Febrile Antigens

REF IVD +2°C +8°C CE

INTENDED USE

Rapid Labs febrile antigens are used for slide and tube agglutination tests that detects the presence of serum agglutinins (O, H) in a patient's serum with typhoid and paratyphoid fever.

SUMMARY

Enteric fever occurs when pathogenic microorganisms like *S.typhi*, *S.paratyphi A*, *S.paratyphi B*, *S. paratyphi C* infect the human body. During the course of the disease, the body responds to this antigenic stimulus by producing antibodies whose titre rises slowly in early stages to a maximum and then slowly falls till it is undetectable. Antibodies to Salmonella organisms may be detected in the patient serum from the second week after onset of infection. Information regarding the titres and whether or not they are rising or falling can be obtained by performing serological tests using Rapid Labs' antigen suspensions. Usually tube titres of 1:80 and above are taken as diagnostically significant, however, for endemic areas higher cut-offs may need to be established.

REAGENTS

Rapid Labs febrile antigens contain ready to use concentrated, smooth antigen suspensions of the bacilli; *S.typhi 'O'*, *S.typhi 'H'*, *S.paratyphi 'AO'*, *S.paratyphi 'BO'*, *S.paratyphi 'AH'*, *S.paratyphi 'BH'*, *S.paratyphi 'CH'*, *S.paratyphi 'CO'* and/ or polyspecific positive control reactive with these antigens.
Each batch of reagents undergoes rigorous quality control at various stages of manufacture for its specificity and performance.

STORAGE AND STABILITY

- Store the reagents at 2-8^{0}C. DO NOT FREEZE. Keep the reagents away from direct sunlight.
- The shelf life of reagents is as per expiry date mentioned on the reagent vial labels. Do not use beyond expiry date.
- Once opened the shelf life of the reagent vial is as described on the reagent vial label provided it is not contaminated.

PRECAUTIONS

- For *in vitro* diagnostic and for professional use only
- The *S.typhi 'O'*, *S.paratyphi 'CO'* reagents contain 0.5% Phenol, *S.typhi 'H'*, *S.paratyphi 'AH'*, *S.paratyphi 'BH'*, *S.paratyphi 'CH'* reagents contain 0.3% Formaldehyde and *S.paratyphi 'AO'*, *S.paratyphi 'BO'* reagents contain 0.7% Ethanol along with 0.1% sodium azide as preservatives.
- Avoid contact with skin and mucosa. Do not breathe vapour.
- In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- Sodium azide may react with lead and copper in plumbing and form highly explosive metal oxides, on disposal flush with large quantities of water.
- The reagent can be damaged due microbial contamination or on exposure to extreme temperatures. It is recommended that the performance of the reagent be verified with positive and negative controls (if available).
- Shake the reagent vials well before use to disperse the antigen suspension uniformly and improve test readability.
- Rapid Labs antigen suspensions are not from human sources hence contamination due to HBsAg and HIV is practically excluded
- Do not use damaged or leaking reagents.
- Wear protective gloves, eye protection and laboratory coats when performing the test.

PRESENTATION OF STAINED FEBRILE ANTIGENS

F-BAA5	Brucella abortus	5 ml
F-BMA5	Brucella melitensis	5 ml
F-POX25	Proteus OX2	5 ml
F-POX195	Proteus OX19	5 ml
F-POXK5	Proteus OXK	5 ml
F-SHA5	Salmonella H paratyphi a	5 ml
F-SHB5	Salmonella H paratyphi b	5 ml
F-SHC5	Salmonella H paratyphi c	5 ml
F-SHD5	Salmonella typhi H	5 ml
F-SOA5	Salmonella O paratyphi A	5 ml
F-SOB5	Salmonella O paratyphi B	5 ml
F-SOC5	Salmonella O paratyphi C	5 ml
F-SOD5	Salmonella typhi O	5 ml
F-FPC1	Positive control	1 ml
F-FNC1	Negative control	1 ml
F-KIT8x5	Febrile antigen kit	8x5 ml antigens
F-KIT8x5C	Febrile antigen kit	8x5 ml plus controls
F-KIT4X5	Febrile antigen kit	4x5ml antigens
F-KIT8X5E	Febrile antigen kit	8x5ml plus controls
F-KIT3X5C	Febrile antigen kit	1x5ml plus controls
F-KIT2C	Febrile antigen kit	2x5ml plus controls

Materials required but not provided

Slide method

- Stop watch
- Micropipettes

Quantitative method

- Timer
- Test tubes
- Pipettes (0.1ml, 1ml)
- Saline
- Incubator (37^{0}C)
- Test tube rack

Specimen & sample preparation

- No special preparation of the patient is required prior to sample collection by approved techniques.
- Do not use haemolysed and turbid samples.
- Do not heat inactive the serum
- Through freshly collected serum is preferable, store samples at 2-8^{0}C in case of delay in testing, for up to 72 hours.

TEST PROCEDURE

Slide screen method

1. Place one drop of positive control onto a reaction circle of the slide.
2. Place 50µl of saline onto the next reaction circle of the slide.
3. Place one drop of patient's serum to be tested onto each of the required number of reaction circles.
4. Add one drop of appropriate Rapid Labs' antigen suspension to the reaction circles containing positive control & saline.
5. Add one drop of appropriate Rapid Labs' antigen suspension to the reaction circles containing the patient's serum.
6. Mix contents of each circle uniformly over the entire circle with separate mixing sticks.
7. Rock the slide gently back and forth, and observe for agglutination macroscopically at **one minute.**

Slide semi-quantitative method

1. Using pipette place 80µl, 40µl, 20µl, 10µl and 5µl of patient's serum to be tested on 5 different reaction circles on the slide. The corresponding titres obtained will be 1:20, 1:40, 1:80, 1:160 & 1:320 respectively.
2. Follow step no 5-7 of slide screen method.

Note: This method is recommended for obtaining quick approximate titres only.

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Quantitative method (test tube method)

1. Take appropriate number of sets (as required; one set for each antigen suspension) of test tubes and label them 1 to 8.
2. Pipette into tube no.1 of all sets 1.9ml of saline.
3. To each of the remaining tubes (2 to 8) add 1ml of saline.
4. To tube no.1 of all sets add 0.1ml of serum sample to be tested and mix well.
5. Transfer 1ml of diluted serum sample from tube no.1 to tube no.2 and mix well.
6. Transfer 1ml of the diluted serum sample from tube no.2 to tube no.3 and mix well. Continue this serial dilution till tube no.7 in each set.
7. Discard 1.0ml of the diluted serum from tube no.7 of each set.
8. The dilutions of the serum samples achieved from tube no.1 to 7 respectively in each set is as follows:
   1:20, 1:40, 1:80, 1:160, 1:320, 1:640, 1:1280. Tube no 8. In all the sets, serves as a saline control.
9. To all the tubes (1 to 8) of each set add one drop of the respective well-mixed Rapid Labs antigen suspension from the reagent vials and mix well.
10. Cover and incubate at 37^{0}C overnight (approx. 18 hours)
11. Dislodge the sedimented button gently and observe for agglutination.

INTERPRETATION OF RESULTS

Slide screen method

Agglutination is a positive test result and indicates presence of the corresponding antibody in the patient's serum

Slide semi-quantitative method

Agglutination is a positive test result. The titre of the patient serum corresponds to the visible agglutination in the test circle with the least amount of serum sample.

Quantitative method (test tube)

The titre of the patient serum using Rapid Labs' antigen suspensions is the highest dilution of the serum sample that gives a visible agglutination.

LIMITATIONS

- Positive results obtained in the slide test should be confirmed with the tube test to establish whether the titres are diagnostically significant or not.
- TAB vaccinated patients may show a high titre of antibodies to each of the antigens. Similarly, an amnestic response to other vaccines and unrelated fevers in case of patients who have had prior infection or immunization may give a false result.
- Agglutinins usually appear by the end of the first week of infection, blood sample taken earlier may give a negative result.
- A rising titre is more significant than a single high titre. It is therefore necessary to evaluate two or more serum samples taken at 4-6 days intervals after the onset of the disease.
- 'O' being a somatic antigen brings about a coarse, compact, granular agglutination whereas 'H' being a flagellar antigen brings about larger, loose, flocculant antigen.
- While the 'O' antigen is species specific, the 'H' antigen is specific to the serotype.
- Serological findings are not intended as a substitute for culture. An appropriate attempt should be made to recover and identify the etiologic organisms through various culture and biochemical tests.
- Generally, antibody titres of 1:80 or more are considered clinically and diagnostically significant. However, the significant titre may vary from population to population and needs to be established for each area.
- False positive results are likely if the test is read more than one minute after mixing on the slide test.
- Any deviation in test procedure could result in variable results.
- Since techniques and standardization vary from lab to lab one tube difference in tube titres can be expected.
- Use a separate disposable tip for each sample to prevent cross contamination.
- After usage the antigen suspension should be recapped and stored at 2-8^{0}C.
- It is recommended that results of the tests should be correlated with clinical findings to arrive at the final diagnosis.
- The performance of the reagents should be validated occasionally using the positive control provided. Good saline may be used as negative control.

PERFORMANCE CHARACTERISTICS

- The positive control antisera should produce 1+ or greater agglutination at 1:80 in the slide and tube test when tested with the Rapid Labs' antigen suspensions.
- The negative control should show no agglutination with any of Rapid Labs' antigen suspensions.
- Generally accepted performance characteristic of this type of test is 70% specificity and sensitivity.
- Reproducibility of Rapid Labs antigen suspensions is 100% (± one double dilution)

BIBILIOGRAPHY

1. Cruickshank R., (1982), Medical Microbiology, 12th Edition, 403. (2).
2. Felix A., (1942), Brit. Med. J., 11, 597-600. (3).

Index of Symbols

	Consult instructions for use	IVD	For *in vitro* diagnostic use only
REF	Catalogue Number	LOT	Lot Number
+2°C +8°C	Store between 2-8°C		Use by
	Manufacturer		Date of manufacture

EC REP Advena Ltd. Tower Business Centre, 2nd Flr., Tower Street, Swatar, BKR 4013 Malta

Manufactured by: Rapid Labs Ltd
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Revision 9 20/06/2022