J. Hyg., Camb. (1985), **94**, 245–262
Printed in Great Britain

Rapid and automated detection of salmonella by electrical measurements

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(*Received* 20 *September* 1984; *accepted* 14 *December* 1984)

SUMMARY

A rapid method for determining the presence of salmonella in food is described. It consists of pre-enrichment in buffered peptone water modified by the addition of dulcitol and trimethylamine oxide, followed by selective enrichment in a selenite–cystine broth with similar modifications. Changes in the conductance of the selective enrichment broth are monitored continuously using a suitable impediometric instrument. Most of the *Salmonella* spp. tested gave a fast ($\sim$ 100 μS/h) and large ($>$ 600 μS) change in conductance, other enteric bacteria much less or no change. The assay is usually complete within 24 h. Samples of foodstuffs, naturally and artificially contaminated with *Salmonella* spp., were all correctly classified. Some strains of *Citrobacter freundii* produced a false positive conductance response, and they could not be selectively eliminated using antibiotics or cyanide. The conductance method is simple and easy to use, gives rapid results and involves less media and subculturing than is required for traditional methods.

INTRODUCTION

D'Aoust (1984) has reviewed the methodology for the detection of salmonella in foods, and states that 'standard cultural methods for the isolation of salmonella in foods are labour intensive and generally require 4–5 days for presumptive identification'. Attempts to speed up the detection of salmonella using alternative methods and other techniques have met with some success, but D'Aoust stresses the need for 'rapid, cost-efficient, pre-enrichment-dependent analytical schemes'.

One property of salmonellae which has not been utilized in their isolation is the ability to reduce trimethylamine-*N*-oxide (TMAO) to trimethylamine (TMA). This reaction supplies the energy required to support anaerobic growth (Kim & Chang, 1974). TMAO is generally present in marine fish and is reduced to TMA during post-mortem bacterial spoilage. Easter, Gibson & Ward (1982) have described an automated assay for TMAO reduction utilizing modern instruments for measuring

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the conductance changes during the growth of microbial cultures. The reduction of TMAO, an uncharged neutral molecule, to the highly basic TMA yields a large change in conductance. The results from conductance assays are generally available in less than one-third of the time taken by conventional assays.

This paper describes how TMAO can be incorporated into one of the standard selective enrichment media to provide a rapid detection system for salmonella in foods using an automated conductance assay.

MATERIALS AND METHODS

Bacteria

The designation and source of the strains used are shown in Table 1. Those from the City Hospital, Aberdeen were recent fresh isolates. All other strains had been in culture for some time, and many had been isolated from foods during routine monitoring.

Assay for TMAO reduction

The conductance assay described by Easter, Gibson & Ward (1982) was used to screen bacteria for their ability to reduce TMAO to TMA in the medium of Wood & Baird (1943).

Colony plate counts

The CLED medium (Oxoid CM 301) was used to enumerate bacteria using either a 0·2 ml spread plate method or the Spiral Plate Maker (Don Whitley Scientific, Shipley).

Conductance measuring instruments

The prototype instrument described by Richards *et al.* (1978), and the commercial version produced by Malthus Instruments, Stoke-on-Trent, described by Baynes, Comrie & Prain (1983), and Porter *et al.* (1983), were both used for all the developmental work described.

In brief, the procedure for using these instruments is as follows. The medium is contained in a 12 ml glass ampoule bearing a pair of platinum electrodes printed on to a ceramic base. The ampoules are kept in a water incubator at constant temperature (±0·002 °C of the set temperature). After inoculation of the medium, the ampoule is clipped to a rack which is connected to the measuring system. The change in conductance (G_B, measured in microsiemens (μS)) is displayed continuously on the strip chart recorder of the 8-channel version and is monitored every 6 min in the 128-channel commercial version. The latter incorporates a microprocessor and a video display screen which monitors the G_B of each sample. The microprocessor is programmed to display on the screen when a significant change in conductance, the detection time, has occurred. The detection time is the time at which the conductance deviates from a flat baseline level to produce a recognizable response (see Fig. 1). In the prototype instruments the data are recorded on paper tape which is fed into an IBM 1130 computer at the end of the experiment.

The final assay system was also tested using the Bactometer M 123 (Bactomatic

Table 1. *Bacteria used in this study*

Organism	No. of strains	Strain designation*
Citrobacter freundii	6	NC1B 11496, L 8, L 9, G 7, G 28, G 29
Citrobacter sp.	1	CH 40
Escherichia coli	26	CH 16–39 (incl.), HP 3, L 5
Klebsiella aerogenes	1	CH 9
K. pneumoniae	2	CH 10, L 6
Proteus mirabilis	3	CH 15, L 7, HP 4
P. morganii	1	CH 14
P. rettgeri	1	HP 6
P. vulgaris	2	CH 13, HP 5
Salmonella agona	4	CH 6, CH 8, G 1, G 14
S. anatum	1	G 15
S. bispebjerg	1	G 16
S. bovis morbificans	1	G 13
S. cerro	1	G 17
S. cubana	1	G 34
S. derby	1	G 8
S. dublin	3	G 9, G 10, G 18
S. eastbourne	2	G 4, G 37
S. enteritidis	3	CH 4, G 5, NCTC 5188
S. enteritidis var. *danysz*	2	G 12, G 19
S. fortune	1	G 33
S. gallinarum	1	HP 2
S. glostrup	1	G 40
S. gloucester	1	G 48
S. hadar	1	NCTC 9877
S. ibaden	1	G 38
S. infantis	5	G 6, G 20, G 27, G 50, NCTC 6703
S. java	1	G 41
S. kuilsrivier	1	G 21
S. lille	1	G 39
S. livingstone	1	G 22
S. mkamba	1	G 2
S. mokola	1	G 44
S. montevideo	1	NCTC 5747
S. napoli	1	G 3
S. neudorf	1	G 43
S. poona	1	G 23
S. senftenberg	2	L 3, G 24
S. singapore	1	G 31
S. sofia	1	G 25
Salmonella sp.	3	CH 1, CH 5, L 4
S. stanleyville	1	G 46
S. stockholm	1	G 47
S. takoradi	1	G 30
S. tennessee	1	G 26
S. thompson	2	L 2, G 32
S. typhimurium	5	L 1, G 11, HP 1, CH 2, NCTC 74
S. vancouver	1	G 45
S. vinohrady	1	G 42
S. virchow	3	CH 3, CH 4, NCTC 5742
S. weltevreden	1	G 51
S. zega	1	G 49.
Shigella sonnei	1	CH 12
Sh. sonnei C	1	CH 11

* Strain designation: CH 1–40, Dr T. M. S. Reid, City Hospital, Grampian Health Board, Aberdeen; G 1–29, gifts donated by several researchers (personal communications); HP 1–6, Dr N. Smith, Hatfield Polytechnic, Hatfield, Herts; L 1–9, laboratory culture at FMBRA; NC1B, National Collection of Industrial Bacteria NCIMB Ltd, Torry Research Station, Aberdeen; NCTC, National Collection of Type Cultures, London.

Inc., Princeton, New Jersey). This instrument measures either conductance and capacitance, or conductance, capacitance or impedance, using stainless steel electrodes that protrude through the base of a plastic disposable module. Each module has 16 sample wells of 2 ml capacity, and is maintained at the required temperature (±0·1 °C) in an electric fan incubator capable of holding four modules. Data are collected and manipulated by a microprocessor and displayed on a video display unit.

Operating procedures. The sample was inoculated into the pre-enrichment broth, buffered peptone water (BPW, Oxoid CM 509) or its modifications, incubated for 3–24 h at 37 °C, and then 0·10–1·00 ml were inoculated into the selective enrichment broth in the ampoule for conductance measurements.

Media

Nutrient broth (Oxoid CM 1) was used for resuscitation of the strains, which were maintained on nutrient agar (Oxoid CM 3) or plate count agar (Oxoid CM 325).

The media used for the detection of salmonella were a pre-enrichment broth and three selective enrichment broths. The basic pre-enrichment medium was BPW either supplemented with 0·1% TMAO HCl (BPW/T), or with 0·5% dulcitol (BPW/D) or with both TMAO and dulcitol (BPW/T/D), and adjusted to pH 7·2. Other pre-enrichment media used were 10% skimmed milk powder (Oxoid L 31) containing 0·002% brilliant green, 0·1% TMAO HCl and 0·5% dulcitol, adjusted to pH 7·2 (SMP) and BPW/T/D with 0·22% Tergitol (BPW/T/D/t). Tergitol was obtained from Union Carbide Ltd, Rickmansworth.

The selective enrichment broths tested were selenite-cystine (SC, Oxoid CM 395 and CM 155), Muller–Kauffman tetrathionate broth (MKT, Oxoid 343) and the Rappaport–Vassiliadis medium RV 10 described by Vassiliadis (1983). For conductance measurements these selective broths were supplemented with 0·5% TMAO HCl and 0·5% dulcitol, and adjusted to the pH recommended.

Foods examined

Samples of ground linseed, coconut and egg albumen, known to be naturally contaminated with salmonella, were tested. Other samples examined were: (*a*) cocoa powder and milk crumb artificially contaminated with *S. napoli* (G3), *S. eastbourne* (G4) and *S. enteritidis* (G5) (serotypes previously isolated from chocolate products); (*b*) milk chocolate inoculated with *S. agona*; (*c*) wheat flour inoculated with *S. typhimurium* (L 1 and NCTC 74); and (*d*) frozen beefburgers inoculated with a strain of *S. hadar* previously isolated from minced beef.

Conventional methods for detecting salmonella in foods

The methods recommended by I.C.M.S.F. (1978) were used for the presumptive detection of salmonella in foods. They consisted of a pre-enrichment period followed by selective enrichment in SC and MKT. BPW/T/D was used for the pre-enrichment of all samples except cocoa powder or milk crumb, and ground linseed, when SMP and BPW/T/D/t were used respectively. The RV 10 medium was also used for selective enrichment at 37 °C.

Selective agars used for the presumptive identification of *Salmonella* spp. were xylose lysine desoxycholate medium (XLD, Oxoid CM 469), brilliant green agar (BGA, Oxoid CM 329), and bismuth sulphite agar (BSA, Oxoid CM 201).

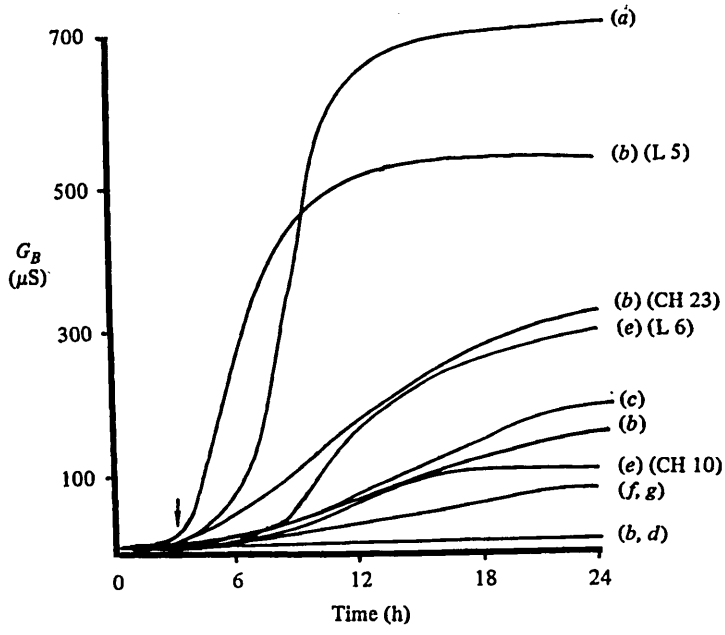

Fig. 1. Conductance changes caused by Enterobacteriaceae in SC/T at 37 °C. SC/T was inoculated with overnight pre-enrichment broths (BPW/T) of the following organisms: (*a*) *Salmonella* spp. (*b*) strains of *E. coli*; (*c*) *P. mirabilis*, *P. vulgaris*; (*d*) *P. rettgeri*; (*e*) *K. pneumoniae*; (*f*) *Citrobacter* spp. (NCIB 11496, CH 40); (*g*) *Sh. sonnei* (CH 11 and 12). ↓ Detection time.

RESULTS

Development of a suitable assay method

Media manipulation

(*a*) *Bacterial reduction of TMAO.* Strains listed in Table 1 were inoculated into Wood & Baird's medium in Malthus conductance ampoules, and tested in the TMAO reduction assay of Easter, Gibson & Ward (1982).

All 66 strains of salmonella tested could reduce TMAO to TMA, thus TMAO reduction seems to be a general property of salmonellas. All of the other bacteria tested could reduce TMAO except some *Proteus* spp., *P. vulgaris* (HP 5), *P. mirabilis* (L 5 and CH 15) and *P. rettgeri* (HP 6). *P. morganii* (CH 14) reduced TMAO, but only very slowly.

(*b*) *TMAO reduction in selective media.* Since the enzyme system necessary for TMAO reduction is inducible in *S. typhimurium* and some other Gram-negative bacteria (Kim & Chang, 1974; Easter *et al.* 1983), TMAO was added to the pre-enrichment broth as well as the selective medium, so the organism has the full complement of enzymes required for TMAO reduction when inoculated into the selective broth. TMAO (0·5%) was also added to three selective media, to give SC/T, MKT/T and RV/T, which were inoculated with an overnight culture of (*a*)

https://doi.org/10.1017/S0022172400061477 Published online by Cambridge University Press

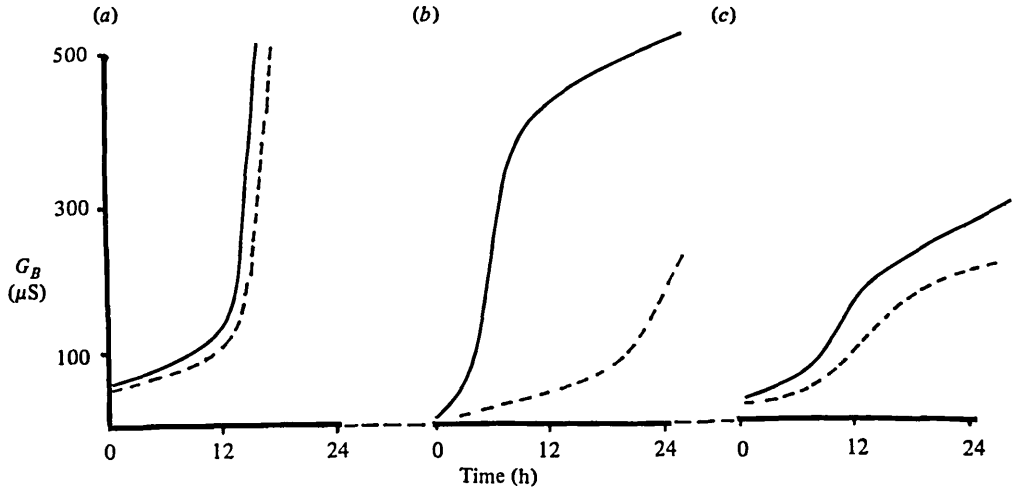

Fig. 2. Effect of omitting lactose from SC/T on the conductance changes caused by *S. typhimurium* (NCTC 74), *E. coli* (L 5) and *K. pneumoniae* (L 6). Overnight pre-enrichment broths of (*a*) *S. typhimurium* (NCTC 74); (*b*) *E. coli* (L 5); and (*c*) *K. pneumoniae* (L 6) in BPW/T were inoculated into SCT with (——) and without (– – –) lactose.

S. typhimurium (L1) in BPW/T and (*b*) 25 g flour in BPW/T with and without added *S. typhimurium* (L1).

TMAO was reduced to TMA in all selective media but the conductance responses differed. SC/T was the best medium for the detection of *S. typhimurium* because it gave the largest and most distinctive conductance change. A large conductance response was produced in the MKT/T broth when inoculated with the pre-enriched flour sample, but this occurred in both the presence and absence of *S. typhimurium*. The baseline response in MKT/T was also noisy due to the high level of electrolytes. The RV/T medium gave a comparatively small conductance change. This was improved by increasing the pH from 5·0 to 5·9, but above pH 6·0 some precipitation occurred, resulting in reduced selectivity. The poor conductance response of *S. typhimurium* in RV/T was probably due to the inhibition of TMAO reduction at pH 6·0 or less (Easter, Gibson & Ward, 1982; Kim & Chang, 1974), and the poor ionization of TMA at pH values below 7·0.

Five other salmonellas (*S. enteritidis*, NCTC 5188; *S. hadar*, NCTC 9877; *S. montevideo*, NCTC 5747; *S. infantis*, NCTC 6703 and *S. virchow*, NCTC 5742) also reduced TMAO in all three selective media. Again the best responses were produced in SC/T, which was therefore chosen for further work in developing the technique. The conductance change caused by *Salmonella* spp. in SC without TMAO was very small.

All the test organisms listed in Table 1 were assayed in SC/T and the conductance changes monitored over 24 h. All *Salmonella* spp. produced a large change in conductance (> 600 μS at 80–140 μS/h, see Fig. 1) except *S. gallinarum* (HP2), which gave a small response of 70 μS. Moderate conductance changes were also produced by some of the other organisms examined, in particular certain strains of *E. coli* (L5, CH23) and *K. pneumoniae* (L6, CH10). It was evident that

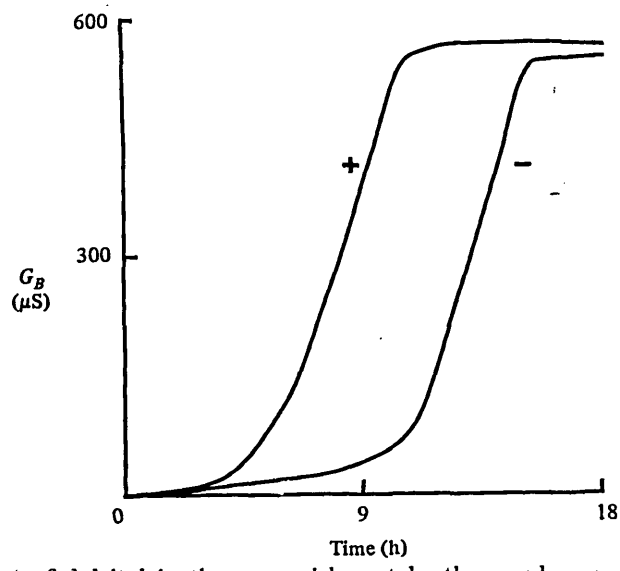

Fig. 3. Effect of dulcitol in the pre-enrichment broth on subsequent conductance changes of *Salmonella* spp. in SC/T/D. Eight *Salmonella* spp. (CH 1–8 inclusive) were pre-enriched for 16 h in BPW/T and BPW/T/D, when 0·2 ml aliquots were inoculated into 10 ml SC/T/D. The conductance response of strain CH 1 is shown after pre-enrichment with (+) and without (−) dulcitol. Similar results were obtained for the other seven species tested.

the specificity of the medium required some improvement in order to make the large conductance change indicative rather than presumptive of the presence of *Salmonella* spp.

(*c*) *Effect of lactose.* Salmonellas are by definition unable to ferment lactose or give a late response, so distinguishing them from other enteric bacteria. Therefore lactose was omitted from the SC/T medium. The effect of this alteration is shown in Fig. 2. As expected, the omission of lactose had no effect on the conductance change of *S. typhimurium* (NCTC 74) but markedly reduced the conductance and rate of change of conductance of *E. coli*, e.g. L5, and slightly diminished that of *K. pneumoniae*. The conductance responses of eight other *Salmonella* spp. (strains CH 1–8) inclusive were unaffected by the omission of lactose.

(*d*) *Effect of dulcitol.* Since most salmonellas can produce acid from dulcitol (Edwards & Ewing, 1972), dulcitol was added to the SC/T medium instead of lactose to give SC/T/D. *Salmonella* spp. produced a larger and faster conductance change than previously obtained with or without lactose. The conductance responses of the other bacteria tested were noticeably smaller when dulcitol replaced lactose, and the rate of change of conductance was also lower. As dulcitol has such a marked positive effect on the assay and the enzymes for dulcitol metabolism are thought to be inducible, dulcitol was also added to the pre-enrichment medium (to give BPW/T/D).

The effect of pre-incubation in the presence and absence of dulcitol on subsequent conductance responses of *Salmonella* spp. in SC/T/D is shown in Fig. 3. It can be

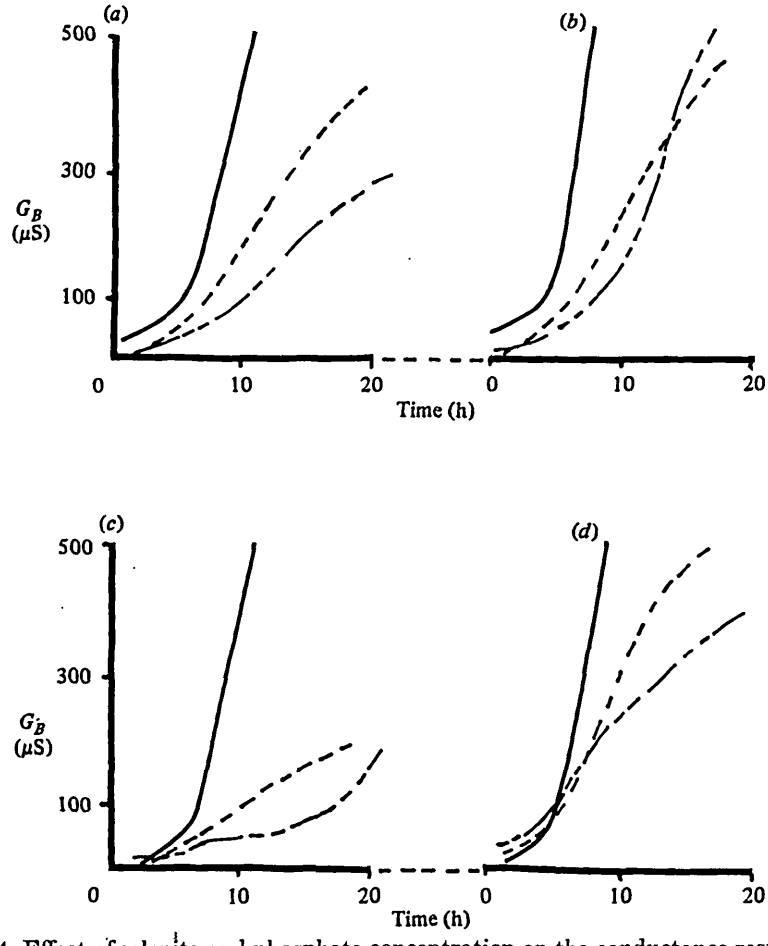

Fig. 4. Effect of selenite and phosphate concentration on the conductance response of *S. typhimurium* (NCTC 74), *E. coli* (L 5) and *K. pneumoniae* (L 6). Overnight cultures of each test organisms in BPW/T/D were inoculated into SC/T/D containing: (*a*) 4 g/l $NaHSeO_3$, 10 g/l $Na_2HPO_4.2H_2O$; (*b*) 2 g/l $NaHSeO_3$, 5 g/l $Na_2HPO_4.2H_2O$; (*c*) 4 g/l $NaHSeO_3$, 5 g/l $Na_2HPO_4.2H_2O$; (*d*) 2 g/l $NaHSeO_3$, 10 g/l $Na_2HPO_4.2H_2O$. ——, *S. typhimurium* (NCTC 74); – – –, *E. coli* (L 5); — — — —, *K. pneumoniae* (L 6).

seen that the presence of dulcitol reduces the detection time giving an earlier response. It had no effect on the other enteric species tested. After overnight incubation, the number of salmonellas in BPW/T/D pre-enrichment was higher than that from BPW/T. However, the effect of a larger inoculum number alone is insufficient to account for the reduction in the detection time of the response.

(*e*) *Effect of selenite and phosphate.* Leifson (1936) recognized that phosphate had 'a most peculiar and striking effect in reducing the toxicity of selenites'. Accordingly the amount of biselenite and phosphate in the SC/T/D medium was varied and its effect on *S. typhimurium* (NCTC 74), *E. coli* (L5) and *K. pneumoniae* (L6) was tested. The results are shown in Fig. 4.

Lowering by half the concentration of selenite and phosphate (Fig. 4*b*) or of selenite alone (Fig. 4*d*), reduced the selectivity of the medium such that the

Table 2. *Effect of inoculum number and duration of pre-enrichment on the detection of* S. typhimurium (*NCTC* 74)

Inoculum of salmonella (no./25 g flour)	Period of pre-enrichment (h)	Conductance DT* (h)	Total detection time (h)
0	3	> 43	46
	6	> 43	49
	9	> 43	52
	12	> 46	58
	18	> 33	52
1·8	3	NT†	—
	6	NT	—
	9	NT	—
	12	NT	—
	18	12·3	30·3
18	3	31·2	34·2
	6	32·8	38·8
	9	11·3	20·3
	12	12·8	24·8
	18	11·7	28·7
180	3	> 42	> 45
	6	24·4	30·4
	9	13·3	22·3
	12	13·2	23·2
	18	9·1	27·1
1800	3	27·8	30·8
	6	22·6	28·6
	9	9·4	18·4
	12	13·2	25·2
	18	14·9	32·9

* DT, detection time. † NT, not tested.

conductance changes caused by non-salmonellas increased. However, reducing the phosphate level alone from 1·0 to 0·5% (Fig. 4*c*) increased its selectivity for salmonellas, since strains of *E. coli* and *K. pneumoniae* gave a poorer response. Subsequently, when some *Salmonella* spp., e.g. *S. napoli*, were assayed in the low-phosphate medium the conductance change ceased after 450 μS, perhaps due to adverse alkaline conditions. However, there was no difficulty in making the correct interpretation of the curve since the rate of change of conductance was relatively unaffected.

Cultural conditions

(*a*) *Duration of pre-enrichment.* A pre-enrichment broth consisting of 25 g salmonella-free flour (containing *ca.* 10^3 bacteria/g) in 100 ml BPW/T was inoculated with *S. typhimurium* (NCTC 74) or *S. virchow* (NCTC 5742) to give a range of 1–1000 salmonella/test. Broths were incubated for 3, 6, 9, 12 and 18 h at 37 °C, after which bacteria were enumerated and conductance assays in SC/T/D were performed and the detection times determined. Incubation periods of 9 and 12 h were achieved by refrigerating a 6 h culture overnight and recommencing incubation for a further 3 and 6 h on the following day after allowing for temperature equilibration.

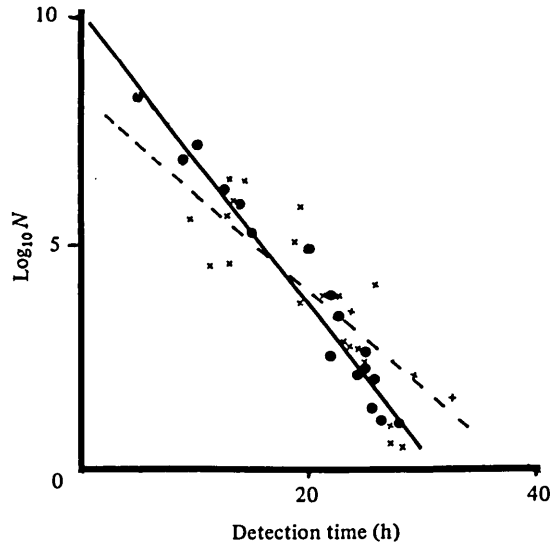

Fig. 5. Relationship between conductance detection time and numbers of *S. typhimurium* (NCTC 74) in SC/T/D after pre-enrichment in BPW/T and BPW/T/D. Pre-enrichment in BPW/T, × -- ×, $r = -0{\cdot}79$; pre-enrichment in BPW/T/D, O—O, $r = -0{\cdot}98$; where r is the correlation coefficient.

The results for *S. typhimurium* are summarized in Table 2. The detection time decreased as the numbers of salmonella present and pre-enrichment period increased. This confirms the inverse relationship between numbers and detection times shown by Richards *et al.* (1978) using non-selective media. It is interesting to note that the total detection time, i.e. period of pre-enrichment plus conductance detection time, was shortest when a 9 h pre-enrichment culture was used, and that this coincided with the end of the logarithmic growth phase (data not shown), irrespective of the initial inoculum level used. Similar results were also obtained for *S. virchow* (NCTC 5742).

The inverse relationship between numbers of salmonella and detection time is shown in Fig. 5. *S. typhimurium* (NCTC 74) in mixed and pure culture was pre-enriched in BPW/T and BPW/T/D, inoculated into SC/T/D for conductance measurements and the detection times determined plotted against numbers. The results show that the conductance method can detect low numbers of salmonella; for example, 400 cells/ml of BPW/T (*ca.* 10 cells/ml SC/D/T) was detected in 34 h. The presence of dulcitol in the pre-enrichment broth (BPW/T/D) improved the sensitivity of the assay, since similar numbers of salmonella were detected in 28 h. Dulcitol also improved the correlation between numbers of salmonella and the detection time, the correlation coefficient for BPW/T being $-0{\cdot}79$ whereas that for BPW/T/D was $-0{\cdot}98$.

(*b*) *Inoculum ratio.* It is generally accepted that the ratio of the volume of the inoculum from pre-enrichment to selective enrichment broths should be 1 : 10. Tests were carried out with *S. typhimurium* (NCTC 74) and *E. coli* (L5) to determine the optimum inoculum of pre-enrichment broth into SC/T/D. Volumes of an overnight pre-enrichment culture in BPW/T (0·10, 0·25, 0·50 and 1·0 ml) were

Table 3. *Effect of inoculum size*

Organism	Inoculum of BPW/T (ml)	Conductance response: DT* (h)	Conductance response: Rate of change (μS/h)
S. typhimurium, NCTC 74	0·10	21·4	115
	0·25	20·1	130
	0·50	19·5	130
	1·00	18·0	120
E. coli (L 5)	0·10	32·0	> 5
	0·25	32·0	10
	0·50	25·4	18
	1·00	21·4	38

* DT, detection time.

inoculated into 10 ml SC/T/D and conductance assays performed. The conductance responses obtained are described in Table 3. *S. typhimurium* gave a large and fast conductance response at all inoculum levels. As expected, the detection time decreased as the inoculum increased, but there was relatively little difference in the detection times with inocula of 0·25 and 0·5 ml. In contrast, the conductance response of *E. coli* was markedly affected by the inoculum size. The detection time increased and the rate of change of conductance was significantly reduced as the inoculum was decreased. Accordingly, an inoculum ratio of 0·25 ml pre-enrichment broth to 10 ml selective enrichment broth was chosen, since this gave a relatively fast detection for *S. typhimurium* and a limited response for *E. coli*, thus reducing the possibility of a false-positive result.

(c) *Incubation temperature.* As it is customary in many laboratories to incubate salmonella enrichment cultures at temperatures above 37 °C, comparison was made of the conductance curves resulting from incubation of the assays at 37 and 43 °C. The higher incubation temperature was inhibitory to *Salmonella* spp. and other enteric bacteria tested. In all cases, the conductance responses were smaller and delayed at the higher temperature, although the differences between the salmonellas and other bacteria remained. Thus there is no advantage in incubating the proposed medium at 43 °C as it becomes too inhibitory, and increases the time required for positive detection.

Proposed method

From the results described above, the media and protocol recommended for the rapid detection of salmonella by conductance measurements are outlined below.

Media. The pre-enrichment broth consists of BPW supplemented with 0·1% TMAO and 0·5% dulcitol adjusted to pH 7·2. The final version of the selective enrichment broth (SC/T/D) contains (g/l): bacteriological peptone (Oxoid L 37), 5; $Na_2HPO_4.2H_2O$, 10; dulcitol, 5; TMAO HCl (BDH Ltd, Poole, Dorset), 5·6; sodium biselenite (Oxoid L 121), 4. The pH is adjusted to 7·2, and the medium steamed for 10 min. It can be stored for up to 1 week at 4 °C. Immediately prior to use, 1 ml L-cystine stock solution is added to each 100 ml of the broth. The stock solution of L-cystine is prepared from L-cystine (0·1 g) in 15 ml N-NaOH and either added to 100 ml sterile distilled water in a sterile flask or filter sterilized.

Table 4. *Detection of salmonella in foods*

Sample	Conventional methods taking 4–7 days	Detection of salmonella using the conductance assay: Typical* salmonella response	Pre-enrichment period (h)	Conductance detection time (h)	Total time to detect (h)
Cocoa powders artificially contaminated with:					
S. napoli	+	+	24	12·0	36·0
S. eastbourne	–	–	24	60	ND
S. enteritidis	–	–	24	60	ND
Control – salmonella-free	–	–	24	60	ND
Dried egg albumen	–	–	18	50	ND
Milk crumb artificially contaminated with:					
†*S. napoli* (0·4/g)	+	+	18	21·4	39·4
†*S. eastborne* (0·4/g)	+	+	18	10·9	28·9
†*S. enteritidis* (0·4/g)	+	+	18	13·7	31·7
Milk chocolate artificially contaminated with *S. agona* at					
0·09/g	+	+	6	17·4	23·4
10^3	+	+	6	14·7	20·7
0·09	+	+	24	10·6	34·6
10^3	+	+	24	3·9	27·9
Frozen beefburgers artificially contaminated with *S. hadar* at:					
0·2/g	+	+	6·5	14·7	21·2
2·0/g	+	+	6·5	14·2	20·7
20·0/g	+	+	6·5	12·6	19·1
Control – salmonella-free	–	–	6·5	18·4	ND
0·2/g	+	+	24	6·5	30·5
2·0/g	+	+	24	6·6	30·6
20·0/g	+	+	24	6·2	30·2
Control – salmonella-free	–	–	24	8·8	ND
†Ground linseed A	–	–	5, 18, 20	28	ND
Ground linseed B	+	+	5	9·5	14·5
Ground linseed B	+	+	18	6·2	24·2
†Ground linseed B	+	+	20	4·7	24·7
Coconut: 5 subsamples from 1 batch tested					
(*a*) 7 December 1983	NT	1/5+	7	14·1	21·2
			24	6·7	30·7
†(*b*) 22 May 1984	–	–	18	46	ND

ND Not detected.
NT Not tested.
* Typical salmonella response: 600 μS at 100 μS/h with TMA production.
† Parallel tests using Bactometer M 123.

Protocol

(i) *Pre-enrichment.* Add 25 g of samples to 225 ml BPW/T/D and incubate for 5–7 h and 24 h, or overnight (16–18 h), at 37 °C.

(ii) *Selective enrichment and conductance measurements.* Inoculate 0·2–0·25 ml of the pre-enrichment culture into 10 ml SC/T/D in a conductance ampoule, connect to the instrument and measure conductance changes at 37 °C. A large response of > 600 μS with a rate of change of $\geqq 100$ μS/h should be regarded as a positive result indicating the presence of salmonella. Ampoules showing a positive response should be streaked on to a selective agar, such as XLD, for confirmation.

Detection of Salmonella *spp. in naturally and artificially contaminated foods*

The authors were offered, from various sources, samples of materials which were known, at least in bulk, to contain some salmonellas. Many of the samples had been stored in a dry state for more than a year. They were examined for the presence of salmonella using the conductance method proposed and by conventional methods. Details of the samples tested and the results obtained are given in Table 4.

Good results were obtained with both naturally and artificially contaminated foods. Samples containing salmonella yielded conductance curves very similar to those obtained from pure cultures, i.e. $> 600\ \mu$S in 24 h with a fast rate of conductance ($\sim 100\ \mu$S/h) and production of TMA. When a positive conductance response was obtained, a sample of the medium from the ampoule was streaked on to XLD agar, and the presence of salmonella was confirmed by the appearance of red colonies with black centres. In some cases no conductance change was obtained. When this occurred the results from conventional testing and subsequent plating of the medium from the conductance ampoule were negative, confirming the absence of salmonella.

Salmonella were not detected by either conventional or conductance methods in two samples of cocoa powder artificially inoculated with *S. eastbourne* or *S. enteritidis*, and in a sample of naturally contaminated dried egg albumen which gave positive results on a previous occasion. The negative results were probably due to either the death of the salmonella during storage or subsample variation. *S. napoli* was detected in cocoa powder after a total of 36 h, including a 24 h pre-enrichment period.

Three samples of milk crumb artificially contaminated with *S. napoli*, *S. eastbourne* and *S. enteritidis* at 0·4 cells/g were also tested. All three gave positive results, indicating that the method can detect 10 salmonellas in a 25 g sample in a total of 29–39 h, depending on species.

Low numbers of salmonella (0·09 and 0·2/g) were detected in artificially contaminated samples of milk chocolate and frozen beefburgers in 19–30 h. Salmonella were also detected in naturally contaminated samples of ground linseed and coconut in 14–30 h. The results obtained with these products show that the use of short pre-enrichment periods will give an overall shorter detection time for salmonella. The total time to detect salmonella was reduced by about 10 h using pre-enrichment periods of 5–7 h instead of overnight or 24 h pre-enrichments.

False positives from C. freundii

In the above test the control samples, i.e. food without salmonellas, all produced very small conductance responses except the frozen beefburger control, which gave a large conductance change of $> 500\ \mu$S. However, the maximum rate of change of conductance was $50\ \mu$S/h, indicating that it was not due to salmonella. Subsequent tests showed that the response was due to *C. freundii*. Another potentially false-positive conductance response due to *C. freundii* was obtained in an experiment involving salmonella-free flour. Consequently the conductance responses of six strains of *C. freundii* in SC/T/D were examined, and the results are shown in Fig. 6.

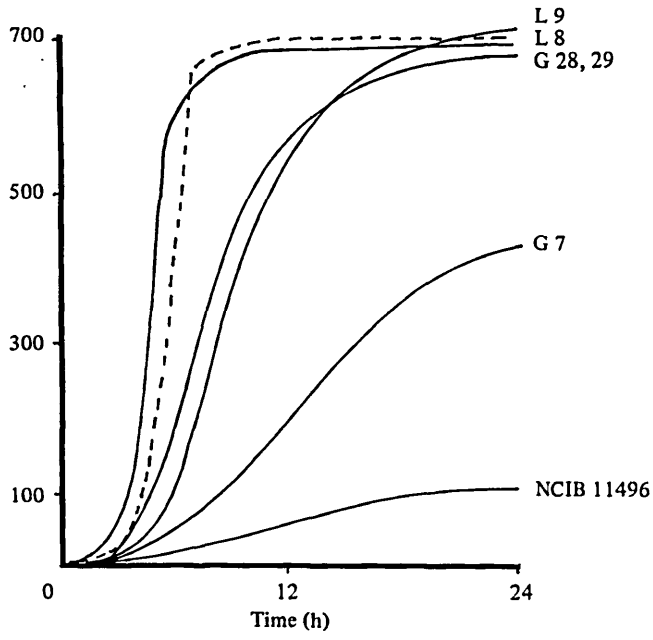

Fig. 6. Comparison of the conductance response of strains of *C. freundii* with *Salmonella* spp. in SC/T/D. Overnight cultures in BPW/T/D were inoculated into SC/T/D and conductance changes were monitored for 24 h. The conductance responses of strains of *C. freundii* (L 8, L 9, G 7, G 28 and 29 and NCIB 11496) are shown in bold lines and the broken line shows the typical response for *Salmonella* spp.

Four of the six strains of *C. freundii* (L 8, L 9, G 28 and G 29) produced a large conductance response in SC/T/D. These responses, especially that of strain L 8, are similar to those produced by salmonellas. The other two strains, L 5 and NCIB 11496, produced a moderate and slow conductance response respectively. Since the responses of some strains of *C. freundii* could be mistaken for those from salmonellas, attempts were made to differentiate the two organisms by selective inhibition.

The conductance responses of three *C. freundii* strains and several *Salmonella* spp. were measured in the presence of antibiotics (tetracycline, streptomycin, kanamycin, chloramphenicol and novobiocin at 10 and 100 μg/ml), sulphonamides (sulphadiazine, sulphanilamide and sulphamandelate (Oxoid SR 87) at 10, 100 and 1000 μg/ml) and KCN (0·05 and 0·075%). There was no definitive selective inhibition between the genera, and thus the possibility of false-positive response from the conductance assay exists.

The selectivity of the SC/T/D medium can be increased by reducing the phosphate concentration from 1·0% to 0·5%. For some strains of *C. freundii* this modification decreased the rate of change of conductance, but not the extent of the conductance change. Thus, close examination of the rate of conductance change in this medium may indicate the presence of a false positive.

Plating out the growth in the conductance ampoule on to XLD agar quickly and easily differentiates most *Citrobacter* spp. (yellow/opaque colonies) from *Salmonella* spp. (red colonies with black centres). However, certain H_2S-positive

strains of *C. freundii* also produce black-centred colonies which could be mistaken for salmonella.

Other impediometric instruments. Towards the end of the developmental work described above, an opportunity arose to test the proposed method using the Bactometer M 123. This instrument can measure the three components of electrical resistance, i.e. conductance, capacitance and impedance. The electrical responses produced by a selection of *Salmonella* spp., *C. freundii*, *E. coli* and *K. pneumoniae* were compared in the Wood & Baird medium and SC/T/D. The effect of anaerobic conditions (i.e. under liquid paraffin), antibiotics and KCN was also tested. Naturally and artificially contaminated foods were examined for the presence of salmonella using the proposed method in both the Bactometer and Malthus instruments.

In the conductance mode, the Bactometer M 123 gave very similar results to that of the Malthus instruments in all experiments. There was no significant difference between the conductance responses in aerobic or anaerobic conditions in the Bactometer, thus a liquid paraffin overlay is not necessary. Both instruments detected salmonella in naturally contaminated ground linseed and milk crumb artificially contaminated with *S. napoli*, *S. eastbourne* and *S. enteritidis* (see Table 4), and similar detection times were obtained.

Similar results were obtained with impedance and conductance measurements using the Bactometer M 123. The baseline signal for impedance was not as flat as that for conductance, however, this did not prevent or mask the detection of salmonella. Capacitance changes were unsuitable since the responses were similar for all organisms tested and gave little differentiation between species.

DISCUSSION

The ability to reduce TMAO to TMA appears to be a general property of *Salmonella* spp. since all the 66 isolates tested could perform the reduction. All the other enteric bacteria tested could also reduce TMAO with the exception of some *Proteus* spp., thus TMAO reduction may be a general property of the Enterobacteriaceae.

The final medium (SC/T/D) detected low numbers of salmonella in pure cultures and in naturally and artificially contaminated foods, and usually gave positive detections in 14–30 h. Leifson (1936) first suggested the use of the selenite medium for the specific isolation of salmonella and noted that the toxicity of selenite depended upon the basal medium. He showed that microaerophilic growth conditions gave the best results, and these are met in the type of ampoule used in the conductance apparatus used, i.e. a deep broth. TMAO reduction also requires similar conditions. Lactose is not metabolized by salmonella, and its presence can allow other organisms such as *E. coli* to grow and produce false-positive responses in the conductance assay. Dulcitol, on the other hand, can be utilized by 90% of salmonella strains, and its presence encourages the growth of many salmonellas. Thus, incorporating TMAO into a selenite–cystine broth, and replacing lactose with dulcitol, encourages the growth of salmonella so that they have a positive competitive advantage over other organisms. Simultaneously, the reduction of TMAO provides a rapid detection system by conductance measurements.

Incorporating TMAO and dulcitol in the BPW pre-enrichment broth reduced

the conductance detection time in the modified selenite broth. This is probably because the enzymes for TMAO reduction (Kim & Chang, 1974; Easter, Gibson & Ward, 1983), and possibly dulcitol utilization are inducible.

The size and shape of the conductance response of the majority of *Salmonella* spp. are so distinctive that it appears to be a characteristic property of the genus. Other species of the Enterobacteriaceae tested which were able to reduce either TMAO or selenite, caused only a small change in conductance and at a far slower rate. The growth rate of salmonellas in SC/T/D is slower than in Wood & Baird's medium, indicating that the selenite still has an inhibitory effect on salmonellas. However, from the response of the organisms tested in the modified selenite medium, it can be stated with confidence that the large change in conductance occurring at the rate shown ($\geqq 100$ μS/h) is a characteristic property of salmonellas, and that when such a curve occurs salmonellas are almost certainly present in the sample. Conversely, any other curve, or lack of curve, indicates the absence of salmonella. Thus at this stage in the isolation procedure for salmonellas there is, for the first time, definite evidence as to their presence.

The conductance method successfully detected salmonella in naturally and artificially contaminated foods, and the results obtained were in complete agreement with conventional methods. However, results were obtained in 14–39 h by the conductance method, compared with 4–7 days for conventional methods. The labour and materials involved in the conductance method were also significantly less than conventional methods. The conductance assay was also tested in another instrument, the Bactometer M 123, and results similar to the Malthus instruments were obtained. Impedance measurements by the Bactometer M 123 could also be used for the detection of salmonella.

The method proposed is very sensitive and can detect low numbers of salmonella in the pre-enrichment broth (see Fig. 5). The mean generation time (MGT) for *S. typhimurium* in BPW/T was 25 min (data not shown), and assuming the MGT for all salmonellas was 25 min, it can be estimated that one salmonella in 25 g food would take 5·5 h (or 13 generations) to reach the detectable level. Thus it is theoretically possible to perform the pre-enrichment stage during the working day (8 h), and run conductance measurements overnight to obtain the results by or during the following day. The results obtained so far suggest that this is the case, and that shorter overall detection times can be achieved by pre-enriching for 5–7 h instead of 16–24 h (Table 4). Hence, it is worthwhile to subculture from pre-enrichment broths after only a few hours and also after 24 h incubation. With automated systems the extra work involved in testing after two incubation periods is minimal.

The only organisms found to give a false-positive response in the conductance test were strains of *C. freundii*. The H_2S-negative strains of *C. freundii* could be differentiated from *Salmonella* spp. by plating out the conductance medium on to XLD agar. However, H_2S-positive strains of *C. freundii* could not be distinguished from salmonellas in this way, and further conventional tests are required for positive identification. Some differentiation between certain strains of *C. freundii* and salmonellas could be achieved by making the medium more inhibitory by reducing the phosphate concentration of SC/T/D from 1·0 to 0·5%. The incorporation of antibiotics or KCN into SC/T/D failed to separate the two organisms.

A more promising approach may be to use bacteriophage such as Felix 01 to eliminate salmonella selectively in a two-tube – i.e. presence and absence – test. In samples naturally contaminated with *C. freundii*, e.g. raw meat and some clinical specimens, the efficacy of the proposed method is slightly reduced, because further confirmatory tests are required for false-positive conductance responses. Nevertheless, the results are still available some 1–2 days sooner than by traditional methods.

We have recently tested a *Salmonella* sp., serotyped *S. arizona*. This organism produced a large conductance change of 500–600 μS but the rate of change of conductance was only 50 μS/h. This was sufficient to distinguish it from other salmonellas. The Arizona group is considered by some workers to be a separate genus and as such the proposed method supports this contention. The response of the Arizona was very similar to some *Citrobacter* species; this might have been anticipated, since all three genera have similar biochemical characteristics.

Latterly one strain of *S. gallinarum* was tested, and although it reduced TMAO in the Wood & Baird medium it did not produce the characteristic response in the modified selenite broth, thus giving a false-negative response. However, strains of the *S. gallinarum–S. pullorum* group are host-adapted avian serotypes that are rarely associated with food-poisoning incidents in man (Wilson & Miles, 1975). Consequently they may be considered to be of little public health significance, and might be an acceptable false negative to the food microbiologist.

There has been little investigation of modern automated microbial systems for the detection of specific organisms in selective media. Silvermann & Munoz (1979) suggested a useful medium for the detection of *E. coli* in sewage and water samples and Petitt (1983) suggested a selective medium for Enterobacteriaceae in foods. Stannard (1983) suggested that conductance or impedance monitoring techniques for salmonella should be applied to tests subsequent to the enrichment culture, including dulcitol fermentation. However, it is apparent from the work described above that it is not necessary to test beyond the modified enrichment culture to establish the presence of salmonellas.

Harvey & Price (1979), in a review of the principles of salmonella isolation, stated that, whilst no optimum method could be prescribed, the most important factor was the ability of the microbiologist to differentiate pathogens from non-pathogens. The authors feel that the method suggested here is a great advance towards this aim. The conductance method is simple to use and gives rapid results. Many authors quote 4–5 d incubation are required before presumptive presence of salmonella can be established, others even 7–10 d. With the proposed method, this stage is usually reached in 1 day and always within 40 h. It also offers savings in labour and materials costs, since it involves less media and subculturing than is required for traditional methods, and automation allows many samples (up to 128) to be tested simultaneously. The conductance method was developed for the rapid detection of salmonella in foods, but it should also find application in the water, medical and pharmaceutical industries.

This work forms part of a research project sponsored by the Ministry of Agriculture, Fisheries & Food (MAFF), to whom thanks are due. The results of the research are the property of the MAFF and are Crown Copyright. The authors

also thank the contributors of bacterial cultures and naturally and artificially contaminated foods, and M.C.E. is grateful to Malthus Instruments Ltd and Bactomatic Inc. for the loan of the conductance instruments.

REFERENCES

Baynes, N. C., Comrie, J. & Prain, J. H. (1983). Detection of bacterial growth by the Malthus conductance meter. *Medical Laboratory Sciences* **40**, 149–158.

D'Aoust, J. V. (1984). Salmonella detection in foods: present status and research needs for the future. *Journal of Food Protection* **47**, 78.

Easter, M. C., Gibson, D. M. & Ward, F. B. (1982). A conductance method for the assay and study of bacterial trimethylamine oxide reduction. *Journal of Applied Bacteriology* **52**, 357–365.

Easter, M. C., Gibson, D. M. & Ward, F. B. (1983). The induction and location of trimethylamine-N-oxide reductase in *Alteromonas* sp. NCMB 400. *Journal of General Microbiology* **129**, 3689–3696.

Edwards, P. R. & Ewing, W. H. (1972). *Identification of Enterobacteriaceae*, 3rd ed. Minneapolis: Burgess.

Harvey, R. M. S. & Price, T. H. (1979). A review. Principles of salmonella isolation. *Journal of Applied Bacteriology* **46**, 27–56.

I.C.M.S.F. (1978). *Microorganisms in Foods.* Vol. I. *Their Significance and Methods of Enumeration*, 2nd ed., pp. 160–172. London: University of Toronto Press.

Kim, K. E. & Chang, G. W. (1974). Trimethylamine oxide reduction by *Salmonella*. *Canadian Journal of Microbiology* **20**, 1745–1748.

Leifson, E. (1936). New selenite enrichment media for the isolation of typhoid and paratyphoid (Salmonella) bacilli. *American Journal of Hygiene* **24**, 423–432.

Petitt, S. B. (1983). Detection of 'coliforms' and Enterobacteriaceae. *Journal of Applied Bacteriology* **55**, vii.

Porter, I. A., Reid, T. M. S., Wood, W. J., Gibson, D. M. & Hobbs, G. (1983). Conductance measurements for determining antibiotic sensitivity. In *Antibiotics Asssessment of Antimicrobial Activity and Resistance* (ed. A. D. Russell & L. B. Quesnel), pp. 49–60. London: Academic Press.

Richards, J. C. S., Jason, A. C., Hobbs, G., Gibson, D. M. & Christie, R. H. (1978). Electronic measurement of bacterial growth. *Journal of Physics E: Scientific Instruments* **11**, 560–568.

Silverman, M. P. & Munoz, E. F. (1979). Automated electrical impedance technique for rapid enumeration of fecal coliforms in effluents from sewage treatment plants. *Applied and Environmental Microbiology* **37**, 521–526.

Stannard, C. J. (1983). Indicator and pathogenic organisms from foods. *Journal of Applied Bacteriology* **55**, vii.

Vassiliadis, P. (1983). The Rappaport-Vassiliadis (RV) enrichment medium for the isolation of salmonellas: an overview. *Journal of Applied Bacteriology* **54**, 69–76.

Wilson, G. S. & Miles, A. (1975). *Topley and Wilson's Principles of Bacteriology, Virology and Immunity*, 6th ed., pp. 944–945. London: Edward Arnold.

Wood, A. J. & Baird, E. A. (1943). Reduction of trimethylamine oxide by bacteria. I. The Enterobacteriaceae. *Journal of the Fisheries Research Board of Canada* **6**, 194–201.