

Phadebact® Salmonella Test

53-1134-02, May-06

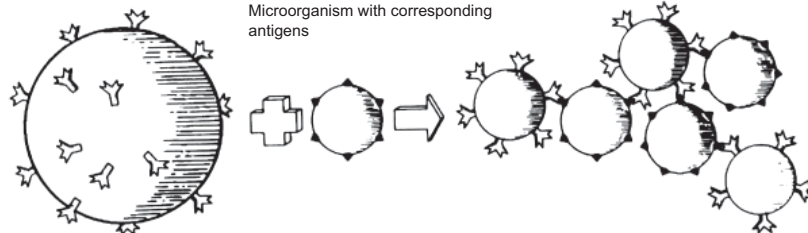
Directions for Use

Bactus AB
Lunastigen 3
SE-141 44 Huddinge
Sweden



Antibody-coated staphylococci

Co-agglutination



INTENDED USE

Phadebact® Salmonella Test is intended for the identification of Salmonella serogroups O:2, O:4, O:7, O:8, O:9 and O:3 (previously A, B, C, C₂₋₃, D and E).

SUMMARY AND EXPLANATION OF THE TEST

Members of the *Enterobacteriaceae* account for a major part of intestinal infections in humans and animals throughout the world. Although many of the *Enterobacteriaceae* have been implicated as a cause of diarrhea, only members of the general *Escherichia*, *Salmonella*, *Shigella* and *Yersinia* are clearly established as enteric pathogens (1).

Human infections with Salmonella can cause various clinical symptoms and involve both intestinal and extraintestinal sites (2). The most common syndrome is that of uncomplicated enterocolitis, in which after an incubation period of 8-48h, the typical patient experiences nausea, vomiting, cramps, diarrhea and fever. Following recovery, some patients may continue to excrete organisms for weeks or months, while carriage for a period of years is uncommon. Salmonellosis is a primary foodborne disease that is more common in summer. Over 40,000 (1987) isolates are reported from humans in the United States each year. Since these isolates are estimated to represent approximately 1% of the people clinically ill - over 4 million cases of symptomatic salmonellosis may occur annually in the United States (3), (4).

The identification of Salmonella is important for both clinical and epidemiological implications. Primarily bacteria belonging to the family *Enterobacteriaceae* are identified by testing for biochemical reactions. Serogroup confirmation of Salmonella isolates is based on somatic O-antigen testing and performed by most clinical laboratories. Complete serological testing, including typing (based on flagellar or capsular antigens) can be obtained by forwarding the isolate to a reference laboratory. Of identified human Salmonella infections about 98% belong to serogroups O:2, 4, 7, 8, 9 and 3 (5). Over 1800 Salmonella serotypes have been reported but 10 serotypes account for over 70% of those isolated from humans (6).

Using conventional polyclonal adsorbed antisera particular care must be taken in the biochemical identification of isolates because of serological crossreactions with other *Enterobacteriaceae*.

Phadebact® Salmonella Test is based on the co-agglutination technique.

Serogroup O-antigens, di- or trisaccharides have been synthesized and used as immunogens for production of highly specific serogroup antisera, thus avoiding the absorption step, giving a reliable and rapid identification. A minimum of laboratory equipment and no special education of laboratory personnel is needed.

PRINCIPLE OF THE PROCEDURE

Phadebact® Salmonella Test is a co-agglutination test containing six Salmonella reagents including serogroups O:2, 4, 7, 8, 9 and 3 respectively. The reagents are composed of highly specific rabbit antibodies which are coupled to the Protein A of non-viable Staphylococci (7). When a drop of suspension containing any of the Salmonella serogroups above is mixed with the corresponding reagent, the specific O-antigen determinants of the cell wall will bind to the antibodies on the surface of the Staphylococci. In this way a co-agglutination lattice is formed which is visible to the naked eye.

REAGENTS

Each Phadebact® Salmonella Test package contains 2 mL reagents sufficient for 50 determinations. The reagents are coloured blue (Methylene blue) to facilitate interpretation of results.

Reactive ingredients

- Salmonella Group O:2 Reagent
Specific antibodies raised in rabbit, bound to non-viable staphylococci, 1 vial.
- Salmonella Group O:4 Reagent
Specific antibodies raised in rabbit, bound to non-viable staphylococci, 1 vial.
- Salmonella Group O:7 Reagent
Specific antibodies raised in rabbit, bound to non-viable staphylococci, 1 vial.
- Salmonella Group O:8 Reagent
Specific antibodies raised in rabbit, bound to non-viable staphylococci, 1 vial.
- Salmonella Group O:9 Reagent
Specific antibodies raised in rabbit, bound to non-viable staphylococci, 1 vial.
- Salmonella Group O:3 Reagent
Specific antibodies raised in rabbit, bound to non-viable staphylococci, 1 vial.

Other components

- Droppers
- Disposable slides
- Directions for Use

Precautions

For *in vitro* diagnostic use.

Warning! The reagents contain sodium azide (NaN₃) as a preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. Please refer to decontamination procedures as outlined by CDC.

Preparation of reagents

The reagents are READY TO USE.

Shelf life and storage

The expiration date is stated on the outer label and on the vials. It is recommended that the kit be stored at 2-8°C. Reagents must be protected from freezing.

SPECIMEN COLLECTION AND HANDLING

Please refer to a standard microbiology textbook regarding information on preparation of primary cultures. After inoculation, the media should be incubated at 35-37°C for 18-24 hours (8). If the primary plate contains enough colonies suspected of being *Salmonella* the co-agglutination can be performed directly. If otherwise, transfer colonies of typical appearance to a nutrient slant and inoculate for another 18-24 hours. There are several suitable media of varying selectivity for primary plating which allow certain enteric pathogenic bacteria to grow and which inhibit the growth of Gram positive and some Gram negative bacteria (8). These media also permit initial differentiation of bacteria by colony morphology (Table 1). Colonies suspected of being *Salmonella* may then be tested with Phadebact® *Salmonella* Test for detection:

- Prepare a heavy suspension of bacteria in 500µl saline or PBS (phosphate buffered saline).
- Drops of this suspension are used for testing.

Table 1.

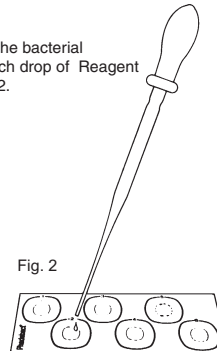
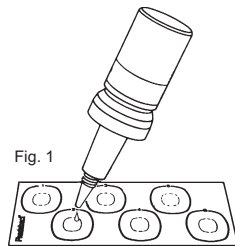
Organism	MacConkey Agar	XLD Agar	Deoxycholate citrate Agar	Bismuth Sulfite Agar	SS Agar	Brilliant Green Agar
<i>Shigella</i>	convex, colourless, 2-3 mm	red, smooth 1-2 mm	colourless, translucent, 2-3 mm	-	colourless, translucent 1-2 mm	-
<i>Salmonella</i>	convex, colourless, 2-3 mm	red, black centre, 1-2 mm	black centre on red colony	black centre, translucent edge, black halo round the colony, metallic sheen (48h)	colourless, translucent 1-2 mm	pink-white, opaque 1-3 mm
<i>S. typhi</i>	convex, colourless, 2-3 mm	red, smooth, colourless	0.5-1 mm with black sheen often with central grey dot	black colonies round (48h)	colourless, translucent 1-2 mm	-
<i>E. coli</i>	red, 2-3 mm	opaque, yellow, colonies	few pink umbilicated colonies, 1-2 mm	-	few small pink colonies	-
<i>Y. enterocolitica</i>	colourless or light pink, 1-2 mm (48h)	-	-	-	colourless, translucent 0.5 mm (48h)	-

Test protocol

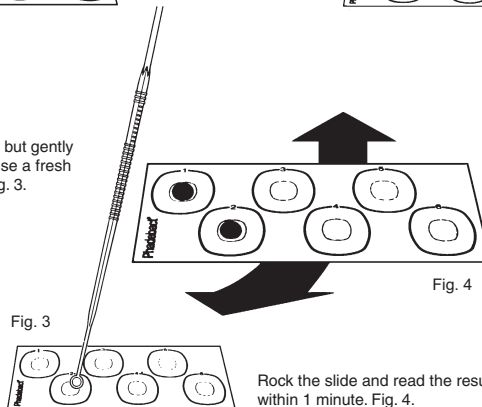
Note! Suspend reagents thoroughly by shaking or vortexing.

Put one drop of each *Salmonella* group reagent on their marked places on the slide. Fig. 1.

Add one drop of the bacterial suspension to each drop of Reagent on the slide. Fig. 2.



Mix the drops thoroughly but gently with a disposable loop. Use a fresh loop for each reagent. Fig. 3.



Rock the slide and read the result within 1 minute. Fig. 4.

Stability of the final reaction mixture

The co-agglutination is stable but good laboratory practice dictates that the result be read within 1 minute while the mixture is still wet (observe the risk of drying out of reagents which may be misinterpreted as a reaction).

Calibration

No calibration is needed.

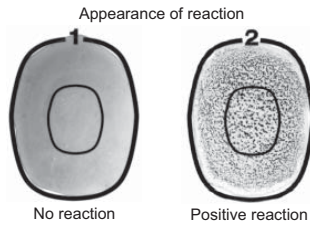
Quality control

Positive control

As a control an established *Salmonella* Group O:2, 4, 7, 8, 9 and 3 strain should be used. The control strain is treated in an identical manner as the unknown bacterium in the test procedure.

Negative control

By simultaneous use of all six Reagents when testing an unknown organism a negative control is built in.



RESULTS

Positive result

A blue precipitate, co-agglutination, seen in one of the six Reagents with no or a very weak reaction in the remaining five Reagents confirms the identity of the specimen as Salmonella Groups O:2, 4, 7, 8, 9 or 3.

Negative result

A very weak or no reaction in all six Reagents constitutes a negative result. A negative result strongly suggests that the bacterium tested is not a Salmonella of serogroups O:2, 4, 7, 8, 9 or 3.

LIMITATIONS OF THE PROCEDURE

- The O:11 (previously F) and O:54 strains did agglutinate in the O:3 reagent. The O:54 reaction is reciprocal (i.e. absorbable) and documented in literature. The O:11 reactivity is relatively weak but distinctive.
- As with all diagnostic tests, a definitive clinical diagnosis should not be based on the result of a single test, but should only be made by the physician after the clinical and laboratory findings have been evaluated.
- The high sensitivity of the test does not exclude the possibility of false negative results.

PERFORMANCE CHARACTERISTICS

Sensitivity and specificity

In total 826 specimens consisting of both clinical isolates and reference strains were investigated (9). The identity of a clinical isolate was assigned to genus/species level by conventional biochemical and serological tests.

The overall performance of Phadebact[®] Salmonella Test was as follows:

Salmonella group	No strains	No positive in CoA					
		O:2	O:4	O:7	O:8	O:9	O:3
O:2	18	18	0	0	0	0	0
O:4	118	0	118	0	0	0	0
O:7	64	0	0	64	0	0	0
O:8	70	0	0	0	70	0	0
O:9	77	0	0	0	0	77	0
O:3	47	0	0	0	0	0	47
O:11	3	0	0	0	0	0	3
O:13-O:50	34	0	0	0	0	0	0
O:51-O:53	3	0	0	0	0	0	0
O:54	1	0	0	0	0	0	1
O:55-O:67	10	0	0	0	0	0	0
Others non O:2,4,7,8,9,3	5	0	0	0	0	0	0
<i>E. coli</i>	200	0	0	0	0	0	0
Other fecal isolates ¹⁾	176	0	0	0	0	0	0

¹⁾Including *Citrobacter* sp., *Morganella* sp., *Proteus* sp., *Serratia* sp., *Shigella* sp.

In total, 394 Salmonella O:2, 4, 7, 8, 9, 3 and 432 other isolates were tested with a sensitivity of 100.0% (394/394) and a specificity of 99.1% (428/432).

WARRANTY

The performance data presented here was obtained using the procedure indicated. Any change or modification in the procedure not recommended by Bactus AB may affect the results, in which event Bactus AB disclaims all warranties, expressed, implied or statutory, including the implied warranty of merchantability and fitness for use. Bactus AB and its authorized distributors, in such event, shall not be liable for any damages, whether direct, indirect or consequential.

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- Data on file, Bactus AB.

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Phadebact® CSF Positive Controls
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