Rose Bengal
Slide Agglutination

PRODUCT CODE
SF015

CLINICAL SIGNIFICANCE
Brucella diagnostic may be assessed either by microorganism isolation in blood or stools, or by titration of specific antibodies in the patient serum. The reagent, because of its formulation in an acid buffer, is reactive with both IgG and IgM antibodies and very useful for the diagnosis of chronic individuals, which present a high level of IgG antibody, difficult to be detected by the reference tube method (Wright).

PRINCIPLE
The Rose Bengal is a slide agglutination test for the qualitative and semi-quantitative detection of antibodies anti-Brucella in human serum. The stained bacterial suspension agglutinates when mixed with samples containing specific IgG or IgM antibodies present in the patient sample.

REAGENTS

Rose Bengal	*Brucella abortus* suspension, strain S99, in lactate buffer 1 mol/L, phenol 5 g/L, Rose Bengal, pH 3.6
Control + Red cap	Animal serum, with an antibody anti- *Br.abortus* concentration ≥ 50 IU/mL. Preservative
Control – Green cap	Animal serum. Preservative

ACCESSORIES
Reaction slide, Mixing pipettes
ADDITIONAL REQUIREMENTS
- Mechanical rotator with adjustable speed at 80-100 r.p.m.
- Vortex mixer.
- Pippetes 50 µL.

STORAGE AND STABILITY
All reagents are ready to use, and will remain stable until the expiration date printed on the label, when stored tightly closed at 2-8ºC and contaminations are prevented during their use. Do not freeze: frozen reagents could change the functionality of the test.
Always keep vials in vertical position. If the position is changed, gently mix to dissolve aggregates that may be present.
Reagents deterioration: Presence of particles
CALIBRATION
The Rose Bengal sensitivity is calibrated against the 2º International Preparation of anti-*Brucella abortus* from NIBS (UK) (WHO).

SPECIMEN AND SAMPLE PREPARATION
Fresh serum. Stable 7 days at 2-8ºC or 3 months at –20ºC.
Samples with presence of fibrin should be centrifuged before use.
Do not use highly hemolyzed or lipemic samples.

PROCEDURES
Qualitative Method
1- Allow the reagents and samples to reach room temperature. The sensitivity of the test may be reduced at low temperatures
2- Place 50 µL of the sample and one drop of each Positive and Negative controls into separate circles on the slide test.
3- Mix the R. Bengal reagent vigorously or on a vortex mixer before using and add one drop next to the sample to be tested.
4- Mix the drops with a stirrer, spreading them over the entire surface of the circle. Use different stirrers for each sample.
5- Place the slide on a mechanical rotator at 80-100 r.p.m. for 4 minutes. False positive results could appear if the test is read later than two minutes

Semi-Quantitative Method
1- Make serial two-fold dilutions of the sample in 9 g/L saline solution.
2- Proceed for each dilution as in the qualitative method.

INTERPRETATION OF RESULTS
Examine macroscopically the presence or absence of visible agglutination immediately after removing the slide from the rotator.
The presence of agglutination indicates an antibody anti-Brucella concentration equal or greater than 25 IU/mL.
The titer, in the semi-quantitative method, is defined as the highest dilution showing a positive result.

CALCULATIONS
The approximate antibody concentration in the patient sample is calculated as follows:

25 x anti-Brucella Titre = IU/mL

QUALITY CONTROL
Positive and Negative controls are recommended to monitor the performance of the procedure, as well as a comparative pattern for a better result interpretation.
All result different from the negative control result, will be considered as a positive.

REFERENCE VALUES
Up to 25 IU/mL.
Each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS
1. **Analytical sensitivity:** 25 (±5) IU/mL, under the described assay conditions
2. **Prozone effect:** No prozone effect was detected up to 1000 IU/mL.
3. **Diagnostic sensitivity:** 100 %.
4. **Diagnostic specificity:** 98 %.

INTERFERENCES
Haemoglobin (10 g/L), lipemia (10 g/L) and rheumatoid factors (300 IU/mL), do not interfere. Bilirubin interferes at 2.5 mg/dL. Other substances may interfere[5].

NOTES
Clinical diagnosis should not be made on findings of a signal test result, but should integrate both clinical and laboratory data.

LIMITATIONS OF THE PROCEDURE
1- False positive results may be obtained in conditions such as, rheumatoid arthritis, scarlet fever, tonsillitis, several streptococcal infections and healthy carriers.
2- Early infections and children from 6 months to 2 years may cause false negative results.
3- Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

SYMBOL ON LABELS

Symbols	Signify	Symbols	Signify
REF	Catalogue Number	SIZE	Pack Size
	Expiry Date	VOL	Volume
	Storage Condition	LOT	Lot Number
	Instruction for Use	IVD	In Vitro Diagnostics
	Manufacturing Date		Manufacturer
	Number of Tests	2	For Single Use Only
EC REP	EC Representative	CE	European conformity

REFERENCES

1.Young E J. Clinical Infectious Diseases 1995; 21: 283-290.
2. Alton GC. Techniques for Brucellosis Laboratory INRA Paris, 1988.
3. Ariza J. Current Opinion in Infectious Diseases 1996; 9: 126-131.
4. Comité mixto FAO/OMS de expertos en Brucelosis. WLD Health Org Tech Rep Ser 1958; 148: 1-60.
5. Young DS. Effects of drugs on clinical laboratory test, 4th ed. AACC Press, 1995

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