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User's Manual

Brucella IgG ELISA Kit

REF **DEIA2402**

Σ **96T**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

The Brucella IgG ELISA Kit is intended for the detection of IgG antibody to Brucella in human serum or plasma.

General Description

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 patogenic 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.

Principles of Testing

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.

Reagents And Materials Provided

1. Microwells coated with Brucella abortus antigen: 12×8×1
2. Sample Diluent: 1 bottle (ready to use) 22 ml
3. Calibrator: 1 Vial (ready to use) 1ml
4. Positive Control: 1 vial (ready to use) 1ml
5. Negative Control: 1 vial (ready to use) 1ml
6. Enzyme conjugate: 1 bottle (ready to use) 12ml
7. TMB Substrate: 1 bottle (ready to use) 12ml
8. Stop Solution: 1 bottle (ready to use) 12ml

9. Wash concentrate 20×: 1 bottle 25ml

Materials Required But Not Supplied

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

Storage

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.

Specimen Collection And Preparation

1. Collect blood specimens and separate the serum.
2. 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.

Reagent Preparation

Prepare 1× Wash buffer by adding the contents of the bottle (25 ml, 20×) to 475 ml of distilled or deionized water. Store at room temperature (20-25°C).

Assay Procedure

Prior to assay, allow reagents stand at room temperature. Gently mix all reagents before use.

1. Bring all specimens and kit reagents to room temperature (20-25°C) and gently mix.
2. Place the desired number of coated strips into the holder.
3. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of test samples, by adding 10 µl the sample to 200 µl sample diluent. Mix well.
4. Dispense 100 µl diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100 µl 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.
5. Remove liquid from all wells. Wash wells three times with 300 µl of 1× wash buffer. Blot on absorbance paper or paper towel.

6. Dispense 100 µl of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
7. Remove enzyme conjugate from all wells. Wash wells three times with 300 µl of 1× wash buffer. Blot on absorbance paper or paper towel.
8. Dispense 100 µl of TMB substrate and incubate for 10 minutes at room temperature.
9. Add 100 µl of stop solution.
10. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm.

Calculation

1. 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.
2. Calculate the cut-off value: Calibrator OD × Calibrator Factor (CF).
3. Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value.

Precautions

1. For Research Use Only. Not for use in diagnostic procedures.
2. For Laboratory use.
3. Not for Internal or External Use in Humans or Animals.
4. There should be no eating or drinking within work area.
5. Always wear gloves and a protective lab coat.
6. No pipetting should be done by mouth. Handle all specimens and reagents as potentially infectious and biohazardous.
7. Do not add sodium azide to samples as preservative.
8. Do not use external controls containing sodium azide.
9. Use disposable pipette tips to avoid contaminating chromogenic substrate reagent. Discard reagent if it turns blue.
10. Do not pour chromogenic substrate back into container after use.
11. Do not freeze reagents.
12. Do not mix reagents from different kit lot numbers.
13. Keep reagents out of direct sunlight.
14. Handle stop reagent with care, since it is corrosive.
15. Bring all reagents to room temperature.
16. Viscous forensic samples should always be diluted in phosphate buffered saline or distilled water prior to pipetting.
17. Ensure the bag containing the micro-plate strips and desiccant is sealed well, if only a few strips are used.

Limitations

1. Lipemic or hemolyzed samples may cause erroneous results.

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