Oxoid Salmonella Test Kit

**1. INTRODUCTION**

The genus Salmonella consists of over 2,400 serotypes, which may cause acute infectious gastroenteritis, frequently associated with food poisoning. Since many Salmonella species infect domestic animals, either clinically or subclinically, cases of Salmonella food poisoning usually originate from animal sources. In contrast, S. typhi and S. paratyphi only infect humans and cause enteric fever. Salmonellae are often present in a sample in small numbers, together with other Enterobacteriaceae. In order to promote the growth of Salmonella, enrichment and/or selective media are used, such as Rappaport Medium, broths for inducing motility (e.g. M broth), and those containing selenite, (RV- CM0669, RV5-CM0866) deoxycholate citrate (DCA – CM0035) or bismuth sulphite.1,2

**2. INTENDED USE**

The Oxoid™ Salmonella Test Kit (DR1108A) is a rapid latex agglutination test for the presumptive identification of Salmonella in selective and/or enrichment cultures. The use of latex technology makes this test more sensitive than direct agglutination methods, so that use of the Oxoid Salmonella Test Kit (DR1108A) is particularly suited to screening cultures due to the high predictive value of a negative result. When screening clinical specimens, these features enable decisions on appropriate patient management to be taken promptly.

Salmonella was confirmed when the reaction card is clean and dry before use. The positive control suspension should be used periodically to check the correct working of the latex reagents. Use a loopful of the non-viable positive control suspension as the test sample. The test latex must give a positive reaction within 30 seconds.

**3. PRINCIPLE OF THE TEST**

Polyvalent antisera are prepared against a wide range of Salmonella flagellar antigens and used to coat latex particles. When mixed with a suspension of Salmonella containing these antigens, the latex particles rapidly agglutinate to form visible clumps. The Oxoid Salmonella Test Kit (DR1108A) detects the majority of common Salmonella species including S. typhimurium and S. enteritidis. To minimise cross reactions with other Enterobacteriaceae, antibodies to the principal somatic antigens are removed during preparation of the antiserum. Consequently, the Oxoid Salmonella Test (DR1108A) reacts predominantly with flagellar antigens although the reagent will react with the non-motile species S. pullorum and S. gallinarum.

**4. MATERIALS SUPPLIED**

The reagents supplied are for **in vitro diagnostic use only**.

Salmonella Latex Reagent (1101): Latex particles coated with rabbit antiserum against Salmonella antigens, preserved with 0.09% merthiolate. 5.0ml

0.85% isotonic saline (1098), preserved with 0.09% sodium azide. 5ml

Positive Control Suspension (FT206): A suspension of non-viable Salmonella preserved with 3% formalin (0.5ml)

Dispersable reaction cards

Additional Requirements

Dispensable mixing sticks

Pasteur pipettes

**5. STORAGE**

The Oxoid Salmonella Test Kit (DR1108A) should be stored at 2-8°C.

**6. PRECAUTIONS**

- Appropriate precautions should be taken when working with potential pathogens.
- Do not mouth pipette.
- Discarded reagents and materials must be autoclaved before disposal.
- Do not cross contaminate solutions or samples.
- Do not use after expiry date printed on outside of carton.
- Ensure the reaction card is clean and dry before use.
- The isotonic saline (1098) is preserved with sodium azide which can form potentially explosive compounds on contact with copper and lead plumbing. On disposal of saline, flush with copious quantities of water to prevent azide build up.

**7. METHOD OF USE**

Allow the Oxoid Salmonella Test Kit (DR1108A) reagents to reach room temperature before use.

### Specimens

Selective and/or enrichment broths or solid media can be tested with the Oxoid Salmonella Test Kit (DR1108A). The recommended procedure for optimum performance of the Kit is shown below:

<table>
<thead>
<tr>
<th>Food samples</th>
<th>Homogenise in 225 ml Resuscitation Broth (e.g. BPW (ISO) CM0109)</th>
<th>Incubate @ 35°C for 18 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) 1 ml in 10 ml Selective Broth</td>
<td>(e.g. MXTm-CM0148-SR0181 or Mntnle Somatic Broth CM0399)</td>
<td>or</td>
</tr>
<tr>
<td>b) 1 ml in 100 ml (or 0.1 ml in 10 ml) of Rappaport Broth</td>
<td>(e.g. RV-CM0669, RV5-CM0866)</td>
<td>Incubate @ 35°C for 18 hours</td>
</tr>
<tr>
<td>Subculture 1 ml in 10 ml M broth</td>
<td>Incubate @ 35°C for 6 hours</td>
<td>Mix and test with the Oxoid Salmonella Test Kit (DR1108A)</td>
</tr>
</tbody>
</table>

**8. PROCEDURE**

### A. Method for Testing Broths

1. Mix the broth culture by gently inverting, and remove 1 drop of culture using a Pasteur pipette.
2. Place a single drop of the broth culture within one circle on the reaction card.
3. Mix the Oxoid Salmonella Latex Reagent (1101) by gently inverting and add 1 drop to the broth on the card. **DO NOT ALLOW THE DROPPER TO TOUCH THE DROP OF CULTURE.** Mix thoroughly using a clean mixing stick or inoculating loop. Gently rock the reaction card 2 or 3 times. Extensive rocking of the slide is not necessary.
4. Examine for agglutination within a maximum of 2 minutes.
5. After reading dispose of the reaction cards in disinfectant. Note: When testing food powders cultured in selective enrichment broths, ensure that the food product alone in enrichment broth does not cause non-specific agglutination. Place one drop of food product in enrichment broth on a reaction card. Observe for any agglutination or clumping which would indicate autoagglutination. Such products cannot be tested with the Oxoid Salmonella Test Kit (DR1108A).

### B. Method for Identification from Selective Solid Media

1. Place a reaction card on the work bench.
2. Add 1 drop of saline (1098) within one circle on the reaction card.
3. Using a mixing stick or inoculating loop emulsify 2-3 mm of suspect colonies in the drop of saline to produce a thick smooth suspension. Suspensions should be made from colonies with morphologies resembling Salmonella species.
4. Observe the suspension for any agglutination or clumping which would indicate autoagglutination. If the suspension remains smooth, proceed to Step 5. (See Limitations of Use, point 1.)
5. Mix the Oxoid Salmonella Latex Reagent (1101) gently by inverting and add a drop to the saline suspension. **DO NOT ALLOW THE DROPPER TO TOUCH THE ORGANISM SUSPENSION.** Mix the Latex Reagent and organism suspension with a clean mixing stick for 30 seconds and gently rock the reaction card two or three times. Excessive rocking of the slide is not necessary. Examine for agglutination within a maximum of two minutes.
6. After reading, discard the used reaction cards into a suitable disinfectant.

**9. INTERPRETATION**

Agglutination within two minutes is a positive result and indicates the presence of Salmonella in the sample. Care must be taken when testing broth cultures that adherence of latex to particulate matter is not interpreted as agglutination. Absence of agglutination indicates that Salmonella is not present in the test culture.

**10. LIMITATIONS OF USE**

- Rough strains of Salmonella are known to cause non-specific autoagglutination in saline alone and therefore cannot be tested with the Oxoid Salmonella Test Kit (DR1108A).
- Some non-motile strains may not be detected by the Oxoid Salmonella Test Kit (DR1108A).
- Some oxidase-positive organisms may give false positive reactions.
- Old stock cultures of Enterobacteriaceae on Nutrient Agar slopes may cause non-specific agglutigation, whereas old stocks of Salmonella may give false negative results. Fresh subcultures should be prepared for testing.
- Identification with the Oxoid Salmonella Test Kit (DR1108A) is presumptively and all positive results should be confirmed by subculture of broths, further confirmatory identification tests and serotyping of pure cultures.

**11. POSITIVE CONTROL SUSPENSION**

The positive control suspension should be used periodically to check the correct working of the latex reagents. Use a loopful of the non-viable positive control suspension as the test sample. The test latex must give a positive reaction within 30 seconds.

**12. PERFORMANCE CHARACTERISTICS**

561 Salmonella isolates were thoroughly characterised by serotyping. All these organisms, as well as 359 isolates representing 13 different non-Salmonella bacteria, were cultured prior to testing with the Oxoid Salmonella Test Kit (DR1108A) at an independent laboratory. Results are shown below:

<table>
<thead>
<tr>
<th>Oxoid Salmonella Test Kit</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>561</td>
</tr>
<tr>
<td>Salmonella -</td>
<td>10</td>
</tr>
<tr>
<td>349</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>571</td>
</tr>
<tr>
<td>349</td>
<td></td>
</tr>
<tr>
<td>920</td>
<td></td>
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</table>

The performance characteristics of The Oxoid Salmonella Test Kit (DR1108A) were: Sensitivity 100.0% and Specificity 97.2%.

The predictive value of a positive result was 98.2% and the predictive value of a negative result was 100.0%.

**Centre 2**

64 food samples suspected of Salmonella contamination were tested for Salmonella at a Food Hygiene laboratory. Homogenised food was incubated for 18 hours in Resuscitation Broth, followed by 18 hours in selective enrichment broth (Cystine mannitol selenite or Rappaports Broth). Broths were subcultured onto selective agar plates for 24-48 hours. Colonies resembling Salmonella were tested with the Oxoid Salmonella Test Kit (DR1108A), or by classical biochemical tests and serotyping.

Results are shown below:

<table>
<thead>
<tr>
<th>Oxoid Salmonella Test Kit</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>53</td>
</tr>
<tr>
<td>Salmonella confirmed by biochemical tests and serotyping</td>
<td>0</td>
</tr>
<tr>
<td>% Sensitivity</td>
<td>100%</td>
</tr>
<tr>
<td>% Specificity</td>
<td>100%</td>
</tr>
</tbody>
</table>

The predictive value of both negative and positive results was 100%
13. REFERENCES

1. Data on file at Oxoid.

2. Personal Communication from Dr A. C. Baird-Parker.

3. Poster presentation: “Validation of a Latex Agglutination Method for Confirmation of Salmonella colonies from Six Selective Media”, I. Fairlamb, T. Organ, Oxoid Ltd., Wade Road, Basingstoke, Hampshire RG24 8PW.

4. Poster presentation: “Evaluation of the Oxoid Salmonella Latex Test as a Screening Test for Salmonella”, A. M. Paccagnella, Provincial Laboratory, BC Centre for Disease Control, Vancouver, Canada.

Symbol Legend

<table>
<thead>
<tr>
<th>Symbol</th>
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<tr>
<td>REF</td>
<td>Catalogue Number</td>
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<tr>
<td>IVD</td>
<td>In Vitro Diagnostic Medical Device</td>
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<td>I</td>
<td>Consult Instructions for Use (IFU)</td>
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<tr>
<td>&quot;N&quot;</td>
<td>Temperature Limitations (Storage temp.)</td>
</tr>
<tr>
<td>&quot;N&quot;</td>
<td>Contains sufficient for &lt;N&gt; tests</td>
</tr>
<tr>
<td>LOT</td>
<td>Batch Code (Lot Number)</td>
</tr>
<tr>
<td>&quot;N&quot;</td>
<td>Use By (Expiration Date)</td>
</tr>
<tr>
<td>&quot;N&quot;</td>
<td>Manufactured by</td>
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</tbody>
</table>

Oxoid Limited, Wade Road, Basingstoke, Hampshire, RG24 8PW, UK

SISTEMAS AVANZADOS DE ANÁLISIS, S.L.
CIF: B-47700026
C/ Cardenal Torquemada, 24
Tel. 983 251 143 • 637 596 017
47010 VALLADOLID
www.analisisonvanzados.com