LORNE LABORATORIES LTD.

GREAT BRITAIN

FEBRILE ANTIGENS

DIRECTIONS FOR USE

Stained Febrile Antigens: For Widal and Weil-Felix Tests

PRINCIPLE

The reagents consist of killed and stained febrile antigens which agglutinate when mixed with human serum samples containing the corresponding antibodies are suitable for both slide and tube agglutination techniques.

INTENDED PURPOSE

The reagents are suitable for the qualitative detection of antibodies against certain Salmonella, Rickettsia and Brucella pathogens present in human serum when tested in accordance with the recommended techniques stated in this IFU.

KIT DESCRIPTION

The bacterial suspensions are diluted in glycine buffer, pH 8.2 preservative. The controls consist of animal serum in preservative. The blue stained antigens are specific to the somatic "O" antigens and the red stained antigens are specific to the flagellar "H" antigens. Suspensions of Proteus OX2, OX19 and OXK are used to detect rickettsial antibodies. Each reagent is supplied in optimal dilution with all recommended techniques stated below without need for further dilution or addition. The reagents do not contain or consist of CMR substances, or endocrine disrupting substances or that could result in sensitisation or an allergic reaction by the user. For lot reference number and expiry date see **Vial Labels**.

STORAGE

Do not freeze. Reagent vials should be stored at 2 - 8ºC. Prolonged storage at temperatures outside this range may result in accelerated loss of reagent reactivity.

SPECIMEN COLLECTION

Specimens should be drawn without anticoagulant using approved phlebotomy techniques. Remove serum from clot by centrifugation. If testing is delayed the serum may be stored at 2-8ºC for up to 8 days or must be frozen at or below –20ºC for up to 3 months.

Do not use plasma or heat inactivated, grossly haemolysed, gross lipaemic or contaminated serum specimens.

PRECAUTIONS

1. The kit is for *in vitro* diagnostic use only.
2. Do not use kit past expiration date (see **Vial and Box Labels**).
3. Discard reagents if clumps or particles are present.
4. The reagents are light *sensitive* and must be stored in the dark.
5. Do not ingest or inhale aerosols, wash any splashes with copious amounts of water.
6. Protective clothing should be worn when handling the reagents, such as disposable gloves and a laboratory coat.
7. The reagents in these kits have been processed to reduce the bioburden but are not supplied sterile. Once a vial has been opened the contents should remain viable up until the expiry date.
8. No known tests can guarantee products derived from human or animal sources are free from infectious agents. Care must be taken in the use and disposal of each vial and its contents.

DISPOSAL OF KIT REAGENT AND DEALING WITH SPILLAGES

For information on disposal of reagents and decontamination of a spillage site see **Material Safety Data Sheets**, available on request.

CONTROLS AND ADVICE

1. It is recommended that known positive and negative controls be tested in parallel with each batch of tests i.e. Positive and Negative Febrile controls. Tests must be considered invalid if controls do not show expected results.
2. All the reagents must be allowed to reach 18-25ºC before use.
3. Shake the reagents gently before use to ensure homogeneity.
4. One drop using the dropper of the vial is approximately 50 µl.
5. The use of the reagents and the interpretation of results must be carried out by properly trained and qualified personnel in accordance with the requirements of the country where the reagents are in use.
6. The user must determine the suitability of the reagents for use in other techniques.
7. When testing for Brucella antibodies it is recommended to reduce the sample volume to 20 µl.
8. In some geographical areas with a high prevalence of febrile antibodies, it is recommended to dilute the sample ¼ in NaCl 9 g/L before performing the assay.

COMPONENTS SUPPLIED

See **Available reagent and kit sizes**.

MATERIALS AND EQUIPMENT REQUIRED BUT NOT SUPPLIED

- Serological Pipette.
- Small Plastic Test Tubes.
- Agglutination Slides.
- Mixing Sticks.
- 37ºC Incubator.
- 9 g/L saline solution.
- Mechanical rotator adjustable to 80-100 rpm.

SLIDE TECHNIQUE (qualitative test)

1. Pipette 50 µl of the sample to be tested and 1 drop of each control into separate circles on the slide (See 7 and 8 of **Controls and Advice).**
2. Add 50 µl of undiluted antigen suspension to each circle next to the sample to be tested.
3. Mix well using a disposable stirrer and spread the mixture over the entire area enclosed by the circle.
4. Place the slide on a mechanical rotator at 80-100 rpm for 1 minute.
5. Read the agglutination in each circle macroscopically immediately after the 1 minute rotation time.

SLIDE TITRATION TECHNIQUE

1. Pipette 80, 40, 20, 10 and 5 µl of undiluted sample to be tested into separate circles on the slide.
2. Pipette 50 µl of the undiluted antigen suspension next to the sample in each circle on the slide.
3. Mix the contents of each circle well using a disposable stirrer and spread the mixture over the entire area enclosed by the circle.
4. Place the slide on a mechanical rotator at 80-100 rpm for 1 minute.
5. Read the agglutination in each circle macroscopically immediately after the 1 minute rotation time.

INTERPRETATION OF SLIDE TITRATION RESULTS

1. Agglutination seen in any circle is indicative of the following results should a tube test be carried out:

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

2. In this way the slide titration test provides an approximation to the expected results from a corresponding tube test.
3. 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 TECHNIQUE (titration)

1. Label 8 small plastic tubes in a rack.
2. Using a pipette dispense 1.9 ml of 9 g/L saline solution 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 using the pipette, making sure not to create any air bubbles.
5. Dispense 1.0 ml from first tube into second tube and mix well.
6. Dispense 1.0 ml from second tube into third tube and mix well.
7. Continue this method of doubling dilutions up to the seventh tube and then discard 1.0 ml from the seventh tube.
8. Prepare one tube as a negative control by adding only saline and therefore should not contain any serum.
9. Add one drop of the appropriate antigen suspension into each tube and mix well.
10. Incubate the tubes as follows:
    - Somatic "O" antigens and Proteus for 4 hours at 50ºC (±1ºC).
    - Flagellar "H" antigens for 2 hours at 50ºC (±1ºC).
    - Brucella Antigen for 24 hours at 37ºC (±1ºC).
11. Examine the tubes after the appropriate incubation time and check for agglutination.

INTERPRETATION OF TUBE RESULTS

1. Tubes should be read immediately after the recommended incubation time to eliminate the possibility of false results.
2. A somatic reaction (O) is characterised by coarse, compact agglutination, which tends to be difficult to disperse, while flagellar reactions (H) have a characteristic loose, flocculant agglutination.
2. Last tube showing signs of agglutination should be taken as titre for that test. For negative results, all tubes should show no agglutination.Titration methods provide semi-quantitative results.
3. Partial or complete agglutination with a variable degree of clearing of the supernatant fluid is recorded as a positive.

REFERENCE RANGES

Salmonellas: Titers ≥ 1/80 (O antibodies) and ≥ 1/160 (H antibodies) indicates recent infection.
Brucellas: Titers ≥ 1/80 indicate infection.
Proteus: A great number of false positive reactions have been reported in healthy individuals with Proteus antigens, especially in slide agglutination test. A titer of less than 1/160 should not be considered significant.The level of "normal" agglutinins to these organisms varies in different countries and different communities. It is recommended that each laboratory establish its own reference range.

STABILITY OF THE REACTIONS

Slide tests should be interpreted immediately after the 1 minute rotation period to avoid the possibility that a negative result may be incorrectly interpreted as positive due to drying of the reagent.

LIMITATIONS

1. It has been found that many serotypes of salmonella possess somatic antigens of the same kind. Agglutination of any of the Salmonella antigens with human serum should not be taken as proof of infection by one organism, but rather than as an infection by an organism of a like antigenic structure.
2. A clinical diagnosis should not rely only on findings of a single test but a combination of clinical symptoms and laboratory data should be used.
3. Hemoglobin (≤ 10 g/L), bilirubin (≤ 20 mg/dL), lipaemia (≤ 10 g/L), rheumatoid factors (≤ 300 IU/mL) do not interfere. Other substances may interfere.
4. False positive or false negative results may also occur due to:
    - Contamination of test materials
    - Improper incubation time or temperature
    - Improper storage of test materials or omission of reagents
    - Deviation from the recommended techniques
    - Early disease, immune-unresponsiveness, prozone (Brucellosis) and antibiotic treatment (false negative results).

SPECIFIC PERFORMANCE CHARACTERISTICS

1. The reagents have been characterised by all the procedures mentioned in the **Recommended Techniques**.
2. Prior to release, each lot of Lorne Febrile Antigens is tested by the **Recommended Techniques** to ensure suitable reactivity.
3. There is no International Reference standard for the sensitivity standardisation of these reagents. That's why Lorne uses an internal control that contains animal serum with antibodies of Salmonellas, Brucellas and Proteus that is tested against commercially available reagents of certified potency.

DISCLAIMER

1. The user is responsible for the performance of the reagents by any method other than those mentioned in the **Recommended Techniques**.
2. Any deviations should be validated prior to use using established laboratory procedures.

BIBLIOGRAPHY

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5. Bradley D Jones. Annu Rev Immunol 1996; 14: 533 – 61.
6. J. Henny et al. Recommendation for the review of biological reference intervals in medical laboratories. Clin Chem Lab Med. 2016 Dec 1;54(12): 1893-1900.doi: 10.1515/cclm-2016-0793.

AVAILABLE REAGENT AND KIT SIZES

A. Febrile Antigens:

	Volume	Catalogue Number	Tests per Vial
Salmonella Typhi H	1x5 mL	502005A	100
Salmonella paratyphi AH	1x5 mL	504005A	100
Salmonella paratyphi BH	1x5 mL	506005A	100
Salmonella paratyphi CH	1x5 mL	508005A	100
Salmonella Typhi O	1x5 mL	510005A	100
Salmonella paratyphi AO	1x5 mL	512005A	100
Salmonella paratyphi BO	1x5 mL	514005A	100
Salmonella paratyphi CO	1x5 mL	516005A	100
Brucella abortus*	1x5 mL	518005A	100
Brucella melitensis	1x5 mL	520005A	100
Proteus OX2	1x5 mL	522005A	100
Proteus OX19	1x5 mL	524005A	100
Proteus OXK	1x5 mL	526005A	100

(*): Useful also for *Brucella suis* antibodies.

B. Febrile Antigen Kits:

	Kit Size	Catalogue Number
Febrile Bacterial Kit + Controls	8 x 5 mL** + 2 x 1mL	532042A

(**) Salmonella Typhi H, Salmonella paratyphi AH, Salmonella paratyphi BH, Salmonella paratyphi CH, Salmonella Typhi O, Salmonella paratyphi AO, Salmonella paratyphi BO, Salmonella paratyphi CO.

C. Febrile Antigen Controls:

	Size	Catalogue Number	Tests per vial
Febrile Positive Control	1 mL	536001A	10
Febrile Negative Control	1 mL	537001A	10

All stained febrile antigens are available in bulk quantities of 500 ml or 1 litre to special order.

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