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Positive Control for Febrile Antigens
Minimum titer 1:80 or Greater

Febrile antigens are bacterial suspensions for use as an aid in the diagnosis of certain febrile diseases such as salmonellosis, rickettsial and brucellosis diseases. These bacterial suspensions may be used in either slide (qualitative) or tube (semi-quantitative) agglutination tests to detect for the presence of bacterial agglutinins associated with bacterial infections.

Principles of the Febrile Agglutination Test

Agglutination tests have been widely used for the detection of antibodies in the patient's serum against various disease-producing microorganisms. The early example of such procedures was the Widal test, devised for the diagnosis of typhoid fever. It employed as an antigen a suspension of killed *Salmonella* Typhi organisms.
The *Proteus* antigens have been widely used in a procedure known as the Weil Felix reaction for the diagnosis of the diseases caused by rickettsial antibodies (*Proteus* antigens are easier to prepare than are the rickettsial antigens).
Essentially the same technique is used in many other diseases; the antigen used is a suspension of the bacteria causing the suspected diseases. The choice of antigens to be used in febrile diseases depends on the disease suspected and also on the geographic area in which the laboratory is situated.

Slide Test Procedure (Qualitative)

All antigens, antisera and test sera should be at room temperature before testing.
1. Obtain a clear, transparent glass slide and divide it into 1 1/2 inch squareswith a wax pencil. A small windowpane can be used for this purpose.
2. Using a suitable pipette, add the following amounts of test sera toconsecutive squares from left to right: 0.08ml; 0.04ml; 0.02ml; 0.01ml;0.005ml. Serum should be clear and unheated. Repeat this procedure withfebrile positive and negative control sera.
3. Shake the antigen bottle gently to insure a uniform suspension.
4. Add one drop of antigen suspension just below each quantity of serum.
5. Mix the test serum and antigen by using a clean toothpick. Proceed fromright to left with the mixing of each ring in the row. Use a new toothpick for each test sample.
6. Gently rock the slides back and forth and observe agglutination for a period for 30-60 seconds, do not rock for more than 60 seconds.

Interpretation of Slide Test Results

The degree of agglutination is recorded as follows:

4+ = 100% of the organisms are agglutinated
3+ = 75% of the organisms are agglutinated
2+ = 50% of the organisms are agglutinated
1+ = 25% of the organisms are agglutinated
± = Less than 25% of the organisms are agglutinated
= No agglutination is observed

The serum dilution giving a 2+ , or 50 % degree agglutination is the end point or titer of that serum.

Tube Test Procedure (Semi-Quantitative)

All antigens, antisera and test sera should be at room temperature before testing.
1. Place 8 test tubes(3x100mm or12x75mm) in a test tube rack.
2. Prepare patient's serum dilutions as following table.

Tube No.	1	2	3	4	5	6	7	8
0.85% NaCl(ml)	1.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Patient's serum (ml)	0.1	→ 1 ml serial dilutions → Discard 1 ml from 7th tube						0
Final dilutions	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	Control

3. Add 1 drop of antigen into each tube and mix well.
4. Shake the rack well to mix the antigen and serum and place in a water bath.
5. Incubate as indicated in following:
   Salmonella "O" antigens at 50 °C for 4 hours
   Salmonella "H" antigens at 50 °C for 2 hours
   Proteus antigens at 50 °C for 4 hours
6. At the end of incubation period, remove the rack from water bath takingcare not disturb the agglutination. Read the results of the control tube first.
7. Remove each tube from rack, hold in front of suitable light source. Read the results and interpret the degree of agglutination.

INTERPRETATION OF TUBE TEST RESULTS

The degree of agglutination is recorded as follows:
4+: All organisms appear agglutinated at the bottom of the tube and supernatant fluid is clear.
3+:75% of organisms are agglutinated and supernatant fluid is slightly cloudy.
2+:50% of organisms are agglutinated and supernatant fluid is moderately cloudy.
1+:25% of organisms are agglutinated and supernatant fluid is cloudy.
±: Less than 25% of the organisms are agglutinated and supernatant fluid is very cloudy.
: No agglutination is observed and suspension appears turbid.

The serum dilution giving a 2+ , or 50 % degree agglutination is the end point or titer of that serum.

LIMITATIONS OF THE PROCEDURE

1. Detectable agglutinins are not always produced in bacterial infection. In some instances, non-specific agglutinins may appear which will react with the febrile antigens. For example, serum from acme narcotic addicts hasbeen reported to contain significant agglutinins to the febrile antigens.
2. Reporting results from a single specimen is of no clinical use because of possible heat exposures, cross-reactions or interfering substances.
3. Results should not be used as a complete substitute for conventional isolation and serological identification of the etiologic agent. Isolation of the organism is required for definitive diagnosis. This test provides apresumptive diagnosis.
4. *Brucella Abortus*, *Brucella Melitensis*, and *Brucella Suis* are antigenically related and will cross react.
5. Cross-reactions may occur in certain diseases. For instance, Tularemia infections may produce agglutinins capable of reacting with *Brucella* antigen.
6. Vaccinations may produce agglutinins capable of reacting with febrile antigens. Typhus vaccinations may produce antibodies to Proteus antigens and Typhoid vaccinations may give rise to antibody specific for *Salmonella* antigens.
7. It is necessary to test several serum specimens from the same patient taken at different intervals to detect semi-quantitative differences in agglutinin content. A four-fold increase in titer between the acute phase specimen anda convalescent phase specimen should be completely evaluated by the physician. All clinical and laboratory results should be evaluated before making a definitive diagnosis.

Febrile antigen	Disease	Appear	Maximum	Significant titre
S.Typhi O	Typhoid Fever	1-2 Weeks	3-5 Weeks	1:80
S.Typhi H	Typhoid Fever	2-3 Weeks	4-5 Weeks	1:80
S. Paratyphi A,B and C	Paratyphoid Fever	2-3 Weeks	4-5 Weeks	1:80
Proteus OX19	Typhoid Fever	1-2 Weeks	2-3 Weeks	1:160
Proteus OX19	Rocky Mountain Spotted Fever	1-2 Weeks	2-3 Weeks	1:160

Rickettsial Disease	OX19	OX2	OXK
Endemic & Murine Typhus	++++	+	-
Rocky Mountain Spotted Fever	++++ or +	+ or ++++	-
Scrub Typhus	-	-	++++
Rickettsial Pox	-	-	-
Q Fever	-	-	-

** Significant in non-vaccinated individuals*
The end point titer may vary (plus or minus) 1 dilution when retesting samples or when testing samples collected at different times from the same patient.

References:

1. Huddleson, J.F., and Abell, E., J. 1928 Infect. Dis., 42:242
2. Alex C. Sonnenwirth. Leorard Jarett 1980. Gradwohl's Clinical Laboratory Methods and Diagnosis, Volume 2, Eighth Edition. The C.V. Mosby Company, Saint Louis.
3. Ewing, W.H. 1986. Edwards and Ewing's Identification of Enterobacteriaceae, 4th edition. Elsevier Science Publishing Co., Inc., New York.

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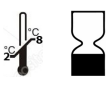

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