MICROPATH ® ANTIGENS/FEBRILE ANTIGEN KITS

Stained bacterial antigens for Widal, Brucella and Weil-Felix tests.
Store at 2°C to 8°C. DO NOT FREEZE.
For in-vitro diagnostic use only.

INTRODUCTION AND INTENDED USE

These products are intended to be used as an in-vitro diagnostic aid in the detection of antibodies to various bacterial pathogens by the slide or tube agglutination method. The reagents are stained, killed and standardised suspensions of pathogenic bacteria supplied in convenient dropper bottles 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 antigens. When this occurs the aggregates become clearly visible to the naked eye.

ANTIGENS AVAILABLE

MICROPATH OA | **Ag** — Ref OD055
Salmonella O – Group A (Paratyphi A-O)
Suspension of bacteria stained and killed.
Working Strength.

MICROPATH OB | **Ag** — Ref OD065
Salmonella O – Group B (Paratyphi B-O)
Suspension of bacteria stained and killed.
Working Strength.

MICROPATH OC | **Ag** — Ref OD075
Salmonella O – Group C (Paratyphi C-O)
Suspension of bacteria stained and killed.
Working Strength.

MICROPATH OD | **Ag** — Ref OD085
Salmonella O – Group D (Typhi O)
Suspension of bacteria stained and killed. Working Strength.

MICROPATH OX2 | **Ag** — Ref OD115
Proteus OX2
Suspension of bacteria stained and killed.
Working Strength.

MICROPATH OX19 | **Ag** — Ref OD125
Proteus OX19
Suspension of bacteria stained and killed. Working Strength.

MICROPATH OXK | **Ag** — Ref OD135
Proteus OXK
Suspension of bacteria stained and killed.
Working Strength.

MICROPATH Ha | **Ag** — Ref OD015
Salmonella H – Group a (Paratyphi A-H)
Suspension of bacteria stained and killed. Working Strength.

MICROPATH Hb | **Ag** — Ref OD025
Salmonella H – Group b (Paratyphi B-H)
Suspension of bacteria stained and killed.
Working Strength.

MICROPATH Hc | **Ag** — Ref OD035
Salmonella H – Group c (Paratyphi C-H)
Suspension of bacteria stained and killed.
Working Strength.

MICROPATH Hd | **Ag** — Ref OD045
Salmonella H – Group d (Typhi H)
Suspension of bacteria stained and killed.
Working Strength.

MICROPATH BRUCELLA ABORTUS | **Ag** — Ref OD095
Brucella abortus
Suspension of bacteria stained and killed. Working Strength.

MICROPATH BRUCELLA MELITENSIS | **Ag** — Ref OD105
Brucella melitensis
Suspension of bacteria stained and killed.
Working Strength.

MICROPATH FEBRILE ANTIGEN KITS AVAILABLE

X 4 Ref OD225	**X 6** Ref OD165	**X 8** Ref OD155	**X 10** Ref OD145
4 Antigens	6 Antigens	8 Antigens	10 Antigens
OA OB OD Hd	OA OB OD Ha Hb Hd	OA OB OC OD Ha Hb Hc Hd	OA OB OC OD Ha Hb Hc Hd B. mel B. ab
1 Card	1 Card	1 Card	1 Card
1 Positive Control	1 Positive Control	1 Positive Control	1 Positive Control
1 Negative Control	1 Negative Control	1 Negative Control	1 Negative Control

Controls:

Control | **+** — 0.5 ml
Serum containing antibodies to the Micropath Range.
Working Strength.
ONLY AVAILABLE IN KIT FORM
ONLY SUITABLE FOR RAPID SLIDE TEST

Control | **-** — 0.5 ml
Serum free of antibodies to the Micropath and Rose Bengal Ranges. Working Strength.
ONLY AVAILABLE IN KIT FORM

MATERIAL REQUIRED BUT NOT PROVIDED

Micropipettes
Small test tubes 75 x 12mm
Physiological Saline (0.9%)
Incubator or water bath
Omega Febrile test slides only, not supplied in individual kits.

PRECAUTIONS

MICROPATH Reagents do not contain dangerous substances as defined by current UK Chemicals (Hazardous Information and Packaging for Supply) regulations. All reagents should, however, be treated as potential biohazards in use and disposal. Final disposal must be in accordance with local legislation. Do not ingest.

MICROPATH 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 adversly 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 enterolitica, Escherichia coli* and *Francisella tularensis.*
A prozone may occasionally occur with the slide procedure. 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 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

A. *Rapid Slide Test*

1. Using a graduated pipette add the following amounts of serum to consecutive circles on a slide for each dilution under test.
   0.08ml 0.04ml 0.02ml 0.01ml 0.005ml
2. Thoroughly resuspend the antigen and add a drop to the appropriate circle on the slide.
3. Mix the drops and spread to cover the entire test circle.
4. Gently and evenly, rock and rotate the test slide for 1 minute whilst examining the test slide for agglutination.
5. Results obtained correspond to tube agglutination titres of 1:20 1:40 1:80 1:160 1:320 respectively.
6. It is advisable to confirm a slide titration by the tube technique.

Any sample showing agglutination should be tested in the tube agglutination test.

B. *Tube Agglutination Test*

1. Prepare a rack of 10 tubes. Add 1.9ml of saline to tube1, and 1.0ml of saline to each of the other tubes.
2. Add 0.1ml of patient's serum to tube 1. Mix well.
3. Withdraw 1.0ml from tube 1 and transfer to tube 2. Continue serial doubling dilutions in this way until tube 9. Discard the 1.0ml withdrawn from tube 9.
4. Add 1 drop of thoroughly resuspended antigen suspensions to each tube in the rack. Do not dilute the suspensions for use. Tubes 1-9 now contain serum diluted from 1/20 to 1/5120. Tube 10 contains only saline and antigen and is the antigen control.
5. Mix well and incubate as follows and examine for agglutination.
   O titrations 50°C for 4 hours
   H titrations 50°C for 2 hours
   Brucella titrations 37°C for 24 hours
   Proteus titrations 50°C for 4 hours

The antigen control should not show any agglutination.

RESULTS

Examine the test slide under a strong light source after 1 minute. Kit controls or known level value samples should be tested with each test run. The kit negative control should give a negative result after 1 minutes. The kit positive control should give a positive result at a titre of 1/2 +/- one double diluition after 1 minute.

Agglutination of the antigen indicates the presence of antibody. Titres in excess of 1/80 are probably significant. A comparison between samples taken 10-14 days apart may be of value in acute illness.

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.

EXPECTED VALUES AND SENSITIVITY

The generally accepted performance characteristics of this type of test is 70% sensitivity and specificity.
Reproducability of the Micropath Range of Reagents is 100% (+/- one double dilution).
Calibrated to major competitors and in house standards.

REFERENCES

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

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