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BRUCELLA A/M

Diagnostic reagent set **(Slide and Tube Tests)** for the in-vitro qualitative screening and semi-quantitative determination of Brucella antibodies present in infected human serum manually.

REF: BS.1/BRA1.100.0100	100 tests for Brucella-A	REF: BS.1/BRS2.100.0200	100 tests for Brucella A+M
REF: BS.1/BRM1.100.0100	100 tests for Brucella-M		

CLINICAL SIGNIFICANCE [1-2]

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 undeulant 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 titers 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 titer of antibodies can be obtained by using specific ***BioScien*** Brucella antigen suspension.

METHOD PRINCIPLE [2]

The smooth, attenuated stained 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 COMPOSITION

The Brucella-A / Brucella-M reagents contain ready to use standardized, attenuated, stained, smooth specific antigen suspensions of Brucella having specific reactivity towards antibodies to Brucella abortus (Brucella-A), and Brucella meltiness (Brucella-M).

PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person. Do not ingest or inhale as reagent (R) contains sodium azide which is classified as dangerous substance for environment.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
  S56: dispose of this material and its container at hazardous or special waste collection point.
  S57: use appropriate container to avoid environmental contamination.
  S61: avoid release in environment.

For further information, refer to the Brucella reagent material safety data sheet.

REAGENT PREPARATION, STORAGE AND STABILITY

BioScien *Brucella* reagent is ready-to-use and is stable until expiration date stated on label when properly stored in an upright position and refrigerated at 2-8°C (do not freeze).

Deterioration

The ***BioScien*** *Brucella* reagent can be damaged due to microbial contamination or on exposure to extreme temperatures. It is recommended that the performance of the reagent be verified with the positive controls.

SPECIMEN COLLECTION AND PRESERVATION [2]

Clean and dry glassware free from detergents must be used for sample collection, freshly collected serum is preferable. Specimen should be free of turbidity and hemolysis. Fresh, uncontaminated serum samples may be stored at 2-8°C in case of delay in testing up to 72 hours.

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Syringe
- Analytical tubes
- Centrifuge
- Stop watch
- Variable Micropipettes
- Physiological saline (as negative control)

ASSAY PROCEDURE

Allow the reagents and samples to reach room temperature. The sensitivity of the test may be reduced at low temperatures. Shake and mix antigens well before dispensing

Qualitative procedure

1. Identify each reaction circle of the slide test to make one positive control for each type, one negative control and the desired number of samples respectively.
2. Place one drop of positive control reagents, 80 µl of physiological saline and 80 µl of patient's serum to be tested onto each reaction circles.
3. Add one drop of Brucella antigen suspension to the reaction circles containing positive controls, physiological saline and patient's serum.
4. Mix contents of each circle uniformly over the entire circle with separate mixing sticks.
5. Rock the slide gently back and forth, and observe for agglutination macroscopically at one minute.

Semi-quantitative method

1. Dispense 80 µl, 40 µl, 20 µl, 10 µl, and 5 µl of patient serum to be tested onto 5 different reaction circles on the slide. The corresponding titers obtained will be 1:20, 1:40, 1:80, 1:160, and 1:320 respectively.
2. Continue with steps 3-5 of the qualitative procedure.

Note: This method is recommended for obtaining quick approximate titers only.

Tube test method

1. Take 8 Test tubes and label them 1 to 8.
2. Pipette 1.9 ml of isotonic saline to tube No.1
3. To each of the remaining tubes (2-7) add 1.0 ml of isotonic saline.
4. the tube No. 1 add 0.1 ml of serum sample to be tested. Mix well.

5. Transfer 1.0 ml of the 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 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.

READING AND INTERPRETATION

Qualitative procedure	
Positive	Agglutination as indication for the presence of Brucella antibody in the patient's serum.
Negative	No agglutination as indication for the absence of Brucella antibody in the patient's serum.
Semi-Quantitative procedure	
Titer	The titer of the patient serum corresponds to the visible agglutination in the test circle with the smallest amount of serum sample.
TUBE TEST procedure	
Positive	The titer of the patient serum is the reciprocal of the last dilution of the serum sample that gives a granular agglutination.
Negative	The appearance of the suspension remains unchanged, which shows a typical swirl when the tube is flicked.

QUALITY CONTROL

The positive controls may included with the test kit to monitor the performance of the reagent. Good physiological saline may be used as a negative control, if the expected results have not been observed, the reagent should not be used. For more information please contact ***BioScien*** technical support.

PERFORMANCE CHARACTERISTICS

Precision (reproducibility and repeatability): Precision of Brucella antigen suspensions is 100% (+/- one double dilution).

Analytical sensitivity: Accurate titer determination of the reference material, under the described assay conditions.

Prozone effect: No prozone effect was detected up to titers 1/160.

Diagnostic sensitivity: 70 %.

Diagnostic specificity: 70 %.

LIMITATIONS OF PROCEDURE

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 Brucella-A and Brucella-M antigen suspensions by rapid slide test and confirmed by the tube test to determine the type of Brucella antibody detected. The higher titer 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 titers are diagnostically significant or not.
4. Agglutinins are found in high proportion of normal individuals and titers less than 1:80 are of doubtful significance. Arising titer is more significant than a single high titer.
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 titers 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 titers 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 immediately recapped and replaced at 2-8 C.
14. 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.

REFERENCES

1. J. G. Collee, J. P. Duguid, A. G. Fraser, Practical Medical Microbiology, 13th Ed.: 525 – 530.
2. G. Galton, L. M. Jones, R. D.angus, J. M. Verger, Techniques for the brucellosis laboratory, ©INRA, Paris, 1988.

SYMBOLS IN PRODUCT LABELLING

IVD	For in-vitro diagnostic use	Σ	Number of <n> test in the pack
LOT	Batch Code/Lot number		Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		

EC REP

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