

AniGen B. Brucella Ab ELISA 2.0

1. Explanation of the Test

Bovine brucellosis is a highly contagious bacterial disease, almost exclusively caused by *Brucella abortus* causing late term-abortion and infertility in cattle. The disease is also a serious zoonosis, causing undulant fever in humans.

The AniGen B. Brucella Ab ELISA is an indirect Enzyme Linked Immunosorbent Assay for the qualitative detection of *Brucella abortus* antibody in serum and plasma.

The AniGen B. Brucella Ab ELISA contains a microplate, which is pre-coated with B. Brucella antigen on the well. During first incubation, if antibodies present in the test sample, Brucella antibodies bind to the antigens in the well. Following this incubation, all unbound materials are removed by aspiration and washing. HRP conjugate added subsequently forms a complex with the Brucella antibodies. Unbound materials are removed by aspiration and washing before the addition of a substrate solution. The residual enzyme activity found in the well will thus be directly proportional to the anti-Brucella concentration in specimens and evidenced by incubating the solid-phase with a substrate solution. The reaction is stopped by addition of the stopping solution and colorimetric reading will be performed by using a spectrophotometer at 450 nm with reference wavelength at 620 nm.

The specially selected *Brucella abortus* antigens are used as capture material in test. These enable the AniGen B. Brucella Ab ELISA to identify to *Brucella abortus* antibodies in specimens, with a high degree of accuracy.

2. Materials Provided (480Tests/Kit)

AniGen B. Brucella Ab ELISA contains following items to perform the assay.

- 1) Microplate coated LPS of *Brucella abortus* 1119-3 ① : 5 plates (96wells/plate) configured in twelve 1x8 strips.
- 2) Standard negative control serum ② : 1 vial (4.0 ml/vial) of normal bovine serum treated with calcium. Sodium azide (0.01%) added as preservatives.
- 3) Standard strong positive control serum ③: 1 vial (4.0 ml/vial) of anti-B.Brucella strong positive bovine serum treated with calcium. Sodium azide (0.01%) added as preservatives.
- 4) Standard weak positive control serum ④ : 1 vial (4.0 ml/vial) of anti-B.Brucella weak positive bovine serum treated with calcium. Sodium azide (0.01%) added as preservatives.
- 5) Standard negative control milk ⑤ : 1 vial (4.0 ml/vial)of normal milk. Sodium azide(0.01%) added as preservatives.
- 6) Standard positive control milk ⑥ : 1 vial (4.0 ml/vial)of anti-B. Brucella positive bovine milk. Sodium azide(0.01%) added as preservatives.
- 7) Sample diluents (5X concentrated) ⑦ : 1 bottle (125 ml/bottle) of phosphate buffered saline, Sodium azide(0.01%) added as preservatives.
- 8) Washing solution (10X concentrated) ⑧ : 2 bottles (250 ml/bottle) of PBS-Tween 20.
Preservative : Thimerosal (0.01%)
Note: Before use, take one bottle content, and then fill-up to 1,000 ml with distilled water. In presence of undissolved crystals, re-suspend the solution by placing the bottle at 37 °C for few minutes.
- 9) Enzyme conjugate : (101X concentrated) ⑨ : 1 vial (1.2 ml/vial) of anti-bovineIgG–HRP.
Preservative : Proclin (0.06%)
- 10) Enzyme conjugate diluent ⑩ : 1 vial (80 ml/vial) of phosphate buffered saline.
Preservative : Proclin (0.06%)
- 11) Substrate (Ready to use) ⑪ : 1 bottle (60 ml/vial) of tetramethyl-benzidine with citrate-phosphate buffer containing H₂O₂.
- 12) Stopping solution ⑫ : 1 bottle (80 ml/bottle) of 1N sulfuric acid. Ready for use.
- 13) Adhesive plate sealer: 10 EA
- 14) Instructions for use.

3. Precautions

In order to obtain reproducible results, the following rules must be observed:

- 1) For in vitro diagnostic use only.
- 2) Do not mix reagent of different lots.
- 3) Use thoroughly clean glassware, free from contamination of metal ion or oxidating substances.
- 4) Use disposable gloves while handling potentially infectious material and performing the assay.
- 5) Substrate and stopping solution should be handled with care. Avoid contact with skin, eyes and mucous membranes. In case of accident rinse thoroughly with running water.

4. Specimen Collection and Storage

1) [Serum]

- ① Collect the whole blood without any anti-coagulant.
- ② Centrifuge whole blood to get serum.

2) [Plasma]

- ① Collect the whole blood using the suitable anti-coagulant.
- ② Centrifuge whole blood to get plasma.

3) [Milk]

- Milk samples must be centrifuged for 15minutes at 2000 Xg to remove the lipid layer.
- 4) If specimens are not immediately tested they should be refrigerated at 2 ~ 8 °C. For storage periods greater than three days, freezing is recommended. They should be brought to room temperature prior to use.
- 5) Specimens containing precipitate may yield inconsistent test results. Such specimens must be clarified prior to assaying.

5. Reagent preparation

- 1) Allow all reagents to come to room temperature (18~25°C) before use.
- 2) **Enzyme conjugate (101X concentrated):** The concentrated enzyme conjugate ⑨ must be diluted 1 : 100 with enzyme conjugate diluents⑩ before use. i.e. add 10 µl of enzyme conjugate ⑨ to 1ml of conjugate diluent ⑩and mix well.
- 3) **Sample diluent (5X concentrated) :** The Sample diluent ⑦ concentrated must be diluted 1 : 4 with distilled/deionized water before use. i.e. add 10 ml of Sample diluent ⑦ to 40 ml of distilled water and mix well.
- 4) **Washing solution (10X concentrated):** The concentrated washing solution⑧ must be diluted 1 : 9 with distilled/deionized water before use. i.e. add 50 ml of Washing solution⑧ to 450 ml of distilled water and mix well. If undissolved crystals are present, re-suspend the solution by warming the bottle at 37 °C for few minute.

6. Procedure of the Test

1) Preparation of Samples

[Serum and Plasma]

- ① Preparation of Samples : Dilute test samples 1:50 with sample diluent (e.g., by diluting 10 µl 1 of sample with 500 µl of sample diluents). **NOTE : Do not dilute controls**
- ② Add 100 µl of the **UNDILUTED 3 Strong Positive controls** into three wells.
- ③ Add 100 µl of the **UNDILUTED 2 Weak Positive controls** into two wells.
- ④ Add 100 µl of the **UNDILUTED 2 Negative controls** into two wells.

[Milk]

- Do not dilute. 100 µl of Negative, Positive Control and the milk sample are required for each sample well .
- 2) Add 100 µl of the DILUTED sample into each well.
 - 3) Cover the microplate with adhesive plate sealer and mix well on vibrating mixer. Mixing is very important to get the reproducible results
 - 4) Incubate the wells at 37 °C for 60 minutes.
 - 5) Wash the wells at 5 times with 350 µl of diluted washing solution. Aspirate all liquid from the wells.
 - 6) Add 100 µl of diluted enzyme conjugate to each well.

- 7) Cover the microplate with adhesive plate sealer.
- 8) Incubate the wells at 37°C for 30 minutes.
- 9) Wash the wells 5 times with 350 µl of diluted washing solution. Aspirate all liquid from the wells.
- 10) Add 100 µl of mixed substrate (Ready to use) to each well.
- 11) Incubate the wells for 15 minutes at room temperature (18~25°C).
- 12) Add 100 µl of stopping solution to each well.
- 13) Read the absorbance of the wells with a bichromatic spectrophotometer at 450 nm with reference wavelength at 620 nm. Reading must be completed within 1 hour from the end of assay.

7. Interpretation of the Test

1) Test validation

[Serum & Plasma]

- ① The OD value of standard negative control serum should be ranged from 0.000~0.2000.
- ② The OD value of standard strong positive control serum should be above 1.000
- ③ The OD value of standard weak positive control serum should be above 0.500

[Milk]

- ① The OD value of standard negative control milk should be ranged from 0.000~0.2000.
- ② The OD value of standard positive control milk should be above 1.000

2) Percent Positivity(%P) value calculation

[Serum & Plasma]

$$\%P = \frac{\text{OD of samples}}{\text{Average OD of standard strong positive control serum}} \times 100$$

[Milk]

$$\%P = \frac{\text{OD of samples}}{\text{Average OD of standard positive control Milk}} \times 100$$

3) Interpretation of serum or plams

[Serum & Plasma] After calculating the %P value, the positive and negative value should be determined based on the following %P criteria.

- ① Positive : %P of sample ≥ 25
- ② Negative : %P of sample < 25

[Milk] After calculating the %P value, the positive and negative value should be determined based on the following %P criteria.

- ① Positive : %P of sample ≥ 15
- ② Negative : %P of sample < 15

4) Example

If the OD of sample is 0.859 and average standard strong positive control serum is 2.500, the %P is 34. This sample is regarded as positive to bovine brucellosis.

8. Limitations and Interferences

1) The test procedure, precautions and interpretation of results sections for this test kit must be followed closely when testing.

2) Samples

- ① Samples containing sodium azide do not affect the test result.
- ② Pasteurized sera samples (no less than 10 hours at 60°C) may lead to diminished reactivity. Therefore should not be used.
- ③ Heat-inactivated sera samples (1 hour at 56°C) do not impair the test.
- ④ Anticoagulants such as heparin, EDTA, and citrate do not affect the test result.
- ⑤ Haemolytic samples should be centrifuged before use to avoid interference by cellular constituents.

⑥ Rheumatoid factors can lead to elevated reactivity if it contained in the samples.

⑦ Lipaemic and icteric samples do not impair the test results.

⑧ This test kit detects antibody to Brucella specific antibodies in serum and plasma samples and thus is useful as a screening procedure.

⑨ Failure to add specimen in the procedure could result in a falsely negative test. Repeat testing should be considered where there is clinical suspicion of infection.

9. Storage and stability

1) The AniGen B. Brucella Ab ELISA kit should be stored at 2~8°C. This test kit is stable through the expiration date printed in the package and in the label of each material / reagent as unopened state.

2) Stability of once opened materials / reagents

Material/Reagent	State	Storage	Stability
Diluted washing solution	1:9 diluted	2~8°C Room temp(18~25°C).	3 months 2 weeks
Enzyme conjugate diluent	1:100 diluted	Room temp(18~25°C). Closed container, protected from light.	4 hours

10. Packaging Unit : 96Test/Kits, 480Tests/kit, 960 Tests/kit

11. Performance characteristics

[Specificity]

AniGen B. Brucella Ab ELISA kit demonstrated 100% specificity in 154 individual bovine serum samples from a brucellosis-free area in Korea.

[Sensitivity]

Serum	Dilution	Expected Result	%P	Interpretation	OD ₄₅₀ SPC	OD ₄₅₀ Neg
SPC	1/16	+	32.8	Pos	2.224	0.051
SPC	1/64	-	7.0	Neg		
OIEISS	1/150	+	27.0	Pos		
OIEISS	1/600	-	8.4	Neg		

*AniGen B. Brucella Ab ELISA kit accurately detected weak and strong positive OIE reference according to EU Directives.

12. Precision

Within-run and between-run precisions have been determined by the testing 10 replicates of three specimens : standard negative serum, standard strong positive serum and standard weak positive serum. The C.V.(%) of negative , weak positive , and strong positive values were within 10% of the time.

13. Bibliography of suggested reading

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