Validation criteria for assay

Negative control should be above 1.0 OD.

Positive control should be above 80 PI

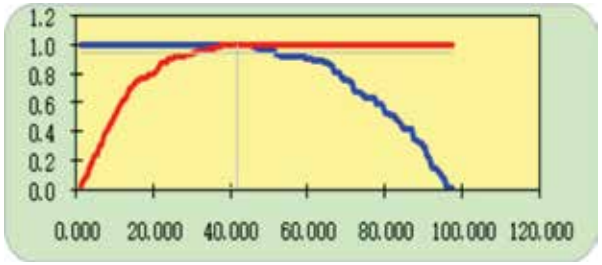
Quality control should be between 40 to 70 PI

**NOTE: Do not use expired kit.

**CAUTION Do not expose the vials beyond 30°C.

Cut-off achieved for Indian bovine population

Percentage Inhibition of 40 is achieved as cut-off for Indian cattle population for brucella serodiagnostics by two graph ROC anlaysis using reference sera samples (n = 200).



Performance Analysis

Brucella culture negative and culture positive animal sera samples were compared with MAb blocking ELISA and had a perfect agreement with k-value 1.

The MAb based blocking ELISA detected brucella specific antibodies in vaccinated animals as early as 5 days post vaccination. The indirect ELISA detected only at 10 days post vaccination.

Salient features of the kit

- Brucella MAb based blocking ELISA has higher sensitivity (100%) and specificity (99%) over indirect ELISA in detecting brucella antibodies.
- This kit is cost-effective, compared to similar imported kits.
- Conventional Rose Bengal test detects only IgM and is less accurate. This kit detects both IgM and IgG antibodies.

For further details contact

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INGENASA

Bru Alert®

Monoclonal based blocking ELISA for diagnosis of brucellosis

(For detecting antibodies against brucella in vaccinated or infected cattle)

Instructions For Use

Please Read Instructions Before Use

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SUMMARY AND EXPLANATION

Bovine brucellosis is a contagious, zoonotic and chronic infectious disease caused by *Brucella abortus*. *B. abortus* is a gram negative, facultative intracellular bacteria with O-polysaccharide (OPS) on the cell surface as part of lipopolysaccharide (LPS) molecule. The disease causes severe economic losses due to abortion, reduced productivity and temporary or permanent infertility in livestock. Diagnosis of brucellosis in cattle is complicated due to the absence of clinical signs except for late abortion. Bacterial isolation by culture is a confirmative test which is time consuming and laborious. Therefore, serological tests are preferred for the diagnosis of brucellosis and the successful diagnostic tests for brucella species are based on the detection of antibodies to the smooth LPS antigen. Monoclonal Antibody (MAb) based blocking ELISA for brucella serology is being recommended to improve the diagnostic specificity and sensitivity of the assay and OIE also considers these test as "prescribed tests for trade".

PRINCIPLE

Brucella ELISA is developed based on the principle of MAb blocking ELISA, where sLPS is precoated on the polystyrene surface of maxisorb microwell modules, to which the brucella specific antibodies of field sera binds and blocks the sLPS epitope. The unbound serum is removed by washing and peroxidise (HRP) conjugated brucella specific Mab is added. The unbound conjugate is washed and TMB substrate is added. The substrate is hydrolysed by the enzyme and subsequent colour development is indicative of the presence of brucella antibodies in the tested bovine sera.

KIT COMPONENTS

1. Brucella sLPS coated microwells – (12 x 8 wells). Ready for use. Microwells should be resealed immediately and stored in the presence of desiccant. Stable at 2-8°C for one year
2. Wash Buffer (10 x)- one bottle : 50ml of 10 x concentrate. Dilute one part wash buffer with 9 parts of distilled water. Diluted buffer may be stored for one week at room temperature
3. Sample diluent- one bottle, 12ml ready to use. Stable at 2-8° for 6 months
4. HRP conjugated brucella specific MAb- one bottle; 12 ml ready to use horseradish peroxidise conjugated MAb with preservative. Stable at 2-8° for 6 months.
5. Substrate- one bottle, 12ml ready to use. Stable at 2-8° for 6 months.
6. Positive control – one vial, 200 µl bovine serum. Stable at 2-8° for 6 months.
7. Negative control - one vial, 200 µl bovine serum. Stable at 2-8° for 6 months.
8. Quality control – one vial, 200 µl of sera Stable at 2-8° for 6 months.
9. Stop solution – one bottle, 15 ml ready to use. Stable at room temperature.

PROCEDURE

1. All reagents should be equilibrated to room temperature (22-25°C).
2. Remove the required number of microwells from the foil sachet and insert into strip holder. Along with the samples, six microwells are required for negative control (N), positive control (P) and quality control (QC) in duplicate. The remaining unused mircowells should be sealed tightly and replaced in the foil sachet.
3. Pipette 100 µl of positive, negative and quality controls to respective control wells.
4. Pipette 90 µl of sample diluents to the sample wells and 10 µl of sera samples to the respective sample wells.
5. Gently mix the contents of the wells, seal the plate and incubate for 1 hour at room temperature (20-25 ° C)
6. Wash 4 times with PBST (300 µl per well).
7. Add 100 µl of conjugate to each well. Seal the plate and incubate for 1 hour at room temperature (20-25 ° C)
8. Wash 4 times with PBST
9. Add 100 µl of substrate (TMB) to each well. Keep the plate for 10 minutes at room temperature.
10. Add 100 µl of stop solution to each well.
11. Read optical density (OD) of each well at 450 nm within 5 minutes after addition of stop solution.

Cut-off analysis

Percentage Inhibition (PI) is calculated by the formula:

PI = 100 – {(Test sample OD/Negative control OD)} X 100.