

MICROPATH® ROSE BENGAL Ref OD265

Stained bacterial antigen test for the detection of Antibodies to Brucella.

Store at 2°C to 8°C. DO NOT FREEZE.

For in-vitro diagnostic use only.

INTRODUCTION AND INTENDED USE

MICROPATH ROSE BENGAL test is a rapid slide test kit for the qualitative and semi quantitative determination of antibodies to Brucella in human serum. The reagent is a stained, killed and standardised suspensions of Brucella abortus bacteria supplied in a convenient dropper bottle for ease of use.

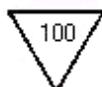
For professional use only.

PRINCIPLE OF THE TEST

The test depends on the ability of antibody in the patient's serum to agglutinate the stained bacterial antigen. When this occurs the aggregates become clearly visible to the naked eye. Calibrated against the NIBSC 2nd International Brucella abortus preparation.

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MICROPATH Rose Bengal	Ag
Brucella abortus bacteria stained, killed and suspended in Lactate Buffer pH 3.6 Working Strength.	
Control	+
Serum containing antibodies to Brucella abortus. Working Strength.	

0.5ml

INSTRUCTION LEAFLET 1

MATERIAL REQUIRED BUT NOT PROVIDED

Micropipettes
Test slide
Stirrers
Physiological Saline (0.9%)

PRECAUTIONS

MICROPATH ROSE BENGAL antigen contains 0.5% phenol which is Toxic in contact with skin and if swallowed. Caution causes burns. In the event of contact with skin wash immediately with plenty of water and seek medical advice. All reagents should be treated as potential biohazards in use and disposal. Final disposal must be in accordance with local legislation. Do not ingest.

MICROPATH ROSE BENGAL controls contain 0.095% sodium azide as a preservative which may be toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive salts. On disposal, flush with large quantities of water.

STORAGE

Reagents must be stored at temperatures between 2°C to 8°C.

The kit will perform within specification until the stated expiry date as determined from date of product manufacture and stated on kit and components. Expiry date is the last day of the month on the bottle and the kit label. Do not use reagents after the expiry date.

Exposure of reagents to excessive temperatures should be avoided. Do not expose to direct sunlight.

DO NOT FREEZE ANY OF THE REAGENTS as this will cause irreversible damage.

SPECIMEN COLLECTION AND PREPARATION

Obtain a sample of venous blood from the patient and allow a clot to form and retract. Centrifuge clotted blood sample and collect clear serum. Fresh serum samples are required.

Do not use haemolysed, contaminated or lipaemic serum for testing as this will adversely affect the results.

Serum may be stored at 2°C to 8°C for up to 48 hours prior to testing. If longer storage is required, store at -20°C for up to 6 weeks. Thawed samples must be mixed prior to testing.

Do not repeatedly freeze-thaw the specimens as this will cause false results.

REAGENT PREPARATION

All reagents should be brought to room temperature (20°C to 25°C) and mixed gently prior to use. Do not induce foaming.

The test slide should be thoroughly cleaned before use as traces of detergent or prior specimen may affect the result.

Recommended Cleaning procedure:

1. Used cards must be immediately immersed in a disinfectant solution. Follow disinfectant manufactures guidelines.
2. The reaction circles must be physically rubbed with non-abrasive material to ensure removal of possible adhering particles.
3. Thoroughly rinse in purified water.
4. Allow reaction card to dry.
5. Spray cards with a 70% alcohol solution.

Allow the alcohol to evaporate prior to re-use.

LIMITATIONS OF USE

The use of samples other than serum has not been validated in this test. There is no reuse protocol for this product.

A low or suspected positive result should be re-assessed. Diagnosis should not be made solely on the findings of one clinical assay. When making an interpretation of the test it is strongly advised to take all clinical data into consideration.

Cross reactions between Brucella antigens and other organisms have been reported. These include *Yersinia enterocolitica*, *Escherichia coli* and *Francisella tularensis*.

A prozone may occasionally occur. If this is suspected, dilute the serum 1/20 in saline and retest. Always include a known positive and negative serum in the test panel as part of the normal laboratory quality control procedure.

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 the MICROPATH Brucella abortus and the MICROPATH Brucella melitensis suspensions by rapid slide test and confirmed by tube agglutination test to determine the type of Brucella antibodies detected. The higher titre detected determines the specific type of Brucella antibodies present.

ASSAY PROCEDURE

Qualitative Method

1. Allow kit reagents and patient serum to come to room temperature.
2. Transfer one drop (50µl) of patient's serum to the test circle on the slide.
3. Shake the reagent, then using the dropper provided, add one drop of suspension to the test circle.
4. Mix the drops ensuring coverage of the test circle with the mixture.
5. Gently and evenly, rock and rotate the test slide for 4 minutes by hand, or on a rotator at 80 to 100 rotations per minute, whilst examining the test slide for agglutination.

Semi Quantitative Method

1. Using isotonic saline prepare serial dilutions of the patients serum (1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and so on)
2. Transfer one drop (50µl) of each serum dilution to the test circle on the slide.
3. Shake the reagent, then using the dropper provided, add one drop of suspension to the test circle.
4. Mix the drops ensuring coverage of the test circle with the mixture.
5. Gently and evenly, rock and rotate the test slide for 4 minutes by hand, or on a rotator at 80 to 100 rotations per minute, whilst examining the test slide for agglutination.

RESULTS AND INTERPRETATION

Examine the test slide macroscopically under a strong light source after 4 minutes. Kit controls or known level value samples should be tested with each test run. The kit negative control should give a negative result after 4 minutes. The kit positive control should give a positive result at a titre of 1/4 +/- one double dilution after 4 minutes. If levels of controls or users known samples do not give expected results, test results must be considered invalid.

Qualitative Method

A positive result is indicated by the obvious agglutination pattern of the latex, in a clear solution. A negative result is indicated by no change in the latex suspension on the test slide. Agglutination of the antigen with undiluted sample indicates the presence of antibody at a concentration of greater than or equal to 25 IU/ml. A comparison between samples taken 10-14 days apart may be of value in acute illness.

MICROPATH ROSE BENGAL has a detection limit of 25 IU/ml of Brucella antibody in the patient's serum. Positive results will be obtained at a serum concentration of 25 IU/ml and above and negative results will be obtained at below 25 IU/ml.

Semi-Quantitative Method

The serum concentration can then be calculated approximately by multiplying the dilution factor (i.e 2, 4, 8 or 16) by the detection limit, i.e. 25, to give the IU/ml concentration e.g. if the agglutination titre appears at 1/8 the approximate serum Brucella antibody concentration is $8 \times 25 = 200$ IU/ml.

Titres of 1000 IU/ml have been detected with MICROPATH ROSE BENGAL with no prozone (hook) effect.

TROUBLESHOOTING

Use a separate disposable tip for each sample to prevent cross contamination.

Replace caps on all reagents immediately after use.

Prior to the start of the assay bring all reagents to room temperature (20°C to 25°C). Gently mix all reagents by gentle inversion or swirling.

For use by operatives with at least a minimum of basic laboratory training.

Do not use damaged or contaminated kit components.

EVALUATION DATA

Reproducibility of the Micropath Range of Reagents is 100% (+/- one double dilution).

Sensitivity 93 %

Specificity 100 %

REFERENCES

1. **Cowan, S. T. and Steel, K. J.** (1965). Manual for the identification of Medical Bacteria, Cambridge University Press.
2. **Lenette, E. H.** (1985). Manual of Clinical Microbiology (4th Edition). American Society for Microbiology Washington DC.

8065 Issue 1 Revised March 2006

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