A one-day selective migration procedure for detecting salmonellae in faeces¹

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SYNOPSIS A one-day procedure for detecting salmonellae was developed and evaluated with clinical faecal specimens. The procedure was based on the selective retardation of enterobacteria in their migration through a semi-solid enrichment medium. It was shown that a pure or almost pure growth of salmonella could often be obtained within 16 to 24 hours by this method. The culture thus obtained could then be used directly for serological confirmation. The method was rapid, economical, and efficient. Clinical trials indicated an increase of 28% in the frequency of positive isolations of salmonella with this procedure as compared with the conventional multistep cultural procedures with no false-positive results. However, the method was unsatisfactory for detecting *Salmonella typhi* due to its slow migration through the semi-solid medium.

Conventional procedures for isolation of salmonellae from faeces consist of direct plating of the faecal specimens onto one or more selective solid media and inoculation into selective liquid enrichment and then onto selective solid media, followed by confirmatory biochemical and serological tests. Such multistep procedures are tedious, time consuming, and uneconomical, especially when the work load is large. Direct detection of salmonellae from food samples and faeces with fluorescent antibody techniques has been tried by a number of workers (Goepfert, Mann, and Hicks, 1970; Gibbs, Patterson, and Murray, 1972; Thomason and McWhorter, 1965). That method is rapid and effective but its value is limited because of the occurrence of false positives and the need for sophisticated instruments. In recent years, the use of semi-solid media as enrichment for isolating salmonellae on the basis of their selective motility has been studied (Stuart and Pivnick, 1965; Banwart, 1968; Harper and Shortridge, 1969; Fung and Kraft, 1970). These workers noted that after passing through the semi-solid media followed by direct plating onto MacConkey agar, salmonellae often isolated in a pure or nearly pure culture state. In their procedures, however, it was necessary to plate onto solid media in order to

obtain single colonies for biochemical and serological confirmatory tests.

Against this background, a search for a simple and more effective technique for the rapid detection of salmonellae was undertaken. Preliminary results showed that after passing through semi-solid enrichment media, salmonellae with well developed H-antigens could easily be detected by the slide agglutination test. This observation formed the basis of a one-day procedure in which the migrated growth was checked directly for salmonella O and H antigenicity.

Materials and Methods

MEDIA

The formula of the semi-solid enrichment medium is as follows:

Tryptone (Oxoid)	..	..	0.5 g
Lab-Lemco powder (Oxoid)	..	..	0.8 g
40% MgCl ₂ .6H ₂ O	..	..	6.0 ml
1% Brilliant green (BDH)	..	..	0.1 ml
Agar (Oxoid No. 3)	..	..	0.2 g
Distilled water	..	..	94.0 ml
pH adjusted to 6.0			

The agar and other constituents were dissolved by gentle boiling and dispensed in about 10-ml quantities in U-tubes with an inner diameter of 10 mm, autoclaved at 15 lb/sq in for 15 min, cooled to room temperature and then stored in a refrigerator.

A liquid enrichment medium of the same composi-

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tion without agar was dispensed in tubes in 3-4 ml quantities and was used for suspending stool specimens.

METHOD

Stool specimens obtained from clinical cases were examined by the following procedure. A small amount (about the size of a match-head) of a specimen of stool was suspended in 3-4 ml of liquid enrichment. To one arm of the U-tube, 0.5 to 1 ml of the suspension was gently added, and to the other 1 ml of an enriched tryptone soya broth (tryptone 1.5%, soya peptone 0.5%, Lab-Lemco powder 0.8%, pH 7.0) was added. The tubes were incubated at 37°C and examined after incubation for 16 to 24 hours. Tubes with no migrated growth appearing in the tryptone soya broth after 24 hours were considered negative. If, on the other hand, migrated growth appeared in the tryptone soya broth, a slide agglutination test was performed with polyvalent H antiserum on a ceramic ring slide. The mixture of growth and antiserum was shaken on a rotator at 80 rotations per min for three minutes. If a positive agglutination reaction was observed, about 0.5 ml of the growth was pipetted out to a small tube, and used for direct agglutination with polyvalent O and group specific O antisera. This usually gave a clear-cut result. In the case of a doubtful agglutination result, the growth was inoculated onto a sector of a nutrient agar plate and incubated for three to four hours. The fresh growth was then tested against salmonella-polyvalent O and group specific O-antisera to identify the O-antigenic group. The type of the fresh growth was identified also using phase 1 and 2 H antisera when required.

To check the efficiency of the procedure and the purity of salmonella isolates, migrated growth which reached the top of the U tube in the tryptone soya broth was also plated onto MacConkey agar and suspected salmonella colonies were picked out for biochemical and serological tests.

Parallel studies with the same specimen by conventional isolation methods were done independently by colleagues in our laboratory.

The procedure was to plate stool specimens directly onto MacConkey agar and salmonella-shigella agar media and inoculate into strontium-selenite broth (Iveson and Mackay-Scollay, 1972), followed by plating on salmonella-shigella agar. From these plates, suspicious colonies of salmonellae were examined biochemically and serologically.

Results

From June 1972 to April 1973, 1675 stool specimens were examined for salmonellae by this one-day

Group	Serotype	Total Number of Strains Detected by Combination of Both Procedures	No. of Strains Detected by	
			One-day Procedure	Routine Procedure
A	<i>S. paratyphi</i> A	1	1	1
B	<i>S. paratyphi</i> B	3	3	2
	<i>S. derby</i>	26	23	19
	<i>S. typhimurium</i>	90	85	66
	Untyped	11	10	7
C	<i>S. paratyphi</i> C	2	2	2
	<i>S. newport</i>	5	5	4
	<i>S. muenchen</i>	1	1	0
	Untyped	2	2	2
D	<i>S. enteritidis</i>	1	1	1
	<i>S. panama</i>	4	4	3
	Untyped	4	3	2
E	<i>S. anatum</i>	18	16	13
	Untyped	9	8	6
R	<i>S. Johannesburg</i>	4	4	3
Total isolated		181	168	131
Percentage of total isolates		100	92.8	72.3

Table Comparison of efficiency of the one-day selective migration and routine procedures in isolating salmonellae from faeces

selective migration procedure in parallel with the conventional routine isolation methods. A total of 181 salmonella strains, other than *S. typhi*, were isolated during this period when both procedures were used. The results presented in the table reveal that when the one-day procedure was applied, 168 (92.8%) salmonella strains were detected and 13 (7.2%) were missed whereas with the routine isolation procedure only 131 (72.3%) strains were isolated and 50 (27.7%) were missed. From the table it appears that the one-day procedure did not discriminate against particular serotypes other than *S. typhi*. The high recovery rate of *S. typhimurium* was attributed to the occurrence of an outbreak in a children's ward during the study. Allowing for the exclusion of *S. typhimurium*, 91 salmonella strains would then be isolated by both procedures. The one-day procedure detected 83 whereas the routine procedure detected 65 strains only.

After the migrated growth at the top of the U tubes (in the tryptone soya broth) was plated onto MacConkey agar and suspected colonies were picked out for biochemical and serological testing, an additional 15 strains of salmonella was isolated to make up a total of 183 (168 + 15). These 15 salmonella strains were missed because they were mixed with other enteric bacteria (often *Citrobacter*) and comprised less than 50% of the total bacterial population. However, from the majority (128 out of 183, 70%) of the positive cases subculture of the migrated growth yielded pure cultures of salmonella; only very occasionally were there a few lactose-positive colonies of other enteric bacteria present amongst the salmonella colonies on the MacConkey agar.

Once a pure culture of salmonella was established in the migrated growth, it remained in the pure state for a further one or two days. This is valuable in case further tests have to be delayed. On the other hand, if the initial numbers of salmonella cells were not predominant, as occasionally observed, they would be gradually overgrown by other enteric bacteria upon further incubation.

It was noted that in most cases only one flagellar phase (either phase 1 or phase 2) of a diphasic salmonella strain could be detected by direct agglutination of the migrated growth in the broth. This variation made direct serotyping of the migrated culture not feasible. However, on subculturing the migrated growth onto nutrient agar medium, the missing phase would frequently reappear.

In 230 out of 1675 stool specimens (13.7%), migrated bacterial growth was observed in the uninoculated end of the U-tubes within 24 hours and in them salmonellae were not detected. Of these 230 migrated growths, three gave positive and six gave doubtfully positive agglutination results with polyvalent H antiserum. Nevertheless, further tests with salmonella polyvalent O antiserum all gave a negative reaction. Subsequent examination showed that eight of them were *Citrobacter* strains.

From the 1675 stool specimens, nine strains of *S. typhi* were isolated by the routine procedure. All of them were missed by the one-day selective migration procedure.

Discussion

The one-day selective migration procedure presented is efficient, time-saving, and economical. From 1675 clinical stool specimens, only 131 salmonella strains, other than *S. typhi*, were isolated with routine methods whilst 168 were detected by the one-day procedure, ie, a 28% increase in efficiency. The one-day procedure shortens the time of investigation from two to four days to 16 to 24 hours. The method also economizes in the use of media and glassware and saves manipulation. In 230 out of the 1675 stool specimens with migrated growth in the uninoculated arms, most could be differentiated immediately from salmonella by direct agglutination and only nine cases required further clarification.

The selective substances responsible for re-

tarding the migration of non-salmonella enterobacteria were magnesium chloride and brilliant green. The concentration of the former was critical whereas that of the latter was less so. An increase in the concentration of $MgCl_2 \cdot 6H_2O$, eg, to 3.2% in the semi-solid enrichment medium, would make it more selective for detecting salmonellae but the time for detection would be prolonged. We found that the concentration (2.4% $MgCl_2 \cdot 6H_2O$) employed in our study was appropriate, although the formula for the medium could be further modified, depending on the purpose of examination and also the nature of specimens.

The most important drawback to the procedure is that it cannot be applied to the detection of *S. typhi* which is the most important salmonella in human infection. As to other salmonellae frequently isolated from clinical faecal specimens, the one-day procedure appeared to have no discrimination against particular serotypes. Another drawback is that when stool specimens were artificially infected with two or more salmonella serotypes, usually only one of them was recovered by the one-day procedure because of differences in migration rate. Nevertheless, the method presented is of practical value for the isolation of salmonellae from stool specimens of gastroenteritis cases or when an outbreak of salmonella food poisoning is encountered, or for screening of salmonellae from food-stuffs or animal feeds for public health measures.

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