

# User-Friendly Chromogenic *Salmonella* Media in Clinical Routine Practice

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## INTRODUCTION

In the clinical microbiology labs, screening of stool samples for the presence of salmonellae on enteric agars ( Hektoën, SS and others) (1) is labor intensive with a low specificity due to colonies resembling *Salmonella* (2) ( lactose/sucrose negative and/or H<sub>2</sub>S positive ); this leads to additional costs and diagnosis delays.

Chromogenic media have been more recently designed and introduced for the specific detections of salmonella-specific enzyme activities. On the **SM-ID** medium ( bioMérieux, Montalieu-Vercieu, France ) *Salmonella* are positive for glucuronate and negative for β-galactosidase activity, resulting in red colored colonies. On the **ASAP** Agar ( AES Laboratoire, Combourg, France ) *Salmonella* cleave a caprylate substrate (C8-esterase activity ) and are negative for β-glucosidase activity, resulting in purple to mauve colonies.

The purpose of this study is to comparatively evaluate, in clinical lab routine, the use, considering handiness and efficiency, of both those chromogenic media and Hektoën enteric agar ( **HKTN** ) (BBL Becton Dickinson, Baltimore, Md. ).

## MATERIAL AND METHODS

Four hundred and eighty five stool specimens were collected from patients of any ages ranging from infants to elders, either on admission or hospitalized. Samples were processed upon arrival by direct plating on each medium. Additionally, a Muller Kauffmann tetrathionate + brilliant green selective enrichment broth (1) was seeded and incubated overnight at 37°C, then a loopful of this broth was streaked on each agar. Every plates were incubated overnight at 37°C and examined at the same time of the daily routine for a same stool sample. The colonies suspect of being *Salmonella enterica subsp. enterica* were spotted according to their aspects and colors as indicated by the manufacturers, then screened and identified as needed by standard procedures ( urease, oxidase, oxidation-fermentation, Api 20E gallery, serotyping ). Additionally, the total numbers of any colonies grown on every plates were recorded in three classes : 10 or less, 10 to 100 and over 100.

## RESULTS

*Salmonella* were recovered from 11 out of the 485 samples ( table 1 ) : 8 following direct plating ( 6 with all three media, 1 with both **HKTN** and **ASAP** and 1 with the **ASAP** only ) and 11 after selective enrichment ( 8 with all three media, 2 with both **HKTN** and **ASAP** and 1 with **ASAP** only.

Following direct plating, suspect colonies which eventually appeared not to be *Salmonella* spp. ( table 2 ) occurred 61 times on the **HKTN** ( including *Shigella sonnei* and *Yersinia enterocolitica* once each ), 28 times on the **SM-ID** ( never *Shigella* nor *Yersinia* ) and 3 times on the **ASAP** ( *Pseudomonas* spp. once and *Aeromonas hydrophila* twice ).

After enrichment, « false suspicions » occurred 101 times on the **HKTN**, 39 times on the **SM-ID** and 7 times on the **ASAP** ( *Pseudomonas* = 5 and *Aeromonas hydrophila* again from the 2 previously positive samples ).

Finally, bacterial growth were routinely examined and recorded : the enumerations of the totals of colonies grown on each plate ( table 3 ) indicate that following direct plating **ASAP** medium let grow less bacteria compared to other media ( p<0.001, chi-2 ). After enrichment, a similar trend appears but non significantly.

Table 3: Microbial growths observed on the varied plates

N° colonies	after direct plating			following enrichment		
	0 - 10	10 - 100	> 100	0 - 10	10 - 100	> 100
<b>HKTN</b> <sup>(a)</sup>		83	235	126	24	335
	167 <sup>(b)</sup>					
<b>SM-ID</b>	141	58	286	117	22	346
<b>ASAP</b>	205	81	199	124	41	320

(a): media, see text – (b): numbers of plates recorded into each class

Table 1 : Recovery of salmonella from the plates

case	<i>Salmonella</i> Serotype	By direct Plating			After selective enrichment		
		HKTN <sup>(a)</sup>	SM-ID	ASAP	HKTN	SM-ID	ASAP
1	Typhimurium	+	+	+	+	+	+
2	Typhimurium	+	+	+	+	+	+
3	Poona	+	+	+	+	+	+
4	Hadar	+	+	+	+	+	+
5	Hadar	+	+	+	+	+	+
6	Typhimurium	-	-	-	+	+	+
7	Typhimurium	+	+	+	+	+	+
8	Brandenburg	-	-	-	+	-	+
9	Typhimurium	-	-	-	+	-	+
10	Typhimurium	-	-	+	+	+	+
11	Enteritidis	+	-	+	-	-	+

(a): media, see text

Table 2 : false suspicions of salmonella from the varied media

Taxonomy group	By direct Plating			After selective enrichment		
	HKTN <sup>(a)</sup>	SM-ID	ASAP	HKTN	SM-ID	ASAP
Urease positive <i>Proteae</i>	30	-	-	61	-	-
<i>Morganella</i> spp.	-	6	-	-	19	-
<i>Klebsiella</i> spp.	-	-	-	-	1	-
<i>Enterobacter</i> spp.	9	9	-	9	11	-
<i>Serratia</i> spp.	-	2	-	-	-	-
<i>Citrobacter freundii</i>	4	1	-	4	2	-
<i>Escherichia coli</i>	4	10	-	-	6	-
<i>Citrobacter diversus</i>	-	-	-	1	-	-
<i>Shigella sonnei</i>	1	-	-	-	-	-
<i>Yersinia enterocolitica</i>	1	-	-	-	-	-
<i>Aeromonas hydrophila</i>	-	-	2	2	-	2
<i>Pseudomonas</i> spp.	12	-	1	24	-	5
Total	61	28	3	101	39	7

(a): media, see text

## DISCUSSION-CONCLUSIONS

The percentage of positive stool specimens is 2.27% in this study. All positive cases were diagnosed on patient admission or in the early days of hospitalization. This is in keeping with the fact that diarrhea that develops in the hospital is not likely to be due to food-borne pathogens ( inpatients at our institution as well as at others will suffer from diarrhea first due to *Clostridium difficile* ).

Compared to the **HKTN**, plating on the chromogenic media actually lowered the needs for subsequent screening tests and microbial identifications.

The **ASAP** agar allowed less bacteria to grow compared to others. This stronger selectivity may account for both the better growth and recovery of the salmonellae on **ASAP** by a better reduction of the competitive microflora.

The use of chromogenic media, especially the **ASAP**, allow accurate detections of salmonella together with valuable reductions of diagnosis delays (1 or 2 days will be gained) and lower laboratory labor and costs.

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## Communications :

ASM Congress (Salt Lake City- USA- 20<sup>th</sup> and 21<sup>st</sup> May2002).  
I35 Congress (Ploufragan- France- 29<sup>th</sup> to 31<sup>st</sup> May 2002).