Comparison between sodium selenite and sodium tetrathionate broths, incubated at 37°C and 42°C for the isolation of Salmonella spp. from faeces of carriers.

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INTRODUCTION.

Salmonella are widely distributed in nature. The primary reservoir for Salmonella is the intestinal tract of many animals including birds, pets, farm and wild animals. Humans become infected through the ingestion of contaminated water or food or through handling. Specific Salmonella serotypes most often produce characteristic clinical manifestations that have been given the syndrome designation: gastroenteritis, enteric fever, bacteremia and vascular infection, localized infection, and chronic carrier state (1).

Isolation procedures usually involve enrichment broths which are particularly helpful in the recovery of organisms from the stools of Salmonella carriers in whom the number of microorganisms may be as low as 200 CFU/g of faeces. Sodium selenite and sodium tetrathionate broths incubated at 37°C are commonly used, followed by subculture within 6-12 hours in selective/differential media, and presumptive identification by biochemical and serological tests (Edwards and Ewing method applied according to the ASM) (2, 3).

Among the techniques for Salmonella isolation in sanitary microbiology, from water and food, the incubation of enrichment broths at 42°C has been used to allow greater recovery of the bacteria when it is found in very small quantities or when it needs to be isolated from very contaminated mediums (4).

Although the use of 42-43°C as incubation temperature of the enrichment broth has been widely used and tested in the isolation of Salmonella in meat products like chicken and lamb, in pork sausages, in water contaminated with sewage water, in streams, puddles and sewers, tissue from domestic animals, chicken and even in pig faeces, we haven’t found any studies on human faeces nor on asymptomatic human carriers’ faeces, where the isolation of the bacteria is difficult (5, 6, 7, 8, 9-12).

The aim of this study was to compare two enrichment broths and the elevated temperature (42°C) of incubation at 37°C, for the recovery of Salmonella spp. from the faeces of asymptomatic children in the city of Merida, Yucatan.
**MATERIALS AND METHODS.**

2,080 samples of faeces from asymptomatic children were studied. The samples were processed within two hours of collection. They were inoculated into two enrichment broths: sodium tetrathionate (Difco) in duplicate and sodium selenite (Bioxon) in duplicate, and streaked on xilose-lisine-desoxicolate (XLD) agar plate for direct isolation.

From each broth, one tube was incubated at 37ºC and the other at 42ºC for the same length of time (3, 4, 13). The XLD agar plate was incubated at 37ºC for 18-24 hours.

After the corresponding time, both broths were streaked on Salmonella-Shigella (SS) agar plates, which were incubated at 37ºC for 18-24 hours. The colonies morphologically suggestive of *Salmonella* were selected and the biochemical tests recommended by the American Society for Microbiology (3) were performed.

The results were evaluated using the X² test (14).

**RESULTS.**

Of the 2,080 samples studied, 87 (44.2%) were positive for the isolation of *Salmonella* spp.

From sodium selenite broth, 52 (2.5%) samples were positive at 37ºC, and 54 (2.6%) at 42ºC. No statistical significant difference was found. The total of positive samples from sodium selenite broth was 57 (2.7%).

From sodium tetrathionate broth, 71 (31.4%) samples were positive at 37ºC, and 73 (3.5%) at 42ºC. No statistically significant difference was found. The total of positive samples from sodium tetrathionate broth was 76 (3.7%).

*Salmonella* was isolated in 9 (0.4%) of the samples from direct isolation on XLD agar plates.

The results of the isolation of *Salmonella* by the different methods are shown in table 1.

A statistically significant difference was also found in the positive samples from direct isolation on XLD agar plates against the total of positive samples (9/87, 10.3%).

The distribution of positive samples according to the broths and the temperatures is shown in Table 2. We can observe that 44 samples (50.6%) were positive for the isolation of *Salmonella* with both broths and at both temperatures.

The remaining 43 samples, which are 49.4% of all positive samples in the isolation of *Salmonella*, were distributed as follows: 12 (13.8%) were positive from sodium selenite broth only, and 7 of those at both temperatures, one at 37ºC and four at 42ºC. 31 (35.6%) samples were positive from sodium tetrathionate broth only: 26 at both temperatures, two at 37ºC and three at 42ºC.

**DISCUSSION.**

Even though we could not find any statistically significant difference between isolation from each enrichment broth incubated at 37ºC or at 42ºC, we did find difference between sodium selenite and sodium tetrathionate broths, since the higher number of positive samples corresponded to sodium tetrathionate broth (table 1), no matter the temperature. It was interesting to observe that the raised temperature suppressed the competing gram-negative bacteria and permitted *Salmonella* to grow in relatively pure culture, thus providing an advantage for isolating and identifying the organisms.

It should be pointed out that direct isolation of *Salmonella* on XLD agar plate gave very poor results compared to the use of enrichment broths, in fact it was the method which gave the lowest results, so the use of at least one enrichment broth is always highly recommended.

In table 2, where the distribution of the positive samples according to the methods we used is noted, we can observe that positive results were better when we used two broths (44/88, 50.6%) at both temperatures, while the samples isolated from just one broth are less: 31/87, 35.6% with only sodium...
tetrathionate and 12/87, 13.8% with only sodium selenite broth.

The X² test was carried out on the total number of positive samples with sodium tetrathionate broth against the total of positive samples in the study, and no statistically significant difference was found (X² = 0.768, p = 0.38), which brings us to believe the same results, statistically speaking, would be obtained using both enrichment broths or just the sodium tetrathionate broth; although it is recommended to use both broths to recover the greatest number of cases with *Salmonella*.

If we observe the positive samples by broth and by temperature, we find that 26 (29.5%) were positive with sodium tetrathionate at both temperatures while only 7 (7.9%) with sodium selenite at both temperatures, which supports the fact that sodium tetrathionate broth is much better enrichment medium

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**Table 1.**

<table>
<thead>
<tr>
<th>Enrichment broth</th>
<th>Positive samples</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium selenite</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 37°C</td>
<td>52</td>
<td>2.5</td>
</tr>
<tr>
<td>At 42°C</td>
<td>54</td>
<td>2.6</td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
<td>2.7</td>
</tr>
<tr>
<td>Sodium tetrathionate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 37°C</td>
<td>71</td>
<td>3.4</td>
</tr>
<tr>
<td>At 42°C</td>
<td>73</td>
<td>3.5</td>
</tr>
<tr>
<td>Total</td>
<td>75</td>
<td>3.7</td>
</tr>
<tr>
<td>Direct isolation on XLD</td>
<td>9</td>
<td>0.4</td>
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<tr>
<td>Total of positive samples</td>
<td>87</td>
<td>4.2</td>
</tr>
</tbody>
</table>

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**Table 2.**

<table>
<thead>
<tr>
<th>Broth/temperature</th>
<th>Number</th>
<th>Number %</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both broths and both temperatures*</td>
<td>44</td>
<td>50.6</td>
<td></td>
</tr>
<tr>
<td>Sodium selenite</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Both temperatures</td>
<td>7</td>
<td>8.0</td>
<td></td>
</tr>
<tr>
<td>37°C only</td>
<td>1</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>42°C only</td>
<td>4</td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>13.8</td>
<td></td>
</tr>
<tr>
<td>Sodium tetrathionate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Both temperatures</td>
<td>26</td>
<td>29.9</td>
<td></td>
</tr>
<tr>
<td>37°C only</td>
<td>2</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>42°C only</td>
<td>3</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>35.6</td>
<td></td>
</tr>
</tbody>
</table>

TOTAL 87 100.0

* 1 positive sample with sodium selenite at 37°C and sodium tetrathionate at 42°C.
than sodium selenite, a fact which has already been demonstrated by other authors (3, 5, 8).

On the other hand we observed positive samples with just one medium and at just one temperature, even though there were only a few: sodium selenite at 37ºC: 1 sample; sodium selenite at 42ºC: 4 samples; sodium tetrathionate at 37ºC: 3 samples, and the combination of sodium selenite at 37ºC plus sodium tetrathionate at 42ºC in just one sample.

The data continues to show that even though a particular method is better from a numerical or statistical point of view, there will always be cases that will not be detected if only one method is used. This is important, especially when working with carriers, due to the characteristics of elimination of bacteria and considering the number of CFU.

We had previously tested the use of pre-enrichment broths trying to improve the isolation of *Salmonella* from carriers using a method for water and food, and in this study we also observed differences in the two methods, although we didn’t find a statistically significant difference (not published data). In this study, although one of the methods (sodium tetrathionate at 42ºC) predominated, we also found samples that were positive with another, for example, one sample was positive to sodium selenite at 37ºC only.

Although there was no significant difference between the positive samples incubated at 37 and at 42ºC, seven samples were only positive in one broth at 42ºC (four with selenite and three with tetrathionate), plus the one that resulted positive in both broths, one at 37ºC and the other at 42ºC. These are few samples in relation to the total number we studied, but each one of them represents a carrier who would not have been detected if that method was not used.

Those considerations lead us to recommend the use of sodium tetrathionate broth incubated at 37ºC as enrichment broth for routine clinical work. On the other hand, if we need to carry out an epidemiological study to detect carriers, the addition of sodium selenite broth and both temperatures would be useful.

It should be noted that from the methodology of sanitary microbiology, we used elevated temperature at 42ºC, but we did not use the Rappaport-Vassiliadis enrichment broth which is recommended in that methodology; instead we used sodium selenite and sodium tetrathionate broths, at elevated incubation temperature.

The usefulness of the Rappaport-Vassiliadis broth at 42-43ºC has been demonstrated in different studies on meat products (5, 8, 9), on sewage water (6, 7), and in a study on pig faeces (9), using it together with brilliant green agar, and this could be our next step. Therefore, we believe it is necessary to continue searching for simple, economic methods to improve or widen the detection of this bacteria within the reach of any laboratory, whether it be for clinical or investigative purposes, without having to use more expensive and sophisticated techniques, such as the molecular ones.

REFERENCES.


