BIOLABO
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MANUFACTURER:
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STAINED FEBRILE ANTIGENS

For Widal Felix tests

by tube and slide agglutination tests.

Available in individual vials:

REF 9905TH	1 x 5 mL	REF 9905TO	1 x 5 mL	REF 9905AH	1 x 5 mL	REF 9905BH	1 x 5 mL
REF 9905BA	1 x 5 mL	REF 9905BM	1 x 5 mL				
REF 9901NC	1 x 1 mL	REF 9901PC	1 x 1 mL				

Complete Kits:

REF 99058 1 x 5 mL of each: 9905TH, 9905TO, 9905AH, 9905AO, 9905BH, 9905BO, 9905CH, 9905CO

REF 9901NC 1 x 1 mL REF 9901PC 1 x 1 mL. (Controls are enclosed in each kits)

TECHNICAL SUPPORT AND ORDERS
Tel: (33) 03 23 25 15 50
support@biolabo.fr
Latest revision : www.biolabo.fr

CE

IVD

Made in France

I: corresponds to significant modifications

INTENDED USE

These reagents are designated for professional use in laboratory (manual method).
I Antigen suspensions are both for the identification and quantitative determination of specific antibodies in human sera following infection with certain Salmonellae and Brucellae pathogens.

GENERALITIES

It has been found that many serotypes of salmonella possess somatic antigens of the same kind. Therefore, agglutination of any of the salmonella antigens with human serum should not be taken as proof of infection by one particular organism, but rather as infection by an organism of a like antigen structure.
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 titer must be demonstrated not just a high titer for a one off test.

PRINCIPLE

Febrile antigens are suitable for both the rapid slide and tube agglutination tests against human sera for the detection of listed agglutinins.
Antigen suspensions are killed bacteria, stained to enhance the reading of agglutination. The blue stained antigens are specific to the somatic O antigens whilst the red stained antigens are specific to the flagellar H antigens.

REAGENTS

REF 9905TH	S. Typhi H (d.H)
REF 9905TO	S. Typhi O (9,12-O)
REF 9905AH	S. Paratyphi AH (a-H)
REF 9905AO	S. Paratyphi AO (1,2,12-O)
REF 9905BH	S. Paratyphi BH (b-H)
REF 9905BO	S. Paratyphi BO (1,4,5-O)
REF 9905CH	S. Paratyphi CH (c-H)
REF 9905CO	S. Paratyphi CO (6,7-O)
REF 9905BA	Brucella Abortus
REF 9905BM	Brucella Melitensis
REF 9901PC	Positive control
REF 9901NC	Negative control

SAFETY CAUTIONS

- Refer to current Material Safety Data Sheet available on request or on www.biolabo.fr
- Verify the integrity of the contents before use.
- Waste disposal: Respect legislation in force in the country.
- All specimens or reagents of biological origin should be handled as potentially infectious. Respect legislation in force in the country.

I Any serious incident that has occurred in connection with the device is notified to the manufacturer and the competent authority of the Member State in which the user and/or patient is based.

REAGENTS PREPARATION

Reagents are ready for use.

STABILITY AND STORAGE

Store at 2-8°C, in the original vial and away from light
Store Upright and do not Freeze.

- Reagents should be discarded if they become contaminated or if particles appears in suspension or do not demonstrate correct activity with the controls.
- When stored and used as described and free from contamination, reagents are stable upon expiry date stated on the label.

SPECIMEN COLLECTION AND HANDLING

Fresh serum. Haematic or lipaemic serum must be discarded.
Antibodies are stable in serum at least for 48 h at 2-8°C.

MATERIAL REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. Small test tubes and/or reactions slide with white background.

QUALITY CONTROL

- REF 9901NC, 9901PC: Negative and positive controls.
- External quality control program.

It is recommended to control in the following cases :

- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument.

If controls do not produce the proper reaction, apply following actions :
1. Repeat the test with the same control.
2. Repeat the test using a new vial of control.
3. Use new vials of reagent and repeat the test.

If controls still do not produce proper reaction, please contact BIOLABO technical support or your local Agent.

MANUAL PROCEDURE

Let stand reagents and specimens at room temperature before use.
Do not modify the test procedure.
Do not dilute or modify the reagents in any way.
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 titer for that test.
For negative results, all tests should remain clear of any agglutination.

1) Rapid slide titration

1. Dispense 80 µL, 40 µL, 20 µL, 10 µL and 5 µL of undiluted serum onto a row of 3 cm diameter circle.
2. Shake the reagent bottle well and add one drop (50 µL) of the undiluted antigen suspension to each dispensed volumes of serum.
3. Mix using a disposable stirring stick.
4. Rotate the slide and read after one minute.

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.

2) Quantitative agglutination Tube test

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

1. Label up 8 small plastic tubes in a rack.
2. Dispense 1,9 mL of 0.85 % saline solution into the first tube and 1 mL into the remaining seven.
3. Using a pipette, dispense 0,1 mL of the patient's undiluted serum into the first tube.
4. Dispense 1 mL from the contents of the first tube into the second tube and mix well.
5. Continue this method of doubling dilutions up to the seventh tube. The eighth tube will contain only saline solution as control and therefore should not contain any serum.
6. Shake the reagent bottle well and add one drop (50 µL) of the appropriate antigen suspension into each tube and mix well.
7. Incubate as follows :
   - Salmonella O antigens : 4 h at 50°C
   - Salmonella H antigens : 2 h at 50°C
   - Brucella antigens : 24 h à 37°C
8. Examine the tubes after the appropriate incubation time and check for agglutination.

It is vitally important that when the tubes are placed in the water bath, the level of the water should come to approximately 2/3 the way up the level of the tube content as this will maintain the convection currents within the tube and thereby obviate false results.

For a better reading, leave overnight in fridge, then let stand at room temperature before reading the test.

PERFORMANCES

The generally accepted performances of Widal Test using stained febrile antigens is 70% specificity and 70% sensibility.

Because serological tests in the diagnosis of Salmonellae infections have important limitations, cultures of appropriate specimens is usually preferred.

RESULTS

1- Rapide slide titration :

Read after 1 minute

Specimen volume	80 µL	40 µL	20 µL	10 µL	5 µL	50 µL CONT -	50 µL CONT+	50 µL CONT+ 1/2
Titer	1/20	1/40	1/80	1/160	1/320	-	-	+/-

2- Titration in tubes :

Tube	1	2	3	4	5	6	7	8
Titer	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	Negative

The last tube showing agglutination corresponds to the titer.
Titers in excess of 1/80 are probably significant

Notes

- Negative control should give negative result
- Positive control should give a positive result at a titer of 1/2 (+/- one double dilution)
- Agglutination of the antigen indicates the presence of antibody
- A comparison between samples taken 10-14 days apart may be of value in acute illness.

LIMITS

The use of specimen other than serum has not been validated for this test. A low or suspected positive result should be retest.
Diagnosis should not be made solely on finding of one clinical assay. When making an interpretation it is strongly recommended to take into account all clinical data.
Cross reactions between Brucella antigens and other organisms have been reported. These includes *Yersinia enterolitica*, *Escherichia coli* et *Francisella tularensis*. A prozone may occasionally occur with the slide procedure. If it is suspected, dilute the serum 1/20 in saline and retest.
Both Brucella abortus and Brucella melitensis share a common Brucella antigen. Specimen which give Positive result Rose Bengale, should be tested with REF 9905BA Brucella abortus and REF 9905BM Brucella melitensis suspension by slide test and confirmed by tube agglutination test to determine the type of Brucella antibodies detected. The higher titer detected determines the specific type of Brucella antibodies present.

REFERENCES

(1) Huddleson. I.F. and Bell (1928), J Infect. Dis. 42 242.
(2) Freter, R. (1980) Man of Cli. Imm. 2nd Ed. A.S.M. Washington DC,p.450-460.
(3) Weil E. and Felix A. Wein Klin., 29 974 (1916).
(4) Cruikshank, R (1965) Med Mic 11th Ed. 907

Manufacturer	Store upright	Expiry date	In vitro diagnostic	Storage temperature	Dematerialized water	Biological risk
Product Reference	See Insert	Batch number	Store away from light	Sufficient for	Dilute with	