GB's BRUCELLA – A & M

(SLIDE & Tube Test Method)

KIT NAME	KIT SIZE	CAT. NO
Brucella Abortus	1 x 5 ml	SBAA01005M
Brucella Melintensis	1 x 5 ml	SBAM01005M

GENUINE BIOSYSTEM

INTRODUCTION

Human Brucellosis (Diurnal, or undulant fever) is a common febrile illness caused by infection with bacteria of some of the Brucella species (abortus, melitensis). This undulant fever is associated with symptoms, which are often variable and non-specific with chills, fever, sweats and anorexia. On exposure the body responds to this antigenic stimulation by producing specific antibodies whose titres rise slowly at early stages and then increases. Specific antibodies to the Brucella species are detectable a few weeks after exposure and are of considerable importance in the diagnosis of Brucellosis. Information regarding the titre of antibodies can be obtained by using specific GB's Brucella A/M antigen suspensions.

METHOD PRINCIPLE

The smooth, attenuated stained GB's BRUCELLA antigen suspensions are mixed with the patient's serum. Specific antibodies to Brucella Antigens if present in the patient serum will react with the antigen suspension to produce an agglutination reaction. No agglutination indicates the absence of specific antibodies to Brucella antigens.

Reagent Name	SBAM01005M
R1 Brucella Abortus	1 x 5 ml
R2 Brucella Melintensis	1 x 5 ml

WORKING REAGENT PREPARATION AND STABILITY

1. Store the reagent at 2-8°C. DO NOT FREEZE.
2. The shelf life of the reagent is as per the expiry date mentioned on the reagent vial labels.

SPECIMEN

Avoid exposure to elevated temperatures and air, as the reagents is highly sensitive to denaturation and drying.

ADDITIONAL MATERIAL REQUIRED

Slide Test Method: Stop watch, Positive control, Isotonic saline and Glass slide with clear/ white background, appropriate Pipettes / Micropipettes, Mixing sticks & a High intensity direct light source.

Quantitative Method: Timer, Test tubes (12 mm x 75 mm), Test tube rack, appropriate Pipettes / Micropipettes, Isotonic saline/0.25% phenol saline, Incubator (37°C)

NOTES:

1. In vitro diagnostic regent for laboratory and professional use only. Not for medicinal use.
2. The reagent contains 0.01% thimerosal as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water.
3. Performance of the reagent must be verified with positive and negative controls and it is recommended that controls be run with each test series.
4. The Reagent can be damaged due to microbial contamination or an exposure to extreme temperature.
5. Shake the reagents vials well before use to dispense the antigen suspension uniformly and improve test realibility.
6. Only a clean and dry glass slides/tubes must be used. Clean the glass slides/tube with clean distilled water and dry.
7. It is necessary to use the calibrated dropper provided in the reagent vial to dispense the reagent drop.
8. GB's Brucella - A & M antigen suspensions are not from human sources, hence contamination due to HBsAg and HIV is practically excluded.
9. Do not use damaged or leaked reagents.

SAMPLE COLLECTION AND STORAGE

1. No special preparation of patient is required prior to sample collection by approved techniques. Do not use hemolysed and turbid serum samples.
2. Clean and dry glassware free from detergents must be used for sample collection.
3. Do not heat inactive in the serum.
4. Though freshly collected serum is preferred, samples can be stored at 2-8° C, for 24 hours, or frozen for 8 days should a delay in testing occur.
5. GB's Brucella antigen suspensions are not from human sources hence contamination due to HBsAg and HIV is practically excluded.

PROCEDURE - SLIDE METHOD

Bring reagent and samples to room temperature before testing. Shake and mix the GB's Brucella antigen suspensions well before dispensing. The procedure for GB's Brucella - A and Brucella - M is identical.

QUALITATIVE METHOD

1. Place one drop of the test sample, positive and negative controls onto separate reaction circles of the glass slide using a sample dispensing pipette.
2. Add one drop of saline onto the next reaction circle of the glass slide.
3. Add one drop of patient serum to be tested on the next reaction circle of glass slide.
4. Add one drop of the appropriate GB's Brucella antigen suspensions in each of the above circles (controls and patient sample which dispensed)
5. Mix contents of each circle uniformly over the entire circle with separate mixing sticks.
6. Gently rock the slide back and froth, observe for agglutination macroscopically at 1 minute against the white background.

SEMI - QUANTITATIVE METHOD

1. Using saline prepare serial dilutions of the test sample positive in the qualitative method 1:2,1:4,1:8,1:16,, 1:32, 1:64, 1:128 and so on.
2. Perform the qualitative test procedure using each dilution as test specimen.
3. The titre is reported is the reciprocal of the highest dilution which shows as positive test result.

PROCEDURE - TEST TUBE METHOD

1. Take 8 test tubes and label them from 1 to 8.
2. Pipette 1.9ml of isotonic saline or preferably 0.25% phenol saline to tube no: 1
3. To each of remaining tube (2-7) add 1.0ml of isotonic saline of preferably 0.25% phenol saline.
4. To the tube no:1 add 0.1ml of serum sample to be tested, Mix well.
5. Transfer 1.0ml of diluted serum from tube no:1 to tube no:2 and mix well
6. Transfer 1.0 ml of the diluted serum from tube no.2 to tube no.3 and mix well. Continue this serial dilution till tube no.7.
7. Discard 1.0 ml of the diluted serum from tube no.7.
8. Pipette 1.0 ml of isotonic saline in tube no.8, which serves as a negative control.
9. To all the tubes, add 1 drop of appropriate GB's Brucella antigen suspensions and mix well.
10. Cover the tubes and incubate at 37 C for 24 hours.
11. Observe for agglutination macroscopically in each tube of the dilution series.

INTERPRETATION OF TEST RESULTS.

Semi- Quantitative Method

Agglutination is a positive test result and indicates the presence of Brucella antibodies in the test sample.

No agglutination is a negative test result and indicates the absence of Brucella antibodies in the test sample.

Semi-Quantitative Method:
The titre of Brucella antibodies is the highest dilution of the test sample giving a positive test result.

REMARKS:

1. Both Brucella abortus and Brucella melitensis share a common Brucella antigen. A sample giving a positive result with the Rose Bengal reagent should be tested using GB's Brucella A/M antigen suspensions by rapid slide test and confirmed by the tube test to determine the type of Brucella antibody detected. The higher titre detected determines the specific type of Brucella antibodies present.
2. In the semi quantitative test the reactions obtained are roughly equivalent to those, which would occur in a tube test.
3. Positive results obtained in the slide test should be confirmed with the tube test to establish whether the titres are diagnostically significant or not.
4. Agglutinins are found in high proportion of normal individuals and titres less than 1:8 are of doubtful significance. A rising titre is more significant than a single high titre.
5. False positive reactions may occur in sera of patients infected with Pasteurella tularensis or vaccinated with vibrio cholerae.
6. Cross-reactions between Brucella antigens and other organisms such as Yersinia enterolitica, Escherichia coli and Francisella tularensis have been reported.
7. False positive results are likely if the test is read more than one minute after mixing on the slide test.
8. Prozoning may sometimes be encountered in serum containing very high titres on slide test.
9. Serological findings are not intended as a substitute for culture. An appropriate attempt should be made to recover and identify the etiologic organisms through various culture and biochemical tests.
10. Since techniques and standardization vary from laboratory to laboratory in tube, difference in titres can be expected.
11. Use a separate disposable tip for each sample to prevent cross contamination.
12. Turbid and contaminated sera should not be used for testing.
13. After usage the antigen suspension should be immediabtly recapped and replaced at 2-8^{0}C.
14. Reagent vials that have leakage/ breakage problem should be discarded.
15. Only qualified and well trained staff should use the reagents.
16. It is recommended that results of the tests should be correlated with clinical findings to arrive at the final diagnosis.
17. The performance of the reagents should be validated periodically using known positive control. Good physiological saline may be used as a negative control.

PERFORMANCE CHARACTERISTICS

1. The positive control antisera should produce 1+ or greater agglutination at 1:8 titre in the slide and tube test when tested with the GB's Brucella - A/M antigen suspensions.
2. The negative control should show no agglutination with any of the Brucella - A/M antigen suspensions.
3. Generally accepted performance characteristic of this type of tests is 70% specificity and sensitivity.
4. Reproducibility of Brucella - A/M antigen suspensions is 100% (+/- one double dilution).

LITERATURE

1. J. G. Collee, J. P. Duguid, A. G. Fraser, Practical Medical Microbiology, 13th Ed.: 525 – 530.
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