LORNE LABORATORIES LTD.

GREAT BRITAIN

RAPID LATEX KIT

DIRECTIONS FOR USE

ROSE BENGAL: For Detection Of Anti-Brucella antibodies.

SUMMARY

The Rose Bengal test is a slide agglutination test for the qualitative and semi-quantitative detection of anti-Brucella antibodies in human 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 that is difficult to detect by the reference tube method (Wright).

INTENDED PURPOSE

The reagent is a test reagent intended to be used to qualitatively and semi-quantitatively determine the presence or absence of Anti-Brucella antibodies in the serum or plasma of patients when tested in accordance with the recommended techniques stated in this IFU.

PRINCIPLE

When used by recommended techniques, a suspension of Brucella abortus in the reagent will agglutinate (clump) in presence of anti-Brucella antibodies. No agglutination (no clumping) generally indicates absence of anti-Brucella antibodies (see **Limitations**).

KIT DESCRIPTION

Lorne Rose Bengal Kit is for the detection of anti-Brucella antibodies. The reagent is a suspension of Brucella abortus strain S99, that agglutinates in the presence of anti-Brucella antibodies. The reagents do not contain or consist of CMR substances, or endocrine disrupting substances or that could result in sensitisation or an allergic reaction by the user. All reagents are supplied at optimal dilution for use with all recommended techniques without the need for further dilution or addition. For lot reference number and expiry date see **Vial Labels**.

STORAGE

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 protected from light and contaminations are prevented during their use. Do not freeze: frozen reagents could change the functionality of the test. Store the vials in a vertical position. If the position is changed, gently mix to dissolve aggregates that may be present.
Reagents deterioration: Presence of particles.

SPECIMEN COLLECTION

Specimens should be drawn with or without anticoagulant using an aseptic phlebotomy technique. If testing is delayed specimens can be stored at 2-8ºC for 7 days or for up to 3 months at or below –20ºC. Specimens must be free from bacterial contamination, fibrin, gross lipaemia and gross haemolysis.

PRECAUTIONS

1. The kit is for *in vitro* diagnostic use only.
2. Do not use kit past expiration date (see **Vial and Box Labels**).
3. Protective clothing should be worn when handling the reagents, such as disposable gloves and a laboratory coat.
4. The Rose Bengal reagent contains 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.
5. The reagents in this kit have been processed to reduce the bio-burden, but are not supplied sterile. Once a vial has been opened the contents should remain viable up until the expiry date.
6. Materials used to produce the kit were tested at source and found to be negative for HIV 1+2 and HBsAg using approved microbiological tests. However, no known tests can guarantee that products derived from human or animal sources are free from infectious agents. Care must be taken in the use and disposal of each vial and its contents.

DISPOSAL OF KIT REAGENT AND DEALING WITH SPILLAGES

For information on disposal of kit reagent and decontamination of a spillage site see **Material Safety Data Sheets**, available on request.

CONTROLS AND ADVICE

1. It is recommended that the Brucella Positive and Negative Controls are tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show expected results.
2. All the reagents must be allowed to reach 18-25ºC before use.
3. Shake the reagents well before use to ensure homogeneity.
4. Do not interchange components between different kits.
5. Use of kit and interpretation of results must be carried out by properly trained and qualified personnel in accordance with the requirements of country where kit is in use. The user must determine the suitability of the kit for use in other techniques.

KIT COMPONENTS SUPPLIED

1) Rose Bengal Reagent (White cap, 2.5 mL): Brucella abortus suspension, strain S99, in lactate buffer 1 mol/L, phenol 5 g/L, Rose Bengal, pH 3.6.
2) Positive Control (Red cap, 1 mL): Animal serum with an anti-Brucella antibody concentration ≥ 50 IU/mL and a preservative.
3) Negative Control (Blue cap, 1 mL): Animal serum and a preservative.
4) Pipette-Stirrers.
5) Reusable Agglutination Slide.

MATERIALS AND EQUIPMENT REQUIRED BUT NOT SUPPLIED

- Mechanical rotator with adjustable speed of 80-100 rpm.
- Pasteur and Graduated Pipettes.
- Vortex mixer.

RECOMMENDED QUALITATIVE TECHNIQUE

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 each of the Positive and Negative controls into separate circles on the slide test.
3. Mix the Rose 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 after 4 minutes.

INTERPRETATION OF QUALITATIVE RESULTS

1. **Positive:** Visible agglutination constitutes a positive result and within the accepted limitations of the test procedure, indicates a level of anti-Brucella antibodies in the specimen ≥ 25 IU/ml.
2. **Negative:** No visible agglutination in a milky liquid constitutes a negative result and within accepted limitations of test procedure, indicates a level of anti-Brucella antibodies in the specimen < 25 IU/ml.

RECOMMENDED SEMI-QUANTITATIVE TECHNIQUE

1. The semi-quantitative test can be performed in the same way as the qualitative test using dilutions of the serum.
2. Make doubling dilutions of serum specimen in 9 g/L saline as follows:

Dilution	Serum	Saline
1/2	100 µl undiluted serum	100 µl
1/4	100 µl 1/2 diluted serum	100 µl
1/8	100 µl 1/4 diluted serum	100 µl
1/16	100 µl 1/8 diluted serum	100 µl

3. Test the specimen dilutions in the same way as for the quantitative technique above.
4. Agglutination of the sera indicates:

Dilution	Brucella antibodies (I.U/ml)
1/2	50 (25 x 2)
1/4	100 (25 x 4)
1/8	200 (25 x 8)
1/16	400 (25 x 16)

5. Normal levels of anti-Brucella antibodies in adults is <25 IU/ml.

RESULTS

The titre is expressed as the reciprocal of the highest dilution showing macroscopic agglutination: e.g. if this occurs in dilution 1/8, the titre is (8 * 25 IU/mL) = 200 IU/mL.

STABILITY OF THE REACTIONS

Slide tests should be interpreted immediately after the 4-minute rotation period to avoid the possibility that a negative result may be incorrectly interpreted as positive due to drying of the reagent.

LIMITATIONS

1. Diagnosis should not be solely based on the results of the Rose Bengal method but also should be complemented with a clinical examination.
2. Hemoglobin (≤ 10 g/L), Rheumatoid factors (≤ 300 IU/mL) and lipemia (≤ 10 g/L), do not interfere. Bilirubin (≥ 2.5 mg/dL) interferes. Other substances may interfere[4].
3. False positive or false negative results may also occur due to:
   a) Contamination of test materials
   b) Improper storage of test materials or omission of reagents
   c) Deviation from the recommended techniques

SPECIFIC PERFORMANCE CHARACTERISTICS

1. The kit has been characterised by all the procedures mentioned in the **Recommended Techniques**.
2. The Rose Bengal sensitivity is calibrated against the 2nd International WHO Preparation of anti-Brucella abortus from NIBSC (UK).
3. Analytical sensitivity: 25 (±5) IU/mL, under the described assay conditions.
4. Prozone effect: No prozone effect was detected up to 1000 IU/mL.
5. Diagnostic sensitivity: 100 %.
6. Diagnostic specificity: 98 %.

DISCLAIMER

1. The user is responsible for the performance of the kit by any method other than those mentioned in the **Recommended Techniques**.
2. Any deviations should be validated prior to using established laboratory procedures.

BIBLIOGRAPHY

1. David S.Jacobs et al. Laboratory Test Handbook, 3rd edition, Lexi-Comp Inc, 1994.

AVAILABLE KIT SIZES

Kit Size	Catalogue Number
50 Tests Per Kit	155050A

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