Improved diagnostic performance of a commercial *Anaplasma* antibody competitive enzyme-linked immunosorbent assay using recombinant major surface protein 5–glutathione S-transferase fusion protein as antigen

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**Introduction**

Anaplasmosis is a tick-borne disease of ruminant livestock in tropical and subtropical regions caused by rickettsia of the genus *Anaplasma*, including *A. marginale*, *A. centrale*, *A. ovis*, and *A. phagocytophilum*. As *Anaplasma* invades and multiplies within mature erythrocytes, acute disease is manifested with anemia, weight loss, abortion, and death in infected cattle. In animals that survive acute disease, *Anaplasma* causes life-long persistent infection. These persistently infected animals are clinically healthy, but serve as reservoirs for continued transmission of the pathogen to other animals. Therefore, control of *Anaplasma* infection is enhanced by identification of carrier cattle using a highly specific and sensitive serodiagnostic assay.

**Hypothesis**

Removal of maltose binding protein (MBP) from the recombinant antigen used for plate coating in the commercial cELISA will further improve the specificity.

**Materials and Methods**

- **Sera from *Anaplasma* noninfected cattle** (*n* = 358) were collected as true negative set from dairy herds maintained in barns free of ticks that transmit *Anaplasma*.
- **Anaplasma-positive sera** (*n* = 135) were obtained as true positive set from cattle with positive results by both serology and nested PCR assays.
- An additional 163 sera were selected as possible false positive set from diagnostic samples submitted to the Washington Animal Disease Diagnostic Laboratory.
- Commercial rMSP5-MBP cELISA and novel rMSP5-GST cELISA were evaluated using three sets of sera in relative diagnostic performance.

**Results**

- The number of 358 sera with significant MBP antibody binding (≥30%I) in *Anaplasma*-negative herds was 139 (38.8%) when tested using the rMSP5-MBP cELISA without MBP adsorption. All but 8 of the MBP binders were rendered negative (<30%) using the rMSP5-MBP cELISA with MBP adsorption, resulting in 97.8% specificity (Figure 1A and B).
- To improve the specificity of the commercial cELISA, a new recombinant antigen designated rMSP5–GST was developed, eliminating MBP from the antigen and obviating the need for MBP adsorption. Using the rMSP5-GST cELISA, only 1 of 358 *Anaplasma*-negative sera, which included the 139 sera with significant (≥30%) MBP binding in the rMSP5-MBP cELISA without MBP adsorption, was positive. This resulted in an improved diagnostic specificity of 99.7% (Figure 1C).

**Conclusion**

- The improved cELISA maintained reliable analytical sensitivity and specificity in addition to producing 100% diagnostic sensitivity and 99.7% diagnostic specificity using the cutoff of 30%I determined by ROC analysis.
- The rMSP5-GST cELISA resolved 3 types of problems observed in the rMSP5-MBP cELISA, including MBP binders, nonspecific binders of unknown mechanism, and sera with %I near the cutoff (25–35%).
- Based on the high diagnostic performance demonstrated in the current study, the rMSP5-GST cELISA appears to be a simpler and more reliable serodiagnostic tool for bovine anaplasmosis with various applications including epidemiological monitoring and disease/disease-free certification.

**References**