Phadebact® Salmonella Test

Directions for Use

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Antibody-coated staphylococci

Microorganism with corresponding antigens

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, C1, C2, 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 Veronia are clearly established as enteric pathogens.

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 cross-reactions with other Enterobacteriaceae.

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

Serogroup O-antigens, di- or trisacharides have been synthesized and used as immunogens for production 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.

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). A minimum of laboratory equipment and no special education of laboratory personnel is needed.

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 incubate 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).
• Drop this suspension on the slide and read the result within 1 minute.

Table 1.

<table>
<thead>
<tr>
<th>Organism</th>
<th>MacConkey Agar</th>
<th>XLD Agar</th>
<th>Deoxycholate Citrate Agar</th>
<th>Bismuth Sulphite Agar</th>
<th>SS Agar</th>
<th>Brilliant Green Agar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shigella</td>
<td>convex, colourless, 2-3 mm</td>
<td>red, smooth 1-2 mm</td>
<td>colourless, translucent, 2-3 mm</td>
<td>black centre on red colony</td>
<td>colourless, translucent 1-2 mm</td>
<td>-</td>
</tr>
<tr>
<td>Salmonella</td>
<td>convex, colourless, 2-3 mm</td>
<td>red, black centre, 1-2 mm</td>
<td>black centre on red colony</td>
<td>black centre, translucent edge, black halo round the colony, metallic sheen (48h)</td>
<td>colourless, translucent 1-2 mm</td>
<td>pink-white, opaque 1-2 mm</td>
</tr>
<tr>
<td>S.typhi</td>
<td>convex, colourless, 2-3 mm</td>
<td>red, smooth, colourless, 0.5-1 mm with black sheen on colony, metallic sheen (48h)</td>
<td>black colonies round (48h)</td>
<td>colourless, translucent 1-2 mm</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>red, 2-3 mm</td>
<td>opaque, yellow, colonies</td>
<td>few pink umbilicated colonies, 1-2 mm</td>
<td>-</td>
<td>few small pink colonies</td>
<td></td>
</tr>
<tr>
<td>Y. enterocolitica</td>
<td>colourless or light pink, 1-2 mm (48h)</td>
<td>-</td>
<td>-</td>
<td>colourless, translucent 0.5 mm (48h)</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th>Fecal isolates group</th>
<th>No of strains</th>
<th>O:2</th>
<th>O:4</th>
<th>O:7</th>
<th>O:8</th>
<th>O:9</th>
<th>O:3</th>
</tr>
</thead>
<tbody>
<tr>
<td>O:2</td>
<td>18</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>O:4</td>
<td>118</td>
<td>0</td>
<td>118</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>O:7</td>
<td>64</td>
<td>0</td>
<td>0</td>
<td>64</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>O:8</td>
<td>70</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>70</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>O:9</td>
<td>77</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>77</td>
<td>0</td>
</tr>
<tr>
<td>O:3</td>
<td>47</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>47</td>
<td>0</td>
</tr>
<tr>
<td>O:11</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>O:13-O:50</td>
<td>34</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>O:51-O:53</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>O:54</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>O:55-O:67</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Others non O:2,4,7,8,9,3</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>E. coli</td>
<td>200</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other fecal isolates</td>
<td>176</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

1) Including Citrobacter sp., Morganella sp., Proteus sp., Seratia 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 MKL Diagnostics AB may affect the results, in which event MKL Diagnostics AB disclaims all warranties, expressed, implied or statutory, including the implied warranty of merchantability and fitness for use.

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Bibliography:
9. Data on file, MKL Diagnostics AB.
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Phadebact® Strep F Test
Phadebact® Strep Positive Controls
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