approach in humans, domestic animals and mosquitoes in Burkina Faso, West Africa**. Microorganisms, 10(10), 2016.

(8) Constant O. *et al*. (2022). **One Health surveillance of West Nile and Usutu viruses: A repeated cross-sectional study exploring seroprevalence and endemicity in Southern France, 2016 to 2020**. Eurosurveillance, 27(25), 2200068.

(9) Niczyporuk J. *et al*. (2015)**. Occurrence of West Nile Virus Antibodies in Wild Birds, Horses, and Humans in Poland**. BioMed Research International vol 2015, Article ID 234181.

(10) Tinto B. *et al*. (2022). **Serological Evidence of Zika Virus Circulation in Burkina Faso**. Pathogens 11, 741.

(11) Molini U. *et al*. (2022). **Neutralising antibodies to West Nile virus detected in horses in Windhoek, Namibia**. Journal of the South African Veterinary Association, 93(1), 1-3.

(12) Selim A. *et al*. (2020). **Seroprevalence and molecular characterization of West Nile Virus in Egypt. Comparative Immunology**, Microbiology and Infectious diseases 71, 101473.

(13) Benjelloun A. *et al*. (2017). Seroprevalence of West Nile virus in horses in different Moroccan regions. Veterinary Medicine and Science, 3, 198–207.

(14) Pallari C.T. *et al*. (2021). **Evidence of West Nile Virus seropositivity in wild birds on the island of Cyprus. Comparative immunology**, Microbiology and infectious diseases, 74, 101592.

(15) Islam A. *et al*. (2020). Serological Evidence of West Nile Virus in Wild Birds in Bangladesh. Vet. Sci. 2020, 7, 164.

(16) Ain-Najwaa M.Y. *et al*. (2020). **Evidence of West Nile virus infection in migratory and resident wild birds in west coast of peninsular Malaysia**. One Health 10, 100134.

Related products

- **West Nile IgG positive equine freeze-dried serum** (product code: MRI-WN): Freeze-dried equine serum containing anti-WNV specific IgG. To be used as internal reference material for quality control. This serum does not contain any infectious material.
- **ID Screen® West Nile IgM Capture ELISA** (product code: WNIGM): IgM Antibody Capture ELISA (MAC) kit for the detection of anti-prE IgM antibodies in horse serum and plasma.
- **West Nile IgM positive equine freeze-dried serum** (product code: MRI-WNIGM): Freeze-dried equine serum containing anti-WNV specific IgM. To be used as internal reference material for quality control. This serum does not contain any infectious material.

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

History of revisions

VERSION	EDIT DATE	REFERENCE	TYPE OF REVISION	REVISION MADE
1014	08/2017	doc549	**Technical modification**: Update of the document following technical modification of the kit	Kit provided with a Stop solution made from a different acid type (substitution of the H2SO4 by a less corrosive acid), with no impact on the test performance.
	02/2024	doc1244	**Modification**: Update of the document following modification of the kit	• New commercial denomination: 'ID Screen® Flavivirus Competition' instead of 'ID Screen® West Nile Multi-species' • Human samples testing now available for Research Use Only (RUO), • Innovative Diagnostics now mentioned as the kit's manufacturer.
			Update: Addition/Edition of validation data	• Updated introduction. • Updated data for diagnostic specificity and sensitivity on equine samples. • Addition of new chapters with their associated data: - Specificity summary - Sensitivity summary - Cut-off determination and predictive values (equine samples) - Analytical sensitivity (equine samples) - Repeatability - Reproducibility - Robustness - Stability • Among the seroconversion kinetics studies, addition of a study performed by the ANSES on horses infected by the WNV or other flaviviruses. • Updated conclusion. • Inclusion of sections for the Acknowledgments, the Related products and the History of revisions.

info@innovative-diagnostics.com | www.innovative-diagnostics.com| + 33 (0)4 67 41 49 33