Posters

Performance of an IDEXX Bovine Pregnancy Test adapted for visual-read results and designed for in-clinic use

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Introduction

While bovine pregnancy diagnosis today is predominately done by palpation and ultrasound, blood testing for pregnancy is growing in popularity as producers and veterinarians identify ways to incorporate it into their practices. Many veterinary clinics that are interested in adopting blood testing, however, find the up-front investment in instrumentation required for an ELISA setup to be cost-prohibitive. To address this need, IDEXX is developing a pregnancy test designed for veterinary clinics that does not require ELISA instrumentation. The plate ELISA is washed manually with a wash bottle and read visually; pregnant samples appear blue, while open samples have no color. This technological advance enables veterinarians to offer cost-effective blood-based pregnancy testing in addition to the palpation and ultrasound services they already provide.

Materials and Methods

The IDEXX Bovine Pregnancy Test for serum or plasma detects pregnancy- associated glycoproteins (PAGs) as markers for pregnancy. The ELISA-based plate assay utilizes an anti-PAG antibody coated onto the solid phase to bind PAGs that may be present in the sample. In conjunction with a horseradish peroxidase (HRP) enzyme conjugate, a second, anti-PAG antibody is used as the detection reagent for bound PAGs. A colorimetric reaction occurs with addition of TMB substrate to indicate the presence of PAG in the sample. The color development was read visually by 2 independent readers as presence or absence of blue color in the sample wells. The presence of blue color was interpreted as a pregnant diagnosis. To enable a manual assay protocol, dropper-tip caps were used to dispense assay reagents, a specialized calibrated micropipette was used to dispense the serum and plasma samples, and a squeezable wash bottle was used to wash the plate. The assay protocol consisted of incubating the sample with the plate for 30 minutes, adding the detector and incubating for 30 minutes, and adding the conjugate and incubating for another 30 minutes, with wash steps in between incubations. The TMB substrate was incubated for 15 minutes prior to addition of stop solution. All steps were carried out at room temperature.

Serum or plasma samples were obtained from 521 cows that were between 28 and 220 days after breeding or > 60 days-in-milk and not bred, and evaluated with the described format. The sensitivity and specificity for the test with the visual read results were calculated by use of transrectal ultrasound results as the reference standard.

Results

The visual-read test resulted in an overall sensitivity of 99.5% (95% confidence Interval, CI; 97.1%-100.2%) and an overall specificity of 93.0% (95% CI; 89.3%-95.5%). For plasma samples (n=321) the sensitivity and specificity of the test was 100% and 93.6%, respectively. For serum samples (n=200), the sensitivity and specificity of the test were 98.6% and 92.9%, respectively. The test accurately detected pregnancy in serum or plasma samples at $\geq$ 28 days of gestation.

Significance

The visual-read pregnancy test provides accurate pregnancy diagnoses comparable to laboratory-based alternatives that require ELISA equipment. Because it does not require a quantitative plate reader, plate washer, or incubator, this test expands the pregnancy testing options for veterinary clinics. Veterinarians and veterinary technicians can now perform accurate blood-based pregnancy testing as early as 28 days post-breeding in the clinic in < 2 hours without significant investment in laboratory equipment.