AN INDIRECT ELISA VALIDATED AS DIAGNOSTIC AND VACCINES QUALITY CONTROL TOOL FOR BOVINE PARAINFLUENZA VIRUS TYPE 3 (BPIV3)

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INTRODUCTION

Serological assays, including enzyme-linked immunosorbent assays (ELISA), provide a useful tool to screen animals for the presence of antibodies (Abs) against a wide range of infectious agents (including viruses that cause respiratory disease in cattle) and are widely used in veterinary medicine to assist in the control and monitoring of disease. The aim of the present study was develop and validate of two indirect enzyme-linked immunosorbent assay (ELISA) based on semipurified bovine parainfluenza virus type 3 (BPIV3) for the detection and quantification of Abs against PI3 in serum sample from cattle and guinea pigs for both purpose diagnostic and typify/ specify the quality of vaccines.

MATERIAL AND METHODS

A total of 85 and 95 sera with known history of bovine and guinea pigs respectively were used throughout all the tests applied in this work. Furthermore, internal positive and negative controls were generated from a pool of samples for each species under study. The validation included the estimation of the diagnostic sensitivity and specificity of the assays, repeatability and intermediate precision within a laboratory over time and the reproducibility in interlaboratory assays.

Statistical methods for ELISA validation

Feasibility studies, initial repeatability and intermediate precision

The repeatability was expressed as the coefficient of variation of the optical density (OD) of the positive control sera obtained in the different runs. The control sera were assayed at a 1/40 dilution in duplicate in every ELISA run, and the mean of both replicates needed to fall within an established admission range for the plate to be considered valid. For full validation, the intermediate precision of the assay was estimated using the data of the bovine and guinea pig positive control sera, collected from ELISA runs conducted for 3 year in the our lab. To estimate the repeatability and intermediate precision of the ELISA for each species an ANOVA for a nested model of variance was carried out.

Diagnostic sensitivity and specificity of the ELISA

The diagnostic sensitivity (DSe) and specificity (DSp) of the ELISA for BPIV3 Ab detection in bovine and guinea pig sera were calculated following the recommendation given by Greiner and Gardner (2000) and The OIE Manual of Terrestrial animals. Briefly, the cut off of the assay and its associated DSe and DSp were estimated based on the frequency distribution of the ELISA values obtained after analyzing the reference populations at a 1/40 dilution. ELISA results were normalized by expressing the raw OD values as the percentage of positivity (PP%) of the high-positive bovine and guinea pig reference control sera

MATERIAL AND METHODS

ROC analysis

The cut-off selection of the assay for both species was also carried out with the aid of the receiver-operating (ROC) analysis [18], using the MedCalc Statistical Software version 18.11.6 (MedCalc Software bvba, Ostend, Belgium).

Detection limit and linearity of the assay

Dose response curves were constructed using a total of 36 replicates of a positive bovine serum and 16 replicates of a positive guinea pig serum, assayed in four-fold dilutions in several plates within different independent ELISA runs. To determine the analytic sensitivity linear regression curves were determined by plotting the ELISA values of those sera expressed as ODc of the positive control of the assay versus the log-transformed dilutions.

To verify the linearity of the system the Mallows's Cp were used to assess the fit of a regression model that has been estimated using ordinary least squares.

Assay reproducibility: inter-laboratory assay

The inter-laboratory assay included data collected in 3 laboratories for bovine and guinea pigs samples. The laboratories were requested to perform five ELISA assays following the supplied protocol, including 6 plates per run and testing the samples in 3 replicates per plate. The reproducibility of the assay was calculated as the coefficient of variation (relative standard deviation) using a nested ANOVA.

RESULTS

Table 1: Validation parameters

Validation parameters	Result		Acceptance criteria
	Bovine	Guinea Pig	
Cut-off (ODc)	0,3	0,25	
Diagnostic specificity	88%	91%	
Diagnostic sensitivity	100%	100%	
Positive control mean ODc	0,7	0,699	
Standard deviation (SD)	0,08	0,09	
N	85	95	
ODc admissible working range of the positive control ($\bar{y}$±1 SD)	0,62-0,78	0,61-0,79	
Repeatability	15,00%	5,60%	CV < 20%
Intermediate precision	20,70%	8,56%	CV < 25%
Linearity	Yes, R^2=0,9, p<0,0001	Yes, R^2=0,85, p<0,0001	
Inter-laboratory assay	(3 labs)	(3 labs)	
Reproducibility	17%	15%	CV < 30%

Feasibility studies, initial repeatability and intermediate precision

The coefficient of variation and the admissible working range of the ODc established for each bovine and guinea pig positive control are detailed in Table 1. The analysis of variance for the proposed nested model (5 assays, 6 plates per assay and 3 replicates per plate) indicated that the assay had good values of repeatability. The coefficient of variation for the bovine and the guinea pig positive controls were 15% and 5.6%, respectively.

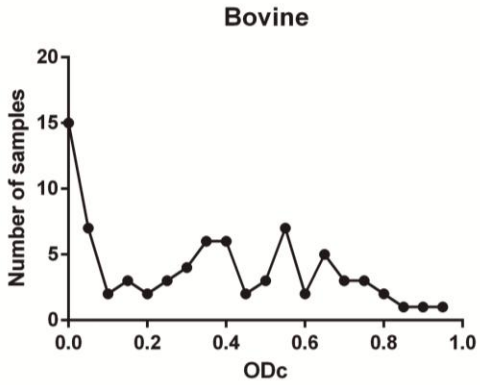
The intermediate precision of the assay given by the overall relative variation for both species was also acceptable (CVbovine = 20.7% and CVguinea pig = 8.56%) and met the OIE requirements (CV<25%).

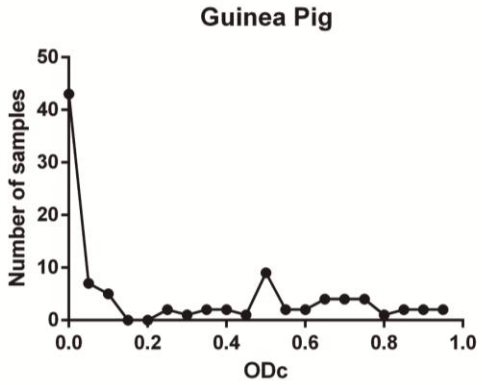
Assay reproducibility: inter-laboratoryassay

The inter-laboratory study data were analyzed with an analysis of variance with 5 ELISA runs, 6 plates and 6 replicates and were collected in three laboratories (defined in 2.6.6 section). The overall mean for the bovine and guinea pig positive controls were y = 0.733 and y = 0.674, both falling within the range of acceptance (Table 1). The reproducibility of the assays expressed as the coefficient of variation was 17% for bovine and 15% for the guinea pig positive control (Table 1).

RESULTS

Figure 1





ELISA Cut-off	Bovine Reference population		
DOC 0,3	+	-	Total
+	43	0	43
-	10	32	42
Total	53	32	85
Kappa 0,764	Dse= 88%	DSp= 100%	

ELISA Cut-off	Guinea pig Reference population		
DOC 0,25	+	-	Total
+	40	0	40
-	9	46	55
Total	49	46	95
Kappa 0,811	Dse= 91%	DSp= 100%	

Area under the ROC curve (AUC)	0,929
Standard Error a	0,041
95% Confidence interval b	0,852 to 0,973
z statistic	10,471
Significance level P (Area=0.5)	<0,0001

a DeLong et al., 1988

b Binomial exact

Area under the ROC curve (AUC)	0,995
Standard Error a	0,00435
95% Confidence interval b	0,952 to 1,000
z statistic	113,803
Significance level P (Area=0.5)	<0,0001

a DeLong et al., 1988

b Binomial exact

Cut-offs of the assay for bovine and guinea pig samples and their associated diagnostic sensitivity (DSe) and specificity (DSp).
Frequency distribution of the ELISA values obtained after analyzing the reference populations at a 1/40 dilution.
ELISA values were expressed as corrected optical density (ODc). (b) ROC analysis, MedCalc® version 18.11.6 statistical software.

Cut-off, diagnostic sensitivity and specificity of the ELISA for bovine and guinea pigs

The frequency of the percent of positivity distribution obtained for the positive and negative reference samples run at 1:40 dilution is depicted in Fig. 1.

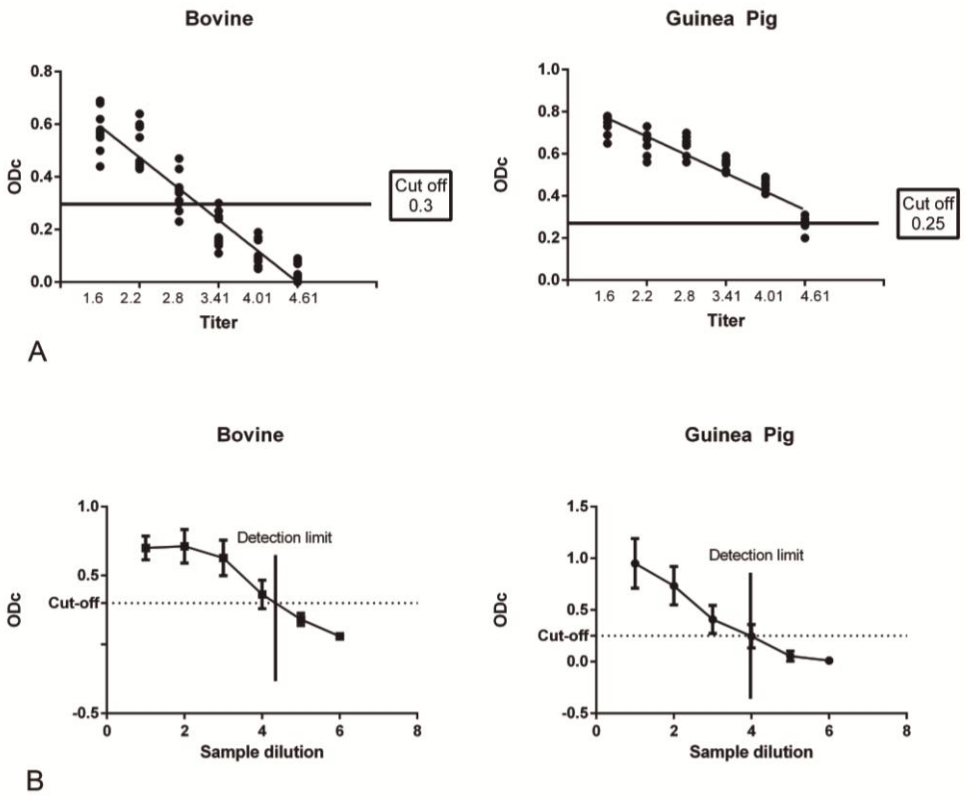
For bovines using a 0.3 ODc cut-off, the assay showed a 88% diagnostic sensitivity and 100% diagnostic specificity.

For guinea pig samples, the frequency analysis of the percent of positivity value distribution of the reference sera showed that a 0.25 ODc cut-off gave a relative sensitivity and specificity of 91% and 100%, respectively.

These cut-off was confirmed by ROC analysis conducted by MedCalc® software as the threshold that gives the highest accuracy.

RESULTS

Figure 2



Dose–response curves limiting dilution analysis of bovine and guinea pig positive control serum, serial four-fold dilutions starting at 1:40. (a) Each point represents the value obtained for each replicate of the diluted simple in each plate of every independent ELISA run, the curve is the linear regression; titers were expressed as the log10. (b) The response detected by ELISA was expressed as the ODc versus the simple dilution. Each line represents the mean ODc value obtained for the different replicates of the diluted simple of every independent ELISA run.

Detection limit and linearity of the assay

The detection limit of the assay was satisfactory and similar in both species. In both cases the regression analysis of the obtained dose–response curves indicated that the assays showed a range of linear behavior, R2= 0.9 and R2=0.85 for bovine and guinea pig respectively.

CONCLUSION

This study reports the development of an alternative tool for the detection antibodies against BPIV3. The additional advantage to the independence of the subjectivity of the operator reading and the dependence of the red blood cells in HAI with respect to the two ELISAs developed, is the adjustment of the range of antibody titers to assess the quality of vaccines with greater precision. In the near future this indirect ELISA could be offered as a commercial kit where the plates will be sensitized, dry and stable over time, making available a serological assay for BPIV3 accessible to our region.

This assay will be useful to evaluate the seroprevalence in cattle and also to evaluate the quality of the vaccines by vaccines in a guinea pig model. It will be a valuable addition to improve the vaccines available in the market and consequently the population immunity to BPIV3 necessary to reduce the high circulation of the virus in the country. The ELISA showed very good diagnostic sensitivity and specificity ;intermediate precision and reproducibility in both species.