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Comparison of Rambach Agar, SM-ID Medium, and Hektoen Enteric Agar for Primary Isolation of Non-typhi Salmonellae from Stool Samples

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Stool samples (n = 504) were streaked simultaneously onto Rambach agar (R agar; E. Merck, Darmstadt, Germany), SM-ID medium (bioMérieux S.A., Montalieu-Vercieu, France), and Hektoen Enteric (HE) agar (BBL Becton-Dickinson, Baltimore, Md.) in order to evaluate the performances of the first two media in comparison with that of the well-established HE agar. Following overnight cultivation at 37°C, 29 samples (5.8%) were positive for non-typhi salmonellae on at least one of the three media. Sensitivities and specificities were 69 and 98%, 79 and 85%, and 100 and 79% for R, SM-ID, and HE agars, respectively. On the basis of the poor sensitivities, R and SM-ID agars are not recommended as primary plating media when screening for non-typhi salmonellae. However, the high specificity of R agar may help to reduce the work load when this medium is used for plating after enrichment.

Rambach agar (E. Merck, Darmstadt, Germany) and SM-ID medium (bioMérieux S.A., Montalieu-Vercieu, France) have recently been introduced as new selective media for the isolation of salmonellae. Several investigators have already reported high rates of sensitivity and specificity for Rambach agar (1, 4, 5, 7) and SM-ID medium (8), but their investigations were essentially based on the use of pure *Salmonella* stock cultures (4, 5, 7) or they tried to isolate *Salmonella* spp. from marine waters (1). In order to complement their findings, we tested the performances of the two new media in comparison with that of Hektoen Enteric (HE) agar (BBL Becton-Dickinson, Baltimore, Md.), our standard medium, when they were used in the routine stool laboratory for direct inoculation of stool specimens. Since *Salmonella typhi* and *Salmonella paratyphi* strains are not expected to be detected on Rambach agar, we restricted our analysis to non-typhi *Salmonella* spp. Disease caused by typhoid serotypes of salmonellae usually starts with a high fever in the absence of diarrhea, and therefore, the causative agents can be isolated from blood cultures rather than stool samples.

Rambach agar allows identification of non-typhi salmonellae because of their red color on this medium (9). While *Salmonella* colonies are bright red, coliforms appear blue, green, violet, or colorless. Since the biochemical characteristics used with Rambach agar (positive propylene glycol metabolism and absence of β-galactosidase) are highly specific for non-typhi salmonellae, only very few false-positive results are expected. A drawback of this medium is that typhoid *Salmonella* serotypes may not be recovered since they are not positive for propylene glycol metabolism.

In SM-ID medium, *Salmonella* colonies are similarly detected by their distinctive red coloration, while coliforms appear blue, violet, or colorless. The biochemical characteristics used with SM-ID medium are acid formation from glucuronate and the absence of β-galactosidase (8). This selection of characteristics makes a greater number of false-positive results more likely compared with the number obtained with Rambach agar, since some strains of *Escherichia coli*, *Shigella* spp., and *Morganella morganii* appear to be identical to *Salmonella* strains in reactions in SM-ID medium. On the other hand, SM-ID medium also allows detection of *S. typhi* and *S. paratyphi* strains, both of which are positive in the glucuronate test.

HE agar is the standard primary plating medium in our routine screening for *Salmonella* spp. and *Shigella* spp. (6). Suspicious colonies are transparent (lactose negative) and have black centers because of the production of H_2S (most *Salmonella* strains) or are green or transparent without a black center (lactose negative, no production of H_2S; most *Shigella* strains and some *Salmonella* strains).

We analyzed 504 stool samples sent to us in Cary Blair transport medium (as described previously [2], but without agar) by direct plating onto HE, SM-ID, and Rambach agars. Samples were processed continuously upon arrival in our laboratory, resulting in incubation times ranging from 16 to 24 h at 37°C. The optimal incubation time for Rambach agar is 24 h. By using a glass stick, one drop of stool specimen (suspended in the liquid transport medium) was inoculated onto each of the test plates and was then fractionated with a new glass stick, thus ensuring that about equal amounts of inoculum were used for each plate. Simultaneously, enrichment was carried out with tetrathionate and GN broths (BBL); this was followed by subculturing onto salmonella-shigella agar (BBL) and HE agar, respectively. Following overnight incubation at 37°C, all primary culture plates were inspected and colonies suspicious of being salmonellae were analyzed further (polyvalent O-1 phage [3]; slide agglutination tests with commercially available antisera for O- and H-antigen agglutination [Behringwerke AG, Marburg, Germany], biochemical testing with an in-house, conventional identification system [10]).

A total of 29 *Salmonella* strains were isolated on at least one of the three primary plating media (4 strains belonged to serogroup OB, 2 strains were serogroup OC, 22 strains were serogroup OD, and 1 strain was serogroup OK). Compared with HE agar, the two new media did not detect an additional *Salmonella* strain, whereas in nine (Rambach agar)

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TABLE 1. Comparison of primary plating media for detection of non-typhi salmonellae[a]

Medium	No.		% SENS	No.		% SPEC
	TP	FN		TN	FP	
Rambach	20	9	69.0	465	10	97.9
SM-ID	23	6	79.3	403	72	84.8
Hektoen	29	0	100.0	377	98	79.4

[a] A total of 504 stool samples were tested. Sensitivities and specificities were calculated by comparing all samples found to be positive for non-typhi salmonellae on at least one primary plate (n = 29). TP, true positive; FN, false negative; SENS, sensitivity; TN, true negative; FP, false positive; SPEC, specificity.

and six (SM-ID medium) cases, the two new media failed to confirm the finding of the *Salmonella* strain that was achieved with HE agar. The sensitivities and specificities (suspicious colonies that were not salmonellae) were 69.0 and 97.9%, 79.3 and 84.8%, and 100 and 79.4% for Rambach, SM-ID, and HE agars, respectively (Table 1).

The use of enrichment procedures as outlined above resulted in the detection of an additional five *Salmonella* spp. from our 504 stool samples. Considering all *Salmonella* isolates, the sensitivities of Rambach, SM-ID, and HE agars were 58.8, 67.6, and 85.3%, respectively. Excluded from the analysis were two strains of *S. paratyphi* type A grown from SM-ID medium exclusively, because as members of a typhoid *Salmonella* serogroup they did not meet the criteria for enrollment in our study.

In order to resolve discordant results and to rule out the possibility of confusion of samples, we repeated the cultivation procedure for 10 of the 11 samples that were found to be positive for salmonellae on only one or two primary media (Table 2). In the interim, stool samples were kept at 4°C. The original results were confirmed in 7 of 10 specimens, whereas in 3 specimens, a *Salmonella* strain was newly isolated on either SM-ID agar or Rambach agar, or both. In addition, six specimens negative on the primary Rambach agar plate were subcultured onto the same medium after enrichment in tetrathionate broth. All six specimens became positive and yielded typical bright red colonies.

The relatively low sensitivity of both Rambach and SM-ID agars was reflected in the distribution of suspect colonies during primary inspection of plates. Several times, when we found up to 10 or 20 suspect colonies on HE agar plates, there were only 2 or even 1 suspect colony among many coliforms on the corresponding SM-ID and Rambach agar plates. It seems to us that HE agar is more effective in inhibiting coliforms without impeding *Salmonella* strains too strongly.

TABLE 2. Stool samples (n = 11) with discrepant results in primary culture[a]

Stool sample no.	Primary culture			Repeat culture		
	H	SM	R	H	SM	R
1	+	−	−	+	−	−
2	+	−	−	+	−	−
3	+	−	+	+	−	+
4	+	+	−	+	+	−
5	+	+	−	+	+	−
6	+	−	−	+	−	−
7	+	+	−	+	+	−
8	+	−	−	+	+	+[b]
9	+	+	−	+	+	+[c]
10	+	−	+	+	+	+
11	+	+	−	—[d]	—	—

[a] H, Hektoen Enteric agar; SM, SM-ID medium; R, Rambach agar.
[b] Colorless colonies of *Salmonella* spp.
[c] Rose-colored colonies of *Salmonella* spp.
[d] —, culture was not repeated.

Freydière and Gille (4) found among 159 non-typhi *Salmonella* spp. 10 strains failed to yield red colonies on Rambach agar within 24 h. Similar results were seen by Gruenewald et al. (5) and Manafi and Sommer (7). This corresponds to the findings in our repeat cultures 8 and 9, in which *Salmonella* colonies were colorless or faintly rose colored (Table 2). However, the significance of this observation seems secondary in explaining the poor sensitivity of Rambach agar when used for direct plating of stool specimens, since six strains of *Salmonella* that were missed on primary Rambach agar plates yielded bright red colonies on Rambach agar when they were cultivated out of the enrichment broth. This means that all six strains behaved biochemically in a typical way, but on primary plating their numbers were too small compared with the competing growth of other coliforms.

Rambach agar is highly specific; we obtained no more than 10 false-positive strains from our 504 samples (*E. coli*, 4; *Citrobacter freundii*, 3; *Citrobacter amalonaticus*, *Klebsiella oxytoca*, and *Enterobacter agglomerans*, 1 each). On repeat cultivation, none of the false-positive strains yielded typical bright red colonies. Only two strains gave reddish colonies; the other strains were colorless, greenish, violet, or blue. Some of our false-positive strains had lower β-galactosidase activities compared with those of the other strains, requiring a full 24-h incubation period (as recommended by the manufacturer) to develop blue pigmentation. Incubation times below 24 h may result in red instead of violet colonies at the time of reading of the plates. In daily practice, however, it is not possible to achieve an exact 24-h incubation as proposed by the manufacturer.

It can be anticipated that we missed some of the *Salmonella* strains grown on SM-ID medium because of their strong glucolytic activity, which could lead to rapid realkalinization of the medium and, consequently, to loss of the red colony coloration (2a). Meanwhile, bioMérieux has changed the formulation of SM-ID medium, but an improved performance of the new formulation in screening salmonellae from stool specimens remains to be demonstrated. Unfortunately, the reformulation of SM-ID medium was done after we finished our study.

On the basis of our results, we cannot recommend the use of either Rambach or SM-ID agar for primary plating of stool specimens, since both of them considerably lack sensitivity. However, Rambach agar might be used for subculturing of enrichment broth cultures, because its markedly higher specificity (compared with that of HE agar) may substantially reduce the work load in a laboratory.

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