PRELIMINARY VALIDATION OF A SOLID - PHASE COMPETITIVE ELISA FOR THE DETECTION OF ANTIBODIES AGAINST WEST NILE DISEASE VIRUS IN HORSE SERA

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Introduction

In 1998, Italy reported the first equine outbreaks of West Nile Disease (WND). From 2001, a national veterinary surveillance system was adopted in areas considered at risk of WNV introduction, based on the passive surveillance of avian mortality and on the repeated serological testing of sentinel horses first in screening ELISA tests available commercially, either in a competitive format or based on the detection of IgG and IgM. Positive samples are then examined in the confirmatory test represented by the plaque neutralization reduction test. A solid-phase competitive ELISA was developed and a preliminarily validation was carried by an interlaboratory trial. Data on the preliminary validation is presented and discussed.

Materials and methods

Nine laboratories were involved in this validation. Pre-adsorbed plates, reagents (Mab and antigen) and a panel of sera were sent to each of the participants. The panel was made up of 20 sera, each replicated twice, and numbered from 1 to 40. The panel derived from the sera of five horses, two experimentally infected, two repeatedly vaccinated and one negative. The order of the sera was different for each laboratory. Each participant was requested to carry out the test three times, each time carried out by a different operator and on different days. The OD of each test was registered and returned in an Excel file. Runs that did not comply with validation's criteria were discarded.

Validation was performed according to WOAH Manual guidelines.

The following parameters were estimated:

Qualitative accuracy, estimated by:

- Sensitivity (Se) and specificity (Sp);
- Cohen K value for each laboratory;
- Weighted Cohen K value for each laboratory;
- Cohen K value for all laboratories gathered together.

For Cohen K value and weighted Cohen K value, expected results, categorized as negative, weak, medium and strong positive, were compared with those obtained. In considering both qualitative and semi-quantitative characteristics of the ELISA test, ***repeatability*** and ***reproducibility*** were estimated using the following parameters:

- Coefficient of variation (CV);
- Accordance;
- Concordance;
- Concordance Odds Ratio (COR);
- K value of all laboratories results gathered together.

K values were calculated by comparing the expected results, positive and negative, with those obtained.

For details regarding the calculation of these parameters, articles by Langton et al., Quatto and Soliani et al. can be consulted (1,2,3,4).

Procedure for the competitive ELISA (C-ELISA)

Microplates ready to use by pre-adsorbtion with a monoclonal antibody (Mab) recognising the domain III (Ed III) of WND virus (WNDV).

- addition of the antigen, a cell-culture cryolysate of inactivated virus;
- incubation for 90 minutes at 37°C;
- in parallel to the first incubation, serum samples and controls are diluted 1:5 and 1:10 on a separate plate;
- samples and controls are then transferred on the adsorbed plate;
- incubation of 60 minutes at 37°C;
- 3 washing cycles;
- addition of the same Mab used for adsorption conjugated with HPRO;
- incubation of 90 minutes at 37°C;
- addition of OPD substrate;
- incubation at room temperature for 15 minutes in the dark;
- addition of sulphuric acid to stop the enzymatic reaction;
- Reading at an optical density (OD) of 492nm.
- Sera are categorized as positive or negative according to percentage inhibition (PI), calculated as the ratio between sample and reaction control, represented by the mean of the OD of the antigen wells. Validation criteria for the ELISA were the following: mean OD of control reaction higher than 1.0; PI of negative control < 50% of OD of control reaction for both dilutions; PI of positive control > 50% of OD of control reaction in both dilutions.

Discussion and conclusions

From the results of the evaluated parameters the test is suitable for screening purposes. Accuracy is highly satisfactory since sensitivity and specificity resulted equal to 100%. Furthermore, K values indicate a degree of concordance almost perfect according to the classification of Landis *et al.* (5). Qualitative repeatability and reproducibility are also satisfactory since CV values are all less than 20%, value set as acceptable limit; accordance and concordance are also close to 100% in more than the half of the sera. COR resulted very close to 1 for all sera, except for two of them with however with a maximum value of 2.53.

An additional advantage of this c-ELISA is that it can be extended to the testing of other species for which it has to be validated. To fulfil the remaining criteria set by WOAH, a second phase of the validation procedure is necessary in which further parameters need to be evaluated, among which are the performances of this test on field samples.

Results

1.Both sensitivity and specificity resulted 100%.

2.K values are shown in figure 1. K for all laboratories resulted equal to 0.76.

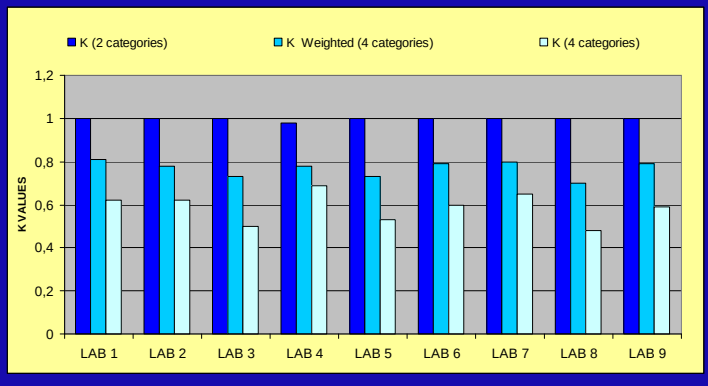
3.Values of accordance, concordance and COR are showed in table 1.

K value of all laboratories together, not compared with expected results, is equal to 0.72.

Table 1: Values of accordance, concordance and COR for each serum and for gathered laboratories

Serum n°	Accordance	Concordance	COR	Serum n°	Accordance	C oncordance	COR
1	86.7	81.7	1.46	**11**	62.1	45.1	2
2	95.3	95.2	1.01	**12**	75.4	74.9	1
3	92.4	90.5	1.3	**13**	64	41.3	2.5
4	100	100	1	**14**	66.9	52.8	1.8
5	76.3	56.9	2.4	**15**	100	100	1
6	95.3	95.2	1	**16**	100	100	1
7	65,9	49.7	1.9	**17**	100	100	1
8	86.7	81.7	1.5	**18**	100	100	1
9	100	100	1	**19**	100	100	1
10	100	100	1	**20**	100	100	1

Figure 1: K values for each laboratory: not weighted with 2 and 4 categories, and weighted with two categories.



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