Performance of VMRD *Brucella ovis* ELISA Reagents

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I. Introduction

Brucella ovis is a causative agent of ovine brucellosis that can result in economic losses to sheep producers due to impaired fertility and occasional reproductive failure. Here we describe the performance of **VMRD's BOC ELISA** reagents for detection of antibody produced in response to *B. ovis* and/or *B. canis* exposure.

II. Method

Ovine and canine serum samples were diluted 1/50 in serum diluting buffer before adding to the antigen coated plate (100 µl per well). Each plate was run with positive control (undiluted) loaded in triplicate and negative control (undiluted) loaded in duplicate. Samples were incubated for 30 min at room temperature before washing four times with 1x wash solution. The peroxidase conjugate was added to the plate (100 µl per well) and incubated for 30 min at room temperature before washing four times with 1x wash buffer. Plates were developed with peroxidase substrate solution (100 µl per well) for 15 min at room temperature before adding stop solution (100 µl per well). Plates were read with a microplate absorbance spectrophotometer set at 450 nm. Sample OD values were converted to S/P ratios using the following equation:

$$S/P = \frac{\text{Sample OD} - \text{Mean Negative Control OD}}{\text{Mean Positive Control OD} - \text{Mean Negative Control OD}}$$

III. Results

Validation and Performance of Reagents on Ovine Samples

An initial cutoff of 0.3 S/P was calculated for ovine samples based upon the mean S/P ratio plus 3 times the standard deviation for 208 NVSL ELISA negative field serum samples **(Figure 2)** as well as results with the international *B. ovis* reference serum standard **(Table 1)**.

Sample	Dilution	Expected*	S/P	Result
OIE Strong Positive	1/16	+	0.79	+
OIE Strong Positive	1/32	+	0.43	+
OIE Strong Positive	1/64	+/-	0.20	-
OIE Strong Positive	1/256	-	0.03	-

* Based on 2014 ANSES ring trial

Table 1: OIE *B. ovis* reference serum in VMRD ELISA

VMRD's BOC ELISA reagents were used to test ovine field serum samples previously characterized by a third-party laboratory using the NVSL ELISA and were classified as positive (n = 100), negative (n = 208) or indeterminant (n = 29). Using a 1.35 S/P cutoff (Maximal Youden Index = 0.967), the sensitivity was 98.0% and specificity 98.7% if indeterminants were included in the negative cohort. Using a 0.75 S/P cutoff the sensitivity was 91.5% and specificity 100% if indeterminants were included in the positive cohort.

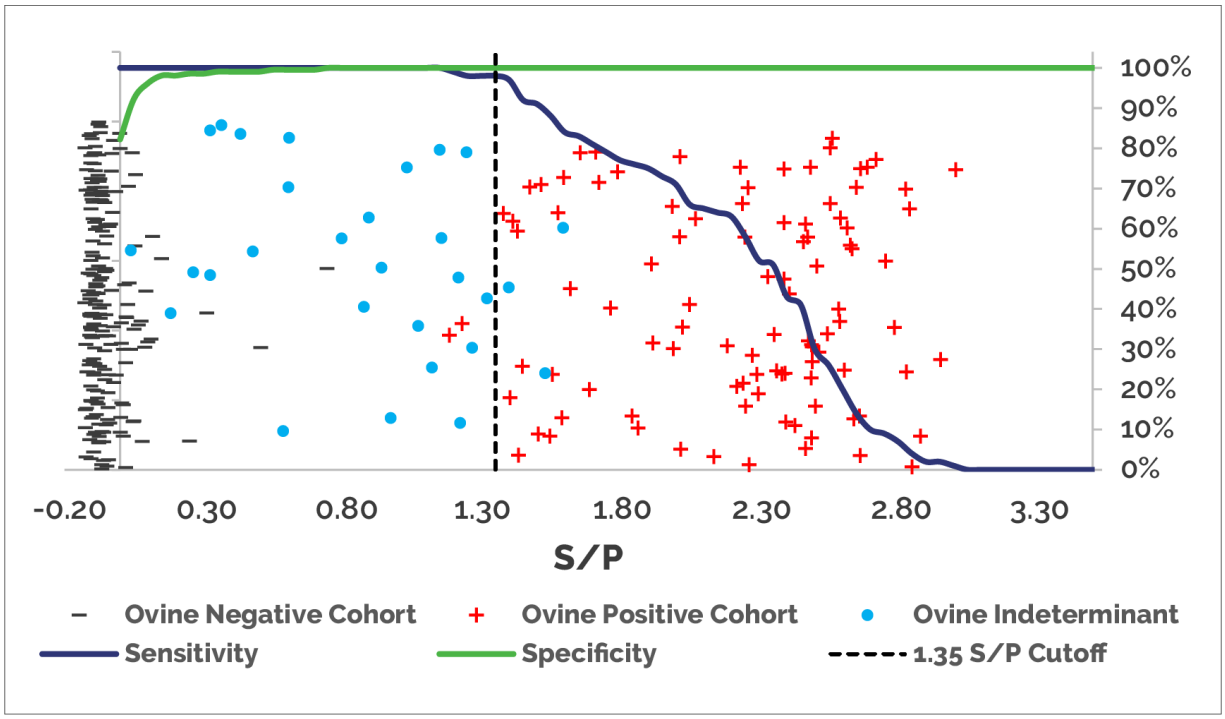

Figure 1: ROC analysis for ovine samples tested in VMRD ELISA

Validation and Performance of Reagents on Canine Samples

To assess diagnostic performance with canine samples, positive (n = 32) and negative (n = 100) samples that had been characterized with VMRD *B. canis* IFA slides were tested on the ELISA reagents. Using a cutoff of 0.8 S/P, the sensitivity and specificity was 96.9% and 100% respectively **(Figure 2)**.

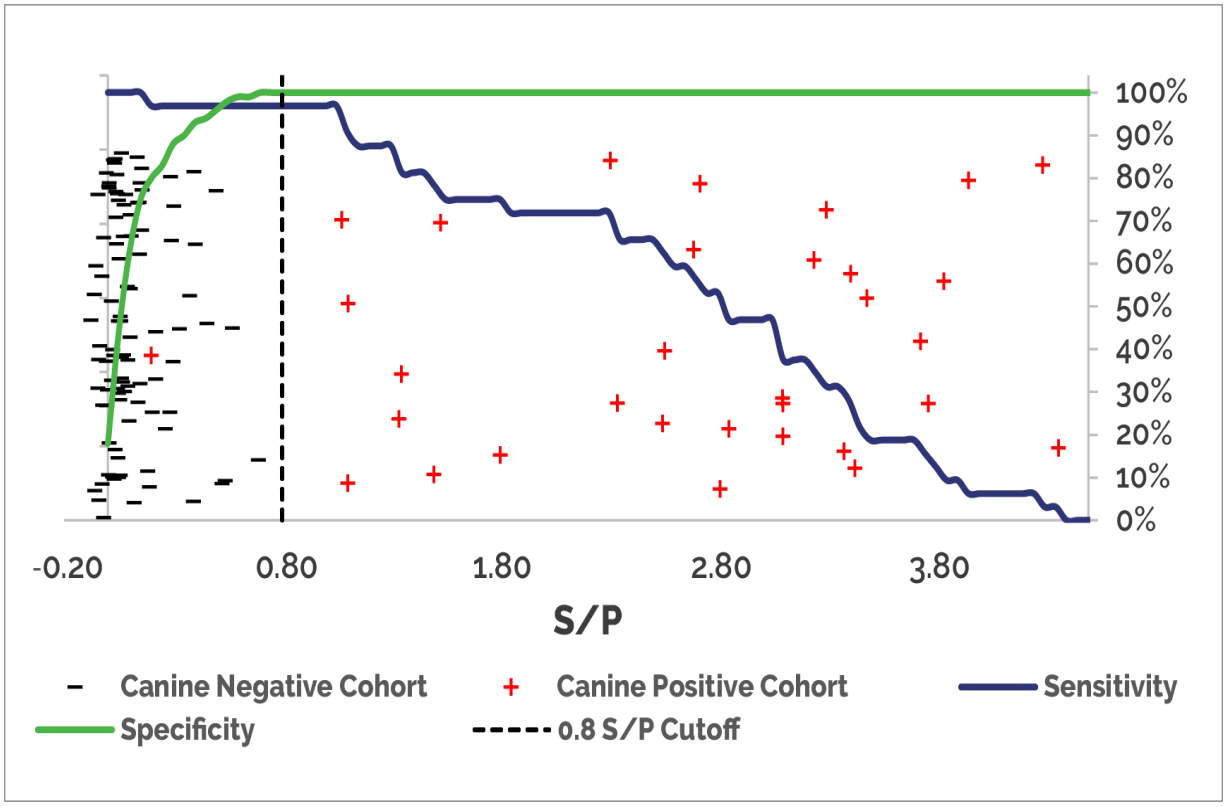

Figure 2: ROC analysis for canine samples tested in VMRD ELISA

IV. Conclusion

VMRD's *Brucella ovis* ELISA reagents accurately detect the OIE *B. ovis* reference serum standard and can be used for detection of antibody produced in response to *B. ovis* or *B. canis* exposure. These ELISA reagents are for **research use only**. It is recommended that any diagnostic interpretation be validated for the particular laboratory and sample population against samples of known disposition and/or a suitable reference assay.