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. The data of the this preliminary validation is presented and discussed.

Materials & methods

The procedure for the competitive ELISA (C-ELISA), object of this validation, is briefly described as follows. 96 well microplate are pre-adsorbed with a monoclonal antibody (Mab), recognising the domain III (Ed III) of WND virus (WNDV), are prepared ready for use. The reaction is started by the addition of the antigen, a cell-culture cryolysate of inactivated virus, on to the plate and its 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. The samples and controls are then transferred on the adsorbed plate containing the antigen and incubated for 60 minutes at 37°C. After washing the plate, the same Mab used for adsorption is added conjugated with horseradish peroxidase. Following another incubation period of 90 minutes at 37°C, ortophenyl-diamine substrate is added and the plate is incubated at room temperature for 15 minutes in the dark. Sulphuric acid is used to stop the enzymatic reaction, which is read using 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.

Validation was performed according to WOAH Manual guidelines. The aim for this ELISA test is for screening purposes.

Nine laboratories were involved in this validation. Pre-adsorbed plates, reagents (Mab and antigen) and a panel of sera were sent to each of them. The latter 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. Validation criteria for the ELISA were the following: mean OD of control reaction higher than 1.0; OD of negative control < 50% of OD of control reaction for both dilutions; OD of positive control > 50% of OD of control reaction in both dilutions. Runs that did not comply with these criteria were discarded.

In analysing all data 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.

K values were calculated by comparing the expected results, positive and negative, with those obtained, for Cohen K value and negative, weak, medium and strong positive for Cohen K value and weighted Cohen K value.

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.

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).

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.

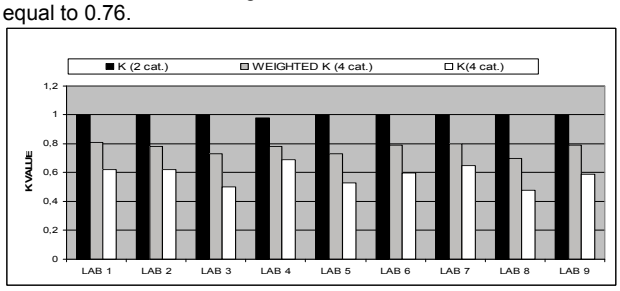

Figure 1: K values for each laboratory: not weighted with 2 and 4 categories, and weighted with two categories.

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

Table 1: Values of Accordance, concordance and COR for each serum and all laboratories together

Serum N°	Accordance	Concordance	COR	Serum N°	Accordance	Concordance	COR
1	86,71	81,75	1,46	**11**	62,14	45,11	2,00
2	95,29	95,24	1,01	**12**	75,43	74,87	1,03
3	92,43	90,48	1,29	**13**	64,00	41,27	2,53
4	100	100	1	**14**	66,86	52,78	1,80
5	76,29	56,88	2,44	**15**	100	100	1
6	95,29	95,24	1,01	**16**	100	100	1
7	65,86	49,74	1,95	**17**	100	100	1
8	86,71	81,75	1,46	**18**	100	100	1
9	100	100	1	**19**	100	100	1
10	100	100	1	**20**	100	100	1

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

Discussion & 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 sera. COR resulted very close to 1 for all sera except for two of them, 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.

References

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