Enzyme-Linked Immunosorbent Assay for Salmonella typhimurium in Food: Feasibility of 1-Day Salmonella Detection

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A microtitration plate, antibody-capture, enzyme-linked immunosorbent assay was developed for detection of Salmonella typhimurium. The assay utilizes a monoclonal detector antibody which shows no cross-reactions with non-Salmonella species and only a slight cross-reaction with one other Salmonella serotype. By using only one cultural stage (in a nonselective, chemically defined medium) prior to the enzyme-linked immunosorbent assay, low numbers of cells in food (10 cells 25 g"1") were detected in 19 h. Non-Salmonella competing organisms did not interfere with detection of S. typhimurium even when present in the ratio of 10^5:1 (non-Salmonella/Salmonella spp.). The assay shows the feasibility of rapid, 1-day testing for Salmonella spp. with antibody technology.

The presence of Salmonella spp. in food is of increasing concern to the food industry, the public, and the regulatory authorities. In the United Kingdom, the number of cases of food-borne illness due to Salmonella are the second highest of any reported gastrointestinal infection and have steadily increased over the last 3 years (2).

The conventional methods for Salmonella detection involving cultural steps are labor intensive and time consuming, taking 4 to 5 days for detection and confirmation (5). Therefore, there is an urgent need for rapid methods which would allow the industry to respond quickly to raw material and product contamination and which would also help with the early release of tested stocks of raw materials and finished products.

Since the first enzyme immunoassay for Salmonella spp. was reported by Krysinski and Heimisch (16), immunochemical techniques have shown considerable potential. Several enzyme-linked immunosorbent assays (ELISAs) have been developed, using both polyclonal antibodies and monoclonal antibodies (MAbs) that will detect most Salmonella serotypes (9, 13, 19). These assays and others have subsequently been developed in kit form and are available commercially. Although they reduce the total assay time by 1 or 2 days, most of the kits actually only replace the agar-plating stage of the cultural assay and even introduce a third, "postenrichment" broth before the sample can be assayed by ELISA (11). Two of these kits have been declared official first action by the Association of Official Analytical Chemists (6, 7), though all of them suffer to some degree from the disadvantages of false-negatives and false-positives (3, 10).

In this paper, we describe an ELISA for Salmonella typhimurium which is both rapid and specific. The assay has been used as a model to show the feasibility of testing for Salmonella spp. within 1 day.

MATERIALS AND METHODS

Preparation of bacterial strains. (i) Bacteria. The following organisms were a gift from the Public Health Laboratory Service, Norwich, United Kingdom (U.K.): S. typhimurium, S. enteritidis, S. breedeney, and S. weltevreden. Other organisms used were S. agona, S. infantis, S. hadar, Klebsiella pneumoniae, Enterobacter aerogenes, and Escherichia coli (all gifts from T. F. Brocklehurst, this laboratory); Erwinia carotovora subsp. carotovora and a nalidixic acid-resistant strain of S. typhimurium (gifts from B. M. Lund, this laboratory); and Citrobacter freundii NCTC 09750, Citrobacter koseri NCTC 10849, and Enterobacter aerogenes NCTC 10006 (obtained from the National Collection of Type Cultures, London, U.K.).

(ii) Cell suspensions for use in ELISA. Salmonella strains were grown for 18 h at 35°C on a rotary platform in a chemically defined medium (SCDM [12]). SCDM was prepared as follows. Solution A contained the following, in grams per liter: (NH₄)₂SO₄, 10.0; Na₂HPO₄, 30; KH₂PO₄, 15.0; NaCl, 15.0; Na₂SO₄, 0.055. Solution B was made up of the following, in grams per liter: MgCl₂·6H₂O, 0.25; CaCl₂, 0.013; FeCl₃, 7H₂O, 0.0006; yeast extract, 0.125; amino acids DL-tryptophan, L-histidine, L-proline, L-threonine, L-arginine, glycine, DL-o-alanine, and L-methionine, 0.31 (each). After being adjusted to pH 7.2, solutions A and B were autoclaved separately; then 100 ml of solution A was added to 400 ml of solution B. Finally, this mixture was supplemented with 10 ml of 25% (wt/vol) sterilized glucose. Cells were harvested by centrifugation, washed twice in saline (8.5 g liter⁻¹), and suspended in saline. The protein content of the suspensions was measured by the method of Lowry et al. (18), after which the suspensions were diluted in saline to 10 mg of protein ml⁻¹ and stored at −70°C.

When necessary, a particular phase of H-antigen expression was selected by prior growth of the organism on soft heart infusion agar (5 g liter⁻¹; Oxoid Ltd., Basingstoke, U.K.) containing thioglycolic acid (20 mg liter⁻¹) and 1% (vol/vol) antiserum to the unwanted phase (Wellcome Diagnostics, Dartford, U.K.). After incubation at 37°C, cells were picked from the leading edge of the spreading growth produced from a central inoculation point and inoculated into SCDM as described above, and the resulting suspensions were checked for the correct phase, using a slide agglutination test.

Non-Salmonella species were grown in nutrient broth (Oxoid Ltd.) for 24 or 48 h at 30°C, and suspensions were prepared as described above.

Production of polyclonal antibodies. Polyclonal antisera were raised in rabbits against S. typhimurium (H. A. Lee, G. M. Wyatt, S. Bramham, and M. R. A. Morgan, unpublished results). Following booster injections, blood was collected from the marginal ear vein after 10 and 14 days.
After centrifugation of blood samples, the plasma was removed and stored at −20°C until used.

Production of MAbs. BALB/c mice were immunized intraperitoneally to produce antibodies against *S. typhimurium* (Lee et al., unpublished results). A test bleed was taken from the tail to test for antibody activity by measuring binding to a cell-coated plate. Once this was found to be positive, the mouse was given one more booster injection and killed 4 days later, and the spleen was removed. To produce MAbs, spleen cells were fused with myeloma cells (X63-Ag8-653) obtained from Flow Laboratories Ltd., Rickmansworth, U.K. (14), in the ratio 5:1 in phosphate-buffered saline (PBS) containing 40% polyethylene glycol by the method of Galfré and Milstein (8). Subsequently, the cells were suspended in OPTI MEM 1 (GIBCO Ltd., Uxbridge, U.K.) containing fetal calf serum (4%), hypoxanthine (100 µmol liter⁻¹), aminopterin (1 µmol liter⁻¹), and thymidine (16 µmol liter⁻¹) and dispensed into three 96-well tissue culture plates (Costar; Northumbria Biologicals Ltd., Cramlington, U.K.). Following 2 weeks of culture, the well supernatants were screened for antibody activity by ELISA. Positive wells were expanded and cloned three times by limiting dilution, using rat erythrocytes (1%) as feeder cells. These operations were performed on a microprocessor-controlled automatic work station, the Biomek 1000 (Beckman Instruments Inc., High Wycombe, U.K.). To obtain large quantities of MAbs, hybridomas were grown in vitro in culture tissue flasks (Flow Laboratories Ltd.). After the cells were removed by centrifugation, culture supernatant was stored at −20°C before use in the assay. MAb subclass determinations were performed by using a mouse MAb-typing kit (Sero- tec, Oxford, U.K.).

Preparation of coated microtitration plates. (i) Cell-coated plates. Cell-coated plates were used for antibody-binding assays to screen for MAb production and to investigate antibody specificity. To prepare the plates, suspensions of whole cells (200 µl of 1 or 10 µg of cell protein ml⁻¹) in phosphate buffer (0.1 mol liter⁻¹; pH 8) containing methyl glyoxal (3 ml liter⁻¹ [11]) were placed in 96-well microtitration plates (Nunc Immunoplate 1; GIBCO Ltd.). After 16 h at 4°C, the plates were washed three times with water, using an automatic plate washer (Titertek Microplate Washer 120; Flow Laboratories Ltd.), before bovine serum albumin (200 µl of 10 g liter⁻¹) in carbonate-bicarbonate buffer (0.05 mol liter⁻¹; pH 9.6) was added to block unreacted sites. This solution was left in the plate wells for 3 h at room temperature before being removed. The plates were washed three times with water and left to dry in air before being stored in the dark in sealed plastic bags containing silica gel as desiccant.

(ii) Antibody-coated plates. For the antibody-capture ELISA for *S. typhimurium*, microtitration plates (Nunc Immunoplate 1; GIBCO Ltd.) were coated with rabbit polyclonal antiserum (200 µl) diluted 1:10⁵ (vol/vol) in carbonate-bicarbonate buffer (0.05 mol liter⁻¹; pH 9.6). This dilution was chosen as the one which gave maximum binding of *S. typhimurium* cells (2 × 10⁵ cells ml⁻¹) at the lowest antibody concentration in the antibody-capture assay. After 16 h at 4°C, the solution was removed and the plate was washed three times with water. Unreacted sites were blocked by adding bovine serum albumin as for the cell-coated plates. Following a 3-h incubation at room temperature, the plates were washed three times in PBS containing 0.05% Tween (PBS-Tween [23]) and stored at −20°C after excess buffer was removed by blotting on absorbent paper.

ELISA protocols. (i) Antibody-binding assay. To screen for MAb production, culture supernatant (150 µl) was transferred from a fusion or clone plate, using the Biomek 1000, to a cell-coated plate. After 3 h at 37°C, the plates were washed five times with PBS-Tween, and then anti-mouse immunoglobulin G-horseradish peroxidase (HRP [Sigma Chemical Co., Poole, U.K.]; 200 µl of a 1:10⁶, vol/vol, dilution in PBS-Tween) was added to the plate. After 1 h at 37°C, the plate was washed again five times with PBS-Tween, and substrate (200 µl of 3.3',5'-tetramethyl benzidine solution; Cambridge Life Sciences, Cambridge, U.K.) was added and left for approximately 15 min at room temperature. The reaction was stopped by adding sulfuric acid (50 µl; 2 mol liter⁻¹), and the well optical densities were read at 450 nm on a plate reader (Multiskan MCC; Flow Laboratories).

To investigate the binding of both polyclonal antibodies and MAbs to other *Salmonella* serotypes and other non-*Salmonella* enterobacteria, cell-coated plates were prepared with suspensions of bacterial strains (1 µg of protein ml⁻¹). Antibody solutions (200 µl), diluted to the required concentrations in PBS-Tween, were added to the cell-coated plates and left for 16 h at 4°C. Following this, the plates were washed, anti-mouse immunoglobulin M-HRP or anti-rabbit immunoglobulin G-HRP was added, and the assay was completed in the same manner as the MAb screening assay already described. Each time a batch of plates was set up to investigate antibody specificity this way, an *S. typhimurium* plate was included as a control.

(ii) Antibody-capture assay. For the identification and quantification of *S. typhimurium*, samples or standard cell suspensions (200 µl) in PBS-Tween or SCDM were added to an antibody-coated plate and left for either 16 h at room temperature (the 22-h assay) or 1 h at 37°C (the 3-h assay). Subsequently, the plate was washed five times in PBS-Tween and MAB IPRN 0402 (200 µl) diluted 1:20 (vol/vol) in PBS-Tween was added. After a reaction time of 3 h at 37°C for the 22-h assay or 1 h at 37°C for the 3-h assay, the plate was washed and the assay was completed by using anti-mouse immunoglobulin M-HRP as described previously. The incubation times were 3 h at 37°C for the 22-h assay or 1 h at 37°C for the 3-h assay.

Growth and detection of *Salmonella* spp. (i) Culture medium. In a preliminary study, various culture media were investigated for optimum growth of *Salmonella* spp., both in pure culture and in the presence of equal numbers of other members of the family *Enterobacteriaceae*. The media tested were M broth (22), M broth modified by replacement of the tryptone with the amino acid mix used in SCDM, and SCDM itself. M broth was prepared by adding the following substances, in grams, to 1 liter of distilled water: yeast extract (Difco Laboratories), 5; tryptone (Difco), 12.5; d-mannose, 2; sodium citrate, 5; NaCl, 5; K₂HPO₄, 5; MnCl₂·4H₂O, 0.14; MnSO₄·H₂O, 0.8; FeSO₄·7H₂O, 0.04; Tween 80, 0.75 (pH 7.0). The solution was autoclaved for 15 min. The broths (200 ml) were inoculated with approximately 100 cells of *S. typhimurium*, *Escherichia coli*, and *C. koseri* ml⁻¹, using a known relationship between optical density and viable count, and incubated at 37°C on a rotary platform. The cultures were sampled at intervals, and viable counts were performed by plating suitable dilutions onto XLD agar (Oxoid Ltd.). ELISA was carried out on the samples as described previously.

(ii) Effect of high numbers of competing organisms. SCDM broths (50 ml) were inoculated with *S. typhimurium* at approximately 1 cell ml⁻¹; to the same broths were added *Escherichia coli* and *Enterobacter aerogenes* at ratios to *S. typhimurium* increasing from 1:1 to 10⁶:1. The broths were
incubated for 24 h and sampled for the ELISA and for differential viable counts of the three organisms by plating on XLD agar as in (i) above. Also, isolation of *Salmonella* spp. by cultural enrichment was carried out. By using the SCDM culture as a pre-enrichment, 1-ml samples were transferred to 9 ml of Muller-Kaufman tetraphionate broth and 0.1-ml samples were transferred to 9.9 ml of Rappaport-Vassiliadis broth. After 24 and 48 h of incubation at 43°C, loopfuls were plated from each broth to modified brilliant green agar and XLD agar (all media from Oxoid Ltd.) and incubated at 37°C.

(iii) Detection of low numbers in food. Samples of chocolate were melted and inoculated with *S. typhimurium* cells (approximately 10 and 10^2 cells 25 g⁻¹), as in (i) above. A viable count was performed on the inoculum to confirm the number of cells added. The samples (25 g) were mixed with SCDM (225 ml) and left to incubate overnight (16 h) at 37°C. The chocolate-SCDM mixture was then analyzed directly in the ELISA and also sampled for enrichment culture as described above.

To assess the effect of the chocolate-SCDM mixture on the performance of the ELISA, standard curves were constructed in chocolate-SCDM (25 g/225 ml) and in chocolate-SCDM diluted in PBS-Tween (1:10; vol/vol). These were prepared with a standard curve in PBS-Tween alone.

(iv) Differentiation of viable and nonviable cells. Stuffing (prepared from sage, onion, and pork) was inoculated with high numbers of a nalidixic acid-resistant strain of *S. typhimurium* (3.8 × 10^8 g⁻¹) and cooked inside chickens for various lengths of time in a microwave oven. After cooking, samples (1 g) of the stuffing were added to buffered peptone-water (9 ml) and treated in a Colworth stomacher. The samples were then diluted in buffered peptone-water to below the sensitivity of the assay (1:10⁶, vol/vol) and measured in the 3-h ELISA before and after overnight incubation of the buffered peptone-water dilutions at 37°C.

Also, viable counts of *Salmonella* spp. in the cooked stuffing were performed on modified brilliant green agar (Oxoid Ltd.) containing nalidixic acid (20 mg liter⁻¹), using a variation of the membrane enrichment technique (15). The sample heated for the longest total time was also selectively enriched and plated as for the chocolate samples, using buffered peptone-water as the pre-enrichment broth.

**RESULTS**

**MAbs.** Several MAbs were raised to *S. typhimurium*, but the results reported all relate to MAb IFRN 0402, which belongs to the immunoglobulin M subclass.

**Antibody cross-reactions.** The cross-reactions of both the polyclonal antibodies and MAbs were studied by constructing dilution curves on cell-coated plates. The curves obtained were all compared with the curve on a plate coated with *S. typhimurium* as control. (An example of this with IFRN 0402 is shown in Fig. 1.) The percent cross-reaction was calculated by dividing the antibody dilution obtained on the cross-reactant-coated plate at half-maximum optical density by the antibody dilution obtained on the *S. typhimurium*-coated plate at half-maximum optical density. The results obtained in this manner are given in Table 1.

MAb IFRN 0402 shows a small degree of cross-reaction with *S. agona* on cell-coated plates. When this serotype is tested in the antibody-capture assay, the percent cross-reaction is 0.14% as calculated by dividing the mass of *S. typhimurium* to give half-maximal binding by the mass of *S. agona* required to give the same amount of binding.

**ELISA standard curves.** Standard curves obtained for the 22-h *S. typhimurium* ELISA and the rapid 3-h ELISA are shown in Fig. 2. Whole cells were used as the standards and were expressed as cell counts per milliliter. The within- and between-plate precision profiles for the 3-h assay calculated by the method of Ekins (4) are shown in Fig. 3. The assay limits of detection, calculated by adding 2 standard deviations to the zero value, were 5 × 10^2 cells ml⁻¹ for the 22-h assay and 10⁵ cells ml⁻¹ for the 3-h assay.

**Investigation of growth media.** *Salmonella* organisms were detected in the 22-h ELISA after 4 h of culture in SCDM, 4 to 6 h in modified M broth, and 6 h in M broth in both pure culture and mixed culture. It was decided to use SCDM for all further work. Growth of *S. typhimurium*, *Escherichia coli* and *C. koseri* in SCDM in mixed culture was identical, all cultures reaching a level of 10^8 cells ml⁻¹ after 11 h.

**Detection of *Salmonella* spp. in the presence of large numbers of competing organisms.** The presence of large numbers of microbial cells could interfere in the assay system in spite of the competitive advantage of the *Salmonella* spp. in enrichment.

![FIG. 1. Dilution curves of IFRN 0402 on an *S. typhimurium* coated (●) and an *S. agona* coated (○) plate. The percent cross-reaction was calculated by comparing the antibody dilutions which give half-maximum optical density (O.D.) (i.e., an optical density of 0.38 unit).](http://aem.asm.org/)

| TABLE 1. Cross-reactions of anti-*S. typhimurium* polyclonal antibodies and MAbs† |
|----------------------------------------|----------------|----------------|
| Cross-reactant                          | % Cross-reaction |                    |
|                                       | Polyclonal antibodies | Mab IFRN 0402 |
| *S. typhimurium* phase 1               | 100             | 100             |
| *S. typhimurium* phase 2               | 45              | 65              |
| *S. hadar* phase 1                     | 32              | 0               |
| *S. hadar* phase 2                     | 11              | 0               |
| *S. enteritidis*                       | 10              | 0               |
| *S. virchow* phase 1                   | 144             | 0               |
| *S. bredeney* phase 1                  | 20              | 0               |
| *S. weltevreden* phase 2               | 30              | 0               |
| *S. infantis* phase 2                  | 9               | 0               |
| *S. agona*                             | 20              | 7               |
| *Enterobacter agglomerans*             | 5               | 0               |
| *E. coli*                              | 6               | 0               |
| *Erwinia carotovora*                   | 10              | 0               |
| *K. pneumoniae*                        | 14              | 0               |
| *C. freundii*                          | 5               | 0               |

† Data were obtained by comparing antibody binding to cell-coated plates.
of antibody specificity: for example, competition from non-
Salmonella spp. at high inoculum levels could suppress the
growth of Salmonella spp. in the nonselective culture.
However, even with an inoculum containing 1 cell ml\(^{-1}\)

FIG. 2. Standard curves for the 3-h *S. typhimurium* assay (●) and the 22-h assay (○). The plates were coated with rabbit polyclonal
antisera (1:10\(^3\)), and the specific MAb, IFRN 0402, was detected with an anti-mouse immunoglobulin M-HRP-labeled antibody. OD, Optical
density.

to a vast excess of non-Salmonella spp. (Table 2), all
samples inoculated with *S. typhimurium* were positive in the
ELISA after 24-h culture. In this experiment, the ELISA
time was 4 h (1-h reaction with the sample, 2-h reaction with the
MAb, and 1 h with anti-mouse immunoglobulin M-HRP
antibody) and the sensitivity was \(6 \times 10^5\) cells ml\(^{-1}\).

Detection of *S. typhimurium* in food. (i) Presence of low
numbers of cells. The chocolate-SCDM mixture alone or
diluted in PBS-Tween (1:10, vol/vol) had a slightly suppressive
effect on the standard curve. The \(6 \times 10^6\)-cell-ml\(^{-1}\)
standard in undiluted chocolate-SCDM read \(3.2 \times 10^6\) cells
ml\(^{-1}\) off the PBS-Tween curve, and the \(6 \times 10^6\)-cell-ml\(^{-1}\)
standard in chocolate-SCDM diluted in PBS-Tween (1:10,
vol/vol) read \(4.8 \times 10^6\) cells ml\(^{-1}\) off the PBS-Tween curve.
The viable count on the inoculum used for the chocolate
samples showed that the samples had been inoculated at 5
and 500 cells g\(^{-1}\). The ELISA results on these samples
after incubation were \(10^5\) and \(7.6 \times 10^6\) cells ml\(^{-1}\), respec-
tively. This correlated with positives in the cultural assay.
The uninoculated samples were negative in both assays.

(ii) Distinction between viable and nonviable Salmonella

\begin{table}[h]
<table>
<thead>
<tr>
<th>No. of cells ml(^{-1}) of inoculum</th>
<th>Final count ml(^{-1})</th>
<th>ELISA</th>
</tr>
</thead>
</table>
| *S. typhi-
murium* | Enterobacter aerogenes | Escherichia coli | *S. typhi-
murium* | Total |
| 1.4 | 1.0 | 0.8 | 4.9 \(\times\) 10^6 | 4.9 \(\times\) 10^6 | + |
| 1.4 | 1.0 | 0.8 | 8.0 \(\times\) 10^6 | 7.8 \(\times\) 10^6 | + |
| 1.4 | 1.0 \(\times\) 10^2 | 0.8 \(\times\) 10^6 | 2.0 \(\times\) 10^7 | 8.6 \(\times\) 10^6 | + |
| 1.4 | 1.0 \(\times\) 10^2 | 0.8 \(\times\) 10^6 | <2.0 \(\times\) 10^6 | 8.0 \(\times\) 10^6 | + |
| 1.4 | 1.0 \(\times\) 10^2 | 0.8 \(\times\) 10^6 | <2.0 \(\times\) 10^6 | 7.9 \(\times\) 10^6 | + |
| 1.4 | 1.0 \(\times\) 10^2 | 0.8 \(\times\) 10^6 | 9.6 \(\times\) 10^6 | + |

TABLE 2. Detection of *S. typhimurium* in the presence of increasing numbers of competing organisms

FIG. 3. Precision profiles for *S. typhimurium* ELISA showing within-assay (-----) and between-assay (—) variation. The
curves were calculated with Immunofit software (Beckman).
TABLE 3. Detection of *S. typhimurium* by ELISA in inoculated and cooked sage and onion stuffing samples diluted 1:10⁴ (vol/vol) in buffered peptone-water

<table>
<thead>
<tr>
<th>Cooking time (min)</th>
<th>Viable count a, b</th>
<th>ELISA result</th>
<th>Before overnight culture</th>
<th>After overnight culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.8 × 10⁶</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>20</td>
<td>1.9 × 10⁶</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>30</td>
<td>2.6 × 10⁵</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>30 + 10 standing</td>
<td>&lt;10</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>30 + 20 standing</td>
<td>0⁶</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

a Viable count performed by a membrane enrichment technique. A nalidixic acid-resistant strain of *S. typhimurium* was used.

b Using selective enrichment broth and agar.

**spp.** All inoculated stuffing samples were negative by ELISA before incubation because of dilution of the sample beyond the assay sensitivity. However, the 0-, 20-, and 30-min cook times gave positive results after overnight incubation (Table 3), with cell counts all greater than the upper limit of detection of the ELISA (4 × 10⁶ cells ml⁻¹). These results correlated with those obtained in the cultural assay and may provide a useful way of assessing the efficiency of a food process, such as cooking or irradiation, in killing *Salmonella* spp.

**DISCUSSION**

This study has shown the potential advantages of using a specific MAbs (IFRN 0402) in an ELISA for *S. typhimurium*. The specificity of the assay is such that there is no need for selective culture and only one incubation step is necessary to allow damaged cells to recover and start multiplying. The specificity is illustrated by the study in Table 2 in which low numbers of *S. typhimurium* cells are incubated with increasing numbers of *Escherichia coli* and *Enterobacter aerogenes*. The results show that, even with 10⁶ competing organisms in the initial inoculum, the ELISA still detects *S. typhimurium*. In this study, the growth of *S. typhimurium* was depressed by the presence of the other organisms. The cell count dropped from 4.9 × 10⁵ ml⁻¹ in sample 1 to <2 × 10⁶ ml⁻¹ in samples 5 and 6 (Table 2) after 24 h of growth, as obtained by the cultural method. However, these low numbers of cells were still detectable by the antibody assay, despite viable counts of 7 × 10⁵ of the two non-*Salmonella* species ml⁻¹. This work shows that there is no cross-reaction with either of these organisms at high levels, confirming the lack of cross-reactivity shown in Table 1, and also the absence of other effects such as physical interference that might be expected at these levels.

Previously reported assays for *Salmonella* spp. (19, 20) have used antibodies with much wider specificities, recognizing a wide range of *Salmonella* serotypes. This approach has the disadvantage that non-*Salmonella* species may also be recognized. The original myeloma antibody, MOPC 467, used by Robison et al. (20) appeared to be fairly specific for *Salmonella* species, as the only reported cross-reacting organisms were *Herellea vaginicola* and *Pasteurella pneumotropica*. However, use of this assay with food samples has given a fairly high false-positive rate (3, 24). In the present study, we have selected an antibody which recognizes an epitope which is more specific. In this way, it is possible to abolish any cross-reaction with non-*Salmonella* species and to omit a selective enrichment step with consequent reductions in assay time.

The standard curves shown in Fig. 2 demonstrate that the assay has a very low limit of detection, being able to pick up as few as 5 × 10² cells ml⁻¹ in a 22-h ELISA and 10⁵ cells ml⁻¹ in a 3-h test. It was found that the benefits gained by growing the cells in SCDM for a given time were greater than increasing the ELISA incubation by the same length of time, and so a format using the 3-h ELISA with a longer cultural stage was preferred. Even better limits of detection may be achieved by using more sensitive novel endpoints in the ELISA such as enzyme amplification systems or chemiluminescence.

The combination of sensitivity and specificity provided in this ELISA resulted in a very rapid assay in which low numbers of unstressed *S. typhimurium* (10 cells 25 g⁻¹) can be detected in as little as 19 h, involving a 16-h culture in SCDM followed by a 3-h ELISA. The assay can be used quantitatively, if necessary, which may give some indication of the original numbers of cells present in the food. Accurate quantification would be difficult because different *S. typhimurium* strains give varying responses in the ELISA, probably due to the effect of the growth medium on expression of epitopes.

The two studies in food show that this antibody-capture ELISA is largely unaffected by the food matrix. The chocolate-SCDM mixture caused slight inhibition of binding, depressing the actual values by <1 log cycle. The stuffing samples, which were diluted 1:10⁴ (vol/vol) in buffered peptone-water, showed no effect on the standard curve. The study with inoculated chocolate shows clearly that low numbers of cells in food can be detected by the assay, and the study with inoculated sage and onion stuffing shows that the assay can be used to distinguish between viable and non-viable *Salmonella* spp., which can provide a useful way of assessing the efficiency of processing in killing *Salmonella* spp.

Work from this laboratory describing an ELISA for *S. enteritidis* in eggs, using a similar assay format, has also been reported (17). We have shown that as few as 10 cells in one egg can be detected in 13 h by using a cultural step in SCDM followed by either a capture-antibody ELISA or a competitive ELISA.

While specific assays such as this one for *S. typhimurium* will be useful in epidemiological and other screening programs for known serotypes, and in clinical diagnostic work, an assay which can detect all types of *Salmonella* spp. is urgently needed by the food industry. With this ELISA as a model, we have shown that the approach of using specific MAbs can offer distinct advantages in terms of sensitivity and specificity, and ultimately of speed. The ease of use and rapidity of this type of assay would allow much larger numbers of samples to be tested than with the present entirely microbiological method; incorporation of the assay into a scheme such as Hazard Analysis Critical Control Point (21) should improve the microbiological standards of foodstuffs.

**ACKNOWLEDGMENTS**

We thank Maggie Knox and Barbara Lund of this institute for some of the cultural data pertaining to the studies with sage and onion stuffing.

**LITERATURE CITED**