**SUMMARY**

Human Brucellosis (J unemployment) is a common febrile illness caused by infection with bacteria of some of the Brucella species (abortus/melitensis). This unemployment is associated with symptoms, which are often variable and non-specific with chills, fever, sweats, and anorexia. 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-RB antigen suspension.

**REAGENT**

The BRUCEL-RB reagent contains smooth, killed buffered suspensions of Brucella abortus strain 99, coloured with rose bengal, standardized against the 2nd International preparation of anti-Brucella abortus from NIBS (UK) (WHO).

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°C. **DO NOT FREEZE**. Frozen reagents could change the functionality of the test.
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 vials is as described on the reagent vial provided it is not contaminated.

**ADDITIONAL MATERIAL REQUIRED**

- Stop watch
- Positive control
- Isotonic saline
- Glass slide with clear / white background
- Appropriate Pipettes / Micropipettes
- Mixing sticks
- A High intensity direct light source.

**PRINCIPLE**

The smooth, coloured, killed BRUCEL-RB antigen suspension is mixed with the patient serum. Specific antibodies to Brucella antigens if present in concentration ≥ 25IU/mL in the patient serum will react with the antigen suspension to produce an agglutination reaction. No agglutination indicates the absence of detectable levels of specific antibodies to Brucella.

**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 IgM and IgG antibodies and very useful for the diagnosis of chronic individuals, which present a high level of IgG antibody.

In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.

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.

1. **NOTE**
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. **PROCEDURE**
4. **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 50 µl of saline onto the next reaction circle of the glass slide.
3. Place 50 µl of patient serum to be tested onto the next reaction circle.
4. Add one drop of well mixed BRUCEL-RB antigen suspension in each of the above circles containing positive control, isotonic saline and 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 four minutes against a white background.

**Semi-quantitative method**

1. Place one drop of BRUCEL-RB antigen suspension to each circle.
2. Mix contents of each circle uniformly over the entire circle with separate mixing sticks.
3. Gently rock the slide back and forth, observe for agglutination macroscopically at four minutes against a white background.

**INTERPRETATION OF RESULTS**

Agglutination is a positive test result and indicates the presence of antibodies to Brucella in concentration ≥ 25IU/mL in the patient serum.
No agglutination is a negative test result and indicates absence of antibodies to Brucella in concentration ≤ 25 IU/mL in the patient serum.

**Semi-quantitative method**

Agglutination is a positive test result. The titre in 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.

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25 \times \text{anti-Brucella titer} = \text{IU/mL}
\]

**PERFORMANCE CHARACTERISTICS**

**Analytical sensitivity:** 25 IU/mL, under described assay conditions.

**Specificity:** 100%

**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.
2. Agglutinins are found in a high proportion of normal individuals and concentration less than 25 IU/mL are of doubtful significance. A rising titre is more significant than a single high titre.
3. False positive reactions may occur in sera of patients infected with Pasteurella tularensis or vaccinated with vibrio cholerae.
4. Cross-reactions between Brucella antigens and other organisms have been reported. These include Yersinia enterocolitica, Escherichia coli (0:157) and Francisella tularensis.
5. False positive results are likely if the tests is read more than four minutes after mixing on the slide test.
6. Freezing or freezing may sometimes be encountered in serum containing very high titres on slide test.
7. 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.
8. Since techniques and standardization vary from laboratory to laboratory on tube difference in titres can be expected.
9. Use a separate disposable pipette for each sample to prevent cross contamination.
10. Turbid and contaminated sera should not be used for testing.
11. After usage the antigen suspension should be immediately recap and replaced at 2-8°C.
12. Reagent vials that have leakage or breakage problem should be discarded.
13. Only qualified and well trained staff should use the reagents.
14. It is recommended that results of the tests should be correlated with clinical findings to arrive at the final diagnosis.
15. The performance of the test should be validated periodically using known positive control. Good physiological saline may be used as a negative control.

**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**

3. Data on file: Tulip Diagnostics (P) Ltd.