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MAST® ASSURE FEBRILE ANTIGENS

Intended use
Stained antigen suspensions for Widal and Weil-Felix tests. For the identification and quantitative detection of specific antibodies in human sera for epidemiological and diagnostic purposes, primarily in the investigation of pyrexia and enteric infections with certain Salmonellae, Rickettsiae and Brucellae pathogens. They are suitable for both the rapid slide and tube agglutination tests.

FOR IN VITRO DIAGNOSTIC USE ONLY.

Contents
The MAST· ASSURE FEBRILE ANTIGENS· are stained (unless marked) antigen suspensions of killed bacteria, stained to enhance the reading of the agglutination tests. Somatic 'O' antigens are coloured blue and flagellar 'H' antigens are coloured red and are available as follows:

Code	Specification	Content
P00002	Salmonella typhi H	5ml
P00004	Salmonella H paratyphi A	5ml
P00006	Salmonella H paratyphi B	5ml
P00008	Salmonella H paratyphi C	5ml
P00010	Salmonella typhi O	5ml
P00012	Salmonella O paratyphi A	5ml
P00014	Salmonella O paratyphi B	5ml
P00016	Salmonella O paratyphi C	5ml
P00018	Brucella abortus	5ml
P00020	Brucella melitensis	5ml
P00022	Proteus OX2	5ml
P00024	Proteus OX19	5ml
P00026	Proteus OXK	5ml
P00030	Positive Polyvalent Febrile Control	1ml
P00032	Negative Febrile Control	1ml

Stability and storage
Store unopened at 2 to 8°C in an upright position until the expiry date shown on the pack label. Once opened, MAST· ASSURE FEBRILE ANTIGENS should be stored at 2 to 8°C and may be used until the expiry date given on the label. Reagents may be light sensitive. **Do not freeze reagents.**

Warnings and precautions
For *in vitro* diagnostic use only. Observe approved biohazard precautions and aseptic techniques. To be used only by adequately trained and qualified laboratory personnel. Sterilise all biohazard waste before disposal. Non disposable apparatus must be sterilised after use by an appropriate method. Spillages of potentially infectious material should be absorbed and disposed of as above. The site of spillage must be sterilised with disinfectant or 70% alcohol. Do not pipette by mouth. Control reagents contain rabbit serum. The products contain sodium azide (0.095% w/w) and Thiomerosal™ (0.0095% w/w) as preservatives. Refer to Product Safety Data sheet. Do not inhale or ingest aerosols – wash splashes with copious amounts of water.
Analytical precautions:
Do not modify the test procedure. Do not dilute or modify the reagents in any way. Allow all reagents and samples to reach room temperature (18 to 30°C) before use. Do not interchange reagents from different kit batches.

Materials required but not provided
Standard microbiological supplies and equipment such as small glass or plastic tubes, pipettes, reaction slides with a white background. Mixing sticks and 37°C and 50°C waterbaths.

Procedure
A. Sample preparation
Use fresh serum samples obtained by centrifugation of clotted blood. Serum samples may be stored at 2 to 8°C for up to 48 hours or frozen for longer term storage. Do not use plasma or heat inactivated, haemolysed, lipaemic or contaminated serum specimens.
B. Rapid Slide Titration Procedure
1. Using a pipette, dispense 80µl, 40µl, 20µl, 10µl and 5µl of undiluted serum onto a row of 3 cm diameter circles on a reaction slide.
2. Shake the antigen reagent bottle well and add one drop of undiluted antigen suspension to each serum aliquot.
3. Mix well using a mixing stick and rotate the slide.
4. Read the agglutination after one minute.

C. Tube Agglutination Procedure
All positive rapid slide titration results should be confirmed using the following technique.
1. Label 8 small plastic tubes in a rack.
2. Using a pipette, dispense 1.9 ml of 0.85% saline into the first tube, and 1.0 ml into the remaining seven.
3. Using a pipette, dispense 0.1 ml of the patient's undiluted serum into the first tube.
4. Mix contents well with the pipette. Do not to create air bubbles.
5. Dispense 1.0 ml from the first tube into the second tube and mix well.
6. Continue this method of doubling dilutions up to the seventh tube and then discard 1.0 ml from the seventh tube. The eighth tube will contain only saline as a control and therefore should not contain any serum.
7. Shake the antigen reagent bottle well before use then add one drop of the appropriate antigen suspension to each tube and mix well.
8. Incubate the tubes as follows:
   - Salmonella "O" antigens and Proteus for 4 hours at 50°C.
   - Salmonella "H" antigens for 2 hours at 50°C.
   - Brucella Antigen for 24 hours at 37°C.
   - Salmonella typhi Vi for 2 hours at 37°C.

   Leave all tubes overnight in a fridge, then allow to reach room temperature before reading the results.
   NB: It is vitally important that when the tubes are placed in a water bath, the level of water should come to about 2/3rd the way up the level of the tube content. This will maintain convection currents within the tube and prevent false results.
9. Examine the tubes after the appropriate incubation time and check for agglutination.

Interpretation of results
Rapid Slide Titration Procedure
Agglutination seen in any circle of the reaction card is indicative of the following results in a corresponding tube test agglutination procedure. In this way the rapid slide titration procedure gives an approximation to the expected results in the tube agglutination procedure.

Volume	80µl	40µl	20µl	10µl	5µl
Results	1:20	1:40	1:80	1:160	1:320

It is important that all dilutions in the slide test are conducted to allow any "prozone" effect (where higher concentrations of the serum may give a negative result but further dilutions may give a positive result) to be recognised and ignored.

Tube Agglutination Procedure
Tubes should be read after the recommended incubation time to eliminate the possibility of false results. A negative result and the saline control should show no change in appearance and should show a characteristic "swirl" when flicked. Tubes must not be shaken. A positive test will show an obvious floccular agglutination throughout the tube. The last tube showing signs of agglutination should be taken as the titre for that test.

Limitations of Use
It has been found that many serotypes of Salmonella possess somatic antigens of the same kind. Therefore, agglutination of any of the Salmonella antigens with human serum should not be taken as proof of infection by one particular organism, but rather as infection by an organism of a similar antigenic structure. Tests should be read after the recommended incubation time to eliminate the possibility of false results.
The last test to show signs of agglutination is taken as the titre for that test. For negative results, all tests should remain clear of agglutination.
Many populations or communities can show high levels of residual antibodies often in excess of 1/80 – 1/160. Patients can also show high levels of residual antibodies from previous infections. For a test to be of clinical significance a rise in titre must be demonstrated not just a high titre for a single test. Chronic liver disease has also been shown to cause a rise in salmonella antibody titre.
Highly lipaemic, haemolytic or contaminated samples should be avoided.

Performance Characteristics
The generally accepted performance capability of the Widal test using febrile antigens is 70% specificity and sensitivity. As serological tests used in the diagnosis of Salmonella infections have important limitations, cultures of appropriate specimens are normally preferred.

Quality control
It is recommended that quality control should be performed at regular intervals with positive and negative febrile controls to verify that the test is working correctly. Do not use reagents if they show signs of deterioration.

References
Bibliography available on request.

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