Evaluation of Selenite F broth as an enrichment step for the isolation of *Salmonella* and *Shigella* in clinical faecal specimens – A retrospective study

**Arleen Donaldson and Mackenzie Nicol**  
Aotea Pathology, Wellington

**ABSTRACT**

**Objectives:** To investigate whether enrichment of clinical faecal specimens in Selenite F broth, for the cultivation of *Salmonella* and *Shigella*, should be discontinued in the community laboratory setting.

**Methods:** A retrospective analysis of all faecal specimens submitted to Aotea Pathology Limited for bacterial culture over a two-year period was analysed using the Labsolutions statistics software. Samples positive for either *Salmonella* or *Shigella* were analysed as being isolated from either the direct Hektoen agar plate, isolated on secondary XLD agar plate following enrichment in Selenite F broth, or both.

**Results:** Direct culture on Hektoen agar yielded a recovery rate of 39% of the *Salmonella* isolated and 67% of the *Shigella* isolated. Secondary sub culture on XLD agar following enrichment in Selenite F broth yielded a recovery rate of 99% of the *Salmonella* isolated and 50% of the *Shigella* isolated.

**Conclusions:** This study shows that enrichment in Selenite F broth should continue to be used and a combination of direct plating and sub-culturing is required in order to maximise the recovery rate of both *Salmonella* and *Shigella* as well as to provide early detection. If enrichment was to be discontinued, potentially a significant proportion of *Salmonella* and *Shigella* isolates may have been missed (61% and 33% respectively).

**Key words:** *Salmonella* F broth, *Salmonella*, *Shigella*.

**INTRODUCTION**

*Salmonella* and *Shigella* are members of the *Enterobacteriaceae* family and are non-lactose fermenting (NLF) Gram-negative bacilli causing gastrointestinal illness in humans and are primarily acquired through ingestion of contaminated food and water (1). *Salmonella* can cause enteritis or enteric fever and the severity of disease depends on the inoculating dose, serotype and predisposing host factors (1, 2). *Shigella* causes dysentery where symptoms include fever, abdominal cramps, bloody diarrhoea and haemolytic uremic syndrome, the most serious complication of shigellosis (2).

Diagnosis in medical laboratories is mainly by faecal culture, however, molecular testing methods have become available in recent years (2). At Aotea Pathology the protocol for the cultivation and isolation of *Salmonella* and *Shigella* from clinical faecal specimens involved directly plating faeces onto Hektoen enteric agar (half plate) and sub-culturing onto Xylose Lysine Deoxycholate agar (XLD) following enrichment in Selenite F broth after overnight incubation (All media supplied by Fort Richard Laboratories Limited). Historically, enrichment broths have been a critical step in enhancing the growth of some pathogens while inhibiting other commensal bacteria and are commonly used in combination with direct plating in medical laboratories to ensure recovery of faecal pathogens from diarrhoeal patients (3). It has been suggested that enrichment broths be used only during outbreaks, or for screening asymptomatic carriage, and that it is not a requirement in hospital laboratories as it’s primary role is to diagnose acute phase of disease (4, 5). Aotea Pathology is also required to diagnose acute diarrhoea. The use of enrichment broths has been discontinued in some laboratories as the yield does not justify the cost and it has been suggested that individual laboratories should look through historic data to help determine whether enrichment broths should remain to be used or discontinued (2).

The aim of this study was to compare direct culture isolates on Hektoen agar to subculture isolates on XLD agar following enrichment in Selenite F broth and to evaluate whether enrichment in Selenite F broth should be discontinued. This was achieved by doing a retrospective study on all positive *Salmonella* and *Shigella* isolated from these media at Aotea Pathology over a two year period.

**METHODS**

A retrospective study comparing direct faecal culture isolates on Hektoen agar vs sub-cultured isolates on XLD agar after enrichment in Selenite F broth for the isolation of *Salmonella* and *Shigella* from clinical faecal specimens received at Aotea Pathology over a two year period (1 January 2013- 31 December 2014) was performed using the Statistics software linked to the Laboratory Information System, Labsolutions.

Positive samples for *Salmonella* or *Shigella* within the chosen timeframe were searched and analysed as to whether the isolate had been isolated from either direct culture, on subculture following the enrichment step, or both. A total of 190,864 faecal specimens were requested for bacterial culture in the two year period and of these, 172 were positive for either *Salmonella* or *Shigella*. Positive isolates from External Quality Assurance samples were also included in this study.
All media was incubated 18-24 hours at 35°C in O2, prior to being sub-cultured or read. NLF colonies on either Hektoen or XLD underwent an intermediate step involving a purity plate onto MacConkey agar and subbed onto a urea slope and incubated 18-24 hours at 35°C in O2. Isolates that were urease negative were then identified by API10S and or API20E (Biomerieux) and serology testing with antisera Salmonella polyvalent O (A-S), Salmonella polyvalent H (phase 1 and 2), and Shigella sonnei (phase 1-2) (manufactured by Remel) was performed on isolates that had included Salmonella spp or Shigella spp in the API profile. Further characterisation and speciation of all positive isolates was performed by Environmental Science and Research Limited laboratory (ESR) at Wallaceville. All media used was manufactured and obtained from Fort Richard Laboratories Limited where they obtain their reagents to make up media from Becton Dickinson.

### RESULTS

There were a total of 154 Salmonella isolates and of these, 2 were recovered from direct culture only on Hektoen agar compared to 94 on XLD agar only (following enrichment in selenite F broth) and 58 were isolated on both direct and subculture plates (Table 1). There were a total of 18 Shigella isolates and of these, 9 were recovered from direct culture only on Hektoen agar compared to 6 from subculture onto XLD agar only (following enrichment in selenite F broth) and 3 were isolated on both direct and subculture plates. When comparing direct culture with subculture only, 60 Salmonella isolates were recovered on Hektoen compared to 152 on XLD (following enrichment) and 12 Shigella isolates were recovered on Hektoen compared to 9 on XLD (following enrichment).

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Direct Hektoen agar only</th>
<th>Subcultured XLD only (following enrichment)</th>
<th>Both direct and subculture plates</th>
<th>Total on Hektoen</th>
<th>Total on XLD (following enrichment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella (Total of 154)</td>
<td>Isolated</td>
<td>2</td>
<td>94</td>
<td>58</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Not isolated</td>
<td>152</td>
<td>60</td>
<td>96</td>
<td>94</td>
</tr>
<tr>
<td>Shigella isolates (Total of 18)</td>
<td>Isolated</td>
<td>9</td>
<td>6</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Not isolated</td>
<td>9</td>
<td>12</td>
<td>15</td>
<td>6</td>
</tr>
</tbody>
</table>

The recovery rate on Hektoen agar was 39% for Salmonella and 67% for Shigella whereas the recovery rate on XLD agar yielded 99% for Salmonella and 50% for Shigella. There is a slightly higher recovery rate of Shigella on Hektoen agar in comparison to XLD but a third of all Shigella isolates recovered were not isolated on Hektoen (Table 2).

### DISCUSSION

The practice of using enrichment broths as part of routine culture has been discontinued in some laboratories and it has been suggested that analysis of historic data should be performed to determine whether enrichment broths should remain in use or be discontinued (2). A previous study evaluating Gram-negative enrichment in faecal cultures showed that the use of Gram-negative enrichment broth was at least as good as direct plating as theoretically, direct plating on both Hektoen and XLD would allow selective isolation of Salmonella and Shigella, making the use of Gram-negative broth unnecessary (4,5).

The aim of this study was to evaluate the possibility of discontinuing the use of Selenite F broth at Aotea Pathology by looking through historic data and comparing direct culture isolates to isolates isolated following subculture from enrichment broth. The results show that enrichment in Selenite F broth potentially maximises the recovery of Salmonella in faecal specimens. The recovery rate on Hektoen agar yielded 39% of Salmonella isolated whereas on XLD agar following enrichment, the yield of Salmonella recovered had increased to 99%. Therefore, if enrichment was to be discontinued, potentially 61% of Salmonella isolates may have been missed, which is a significant proportion. Direct culture onto Hektoen agar did have a slightly higher recovery rate of Shigella, 67% in comparison to 50% isolated on XLD agar following enrichment. However, surprisingly a third of all isolates were still not isolated on Hektoen and this suggests that both primary and subculturering following enrichment is still required in order to maximise the recovery rate of Shigella. Direct plating onto Hektoen, in combination with subculture following enrichment, should also continue to be used for the isolation of Salmonella. Of the 154 Salmonella isolated, 58 were isolated on both direct culture and subculture resulting in earlier identification and therefore reporting of the result. Using a combination of both methods allows early identification of pathogens that is important for public health interventions and to minimise further transmission, especially during outbreaks (7). This result is similar in outcome to a study done in the evaluation of primary inoculation on XLD and following enrichment in Selenite broth. The results from that study showed that direct plating enhances the speed, but not the sensitivity of Salmonella enterica (7). Another study evaluating a variety of chromogenic agars and Hektoen agar also showed the importance of using selenite broth for the recovery of Salmonella following prolonged incubation of 48 hours, and that direct plating identified Salmonella a day earlier in 50% of cases (8).

One of the major limitations of our study was the difference in media used between direct and subculture plates.
As a result, specificity and sensitivity of Selenite F broth could not be calculated and would not have been an appropriate measure in comparison as it would have been difficult to differentiate as to whether the increased performance in the recovery of Salmonella and Shigella on subculture was due to XLD or Selenite F broth. A study evaluating the use of a variety of enrichment broths and plating media, including Hektoen and XLD for the isolation of Salmonella spp. had shown no statistical significance in Salmonella positive stools between Hektoen and XLD from direct plating (9). In an external performance evaluation performed by BD in comparing a chromogenic plating medium to XLD and Hektoen, the sensitivity and specificity of Hektoen and XLD were similar (10). Fort Richard Laboratories obtain their media ingredients from BD and therefore if it was assumed that both agars are similar in performance, then these results would indicate that enrichment in Selenite F broth is beneficial for the maximum recovery of Salmonella in clinical specimens, and therefore should not be discontinued in the laboratory.

As many members of the Enterobacteriaceae are NLF, it is possible to have enteric flora overgrown or mixed in populations with either Salmonella or Shigella on Hektoen agar, especially when they are present in low numbers. Therefore, there may have been a few colonies on Hektoen that were mixed in with other NLF colonies that may have potentially been missed as a similar colonial morphology was picked instead. Selenite F broth was developed to maximise the chance of isolating and recovering Salmonella as selenite is inhibitory for enteric flora and thus enrichment can result in isolation without the overwhelming growth of many enteric flora as long as the incubation period does not exceed 24 hours (6,11). Therefore, due to the inhibitory effects on enteric flora, Selenite F broth is essential to ensure a greater chance of recovering both pathogens. According to BD, the use of Selenite F broth on its own for subculture is not recommended and should be used in conjunction with other selective media to increase the probability of isolating pathogens when present in low numbers (6).

CONCLUSION

Our results showed that direct plating onto Hektoen yields a lower recovery rate in Salmonella and the use of Selenite F broth results in an increased yield of Salmonella isolated. If enrichment is to be discontinued, potentially a significant proportion of Salmonella isolates (61%) may have been missed. However, in order to achieve greater isolation rates, a combination of both direct plating and subculture following enrichment should be performed in order to maximise the recovery rate. Our results also show that a combination of both methods is also required in order to maximise the recovery of Shigella. If enrichment is to be discontinued, a third of Shigella isolates may have been missed. Direct plating and sub-culturing following enrichment can provide earlier detection and isolation of either pathogen in addition to increased recovery rates. However, due to limitations in our study, ideally a further study where both media were tested in parallel should be performed where the sensitivity and specificity can be calculated in order to truly evaluate the effectiveness of Selenite F broth, and whether there are potential variations in agar performance between Hektoen and XLD.

ACKNOWLEDGEMENTS

This study was undertaken as part of the Microbiology 400 level paper required for the Graduate Diploma programme in Medical Laboratory Science qualification at Massey University by Arleen Donaldson. We wish to acknowledge and thank the senior management team at Aotea Pathology for funding the Graduate Diploma study. We also wish to acknowledge and thank Associate Professor Mary Nulsen from Massey University for her support and supervision of the Microbiology paper.

AUTHOR INFORMATION

Arleen Donaldson, BBMedSci GradDipSci, Medical Laboratory Scientist
Mackenzie Nicol, MSc, Medical Laboratory Scientist and Head of Microbiology

Aotea Pathology Ltd, Wellington

Current address, SCL, Wellington

Correspondence: Arleen Donaldson. Microbiology, SCL, Wellington.
Email: Arleen.Donaldson@wellingtonscl.co.nz

REFERENCES

4. Lue YA. Is enrichment broth necessary for stool cultures? Clin Microbiol Newsletter 1986; 8:5-6