

*Rev Biomed* 2003; 14:215-220.

***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.***

**Original Article**

Javier J. Flores-Abuxapqui, Miguel A. Puc-Franco, Mario R. Heredia-Navarrete, María de la L. Vivas-Rosel, José Franco-Monsreal.

Laboratorio de Microbiología, Centro de Investigaciones Regionales "Dr. Hideyo Noguchi", Universidad Autónoma de Yucatán, Mérida, Yucatán, México.

**RESUMEN.**

**Comparación entre medios de cultivo de selenito de sodio y tetratiónato de sodio, ambos incubados a 37°C y 42°C para el aislamiento de *Salmonella* spp de las heces fecales de portadores.**

**Introducción.** Los caldos de enriquecimiento son útiles para la recuperación de *Salmonella* a partir de portadores, en los cuales el número de microorganismos puede ser tan bajo como 200 UFC/g de heces. En las técnicas de aislamiento de salmonela en microbiología sanitaria, la incubación de los caldos de enriquecimiento a 42°C es utilizada para mejorar la recuperación de la bacteria cuando se encuentra en muy pequeñas cantidades o cuando tiene que aislarse a partir de medios muy contaminados.

**Material y Métodos.** Se estudiaron 2,080 muestras de heces de niños sin diarrea. Se inocularon caldos selenito de sodio y tetratiónato de sodio, y ambos se

incubaron a 37 y 42°C, y placas de agar XLD para aislamiento directo.

**Resultados.** Ochenta y siete muestras (4.2%) fueron positivas al aislamiento de *Salmonella* spp. Cincuenta y dos muestras (2.5%) fueron positivas con selenito de sodio incubado a 37°C, y 54 (2.6%) a 42°C. El total de muestras positivas con el caldo selenito de sodio fue de 57 (2.7%). Setenta y una muestras (3.4%) fueron positivas con caldo tetratiónato de sodio a 37°C y 73 (3.5%) a 42°C. El total de muestras positivas con caldo tetratiónato de sodio fue de 76 (3.7%). En la siembra directa en agar XLD se aisló *Salmonella* en 9 (0.4%) muestras.

**Discusión.** Encontramos que como medio de enriquecimiento el caldo tetratiónato de sodio es mejor que el caldo selenito de sodio, y lo recomendamos para el trabajo clínico de rutina. Por otro lado, si es necesario llevar a cabo un estudio epidemiológico para detectar portadores, puede ser útil añadir el caldo

Corresponding address: Javier J. Flores-Abuxapqui. Avenida Itzáez # 490 x 59. C.P. 97000. Mérida, Yucatán. México.  
Tel. (999) 924-57-55. Fax (999) 923-61-20. E-mail: fabuxap@tunku.uady.mx  
Received June 19, 2003; Accepted September 9, 2003.

This paper is also available at <http://www.uady.mx/sitios/biomedic/revbiomed/pdf/rb031441.pdf>

**Vol. 14/No. 4/Octubre-Diciembre, 2003**

selenito de sodio y utilizar dos temperaturas para el aislamiento *Salmonella*.

(*Rev Biomed* 2003; 14:215-220)

**Palabras clave:** *Salmonella*, caldos de enriquecimiento, portadores.

## SUMMARY.

**Introduction.** Enrichment broths are particularly helpful in the recovery of *Salmonella* from carriers in whom the number of microorganisms may be as low as 200CFU/g of faeces. In the techniques of isolation of *Salmonella* in sanitary microbiology, the incubation of enrichment broths at 42°C has been used to allow a greater recovery of the bacteria when it exists in very small quantities or when it needs to be isolated from very contaminated sources.

**Material and Methods.** 2,080 samples of faeces from children without diarrhea were studied. They were inoculated in sodium selenite and sodium tetrathionate broths, which were incubated at 37 and 42°C, and streaked on XLD agar plates.

**Results.** Eighty seven (4.2%) samples were positive for the isolation of *Salmonella* spp. Fifty two (2.5%) samples were positive with sodium selenite broth incubated at 37°C, and 54 (2.6%) at 42°C. The total of positive samples with sodium selenite broth was 57 (2.7%). Seventy one (3.4%) samples were positive with sodium tetrathionate broth at 37°C, and 73 (3.5%) at 42°C. The total of positive samples with sodium tetrathionate broth was 76 (3.7%). *Salmonella* was isolated in 9 (0.4%) samples streaked directly on XLD agar plates.

**Discussion.** We found that sodium tetrathionate broth is better than sodium selenite as enrichment medium, and we recommend it for routine clinical work. On the other hand, if we carry out an epidemiological study to detect carriers, the addition of sodium selenite broth at both temperatures would be useful.

(*Rev Biomed* 2003; 14:215-220)

**Key words:** *Salmonella*, enrichment broths, carriers.

## 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 of incubation at 37°C, for the recovery of *Salmonella* spp. from the faeces of asymptomatic children in the city of Merida, Yucatan.

---

*Selenite and Tetrathionate for the isolation of Salmonella.***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 37°C and the other at 42°C for the same length of time (3, 4, 13). The XLD agar plate was incubated at 37°C for 18-24 hours.

After the corresponding time, both broths were streaked on *Salmonella-Shigella* (SS) agar plates, which were incubated at 37°C 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 X<sup>2</sup> 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 37°C, and 54 (2.6%) at 42°C. 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 37°C, and 73 (3.5%) at 42°C. 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 found between both enrichment broths, as much at 37°C as at 42°C, as well as in the total number of positive samples from the sodium selenite broth against those from the sodium tetrathionate broth.

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 37°C and four at 42°C. 31 (35.6%) samples were positive from sodium tetrathionate broth only: 26 at both temperatures, two at 37°C and three at 42°C.

**DISCUSSION.**

Even though we could not find any statistically significant difference between isolation from each enrichment broth incubated at 37°C or at 42°C, 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

**Table 1.**  
Isolation of *Salmonella* from two enrichment broths and two temperatures, from 2,080 samples of faeces of asymptomatic children.

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

tetrathionate and 12/87, 13.8% with only sodium selenite broth.

The X<sup>2</sup> 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 (X<sup>2</sup> = 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

**Table 2.**  
Distribution of positive samples by enrichment broth and temperature.

Broth/temperature	Number	Number	%	%
<b>Both broths and both temperatures*</b>		44		50.6
<b>Sodium selenite</b>				
<b>Both temperatures</b>	7		8.0	
<b>37°C only</b>	1		1.1	
<b>42°C only</b>	4		4.6	
<b>Total</b>		12		13.8
<b>Sodium tetrathionate</b>				
<b>Both temperatures</b>	26		29.9	
<b>37°C only</b>	2		2.3	
<b>42°C only</b>	3		3.4	
<b>Total</b>		31		35.6
<b>TOTAL</b>		87		100.0

\* 1 positive sample with sodium selenite at 37°C and sodium tetrathionate at 42°C.

*Selenite and Tetrathionate for the isolation of Salmonella.*

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 37°C: 1 sample; sodium selenite at 42°C: 4 samples; sodium tetrathionate at 37°C: 3 samples, and the combination of sodium selenite at 37°C plus sodium tetrathionate at 42°C 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 42°C) predominated, we also found samples that were positive with another, for example, one sample was positive to sodium selenite at 37°C only.

Although there was no significant difference between the positive samples incubated at 37 and at 42°C, seven samples were only positive in one broth at 42°C (four with selenite and three with tetrathionate), plus the one that resulted positive in both broths, one at 37°C and the other at 42°C. 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 37°C 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 42°C, 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 42-43°C 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.

- 1.- Miller SI, Alpuche AC. Infección por *Salmonella*. In: Enfermedades diarreicas en el niño. 10ª. ed. México: Editorial Interamericana Mc Graw Hill; 1996. p. 140-2.
- 2.- Joklik WK, Willet HP, Amos DB. Microbiología de Zinsser. 18a. ed., Buenos Aires: Ed. Médica Panamericana; 1987. p. 688.
- 3.- Murray PR, Baron EJ, Pfaller MA, Tenover FC, Tenover FC. Manual of Clinical Microbiology. 7<sup>th</sup> ed. Washington D.C.: American Society for Microbiology, 1999. p. 467-71.
- 4.- Fernández EE. Microbiología Sanitaria. Agua y Alimentos. Vol. I, EDUG/Universidad de Guadalajara, Jal. 1981. p. 647.
- 5.- Arroyo G, Arroyo JA. Efficiency of different enrichment and isolation procedures for the detection of *Salmonella* serotypes in edible offal. J Appl Bacteriol 1995; 79:360-7.
- 6.- Harvey RW, Price TH. A comparison of the R2 modification of Rappaport's enrichment medium with strontium chloride B for *Salmonella* isolation from sewage polluted natural water. J Hyg (London). 1982; 89:111-7.
- 7.- Harvey RW, Price TH. A comparison of Two modifications of Rappaport's enrichment medium (R25 and RV) for the isolation of salmonellas from sewage polluted natural water. J Hyg (London). 1983; 91: 451-8.

*JJ Flores-Abuxapqui, MA Puc-Franco, MR Heredia-Navarrete, M de la L Vivas-Rosel, et al.*

8.- Roberts D, Boag K, Hall ML, Shipp CR. The isolation of salmonellas from British pork sausages and sausage meat. J Hyg (London). 1975; 75:173-84.

9.- Schlater LK. Isolation and identification of *Salmonellae* from veterinary specimens. National Veterinary Services Laboratories, Veterinary Services. Animal and Plant Health Inspection Service, US Department of Agriculture. Ames IA 1987.

10.- Trichopoulos D, Papadakis JA, Karalis D, Vassiliadis P. Incubation at raised temperature of enrichment media, combined with secondary enrichment in Rappaport's medium, for the isolation of *Salmonella* from sewage. J Hyg (London) 1975; 74:205-13.

11.- Vassiliadis P, Kalapothaki V, Mavrommati C, Trichopoulos D. A comparison of the original Rappaport medium (R medium) and the Rappaport-Vassiliadis medium (R-V medium) in the isolation of *Salmonella* from meat products. J Hyg (London) 1984; 93: 51-8.

12.- Vassiliadis P, Trichopoulos D, Papadakis J, Kalapothaki V, Zavitsanos X. *Salmonella* isolation with Rappaport's enrichment medium of different compositions. Zentralbl Bakteriol Mikrobiol Hyg 1981; 173: 382-9.

13.- López RA, Avilés RD, Eusebio HMG. Manual de Laboratorio de Microbiología Sanitaria. México: Escuela Nacional de Ciencias Biológicas-Instituto Politécnico Nacional; 1991. p. 126.

14.- Daniel WW. Bioestadística. Bases para el análisis de las ciencias de la salud, México: Editorial UTEHA (Limusa); 1996. p. 639-93.