BRUCELLA (A+M)

METHOD – IMMUNO AGGLUTINATION
PRODUCT CODE – AB09

ANAMOL
THE ORIGINAL MAKERS

INSTRUCTIONS FOR USE

INTENDED USE: Test for qualitative determination of antibodies against Brucella pathogen in human sera.

PRINCIPLE

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 antigen suspensions.

The smooth, attenuated stained 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 melitensis (Brucella-M).

SAMPLE COLLECTION AND PRESERVATION

1. No special preparation of the patient is required prior to sample collection by approval techniques. Do not use haemolysed and turbid samples.
2. Clean and dry glassware free from detergents must be used for sample collection.
3. Do not heat inactive the serum.
4. Though freshly collected serum is preferable, store samples at 2-8°C in case of delay in testing, for up to 72 hours.

REAGENT PREPARATION AND STORAGE

The shelf life of reagents is as per the expiry date mentioned on the reagent vial labels. Do not use beyond expiry dated.

ADDITIONAL MATERIAL REQUIRED

Slide test method: Stop watch, Variable Micropipettes, Controls.

Quantitative method: Timer, Kahn tubes / test tubes, pipettes (0.1 ml, 1 ml), Physiological saline, incubator (37°C), Test tube rack.

REAGENT STABILITY

All reagents are stable up to the expiry date mentioned on the label when stored at 2-8°C. Do not freeze.

ASSAY PROCEDURE

A. SLIDE SCREEN METHOD

1. Place one drop of positive control onto a reaction circle of the glass slide.
2. Place 50 µl of physiological saline on to the next reaction circle of the glass slide.
3. Place one drop of patient's serum to be tested on to each of the required number of reaction circles.
4. Add one drop of appropriated brucella antigen suspension to the reaction circles containing positive control & physiological saline.
5. Add one drop of appropriate brucella antigen suspensions to the reaction circles containing the patient's serum.
6. Mix contents of each circle uniformly over the entire circle with separate mixing sticks.
7. Rock the slide gently back and forth, and observe for agglutination **macroscopically at one minute.**

B. SLIDE SEMI-QUANTITATIVE METHOD

1. Using a pipette add 80 µl, 40 µl, 20 µl, 10 µl and 5 µl of patient serum to be tested on 5 different reaction circles on the glass slide. The corresponding titres obtained will be 1:20, 1:40, 1:80, 1:160 & 1:320 respectively.
2. Add one drop of appropriate brucella antigen suspensions to each of the reaction circles containing the patient's serum.
3. Mix contents of each circle uniformly over the entire circle with separate mixing sticks.
4. Rock the slide gently back and forth, and observe for agglutination macroscopically at one minute.

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

C. QUANTITATIVE METHOD

Tube Test Procedure

1. Take appropriate number of sets (as required; one set for each antigen suspension) of 8 Kahn tubes / test tubes and label them 1 to 8.
2. Pipette in to tube no. 1 of all sets 1.9 ml of physiological saline.
3. To each of the remaining tubes (2 to 8) add 1 ml of physiological saline.
4. To tube no.1, of all sets add 0.1 ml of serum sample to be tested and mix well.
5. Transfer 1 ml of diluted serum sample from tube no. 1 to tube no. 2 and mix well.

6. Transfer 1 ml of the diluted serum sample from tube No.2 to tube no.3 and mix well. Continue this serial dilution till tube No.7 in each set.
7. Discard 1.0 ml of the diluted serum from tube no. 7 of each set.
8. Now the dilutions of the serum sample achieved from tube no. 1 to 7 respectively in each set is as follows: 1:20, 1:40, 1:80, 1:160, 1:320, 1:640, 1:1280 tube no. 8 in all sets, serves as a saline control.
9. To all tubes (1 to 8) of each set add one drop of the respective well mixed Brucella antigen suspensions from the reagent vials and mix well.
10. Cover and incubate at 37 ^{0}C overnight (approximately 18 hours).
11. Dislodge the sediment button gently and observe for agglutination

INTERPRETATION OF RESULTS:

Slide Screen Method:

Agglutination is a positive test result and indicates presence of the corresponding antibody in the patient's serum.
No agglutination is a negative test result and indicates absence of the corresponding antibody in the patient serum.

Quantitative method

The titre of the patient serum using Brucella antigen suspensions is the highest dilution of the serum sample that gives a visible agglutination.

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 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 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. Agglutinations are found in high proportion of normal individuals and titres less than 1:80 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 immediately recapped and replaced at 2-8 ^{0}C.
14. Reagent vials that have leakage/ breakage problem should be discarded.
15. 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 anti-sera should produce 1+ or greater agglutination at 1: 80 titre in the slide and tube test when tested with the Brucella antigen suspensions.
2. The negative control should show no agglutination with any of the Brucella antigen suspensions.
3. Generally accepted performance characteristic of this type of test is 70% specificity and sensitivity.
4. Reproducibility of Brucella antigen suspensions is 100% (+/- one double dilution).

BIBLIOGRAPHY

1. Cruickshank R., (1982), Medical Microbiology, 12th Edition, 403.
2. Felix. (1942), Brit, Med.J., 11, 597.

SYMBOLS: Read Instruction for use | IVD In Vitro Diagnostic Use Only | Manufactured by | Expiry Date | Storage Temperature

ANAMOL LABORATORIES PVT. LTD.
61, Genesis Industrial Township, Kolgaon,
Palghar – 401 404. India.
Customer Care: +91-9823388695.
admin@anamollabs.com
exports@anamollabs.com
www.anamollabs.com
ISO 9001 : 2015
ISO 13485 : 2003
GMP
CE