Veterinary Medical Research & Development

FOR VETERINARY USEONLY

FOR USE OUTSIDE OF THE UNITED STATES ONLY

BRUCELLA ABORTUS/MELITENSIS/SUIS DIVA ANTIBODY TEST

For use in the detection of antibodies against *Brucella abortus* and *melitensis* and to screen for antibodies against *Brucella suis*

General Description

This enzyme-linked immunosorbent assay detects antibodies to the dominant antibody binding epitopes present in the O-polysaccharide (OPS) of *Brucella spp.* Sample *Brucella spp.* OPS antibodies bind to synthetic *Brucella spp.* OPS derived-antigen coated on the plastic wells. Non-specific antibody is washed away and plate-bound *Brucella spp.* OPS-specific antibody captures the peroxidase conjugate. The presence of bound peroxidase conjugate is quantified by the addition of an enzyme substrate with subsequent color product development. Strong color development indicates the presence of antibody to *Brucella spp.* OPS in the sample. Weak or no color development indicates the absence of detectable antibody to *Brucella spp.* OPS.

Components Needed To Run Test

- Antigen-Coated Plates (Cat No. BSAG-PLATE)
- Positive Control (Cat No. BSAG-PC)
- Negative Control (Cat No. BSAG-NC)
- Peroxidase Conjugate (Cat No. BSAG-CJ)
- Serum Diluting Buffer (Cat No. BSAG-SDB)
- 10X Wash Solution Concentrate (Cat No. BSAG-WASH)
- Substrate Solution (Cat No. BSAG-TMB)
- Stop Solution (Cat No. BSAG-SS)

Additional Materials Required

Single and multichannel adjustable-volume pipettors and disposable plastic tips, test tubes or non-antigen-coated transfer plate(s), ELISA microplate absorbance spectrophotometer with a 450 nm filter, deionized or double distilled water, paper towels, graduated cylinder, timer, multichannel pipettor reservoirs, wash bottle, manual multichannel washing device or automatic plate washer

Storage and Stability

Store all reagents at 2-8°C. **Do not freeze.** Reagents will remain stable until the expiration date when stored as instructed.

Preparation

a. **Warm reagents:** Bring the samples, reagents and plate(s) to room temperature (23 ± 2°C) prior to starting the test.

b. **Prepare wash solution:** Prepare 1X Wash Solution by diluting 1 part of the 10X Wash Solution Concentrate with 9 parts of deionized or distilled water. At least 1.6 ml are needed per well. Allow extra for reservoirs, washing equipment, tubing, etc.

c. **Prepare samples:** Serum samples must be diluted in Serum Diluting Buffer for use in the test. It is recommended that these dilutions be made in a non-antigen-coated transfer plate or in test tubes. At least 115 µl of each diluted sample is required per well because of transfer loss. Mix transfer plate wells by micropipettor action and mix tubes by vortexing.

   Individual serum samples are diluted 1/50.

   Pooled bovine serum samples (up to 10 individual animals) are diluted 1/10.

   Bovine milk samples are not diluted.

d. **Prepare plates:** Remove the plate(s) from the foil pouch(es). If applicable: Return any unused strips to the pouch and securely seal it. Place strips to be used in the frame and number the top of each strip to maintain orientation. Always mark the strips in case they dislodge from the frame during washing.

Test Procedure

1. **Load controls and samples:** Positive and Negative Controls are provided READY-TO-USE. Load Positive Control in triplicate and Negative Control in duplicate, regardless of the number of samples to be tested. When whole plates are used, it is best to put the controls in wells on different areas of the plate. The controls must be loaded every time the assay is performed and on each plate if multiple plates are used. Using a pipettor set at 100 µl, load controls and samples into the Antigen-Coated Plate. Samples and controls should be loaded into the Antigen-Coated Plate as quickly as possible. When running more than two strips, we recommend that samples and controls be first loaded into a transfer plate and then transferred to the Antigen-Coated Plate using multi-channel pipetting equipment. Tap the side of the loaded assay plate several times to make sure the samples coat the bottom of the wells. Use care not to spill samples from well to well.

   When running individual serum samples, incubate plates for 30 minutes at room temperature (23 ± 2°C).

   When running pooled serum or milk samples, incubated plates for 60

minutes at 37 ± 2°C. To prevent evaporation, cover plate(s) with sealing tape, Parafilm®, or equivalent during the incubation.

2. **Wash wells:** After the sample incubation, wash the plate(s) 4 times:

   If an automatic washer is used, place the plate on the washing apparatus and
   wash plate 4 times, filling the wells each time with 1X Wash Solution.

   If manual washing is used, dump well contents and remove remaining samples and controls by sharply striking the inverted plate 4 times on a clean paper towel, striking a clean area each time. Immediately fill each well with at least 200 µl of 1X Wash Solution using a multichannel filling device or a wash bottle. Empty the wash solution from the plate and strike the inverted plate sharply on a clean paper towel as above. Fill and empty the plate by the same method 3 additional times for a total of 4 washes.
3. **Add conjugate:** Add 100 µl of Peroxidase Conjugate to each well. Tap the side of the loaded assay plate several times to make sure the conjugate coats the bottom of the wells. Incubate for 30 minutes at room temperature (23 ± 2°C).
4. **Wash wells:** After the 30-minute incubation, wash the plate(s) 4 times as in Step 2.
5. **Add substrate solution:** Add 100 µl of Substrate Solution to each well. Tap the side of the loaded assay plate several times to make sure that the substrate coats the bottom of the wells. Incubate 15 minutes at room temperature (23 ± 2°C). Avoid leaving the plate in direct sunlight. *Do not empty wells*.
6. **Add stop solution:** Add 100 µl of Stop Solution to each well. Tap the side of the loaded assay plate several times to mix the Substrate Solution and the Stop Solution. *Do not empty wells*.
7. **Read and record the test results:** Immediately after adding the Stop Solution, the plate should be read on a microplate absorbance spectrophotometer. Set the optical density (OD) reading wavelength to 450 nm.
8. Return all remaining reagents to 2-8°C for storage.

Calculations

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

Test Validation

- The **Positive Controls** must produce a mean O.D. ≥0.500.
- The **Negative Control** must produce a mean O.D. <0.250.

PO Box 502
Pullman, WA 99163 USA

P: 509.334.5815
F: 509.332.5356

support@vmrd.com
www.vmrd.com

Interpreting the Results

- Bovine individual serum samples are positive for *Brucella spp.* antibody if the S/P ratio is ≥0.4.
- Bovine individual serum samples are negative for *Brucella spp.* antibody if the S/P ratio is <0.4.
- Bovine pooled serum or milk samples are positive for *Brucella spp.* antibody if the S/P ratio is ≥0.3.
- Bovine pooled serum or milk samples are negative for *Brucella spp.* antibody if the S/P ratio is <0.3.
- Small ruminant serum samples are positive for *Brucella spp.* antibody if the S/P ratio is ≥0.5.
- Small ruminant serum samples are negative for *Brucella spp.* antibody if the S/P ratio is <0.5.
- Porcine serum samples are positive for *Brucella spp.* antibody if the S/P ratio is ≥1.0.
- Porcine serum samples are negative for *Brucella spp.* antibody if the S/P ratio is <1.0.

Precautions

All reagents should be handled and disposed of as potentially hazardous. Do not eat, drink, or smoke where serum samples and reagents are handled. Do not pipette by mouth. Some reagents may be harmful if ingested. If ingested, seek medical attention. Do not use expired or contaminated reagents.

Positive Control contains sodium azide as a preservative.

Negative Control contains sodium azide as a preservative.

Peroxidase Conjugate contains ProClin 300, methylisothiazolinone, bromonitrodioxane, and thimerosal as preservatives.

Serum Diluting Buffer contains sodium azide as a preservative.

Assay Limitations: This assay may give false positive or false negative results. Test results should be interpreted in the context of all available clinical, historical, and epidemiological information relevant to the animals under test. Further confirmatory testing may be required to validate results.

PO Box 502
Pullman, WA 99163 USA

P: 509.334.5815
F: 509.332.5356

support@vmrd.com
www.vmrd.com