



GENESIS

Instructions For Use

Brucella A&M (Agglutination Method)

Product	Cat no.	TEST
Brucella (With Positive Control)	5102 101	100 TEST
Brucella (A)	5102 201	100 TEST
Brucella (M)	5102 301	100 TEST

INTENDED USE

Brucella A & M Reagent (Slide and tube Agglutination Test) is intended for detection of antibodies to *Brucella abortus*/ *melitensis*

SUMMARY

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 BRUCELLA antigen suspensions.

REAGENT

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):-

	Reagents:-
1	Brucella (A)
2	Brucella (M)
3	Positive Control

REAGENT STORAGE AND STABILITY

1. Store the reagent at 2-8°C. DO NOT FREEZE.
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.

ADDITIONAL MATERIAL REQUIRED

Slide test method:

Stop watch, Positive control, physiological 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, physiological saline / 0.25% phenol saline, Incubator(37°C).

PRINCIPLE

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.

SAMPLE COLLECTION AND STORAGE

1. No special preparation of patient is required prior to sample collection by approved techniques.
2. Do not use hemolysed and turbid serum samples.
3. Clean and dry glassware free from detergents must be used for sample collection.
4. Do not heat (inactivate the serum).
5. 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.

PROCEDURE

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

Symbols in Product Labeling			
	Authorized Representative		Expiration date
	For in-vitro diagnostic use		CAUTION, consult instructions for use
	Catalogue number		Manufactured by
	Lot number		Temperature Limit
	Consult instructions for use		

SLIDE TEST METHOD

Qualitative method

1. Place one drop of positive control onto a reaction circle of the slide.
2. Place 40 µl of physiological saline onto the next reaction circle of the slide.
3. Place one drop of patient's serum to be tested onto each of the required number of reaction circles.
4. Add one drop of appropriate 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**.

Semi-quantitative method

1. Using a pipette place 80 µl, 40 µl, 20 µl, 10 µl and 5 µl of patient 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 BRUCELLA 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 physiological 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 physiological 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 physiological 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.

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 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 test is 70% specificity and sensitivity.
4. Reproducibility of BRUCELLA-A/M antigen suspensions is 100% (+/- one double dilution)

BIBLIOGRAPHY

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.

