

CALDON BIOTECH INC. Brucella IgG ELISA

Catalog No. BA052G
(96 tests)

NAME AND INTENDED USE

The CALDON BIOTECH INC (CBI), Brucella IgG ELISA is intended for the use in evaluating of patients with suspected Brucella infection.

SUMMARY AND EXPLANATION OF THE TEST

Brucella is a gram negative coccobacilli capable of infecting a wide range of animal and man. Of the three species causing human infection, *B. melitensis* is the most pathogenic followed by *B. suis* and *B. abortus*. Brucellosis is transmitted through contaminated and untreated milk and milk products and by direct contact with infected animals (cattle, sheep, goats, pigs, camels, buffaloes, and, very recently, seals), animal carcasses, and abortion materials. Worldwide, millions of individual are at risk, especially in developing countries where the infection in animals has not been brought under control, heat treatment procedures of milk (e.g. pasteurization) are not routinely applied, and food habits such as consumption of raw milk. The incubation period of brucellosis is usually one to three weeks, but sometimes may be several months. The illness may be mild and self-limiting or severe. The disease is accompanied by continued, intermittent, or irregular fever, headache, weight loss and generalized aching and fatigue. Urogenital symptoms may dominate the clinical presentation in some patients. This method uses *B. abortus* outer membrane, which is shared by the other species. Brucella IgG and IgA antibodies persist for many years after infection. A significant increase in Brucella IgG level is in patients with symptoms of brucellosis is indicative of recent exposure. IgM antibodies are present in acute brucellosis and also found in about 33% of patients with chronic brucellosis.

PRINCIPLE OF THE TEST

Diluted patient serum is added to wells coated with purified antigen. IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess

enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample.

MATERIALS PROVIDED

- Microwell Strips: Brucella antigen coated wells (12 x 8 x 1 wells)
- Sample Diluent: 1 bottle (22 mL). Ready to use.
- Enzyme Conjugate: 1 bottle (12 mL). Ready to use.
- TMB substrate: 1 bottle (12 mL). Ready to use.
- Stop solution: 1N H₂SO₄; 1 bottle (12 mL). Ready to use.
- Calibrator: Yellow Cap. (1.50 mL/vial). Ready to use.
- Positive Control: Red Cap. (1.50 mL/vial). Ready to use.
- Negative Control: Blue Cap. (1.50 mL/vial). Ready to use.
- Wash Concentrate: 1 bottle (50 mL): 20X Concentrate.

STORAGE AND STABILITY

1. Store the kit at 2-8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage or usage.

WARNINGS AND PRECAUTIONS

1. Potential biohazardous materials: The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984.
2. Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.

4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. Control sera and sample diluent contain preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

SPECIMEN COLLECTION AND HANDLING

- Collect blood specimens and separate the serum.
- Specimens may be refrigerated at 2–8° C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing of serum sample.

PREPARATION FOR ASSAY

1. Bring all specimens and kit reagents to room temperature (20-25°C) and gently mix.
2. Prepare washing buffer by adding the contents of the bottle (50 mL, 20X Wash concentrate) to 950 mL of distilled or deionized water in one-liter container. Store at room temperature.

ASSAY PROCEDURE

1. Place the desired number of coated strips into the holder.
2. **Positive control, negative control and calibrator are ready to use.** Prepare 1:21 dilution of test samples, by adding 10 µL of the sample to 200 µL of sample diluent. Mix well.
3. Dispense 100 µL of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100µL sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature.
4. Remove liquid from all wells. Repeat washing three times with wash buffer.
5. Dispense 100 µL of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Repeat washing three times with wash buffer.
7. Dispense 100 µL of TMB substrate solution and incubate for 10 minutes at room temperature.
8. Add 100 µL of 1N H₂SO₄ to stop reaction.
9. Read O.D. within 30 min at 450 nm using microwell reader.

CALCULATION OF RESULTS

- Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit.
- Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF).
- Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value.

Example of typical results:

Calibrator mean OD = 0.8
 Calibrator Factor (CF) = 0.5
 Cut-off Value = 0.8 x 0.5 = 0.400
 Positive control O.D. = 1.2
 Ab Index = 1.2 / 0.4 = 3
 Patient sample O.D. = 1.6
 Ab Index = 1.6 / 0.4 = 4.0

QUALITY CONTROL

The test run may be considered valid provided the following criteria are met:

- The O.D. of the Calibrator should be greater than 0.250.
- The Ab index for Negative control should be less than 0.9.
- The Ab Index for Positive control should be greater than 1.2.

INTERPRETATION

The following is intended as a guide to interpretation of CBI Brucella IgG test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

Antibody Index Interpretation

<0.9 No detectable antibody to Brucella IgG by ELISA.

0.9-1.1 Borderline positive. Follow-up testing is recommend if clinically indicated.

>1.1 Indicative of past or current Brucella infection.

LIMITATIONS OF THE TEST

1. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
2. Lipemic or hemolyzed samples may cause erroneous results.

PERFORMANCE CHARACTERISTICS

1. Sensitivity and Specificity

92 patient sera were tested by both CBI Brucella IgG ELISA and a reference ELISA method. 14 sera were positive and 77 were

negative by both methods (99% agreement).
The results are summarized below:

	CBI Brucella IgG		
	ELISA		
	+	-	Total
Reference ELISA	14		0
+ Kit	14		
+ Kit	1		77
- Kit	78		
- Kit	15		77
- Kit	92		
Total			

2. Precision Intra Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	16	1.31	0.071	5.41
2	16	0.86	0.052	6.04
3	16	0.24	0.015	6.25

Inter Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	10	1.92	0.21	10.93
2	10	1.44	0.17	11.80
3	10	0.25	0.032	12.80

REFERENCES

- Gad El-Rab MO; Kambal AM. Evaluation of a Brucella enzyme immunoassay test (ELISA) in comparison with bacteriological culture and agglutination. J Infect 1998; 36(2):197-201.
- Mikolon AB; Gardner IA; Hietala SK; Hernandez de Anda J; Chamizo Pestana E; Hennager SG; Edmondson AJ. Evaluation of North American antibody detection tests for diagnosis of brucellosis in goats. J Clin Microbiol 1998; 36(6):1716-22.
- Bowden RA; Cloeckart A; Zygmunt MS; Bernard S; Dubray G. Surface

exposure of outer membrane protein and lipopolysaccharide epitopes in Brucella species studied by enzyme-linked

- immunosorbent assay and flow cytometry. Infect Immun 1995; 63(10):3945-52.
- Baldi PC; Miguel SE; Fossati CA; Wallach JC. Serological follow-up of human brucellosis by measuring IgG antibodies to lipopolysaccharide and cytoplasmic proteins of Brucella species. Clin Infect Dis 1996;22(3):446-55
- Casao MA; Leiva J; Diaz R; Gamazo C. Anti-phosphatidylcholine antibodies in patients with brucellosis. J Med Microbiol 1998; 47(1):49-54.