



**ATLAS FEBRILE ANTIGENS  
SLIDE/TUBE TEST**

**A qualitative and semi-quantitative test for the detection of bacterial agglutinins in bacterial infections**

**For *In-Vitro* and professional use only  
Store at 2 to 8° C**

**INTENDED USE**

For the qualitative and semi-quantitative detection of bacterial agglutinins in bacterial infection.

**INTRODUCTION & PRINCIPLES**

Antibodies are formed in human infection cases with various microbiological agents. Mixing these antibodies with the corresponding homologous antigens causes agglutination under controlled conditions. The agglutination is macroscopically visible. For screening purposes, the antigens can be used in the qualitative rapid slide test. Positive results can be confirmed with the quantitative tube test to verify the antibody titer.

**MATERIALS**

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ATLAS Febrile Kit contains the following antigens:

- Brucella.
- Proteus.
- Salmonella O.
- Salmonella H.

(All febrile antigens contain phenol 0.5% preservative, except Salmonella H antigen which contains formalin 0.5%.)

The kit also contains:

- Positive control antiserum for Brucella abortus, Salmonella H groups A,B,D Salmonella O groups A,B,D, Proteus OX19.
- Negative control antiserum.

The controls are stabilized with glycerine and contain 0.01% merthiolate.

**NOTE**

(This package insert also used for individual febrile antigens).

**MATERIALS NEEDED BUT NOT PROVIDED**

- Pipettes.
- Sodium Chloride 0.85% NaCl preservative free and light source.

**Additional requirements for slide tests:**

- Glass slides.
- Applicator stick and wax pencil.

**Additional requirements for tube tests:**

- Test tubes 13x100mm.
- Tube rack.
- Glass dilution flask.
- Water bath.

**PRECAUTIONS**

- Reagents should be stored in an upright position and refrigerated between 2 to 8° C. Never Freeze.
- Reagents should be brought to room temperature and mixed well to obtain a uniform suspension of antigens.
- The antigens are intended for *In-Vitro* diagnostic use only.
- Always include positive and negative antisera controls as well as a saline control in each test procedure.

**PREPARING THE SPECIMEN**

ATLAS Febrile antigens can be used with serum stored 2 to 8° C. The test requires serum collected from 5-10 ml of whole blood sample. The serum should be separated quickly to avoid any excess hemolysis and should not be inactivated. It should also be clear and free from bacterial contamination.

**PROCEDURES**

**1. QUALITATIVE PROCEDURES**

**Rapid Slide Test:**

1. Bring reagents and samples to room temperature. The sensitivity of the test may be reduced at low temperatures.
2. Place 50 uL of the sample to be tested (Note 1 and 2) and 1 drop of each control into separate circles on the slide test.
3. Swirl the antigen vial gently before using. Add 1 drop (50 uL) of antigen to each circle next to the sample to be tested .
4. Mix with a stirrer and spread over the entire area enclosed by the circle .
5. Place the slide on a mechanical rotator at 80-100 r.p.m., for **1 minute**.
6. Read the results immediately in good indirect light noting the agglutination where visible.

**Slide Titer Test:**

1. Bring reagents to room temperature.
2. Using the wax pencil, divide a clean transparent glass slide into 5 circles of 3cm in diameter.
3. Place 80ul, 40ul, 20ul, 10ul, 5ul of test serum (clear & unheated) into these circles consecutively.
4. Shake the antigen well and add one drop onto every circle. The dilution of the circles are 1:20, 1:40, 1:80, 1:160, 1:320 respectively. Mix using the applicator stick.
5. Repeat steps 5-6 above.

**\*Reading the qualitative results**

**Results are interpreted according to the degree of agglutination**

Degree of agglutination	Result
100% in clear supernatant fluid	4+
75% in slightly cloudy fluid	3+
50% in moderately cloudy supernatant fluid	2+
25% in cloudy supernatant fluid	1+
No agglutination	Negative.

The titer in this method is approximate and determined at 50% agglutination. The quantitative procedure is more recommended for determining the sample titer. Results should be read at one minute.

**2. SEMI-QUANTITATIVE PROCEDURE**

1. Prepare 10 test tubes on a rack.
2. In tube 1, add 950ul of NaCl 0.85% solution.
3. In tubes 2-10, add 500ul of NaCl 0.85% solution.
4. Place 50ul of the serum sample in tube 1 and mix well.
5. Starting from tube 1, prepare a two fold serial dilutions by transferring 500ul from one tube to the next tube. Mix well after each transfer. Discard 500ul from the last tube.
6. Repeat step 5 for the positive and negative controls.
7. In a new test tube labeled 'Saline Control', place 500ul of 0.85% NaCl solution.
8. Mix the antigens well.
9. Add one drop of antigen to each test tubes and shake the rack well. Final serum dilution is 1:20, ..., 1:10240,
10. Incubate the samples in a water bath as follows:

Antigen	Incubation Time	Temperature
Salmonella O antigens	24 hours	37°C
Salmonella H antigens	24 hours	37°C
Proteus antigens	24 hours	37°C
Brucella antigens	24 hours	37°C

11. At the end of the incubation period, gently remove the rack from the water bath to avoid disturbing the suspensions.
12. Examine each tube in turn and observe the agglutination. Interpret the results as in the qualitative test.

**Reading the quantitative results**

The dilution of the tubes are as follows:

1	2	3	4	5
1:20	1:40	1:80	1:160	1:320
6	7	8	9	10
1:640	1:1280	1:2560	1:5120	1:10240

The titer of the sample is read according to the dilution of the test tube with 2+ (50%) agglutination level.

Repeat the test if there is an agglutination in the saline or the negative control or if there is no agglutination in the positive control.

**NOTES**

1. When testing for Brucella antibodies it is recommended to reduce sample volume to 20ul up in order to avoid prozone.
2. In some geographical areas with a high prevalence of febrile antibodies, it is recommended to dilute the sample 1/4 in NaCl 9 g/l before to perform the assay.
3. The incubation procedure may be accelerated incubating as follows:
  - Somatic (O) and Proteus antigens: 48-50°C for 4 h.
  - Flagellar (H) antigens: 48-50°C for 2 h.
4. A single positive result has less significance than the demonstration of a rising or falling antibodies titer as evidence of infection. A clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.
5. A somatic reaction (O) is characterized by coarse, compact agglutination, which tends to be difficult to disperse, while flagellar (H) has a characteristic loose, flocculant agglutination.

**LIMITATION**

- In some non-infected cases, non-specific agglutinins may appear and react with the Febrile antigens giving false results.
- Some vaccination may also produce agglutinins that react with Febrile antigens resulting in false results.
- Physicians should always evaluate all clinical and laboratory findings before making a definitive diagnosis.

Disease	Antigen Reagent	Antibody Presence	Peak Production	Titer
Brucellosis	B.Abortus	2-3 weeks	3-5 weeks	+1:80
Paratyphoid Fever	Salmonella Flag a	2-3 weeks	4-5 weeks	+1:80
Paratyphoid Fever	Salmonella Flag b	2-3 weeks	4-5 weeks	1:80
Rocky Mt. Spotted fever	Proteus OX 19	2-3 weeks	2-3 weeks	+1:160
Typhoid fever	Typhoid H	2-3 weeks	4-5 weeks	1:80
Typhoid fever	Typhoid O	1-2 weeks	4-5 weeks	1:80
Typhus	Proteus OX 19	1-2 weeks	2-3 weeks	+1:160

**Significant in non-vaccinated individuals**

**REFERENCES**

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