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BRUCEL® - A/M

SLIDE AND TUBE TEST FOR DETECTION OF ANTIBODIES TO *Brucella abortus/melitensis*

INTENDED USE

BRUCEL®-A/M is slide and tube test for the detection of specific antibodies to *Brucella abortus* and *Brucella melitensis* in human and animal serum.

SUMMARY

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 Tulip BRUCEL® antigen suspensions.

REAGENT

The BRUCEL®-A / BRUCEL®-M reagents contain ready to use standardized, attenuated, stained, smooth specific antigen suspensions of *Brucella* having specific reactivity towards antibodies to *Brucella abortus* (BRUCEL®-A), and *Brucella melitensis* (BRUCEL®-M). Each batch of reagents undergoes rigorous quality control at various stages of manufacture for its specificity and performance.

REAGENT STORAGE AND STABILITY

1. Store the reagent at 2-8^{0}C. DO NOT FREEZE. Keep the reagents away from direct sunlight.
2. The shelf life of the reagents is as per the expiry date mentioned on the reagent vial labels. Do not use beyond expiry date.
3. Once opened the shelf life of the reagent vial is as described on the reagent vial label provided it is not contaminated.

PRESENTATION

Σ	5 ml	5 ml
REF	105610005	105620005
Reagent	BRUCEL®A	BRUCEL® M
PACKAGE INSERT	1	1

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 x 75 mm), Test tube rack, appropriate Pipettes / Micropipettes, Isotonic saline / 0.25% phenol saline, Incubator(37^{0}C).

PRINCIPLE

The smooth, attenuated stained BRUCEL® antigen suspensions are mixed with the serum. Specific antibodies to *Brucella* antigens if present in the serum will react with the antigen suspension to produce an agglutination reaction. No agglutination indicates the absence of specific antibodies to *Brucella* antigens.

NOTE

1. In vitro diagnostic reagent 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. The 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 and negative controls.
4. Shake the reagent vials well before use to disperse the antigen suspension uniformly and improve test readability.
5. Only a clean and dry glass slides / tubes must be used. Clean the glass slides / tubes with distilled water and dry. It is necessary to use the calibrated dropper provided in the reagent vial to dispense a reagent drop.
6. BRUCEL®-A/M antigen suspensions are not from human sources hence contamination due to HBsAg and HIV is practically excluded.
7. Do not use damaged or leaking 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 inactivate the serum.
4. Though freshly collected serum is preferred, samples can be stored at 2-8^{0}C, for 24 hours, or frozen for 8 days should a delay in testing occur.
5. BRUCEL® antigen suspensions are not from human sources hence contamination due to HBsAg and HIV is practically excluded.

PROCEDURE

1. Bring all reagents to room temperature.
2. Shake and mix the BRUCEL® antigen suspensions well before dispensing.
3. The procedure for BRUCEL®-A and BRUCEL®-M is identical.

SLIDE TEST METHOD

Qualitative method

1. Place one drop of positive control (available as BRUCELLOSIS POSITIVE CONTROL, REF 110200005 and 110200001) onto the reaction circle of glass slide.
2. Place ~ 80 µl of saline onto the next reaction circle of the glass slide.
3. Place ~ 80 µl of the serum to be tested onto the next reaction circle.
4. Add one drop of the appropriate BRUCEL® antigen suspensions in each of the above circles (containing positive control, saline, and the patient serum to be tested).
5. Mix contents of each circle uniformly over the entire circle with separate mixing sticks.
6. Gently rock the slide back and forth, observe for agglutination macroscopically **at one minute** against a white background.

Semi-quantitative method

1. Using a pipette place 80 µl, 40 µl, 20 µl, 10 µl and 5 µl of the serum to be tested on 5 different circles on the glass slide. The corresponding titres obtained will be 1:20, 1:40, 1:80, 1:160, and 1:320 respectively.
2. Place one drop of appropriate BRUCEL® antigen suspensions to each circle.
3. Mix contents of each circle uniformly over the entire circle with separate mixing sticks.
4. Gently rock the slide back and forth, observe for agglutination macroscopically **at one minute** against a white background.

TUBE TEST METHOD

1. Take 8 Test tubes and label them 1 to 8.
2. Pipette 1.9 ml of isotonic saline or preferably 0.25% phenol saline to tube No.1.
3. To each of the remaining tubes (2-7) add 1.0 ml of isotonic saline or preferably 0.25% phenol saline.
4. To 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 BRUCEL® 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 RESULTS

SLIDE TEST METHOD

Qualitative method

Agglutination is a positive test result and indicates the presence of specific antibodies to *Brucella* in the serum.
No agglutination is a negative test result and indicates absence of specific antibodies to *Brucella* in the serum.

Semi-Quantitative method

Agglutination is a positive test result. The titre of the serum corresponds to the visible agglutination in the test circle with the minimum amount of serum sample.

TUBE TEST METHOD

The titre of the serum is the reciprocal of the last dilution of the serum sample that gives a granular agglutination.
In negative reaction, the appearance of the suspension remains unchanged, which shows a typical swirl when the tube is flicked.

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 BRUCEL®-A and BRUCEL®-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 semiquantitative 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 1:80 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°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: 80 titre in the slide and tube test when tested with the BRUCEL®-A/M antigen suspensions.

2. The negative control should show no agglutination with any of the BRUCEL®-A/M antigen suspensions.
3. Generally accepted performance characteristic of this type of test is 70% specificity and sensitivity.
4. Reproducibility of BRUCEL®-A/M antigen suspensions is 100% (+/- one double dilution).

WARRANTY

This product is designed to perform as described on the label and the package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

(1). J. G. Collee, J. P. Duguid, A. G. Fraser, Practical Medical Microbiology, 14th Ed.: 473 – 478. (2). G. Galton, L. M. Jones, R. D. Angus, J. M. Verger, Techniques for the brucellosis laboratory, © INRA, Paris, 1988.(3). Data on file: Tulip Diagnostics (P) Ltd.

SYMBOL KEYS

Temperature limitation	Manufacturer	Contains sufficient for <n> tests
Use by	Consult Instructions for use	This way up
Date of Manufacture	REF Catalogue Number	PS Production Site
LOT Batch Number/ Lot Number	IVD *In vitro* Diagnostic Medical Device	EC REP Authorised Representative in the European Community

Manufactured by

TULIP DIAGNOSTICS (P) LTD.

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B-A/M/0917/VER-04