Bacterial Antigens & Controls

Slide & Tube Agglutination

IVD CE

Presentation of stained Bacterial Antigens & Controls

PRODUCT CODE	DESCRIPTION
SF001	SALMONELLA H GROUP D ANTIGEN (S.Typhi H)
SF002	SALMONELLA H GROUP A ANTIGEN (S.Para Typhi AH)
SF003	SALMONELLA H GROUP B ANTIGEN (S.Para Typhi BH)
SF004	SALMONELLA H GROUP C ANTIGEN (S.Para Typhi CH)
SF005	SALMONELLA O GROUP D ANTIGEN (S.Typhi O)
SF006	SALMONELLA O GROUP A ANTIGEN (S.Para Typhi AO)
SF007	SALMONELLA O GROUP B ANTIGEN (S.Para Typhi BO)
SF008	SALMONELLA O GROUP C ANTIGEN (S.Para Typhi CO)
SF009	Salmonella (Febrile) Positive Control
SF010	Salmonella (Febrile) Negative Control
SF011	Febrile Antigen Kit comprising 6 specified antigens with positive & negative control (H, AH, BH, O, AO, BO)
SF012	BRUCELLA ABORTUS ANTIGEN
SF013	BRUCELLA MELITENSIS ANTIGEN
SF014	BRUCELLA POSITIVE CONTROL
SF018	Febrile Antigen Kit comprising 8 specified antigens with positive & negative control (H, AH, BH, CH, O, AO, BO,CO)
SF019	Febrile Antigen Kit comprising 4 specified antigens with positive & negative control (H, AH, BH, O)

INTENDED USE

This reagent is intended for *in vitro* qualitative & semi quantitative determination of Febrile Antibodies

This reagent is intended for *in vitro* qualitative & semi quantitative determination of Febrile Antibodies, Bio Research-stained antigen suspensions may be used to identify and quantitative determination of specific antibodies in human sera following infections with certain Salmonella and Brucella pathogens. Bio Research-stained febrile antigens are suitable for both the rapid and tube agglutination test against human sera for the detection of these agglutinins.

Bio Research-stained antigen suspensions are killed bacteria stained to enhance the reading of agglutination test; The blue stained antigens are specific to the somatic 'O' antigens whilst the red stained antigens are specific to flagellar 'H' antigens.

CLINICAL SIGNIFICANCE

Febrile diseases diagnostic may be assessed either by microorganism isolation in blood, stools or urine, or by titration of specific antibodies, somatic (O) and flagellar (H). The detection of these antibodies forms the basis for the long-established Widal test. This test dictates that a serum with high levels of agglutinating antibodies to O and H >1/100 is indicative of the infection with these microorganisms.

PRINCIPLE

When the colored, smooth Bio Research bacterial antigen suspension are mixed/incubated with patient serum, anti-salmonella antibodies present in the patient serum react with the antigen suspension to give agglutination.

Agglutination is a positive test result, indicating presence of anti-salmonella antibodies in the patient serum. No agglutination is a negative test result indicating absence of anti-salmonella antibodies

REAGENTS COMPOSITION

-Bacterial Antigens: Suspensions of Salmonellas, Brucellas and Proteus in glycine buffer, pH 8.2. Preservative

-Controls: Animal serum. Preservative

PREPARATION AND STABILITY

Antigen suspensions: Ready to use. It should be gently mixed before to use.
Controls: Ready to use

Reagent's deterioration: Presence of particles and clumps.

All the components of the kit are stable until the expiration date on the label when stored at 2-8°C. Do not freeze.

SPECIMEN AND SAMPLE PREPARATION

Use fresh serum obtained by centrifugation of clotted blood. The sample may be stored at 2-8°C for 48 hours before performing the test. For longer period of time the serum must be frozen. Haematic, lipaemic or contaminated serum must be discarded.

STORAGE

Store up right at 2-8°C. Light sensitive. Don't freeze Reagent should be discarded if they contaminated or do not demonstrate correct activity with the controls.

The reagents in each kit have been standardized to produce the proper reaction and reagents should not be interchanged with those from other batches.

PROCEDURE

SLIDE AGGLUTINATION METHOD (QUALITATIVE TEST)

1. Bring the reagent and samples to the room temperature. The sensitivity of the test may be reduced at low temperatures.
2. Place 50µL of sample to be tested and 1 drop of control into separate circle of the slide test.
3. Mix antigen vial vigorously before using, add 1 drop (50µL) of antigen to each circle.
4. Mix the contents of each circle with a separate stick and spread to the entire circle area.
5. Rotate the slide by hand for one minute and observe agglutination. If agglutination visible within one minute, then proceed slide titration.

RAPID SLIDE TITRATION

1. using a pipette dispense 0.08ml, 0.04ml, 0.02ml, 0.01ml and 0.005ml of undiluted serum on to a row of 3cm diameter circles.
2. Shake the reagent bottle well and add one drop of the undiluted antigen suspension to each serum aliquot.
3. Mix well using a stirring stick and rotate the slide.

Read after one minute

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

0.08ml=1:20, 0.04ml=1:40, 0.02ml=1:80

0.01ml=1:160, 0.005ml=1:320

In this way the rapid slide test provides an approximation to the expected result from a corresponding tube test.

NOTE: 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 my give positive results.

TUBE AGGLUTINATION TEST

1. Label up 8 small plastic tubes in a rack.
2. using a pipette, dispense 1.9ml of 0.85% saline into the first tube, and 1ml into the remaining seven.
3. using a pipette, dispense 0.1ml of the patient's undiluted serum into the first tube. Mix well using the larger pipette volume and tip (ie set to 1.0ml).
4. using the pipette, dispense 1ml from the first tube into the second tube. Mix well.
5. Continue this method of doubling dilutions up to the seventh tube. Discard 1.0ml from the seventh tube.

The eighth tube will contain only saline as a control and therefore should not contain any serum.

6. Shake the reagent bottle well and add 1 drop of the appropriate antigen suspension into each tube and mix well.
7. Incubate as follows:

•Salmonella "O" antigens and proteus =50°C for 4 hours
•Salmonella "H" antigens =50°C for 2 hours
•Brucella antigen =37°C for 24 hours
•Typhi VI =37°C for 2 hours and

Leave overnight in fridge, and then allow reaching room temperature before reading.

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

8. Examine the tubes after the appropriate incubation time and check for agglutination. The titer to be taken is the last tube to show agglutination.

INTERPRETATION OF RESULTS

•It has been found that many serotypes of salmonella possess somatic antigens of the same kind. Therefore, agglutination of any of the Salmonella antigen 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 antigenic structure.
•Tubes should be read after the recommended incubation time to eliminate the possibility of false result.
•The last tube showing signs of agglutination should be taken as the titer for that test. For negative results, all the tubes should remain clear of any agglutination.
•Titers in excess of 1:80 are usually significant, and may reflect recent infection. But low titers can be found in patients.
•DON'T INGEST OR INHALE AEROSOLS-WASH SPLASHES WITH COPIOUS AMOUNT OF WATER.

PERFORMANCE CHARACTERISTICS

The generally accepted performance capabilities of the Widal test using stained febrile antigens are 70% specificity and sensitivity. Because serological tests in the diagnosis of Salmonella infections have important limitation, cultures of appropriate specimens is usually preferred.

SYMBOL ON LABELS

Symbols	Signify	Symbols	Signify
REF	Catalogue Number	SIZE	Pack Size
	Expiry Date	VOL	Volume
	Storage Condition	LOT	Lot Number
	Instruction for Use	IVD	In Vitro Diagnostics
	Manufacturing Date		Manufacturer
	Number of Tests	2	For Single Use Only
EC REP	EC Representative	CE	European conformity

REFERENCES

1- Huddleson, IF and Bell (1928). Infect. Des,42 242
2- Freter , R, (1980) Man. Of Clin. Imm. 2nd Ed.A.S.M, Washington DC 460-453
3- Weil, E. and Felix, A Wein. Klin, 29 974 (1916)
4- Cruickshank, R. (1965) Med. Mic. 11th Ed, 907

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