Validation report

ID Screen® West Nile IgM Capture

Capture ELISA (biwell format) for the detection of IgM antibodies directed against the West Nile Virus in horse serum or plasma.

- **Sensitive and specific detection** of WNV IgM antibodies which indicated recent infection
- **Rapid and convenient:** all reagents are supplied ready-to-use, samples are now diluted in the pre-plate, and all reagents may be transported at room temperature and stored at 4°C

Introduction

West Nile virus (WNV) is a mosquito-borne zoonotic arbovirus belonging to the genus *Flavivirus*. The arbovirus is maintained in nature by cycling through birds and mosquitoes. Among mammals, clinical disease is primarily exhibited in horses and humans.

Serology may be used for disease diagnosis. The IgM Antibody Capture (MAC) ELISA is particularly useful to detect antibodies further to recent natural exposure to the WNV. Equine WNV-specific IgM antibodies are usually detectable from 4-8 days post infection to 2-4 months post-infection (see Figure 1).

The **ID Screen® West Nile IgM Capture** ELISA is a rapid and convenient test.

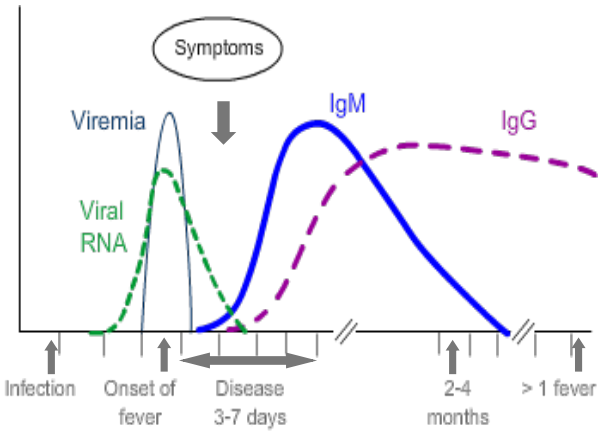
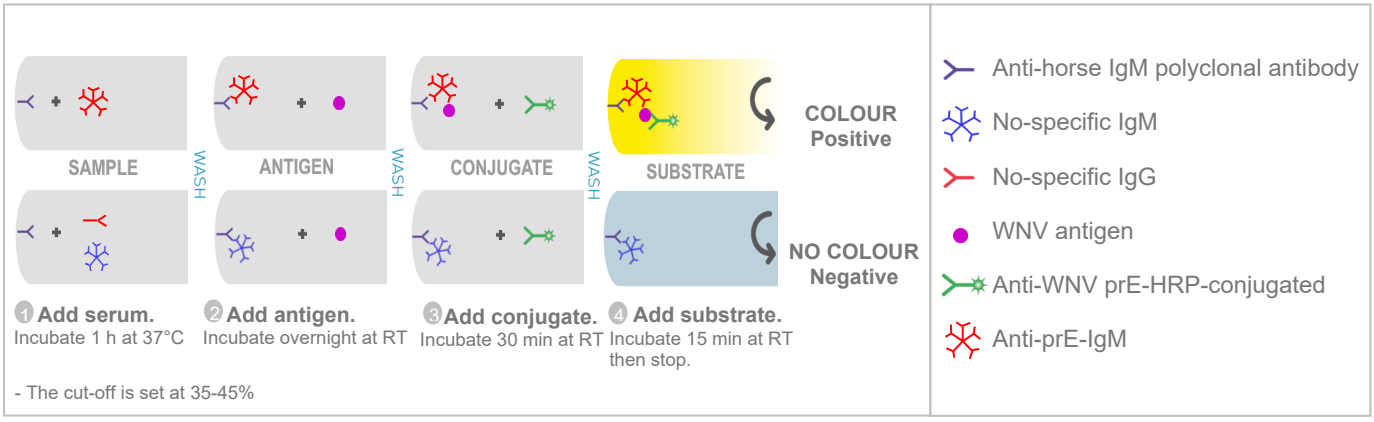


Figure 1: Viremia and antibody Kinetics in West Nile virus infection (Ref 1).

Method: IgM Antibody Capture (MAC) ELISA



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Analytical Sensitivity

As no international standard exists for WNV IgM diagnosis, IDvet has developed a freeze-dried horse serum (serum from naturally infected horse) *, to be used as internal reference material for quality control.

Using this internal standard, IDvet is able to guarantee that the kit's analytical sensitivity remains constant between run, batches and operators.

Serial dilutions of the freeze-dried horse serum were tested 20 times.

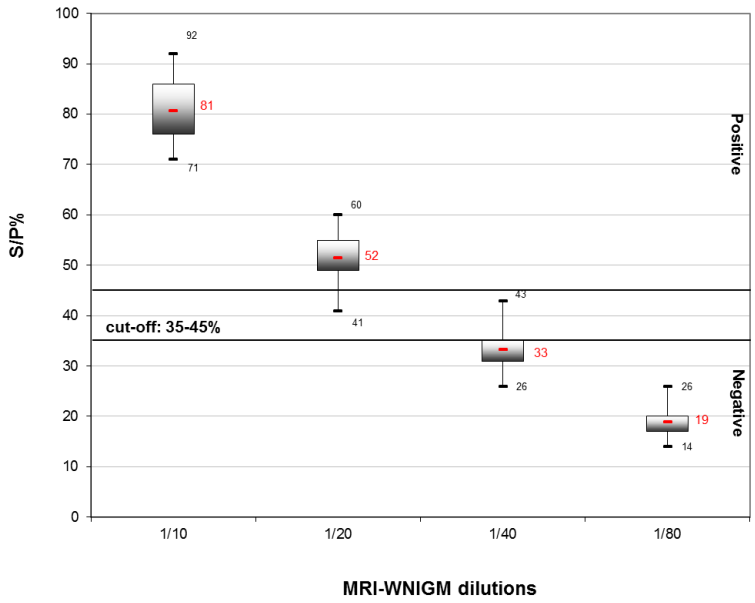


Figure 1: Box-and-whisker plot represents S/P% values for serial dilutions of the MRI-WNIGM from 1:10 to 1:80. (Line indicates average value, box indicates interquartile range and bars indicate range).

Results (Figure 1):

- The IDvet freeze-dried bovine serum was found positive up to dilution 1:20.

** This internal standard is available for purchase, product code: MRI-WNIGM batch 002.*

Specificity

Disease free sera

Specificity was tested on 208 horse sera from disease-free regions (Calvados, France and England).

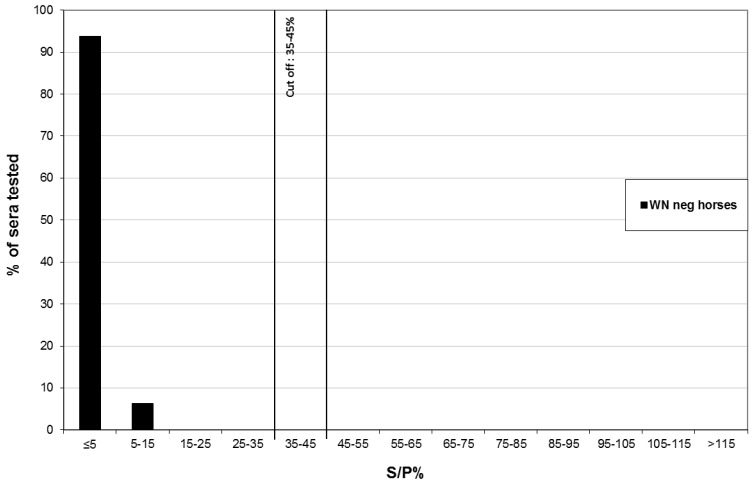


Figure 2: S/P% distribution for disease-free sera (n=208).

Results (Figure 2):

- All sera were found negative.

  Measured specificity: 100% ($CI_{95\%}$: 98.19 - 100%), n=208.

WNV IgG-positive / IgM-negative sera

The following sera were tested:

- 8 WNV VNT-positive sera from the EU
- 10 vaccinated sera from the USA

These sera were confirmed IgG-positive by the ID Screen® West Nile Competitive ELISA.

Animals from the EU were considered IgM –negative as animals were in a late stage of infection when they were bled.

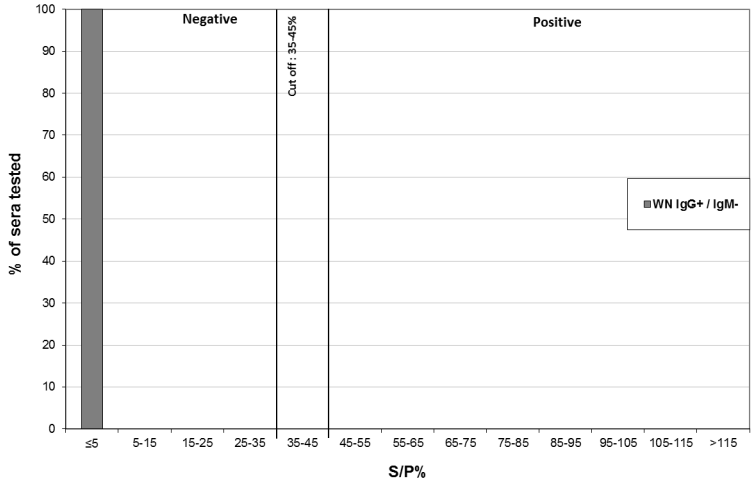


Figure 3: S/P% distribution for disease-free sera and WNV IgG +/ IgM – sera (n=18).

Results (Figure 3):

- These IgG-positive sera were found negative with the ID Screen® West Nile IgM Capture ELISA.

Exclusivity

Sera from animals infected with the five following flavivirus strains were kindly provided by ANSES (Animal Health laboratory, Maisons-Alfort, France) and tested to assess the ID Screen® West Nile IgM Capture ELISA specificity.

- USUV: Usutu Virus
- WNV lineage 1: West Nile Virus lineage 1
- WNV lineage 2: West Nile Virus lineage 2
- TBEV: Tick-Borne Encephalitis Virus
- JEV: Japanese Encephalitis Virus

Each horse was infected at D0 with a strain of flavivirus (dose 10^7 PFU, 1 ml subcutaneous injection). Horses were sampled at 0, 8, 20, 35 and 58 days post-infection and displayed clinical signs of the disease during the experiment.

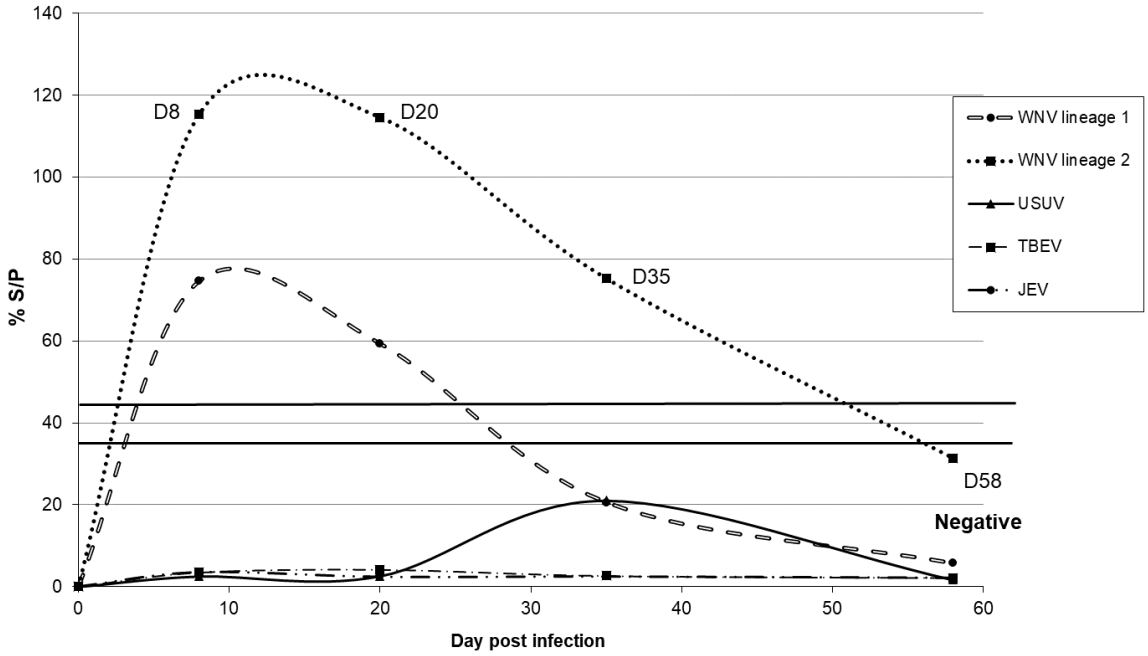


Figure 4: IgM detection during the flavivirus experimental infection

Results (Figure 4):

- All animals infected by viruses from West Nile Virus lineages were found IgM-positive at D8.
- Animals infected by USUV, TBEV and JEV were found negative
- **The ID Screen® ELISA is specific to West Nile Virus and demonstrates no cross reaction with other flavivirus**

Comparison with another WNV MAC ELISA

A panel of 46 positive and negative sera from the EURL (European Union Reference Laboratory) was tested in parallel on the European Community and French National Reference Laboratory (CRL & NRL) in-house MAC ELISA, and on the ID Screen® ELISA.

		ID Screen® West Nile IgM Capture		
		Positive	Negative	**Total**
CRL / NRL West Nile MAC technique	Positive	22	0	22
	Negative	0	24	24
	Total	22	24	46

Table 1: Correlation between NRL and ID Screen® ELISA results.

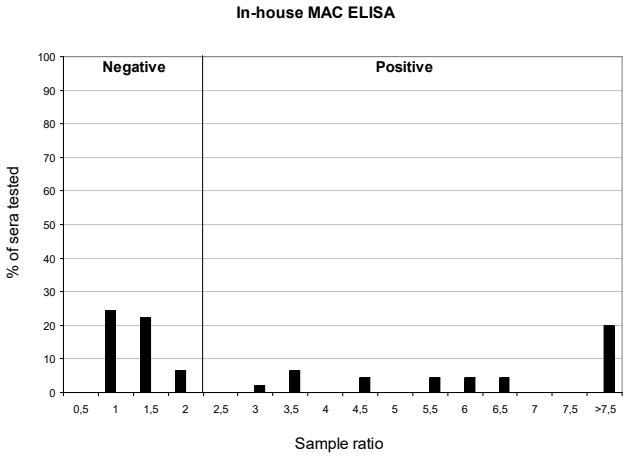


Figure 5: NRL ELISA results on the EURL panel, n=46

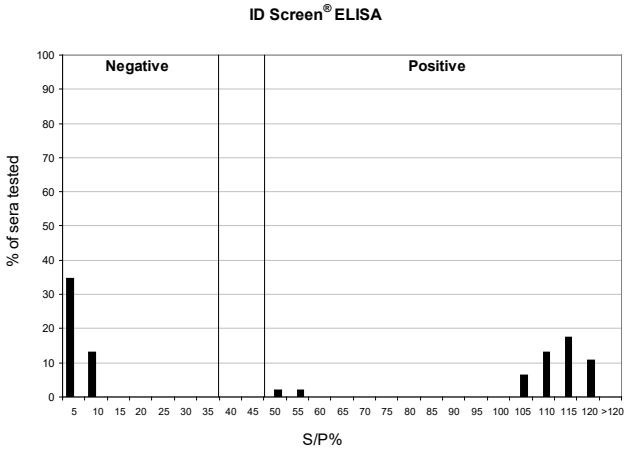
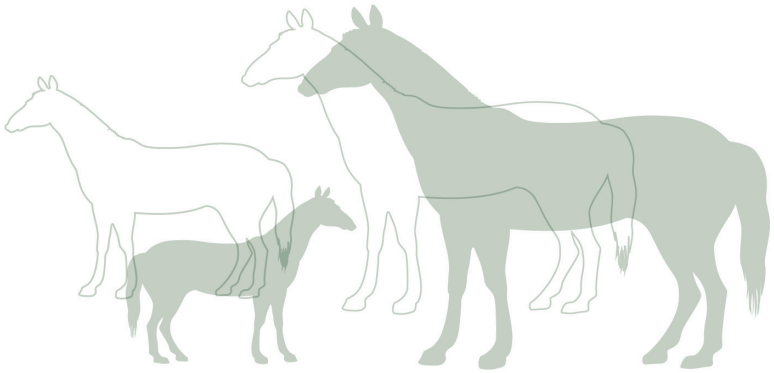


Figure 6: ID Screen® ELISA results on the EURL panel. n=46

Results (Table 1, Figures 5 & 6):

- Test agreement was 100%.
- The ID Screen® test allows for a better separation between positive and negative sera.

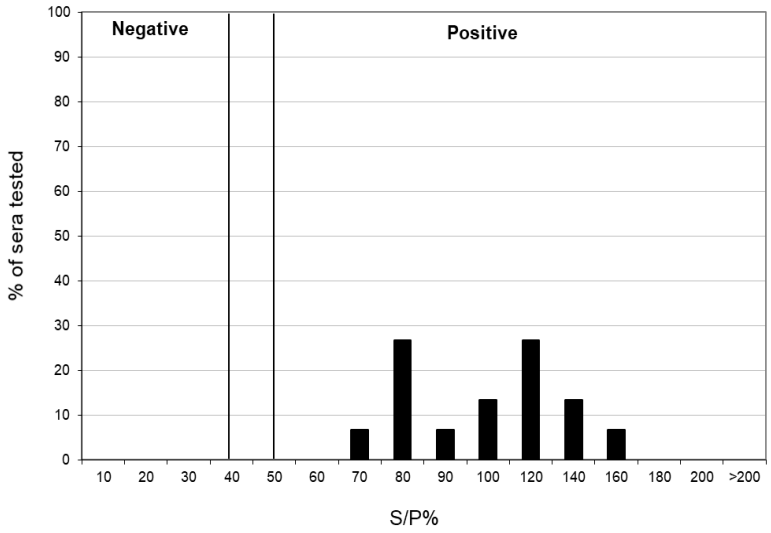


Sensitivity

WNV IgM positive sera from different origins

15 WNV IgM-positive sera (lineages 1 and 2) from animals at early stage of infection and that were later confirmed as WNV positive by observation of pathological signs and/or seroconversion with IgG production, were analysed.

These samples were provided by different EU countries.



Results (Figure 7):

- All sera gave positive results.

Overall sensitivity

Based on WNV IgM-positive samples obtained from experimental infection (n=2), comparison with WNV MAC ELISA (n=22) and from the field (n=15), the **measured sensitivity** of the ID Screen® test is of **100% (n=39).**

Repetability

Repeatability was evaluated by calculating the coefficient of variation (CV) for 18 repetitions of the positive control and 30 repetitions of a positive sample. The CV obtained must be less than 10 and 15%, respectively.

OD values at 450 nm											
0,095	0,898	0,097	0,884	0,099	0,893	0,080	1,012	0,100	0,876	0,108	0,913
0,098	0,857	0,096	0,871	0,097	0,847	0,077	0,905	0,100	0,850	0,107	0,887
0,096	0,860	0,093	0,839	0,095	0,822	0,075	0,885	0,096	0,832	0,105	0,861
0,075	0,902	0,076	0,884	0,079	0,867	0,073	0,810	0,076	0,822	0,083	0,913
0,074	0,955	0,078	0,916	0,072	0,920	0,075	0,913	0,073	0,928	0,086	1,044
0,100	0,987	0,080	0,832	0,089	0,853	0,080	0,936	0,089	0,833	0,103	0,900
0,106	0,883	0,085	0,866	0,088	0,857	0,072	0,904	0,084	0,830	0,100	0,867
0,093	0,902	0,082	0,884	0,084	0,897	0,071	0,966	0,081	0,888	0,094	0,923

Net OD at 450 nm					
0,803	0,787	0,794	0,932	0,776	0,805
0,759	0,775	0,750	0,828	0,750	0,780
0,764	0,746	0,727	0,810	0,736	0,756
0,827	0,808	0,788	0,737	0,746	0,830
0,881	0,838	0,848	0,838	0,855	0,958
0,887	0,752	0,764	0,856	0,744	0,797
0,777	0,781	0,769	0,832	0,746	0,767
0,809	0,802	0,813	0,895	0,807	0,829

	Average	Standard deviation	Minimum	Maximum	CV%
C+	0,839	0,055	0,737	0,958	7
MRI-WNIGM-002 1:8	0,778	0,033	0,727	0,887	4

Table 2: Repeatability study for the ID Screen® ELISA (results expressed as OD values)

Results (Table 2):

- The CV% obtained for intra-plate repeatability for the positive control and positive sample was less than 10%, indicating excellent intra-plate repeatability.

Robustness

1) The ID Screen® test robustness was evaluated by 3 operators in 3 independent runs.

Results:

- For each run:
  - Validation criteria described in the insert for both positive and negative controls were met.
  - The S/P% values for negative control, positive control and borderline samples were equivalent, regardless of the test conditions.

2) The ID Screen® test 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: 60 minutes ± 5 minutes at 37°C (± 3°C);

– Antigen incubation: Overnight (16 - 20h) 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_{PC}) is greater than 0.350 ($OD_{PC} > 0.350$).

– The ratio of the mean values of the Positive and Negative Controls (OD_{PC} and OD_{NC}) is more than 3.

Optical densities at 450nm obtained in each condition for both negative and positive controls are detailed in the table 3 below.

Sample/Antigen/Conjugate/Substrate Incubation Time	**60min / 18h / 30min / 15min**	**55min / 16h / 27min / 13min**	**65min / 20h / 33min / 17min**
Sample/Antigen/Conjugate/Substrate Temperature of incubation	**37° / 21° / 21° / 21°**	**34° / 16° / 16° / 16°**	**40° / 26° / 26° / 26°**
Negative control	0,039	0,022	0,064
Negative control	0,042	0,019	0,075
Positive control	1,439	0,802	1,683
Positive control	1,462	0,860	1,728
Net OD_{PC} > 0,350	√	√	√
Net OD_{PC} / $\lvert OD_{NC} \rvert$ > 3	√	√	√

Table 3: Robustness study for the ID Screen® ELISA (results expressed as OD values at 450nm).

Results (Table 3):

- For each time and temperature condition, the test validation criteria for both the positive and negative controls were met.

3) 3 dilutions of the MRI-WNIGM-002 and 2 negative samples were tested. S/P values obtained for these samples are detailed in Table 4 below.

Sample / Antigen / Conjugate / Substrate Incubation Time	60min / 18h / 30min / 15min	55min / 16h / 27min / 13min	65min / 20h / 33min / 17min
Sample / Antigen / Conjugate / Substrate Temperature of incubation	**37° / 21° / 21° / 21°**	**34° / 16° / 16° / 16°**	**40° / 26° / 26° / 26°**
MRI-WNIGM diluted 1:20	54	56	60
MRI-WNIGM diluted 1:40	32	31	43
MRI-WNIGM diluted 1:80	25	19	33
Negative sample 1	1	0	2
Negative sample 2	6	6	11

Table 4: Robustness study for the ID Screen® ELISA (results expressed as OD values at 450nm).

Results (Table 4):

- For each time and temperature conditions, the S/P% values obtained for each condition 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, 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 four weeks.

Results are shown in Figure 7 below.

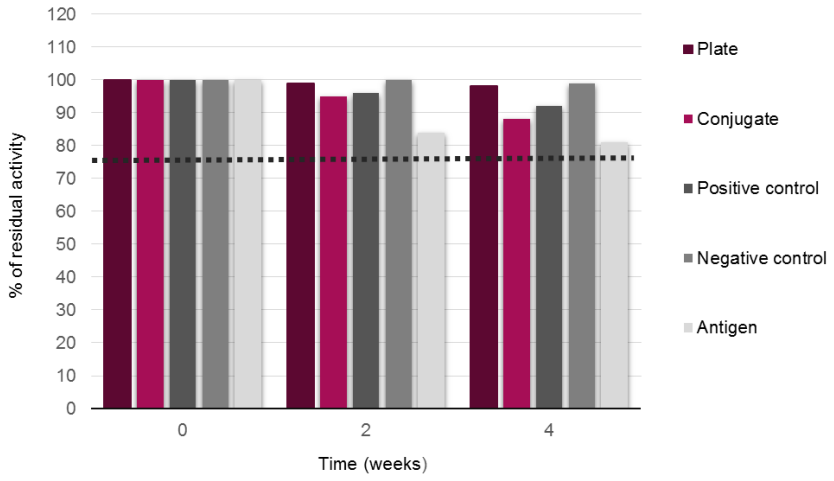


Figure 8: Percentage of residual activity of the plates, positive control and conjugate after stability testing at 37°C.

Results (Figure 8):

- After 4 weeks, the plate, antigen, conjugate, positive and negative controls showed a residual activity of 98%, 81%, 88%, 92% and 99% respectively, indicating high component stability.

Conclusion

The **ID Screen® West Nile IgM Capture** ELISA:

- detects WNV-specific IgM antibodies, indicating recent infection and viral circulation
- demonstrates high specificity on both disease-free and IgG-positive sera
- shows high sensitivity on IgM-positive samples
- shows improved separation of positive and negative samples when compared with a NRL ELISA on the EURL panel.

References

1) *Zeller, H.G. and Schuffenecker, I.* ***West Nile Virus: An Overview of Its Spread in Europe and the Mediterranean Basin in Constrast to Ist Spread in the Americas.*** *Eur J Clin Microbiol Infect Dis (2004) 23:147-156.*

Related Products:

ID Screen ® ELISA:

- **ID Screen ® West Nile Competition Multi-species (product code: WNC)**: Competitive ELISA for the detection of West Nile Virus anti-pr-E antibodies in horse and avian sera.

Internal reference material:

- **Freeze-dried West Nile IgG reference serum (product code: MRI-WN):** Freeze-dried equine serum containing anti-West Nile virus specific antibodies.
- **Freeze-dried West Nile IgM reference serum (product code: MRI-WNIGM):** Freeze-dried equine serum containing anti-West Nile virus IgM specific antibodies.

History of revisions

Version	Edit date	Reference	Type of revision	Revision made
0916	08/2017	DOC556	**Not applicable** (first version)	N/A
	09/2018	DOC678	**Update:** Addition/Edition of validation data	Inclusion of a new paragraph dedicated to **Exclusivity.**
	08/2019	DOC796	**Update:** Addition/Edition of validation data	Addition of related products
0919	11/2019	DOC843	**Technical modification:** Update of the document following technical modification of the kit	To improve test repeatability, samples are now diluted in a pre-dilution microplate prior to transfer into the ELISA microplate. The performances of the test remain constant.
			Update: Addition/Edition of validation data	Inclusion of: -additional data from WNV IgM-positive samples from the field. - a summary of the data relative to diagnostic sensitivity.
0919	12/2020	DOC977	Correction of anomalies in the document	Stability page 9, fig 8: Inconsistency with terms weeks and months.

Phone + 33(0)4 67 41 49 33 Fax + 33(0)4 67 45 36 95
info@id-vet.com www.id-vet.com