Reactivos GPL
Barcelona, España

CE

- ROSE BENGAL-

ROSE BENGAL
Agglutination test

Store at: +2+8ºC.

Presentation:
Cod. SE024 100 Test.

Procedure

Diagnostic reagent for qualitative measurement of Brucella antibodies.

Only for *in vitro use in clinical laboratory* (IVD)

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

REAGENTS COMPOSITION

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

PRECAUTIONS
Phenol: Toxic (T). R24/25: Toxic in contact with skin and if swallowed. R34: Causes burns. S28.2: After contact with the skin, wash immediately with plenty of water. S45: In case of accident, seek medical advice immediately.

Good laboratory safety practices should be followed when handling laboratory reagents or human samples.

REAGENT PREPARATION 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. If appear particles and turbidity do not use.
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.

All the reagents of the kit are stable up to the end of the indicated month and year of expiry. Store tightly closed at 2-8ºC. Do not use reagents over the expiration date.

CALIBRATION
The Rose Bengal sensitivity is calibrated against the 2º International Preparation of anti-Brucella abortus from NIBS (UK)(WHO).

SPECIMEN
Fresh serum. Stable 7 days at 2-8ºC or 3 months at –20ºC.
The samples with particles or fibrin should be centrifuged to eliminate them. Do not use haemolized or lipemic samples.

Discard contaminated specimen.

MATERIAL REQUIRED BUT NOT PROVIDED
- Mechanical rotator with adjustable speed at 80-100 r.p.m.
- Vortex mixer.
- Pippetes 50 µL

General laboratory equipment

TEST PROCEDURE
Qualitative method
1. Allow the reagents and sample 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 control 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 both drops with a stirrer, spreading them over the entire surface of the circle. Use different stirrers for each sample.
5. Rotate the slide with a mechanical rotator at 80-100 r.p.m. for 4 minutes. False positive results could appear if the test is read later than four minutes.

Semi-quantitative method
1. Make two fold dilutions of the sample in 9 gr/L Saline Solution.
2. Test each dilution as described in the qualitative method.

READING AND INTERPRETATION
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 Titer = 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.

Serum controls Brucella are recommended for internal quality control. Each laboratory should establish its own Quality Control scheme and corrective actions.

REFERENCE VALUES
Up to 25 IU/mL.

It is suggested that each laboratory establish its own reference range.

CLINICAL SIGNIFICANCE
Brucella diagnostic may be assessed either by micro organism 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).

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENT PERFORMANCE
- *Analytical sensitivity:* 25 (± 5) IU/mL, under the described assay conditions
- *Prozone effect:* No prozone effect was detected up to 1000 IU/mL.
- *Diagnostic sensitivity*: 100 %
- *Diagnostic specificity*: 198 %

INTERFERING SUBSTANCES
Interferences:
- Hemoglobin (10 g/L), lipemia (10 g/L), rheumatoid factors (300 IU/mL) do not interfere. Bilirubin interferes at 2.5 mg/dL.
- Other substances may interfere[5].

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

BIBLIOGRAPHY

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