A SIMPLE SINGLE-STEP IMMUNOIMMOBILISATION METHOD FOR THE DETECTION OF *SALMONELLA* IN THE PRESENCE OF LARGE NUMBERS OF OTHER BACTERIA

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PLATE IX

NUMEROUS attempts have been made to simplify and improve the methods of detection of *Salmonella* (Rappaport, Konforti and Navon, 1956; Breed, Murray and Smith, 1957; Edwards and Ewing, 1962; Georgeala and Boothroyd, 1964; Haglund *et al.*, 1964; Silliker, Diebel and Chiu, 1964; Silliker *et al.*, 1965; Raj, 1966; Silliker, Schmall and Chiu, 1966; Insalata, Schulte and Berman, 1967; Abrahamsson, Patterson and Rueman, 1968). At present, however, most laboratories find it necessary to use the method recommended by Edwards and Ewing (1962).

Recently, a disk-immunoimmobilisation method for the typing of pure isolates of *Salmonella* was described (Mohit, 1968). We now report a similar procedure for the detection of *Salmonella* in human faeces. This method combines the use of the motility of these bacteria in soft agar, their selective growth in *Salmonella-Shigella* (S-S) medium and their immunoimmobilisation by polyvalent H antiserum in a single step.

MATERIALS AND METHODS

Preparation of media. S-S motility medium (SSM) was prepared by decreasing the agar content in the formula of S-S Medium (Difco, no. B74) to 3 g per l. The pH of the medium was adjusted to 7.0 by addition of NaOH. SSM (15 ml) was poured into a sterile 10-cm petri dish and cooled to room temperature. The lid of the petri dish was sealed on its plate by stopcock grease to prevent evaporation and hardening of agar during the incubation period. This precaution was found to be essential for the success of the technique.

Antiserum and preparation of disks. *Salmonella* polyvalent H antiserum was obtained from the National Communicable Disease Center, National Institutes of Health (NIH), Atlanta, Georgia, USA. Antiserum disks were prepared by impregnating absorbent paper disks (BBL no. 18-104v) with 1 drop of a 1 in 10 dilution of polyvalent H antiserum in Tris-albumin buffer (Mohit, 1968). In general, the disks were freshly prepared; however, they can be stored at 4°C for 10 days without losing activity.

Bacteria. *Salmonella* cultures were either isolated from NIH Clinical Center patients or referred to this laboratory from other hospitals.

Experimental procedure. The specimen (0.02 ml) was inoculated on one side of an SSM plate and an antiserum disk was placed 7 cm from the site of the inoculum. The plate was left at room temperature (24°C) and examined at 24 and 48 h for a semicircular line at the limit of opacity round the disk, caused by the growth and spreading of *Salmonella* and its immune immobilisation ("line of immobilisation": see figure).

The immunoimmobilisation method was compared with a standard method for detection of *Salmonella* in the following way. Fresh faeces (5 g) was suspended in 25 ml of saline.

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A pure broth culture of *Salmonella* was serially diluted with the faecal suspension. To a duplicate set of SSM plates and S-S agar plates, 0.02 ml of each dilution of *Salmonella* in faeces was applied. The SSM plates were examined for the presence of lines of immobilisation. The S-S agar plates were used for the detection of *Salmonella* according to a standard method (Edwards and Ewing, 1962). The number of salmonellae detected by the two methods was determined by performing duplicate bacterial counts (agar pour-plate method) on the original *Salmonella* broth culture. In addition, stool specimens from 60 patients were screened by both methods.

RESULTS

Seven strains of *Salmonella* were tested in 12 experiments by the two methods; the table shows the sensitivity of each method for their detection. The immunoimmobilisation method was consistently more sensitive than the standard method. With the exception of *S. typhimurium* with the immunoimmobilisation method, wide variations in sensitivity were found with both methods when the same *Salmonella* was suspended in faeces from different persons. In the case of the 60 stool specimens screened by both methods, *Salmonella* was detected in two specimens by both. No false positives were noted.

Isolation of *Salmonella* detected by the immunoimmobilisation method was accomplished by removing a loopful of bacteria from the line of immobilisation and inoculating an S-S agar plate with it. The colonies grown on the latter were then tested by the standard method. All such colonies proved to be of *Salmonella*.

DISCUSSION

The need for a simple, sensitive method for the detection of *Salmonella* is apparent from the large number of investigations devoted to devising such methods (Ino and Graber, 1955; Rappaport *et al.*, 1956; Georgeala and Boothroyd, 1964; Haglund *et al.*, 1964; Silliker *et al.*, 1964; Raj, 1966; Insalata *et al.*, 1967; Abrahamsson *et al.*, 1968). An increase in the incidence of salmonellosis and of *Salmonella* contaminants in food and other materials has further accentuated this need (Insalata *et al.*, 1967; Abrahamsson *et al.*, 1968).

TABLE
*Sensitivity of detection of Salmonella by immunoimmobilisation
compared with that of a standard method*

Experiment number	<i>Salmonella</i> species	Least number of <i>Salmonella</i> detected by the		Sensitivity ratio*
		immunoimmobilisation method	standard method	
1	<i>S. typhimurium</i>	1	10	10
2	<i>S. typhimurium</i>	1	1000	1000
3	<i>S. typhimurium</i>	1	10	10
4	<i>S. typhimurium</i>	1	10,000	10,000
5	<i>S. typhimurium</i>	1	10	10
6	<i>S. chester</i>	30	3000	100
7	<i>S. paratyphi B</i>	300	3000	10
8	<i>S. montevideo</i>	3	300	100
9	<i>S. enteritidis</i>	240	2400	10
10	<i>S. blockley</i>	320	3200	10
11	<i>S. typhimurium</i>	1	10	10
12	<i>S. heidelberg</i>	2	200	100

* Sensitivity ratio = $\frac{\text{least number of bacteria detected by standard method}}{\text{least number of bacteria detected by immunoimmobilisation}}$

SALMONELLA IMMUNOIMMOBILISATION

FIGURE—Demonstration of growth of *Salmonella* on S-S motility medium immobilised around a disk impregnated with polyvalent *Salmonella* H antiserum.

The disk immunoimmobilisation method described in this report appears to be a simple and highly sensitive technique for detecting *Salmonella* in faecal samples. In most cases, as few as one *Salmonella* could be detected in the presence of a large number of bacteria in the stool. In this study, the volume of the specimen applied to each plate was 0.02 ml. This small inoculum was necessary for individual colony isolation required by the standard method. When low *Salmonella* concentrations are expected, as in stools of carrier individuals, the volume of inoculum in the immunoimmobilisation procedure may easily be increased 50-fold and the probability of detection of *Salmonella* should be correspondingly increased.

No reason can be advanced for the variation in sensitivity of detection of a *Salmonella* according to the sample of faecal suspension with which it was diluted. Detailed bacteriological studies of different samples showed no apparent relationship between the nature of the bacterial flora they contained and the sensitivity of detection of the test organism.

No false positives were noted in this investigation. This is not surprising, because it has been demonstrated that H antisera are highly specific for the detection of *Salmonella* (Silliker *et al.*, 1966).

It was further noted that when a salmonella was mixed with pure cultures of motile *Proteus* and *Citrobacter* in the ratio of 1 in 10,000 it was easily detected. No false positive results were obtained. Thus, although both *Salmonella* and certain other motile bacteria will grow on selective motility medium and spread toward the antiserum disk, the spreading of *Salmonella* alone is inhibited by the anti-flagellar antibodies and its presence revealed by the formation of a line of immobilisation.

An inherent disadvantage of the technique is its inability to detect non-motile *Salmonella*. *S. gallinarum* and its variant, *S. pullorum*, are the only non-motile species of *Salmonella*; however, these are essentially fowl pathogens and are not significant in human salmonellosis (Breed *et al.*, 1957). We do not propose that our method should be used as a substitute for other conventional methods. However, we do suggest that the technique devised in this study can shorten the time taken to detect salmonellae from faecal or other samples without loss of sensitivity, and that combining it with conventional methods provides a more efficient form of investigation.

SUMMARY

A single-step disk immunoimmobilisation method for the detection of motile salmonellae is described. It combines in a single step the use of the motility of these bacteria in soft agar, their selective growth on *Salmonella-Shigella* media, and their immobilisation by polyvalent H antiserum. The method is shown to be 10 to 10,000 times more sensitive than a standard method for detection of *Salmonella* under the experimental conditions used. The time required for detection is shortened to 24-48 h.

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