PULSE SCIENTIFIC INC.

Unit 18, 5100 South Service Road
Burlington, Ont. Canada L7L 6A5
Tel: (905) 333-8188
Fax: (905) 333-0500

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Proteus Febrile Antigens

INTENDED USE

The Pulse Febrile antigen suspensions are for the identification and quantitative determination of specific antibodies in human sera following infection with certain Rickettsiae pathogens.

SUMMARY

Titration of patient's antibodies by agglutination of known bacteria suspensions has proven to be of value in the evaluation of certain diseases. The classic example is the Widal Test. This method is based upon the identification of the greatest dilution of the patient's serum, which will cause agglutination of a known bacterial suspension, which was prepared in a standard way. Antibodies produced as a response to a "febrile" infection can be detected in a similar fashion. The term "febrile" is associated with a number of organisms, which produce a fever in the host.

PRINCIPLES

In the course of human infection with any pathogenic microbiological agent, a variety of antibodies are formed. Among these antibodies are the agglutinins. An agglutinin, when combined with homologous antigen (agglutinogen) under properly controlled conditions, is capable of causing agglutination. This agglutination forms clumps of bacteria, which become macroscopically visible. Pulse febrile antigens are suitable for both the rapid slide and tube agglutination tests against human sera for the detection of these agglutinins. Pulse antigen suspensions are killed bacteria, stained to enhance the reading of agglutination tests.

MATERIALS SUPPLIED

Febrile Antigens (supplied as individual items):

Cat.#	Description	Cat.#	Description	Cat.#	Description
24114	Proteus OX2	24115	Proteus OX19	24116	Proteus OXK

MATERIALS REQUIRED BUT NOT PROVIDED

Small test tubes, pipets, reaction slides with white background.

STORAGE & STABILITY

Store upright at 2°C - 8°C. Light Sensitive. Do not freeze. Under these conditions, kit performance characteristics will be maintained until the expiry date printed on kit label. Reagents should be discarded if they become contaminated or do not demonstrate correct activity with the controls.
The reagents in each kit have been standardized to produce the proper reaction and reagents should not be interchanged with those from other batches.

PRECAUTIONS

1. For *in vitro* diagnostic use only.
2. For laboratory use only.

Health and Safety warnings:

1. All patient samples and reagents should be treated as potentially infectious and the user must wear protective gloves, eye protection and laboratory coats when performing the test.
2. Disposable apparatus must be treated as biohazardous waste and autoclaved or incinerated.
3. Spillages of potentially infectious material should be absorbed and disposed of as above. The site of spillage must be sterilized with disinfectant or 70% alcohol.
4. Control reagents contain rabbit serum. The product also contains aqueous buffer salts including sodium azide and Thiomersal as preservatives.
5. Do not pipette by mouth. Do not inhale or ingest aerosols – wash splashes with copious amounts of water.

Analytical precautions:

1. Do not modify the test procedure.
2. Do not dilute or modify the reagents in any way.
3. Allow all reagents and samples to reach room temperature (18 - 30ºC) before use.
4. Do not interchange reagents from different kit batches.

SPECIMEN COLLECTION

Use fresh serum obtained by centrifugation of clotted blood. The sample may be stored at 2-8° C for 48 hours before performing the test. For longer periods of time the serum must be frozen. Haematic, lipaemic or contaminated serum must be discarded.

PROCEDURE

RAPID SLIDE TITRATION

1. Using a pipettor, dispense 0.08 ml, 0.04 ml, 0.02 ml, 0.01 ml and 0.005 ml of undiluted serum onto a row of 3 cm diameter circles.
2. Shake the reagent bottle well and add one drop of the undiluted antigen suspension to each serum aliquot.
3. Mix well using a stirring stick and rotate the slide.

Read after one minute

Agglutination seen in any circle is indicative of the following results. A tube test should be carried out: 0.08 ml = 1:20, 0.04 ml = 1:40, 0.02 ml = 1:80, 0.01 ml = 1:160, 0.005 ml = 1:320. In this way the rapid slide test provides an approximation to the expected results from a corresponding tube test. **NOTE**: It is necessary to perform all dilutions in the slide test to obviate the 'prozone' effect where higher concentrations of the serum may give a negative result but further dilutions may give a positive result.

TUBE AGGLUTINATION TEST

All positive results obtained through a slide test should be confirmed using the following technique:

1. Label up 8 small plastic tubes in a rack.
2. Using a pipette, dispense 1.9ml of 0.85% saline into the first tube, and 1.0ml into the remaining seven.
3. Using a pipette, dispense 0.1ml of the patients' undiluted serum into the first tube. Mix well using the larger pipette volume and tip. (*e.g. set to 1.0ml*)
4. Using the pipette, dispense 1.0ml from the first tube into the second tube. Mix well.
5. Continue this method of doubling dilutions up to the seventh tube. Discard 1.0ml from the seventh tube. The eighth tube will contain only saline as a control and therefore should not contain any serum.
6. Shake the reagent bottle well and add 1 drop of the appropriate antigen suspension into each tube and mix well.
7. Incubate as follows: Proteus Antigens = 50°C for 4 hours (Leave overnight in fridge, then allow to reach room temperature before reading.) It is vitally important that when the tubes are placed in a water bath, the level of water should come to approximately 2/3rd the way up the level of the tube content. This will maintain convection currents within the tube and thereby obviate false results.
8. Examine the tubes after the appropriate incubation time and check for agglutination. The titre to be taken is the last tube to show agglutination.

RESULTS

Tests should be read after the recommended incubation time to eliminate the possibility of false results. The last test showing signs of agglutination should be taken as the titre for that test. For negative results, all tests should remain clear of any 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, but not just a high titre for one of the test.

QUALITY CONTROL AND PERFORMANCE CHARACTERISTICS

Known Positive and Negative Controls should be run at regular intervals to confirm that the test is working satisfactorily.

REFERENCES

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