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Assessment of an enzyme immunoassay for the detection of salmonellas in foods and animal feeding stuffs

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SUMMARY

The *Salmonella* Bio-EnzaBead Screening Kit, in its modified form with both the MOPC 467 and the 6H4 antibodies, was used for the detection of salmonellas in naturally contaminated foods and animal feeding stuffs in parallel with a traditional cultural procedure.

Initial results showed an 82% agreement between the enzyme immunoassay (EIA) and cultural methods when using the criterion recommended by the manufacturer as a cut-off for all types of foods. By adjusting the cut-off for each type of food, the number of EIA positive, culture negative samples was reduced although the number of EIA negative, culture positive samples increased. The EIA may be more sensitive than the cultural methods as in many cases the EIA positive, culture negative results could be real positives which were not detected by the cultural methods.

The screening kit provides a simple and convenient method for the detection of salmonella in foods and feeds and a presumptive positive result can be reported within 48 h. The advantages and disadvantages of the method are discussed.

INTRODUCTION

As salmonellas continue to be the major cause of food-poisoning in England and Wales and in many other countries, there is an increasing microbiological surveillance of foods by the food industry and Government authorities. To support this increased monitoring, it is necessary that there are rapid and sensitive methods available for the detection of *Salmonella* spp. in a wide range of foods and animal feeds.

Traditional methods used for the detection and isolation of salmonellas from foods and feeds have been based on culture of the organisms. The main disadvantages of these cultural methods are that they are labour intensive and time-consuming. Although a salmonella-positive result can be confirmed within 3 days, it takes up to 5 days to report that salmonellas were not found in a sample. The traditional culture methods were originally devised for the detection of salmonella in clinical specimens and not in foods. In food specimens salmonellas are often in a stressed condition and can be isolated only after resuscitation and enrichment.

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The number of salmonellas in foods may be as low as 1 organism or even less per 25 g of sample so detection methods must be appropriately sensitive.

For many years there has been an increasing interest in more rapid and economical methods for salmonella detection in foods and animal feeds. The techniques studied include the use of bacteriophages (Cherry *et al.* 1954), fluorescent antibody staining (Thomason, 1971), enrichment serology (Sperber & Deibel, 1969), radiometry (Stewart, Eyles & Murrell, 1980), DNA probes (Fitts *et al.* 1983) and immunoassays. All these methods are reviewed by Ibrahim & Fleet (1985).

Studies on enzyme immunoassays (EIAs) for salmonella detection over the last decade have improved their specificity and sensitivity (Krysinski & Heimsch, 1977; Swaminathan & Ayres, 1980; Minnich, Hartman & Heimsch, 1982; Anderson & Hartman, 1985; Swaminathan, Alcixo & Minnich, 1985). Early EIAs showed high rates of false-positive reactions mainly due to cross-reactivity with other members of the *Enterobacteriaceae*. The procedures were improved by the development of an immunoglobulin A monoclonal antibody (MOPC 467) which was specific for a flagellar determinant located on most but not all salmonella strains (Robison, Pretzman & Mattingly, 1983; Mattingly & Gehle, 1984). To improve the level of salmonella detection an IgG_{2b} hybridoma antibody (6H4) recognizing a non-flagellar antigen on salmonella was raised and when both MOPC 467 and 6H4 were used together in the EIA (Mattingly, 1984) all serotypes tested were detected. The attachment of the antibodies to a solid phase such as a microtitration plate (Minnich, Hartman & Heimsch, 1982) or metal beads (Mattingly & Gehle, 1984) dispensed with the need for centrifugation.

An EIA kit is now marketed as the *Salmonella* Bio-EnzaBead Screening Kit (Bionetics Laboratory Products Division, Organon Teknika Corporation, 800 Capitola Drive, Durham, N. Carolina, 27713, USA). It is commercially available in the United States where it has undergone an Association of Official Analytical Chemists (AOAC) collaborative evaluation and has been granted official first action approval (AOAC, 1986; Andrews, 1986). Currently the kit is being introduced into Europe (Organon Teknika N.V., Veedijk, 2300, Turnhout, Belgium) and is available in the UK (Biokits Limited, Deeside Industrial Park, Deeside, Clwyd, North Wales).

The kit is based on a solid phase EIA using both the MOPC 467 and the 6H4 antibodies attached to the surface of ferrous metal beads which can be easily moved from one reaction mixture to another using a transfer device employing a magnetic force. The test detects the uptake of salmonella antigens onto the antibody-coated beads by means of peroxidase anti-salmonella conjugate.

Most of the foods tested in previous evaluations of the *Salmonella* Bio-EnzaBead Screening Kit were artificially contaminated (Eckner *et al.* 1986; Flowers *et al.* 1986) The present study was undertaken to assess the performance of the kit for the detection of salmonellas in naturally contaminated foods and animal feeding stuffs and is an extension of previously published work (Todd *et al.* 1986).

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MATERIALS AND METHODS

Test samples

Two hundred and eleven samples of foods and animal feeding stuffs naturally contaminated with salmonellas were obtained from Public Health Laboratory Service laboratories and Ministry of Agriculture, Fisheries and Food Veterinary Investigation Centres. They were all examined for the presence of *Salmonella* spp. using the EIA kit in parallel with a conventional cultural procedure.

Cultural procedure

Samples of food (25 g) were pre-enriched in 1 % buffered peptone water (225 ml) (Edel & Kampelmacher, 1973) for 24 ± 2 h at 35 °C.

Two selective enrichment broths were used, Rolfe's tetrathionate (100 ml) (Rolfe, 1946) inoculated with 10 ml of pre-enrichment culture and Rappaport-Vassiliadis (10 ml) (Vassiliadis, 1983) inoculated with 0·1 ml. Both were incubated at 43 °C for up to 48 h. After 24 and 48 h incubation the selective enrichment broths were subcultured onto brilliant green (Oxoid CM329) and deoxycholate citrate sucrose agars (Hynes, 1942) and incubated at 37 °C for up to 48 h.

Suspect colonies were confirmed as salmonella using standard biochemical and serological tests.

Preparation of EIA test sample

Aliquots (0·5 ml) from each of the selective enrichment broths were transferred into a 10 ml volume of sterile M broth (Sperber & Deibel, 1969) and incubated at 35 °C for 6 h. After incubation, 10 ml of the M broth were centrifuged at 1500 ***g*** for 20 min and the remainder stored at 4 °C. The supernatants were discarded and the cell pellets resuspended in 1 ml of phosphate buffered saline (PBS), pH 7·5, using a vortex mixer. The suspensions were heated in a boiling water bath for 20 min, cooled to room temperature and stored at 25 °C for up to 3 days.

Salmonella *Bio-EnzaBead Screening Kit*

Each *Salmonella* Bio-EnzaBead Screening Kit contains sufficient reagents to carry out up to 96 tests and contains positive and negative controls, antibody-coated beads, conjugate, substrate (2,2′ -Azino-[3-ethyl-benzthiazoline-sulfonate], ABTS), stop solution, various diluents and microtitre plates: not supplied (but available from the same supplier) are the magnetic transfer device, incubator with 100 rpm rotator, the bead dispenser and the plate reader with a 405 nm filter.

EIA test procedure

Full instructions for the use of the kit and the operation of the magnetic transfer device are given in the package insert. The magnetic transfer device was used to transfer the beads from plate to plate and during the washing procedure.

Briefly, the EIA test procedure was as follows: control antigens and test samples were dispensed into the plates and incubated with the beads for 20 min at 37 °C with agitation. The beads were washed 12 times in the wash solution and incubated in the conjugate for 20 min at 37 °C with agitation. The beads were washed 12 times in two wash solutions and were transferred to the substrate solution where they

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were incubated at room temperature for 10 min. The reaction was terminated with the stop solution and the optical density (OD) of each well was read at 405 nm with the EIA plate reader blanked on a well containing substrate only.

For the test to be valid the average optical density readings of the duplicate negative controls must be $< 0{\cdot}120$ and the positive control should be $> 0{\cdot}2$. According to the manufacturer's instructions all samples with readings of $\geqslant 0{\cdot}2$ are classed as presumptive salmonella-positive. As the test is only a screening one all EIA positive results must be confirmed culturally from the remaining M broths stored at 4 °C and from the selective enrichment broths.

RESULTS

A total of 211 samples were tested by EIA and conventional cultural methods of which 110 were human foods and 101 were animal feeds and pet foods. Table 1 presents the results obtained using the criterion of a positive sample being one with an OD at 405 nm of $\geqslant 0{\cdot}200$.

A total of 134 samples were salmonella-positive by both methods and 39 were negative by both methods even though they had originally been found to be positive by the cultural procedure. This discrepancy may have been due to death of the salmonella during storage or because the portion of the food retested did not contain the organism.

Two groups of samples in Table 1 were studied further. The first contained samples which gave a positive EIA result but from which no salmonellas were isolated culturally. The group included 35 foods and feeds and there was no association with a particular food or contaminating serotype. Twenty-two of the samples in the group were retested by both methods (Table 2). On retesting many of the foods which were originally EIA positive, culture negative had become negative by both methods. The changes are summarized in Table 3.

The other group of foods investigated in more detail contained three samples found to be EIA negative, culture positive (Table 1). The foods were herbal tea containing a subgenus II strain *Salmonella mjimwema*, desiccated coconut containing a sucrose-fermenting *S. senftenberg* and chocolate containing *S. napoli*. When the tea and coconut were retested (Table 2), the tea was found to be positive and the coconut negative by both methods. The chocolate was not retested as there was insufficient sample left.

To investigate the possibility that different foods and feeds affected the performance and results given by the EIA kit, foods in which salmonella had not been detected on culture were tested with the kit. The mean extinction for each negative food (E) was calculated together with the standard deviation (S.D.) (Table 4). A sample was defined as positive when its reading exceeded the $E + 3 \times Sd$ value for that particular food type. The results of foods analysed in this way are shown in Table 5.

Preliminary work with adjusted cut-off points had a sample size of three in most cases. If a larger sample size had been used, i.e. > 25 then the figures could be quoted with 99·7 % confidence. The results show that adjusting the cut-off for each specific food improves the correlation between the EIA and cultural methods, increasing the number of foods which are negative by both methods and decreasing

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Table 1. *Comparison of EIA and cultural procedures for the detection of salmonella in naturally contaminated foods and feeds*

Type of food	No. tested	No. serotypes present	Positive by both methods	Negative by both methods	EIA positive, culture negative	EIA negative, culture positive
Egg	36	10	30	3	3	0
Poultry	29	9	17	5	7	0
Sausages	11	6	5	4	2	0
Herbal tea	8	5	6	1	0	1
Dried milk	6	1	3	3	0	0
Pasta	5	3	4	0	1	0
Shrimps	4	2	1	0	3	0
Frogs' legs	2	2	2	0	0	0
Ham	2	1	0	0	2	0
Chocolate	2	1	1	0	0	1
Coconut	1	1	0	0	0	1
Pâté	1	1	1	0	0	0
Gelatine	1	1	1	0	0	0
Cheese	1	1	1	0	0	0
Beef	1	1	1	0	0	0
Animal feeds and pet food	101	> 39	61	23	17	0
Total	211		134	39	35	3

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Table 2. *Results of retested samples showing discrepancies between EIA and culture methods*

	Original results		Retest results			
Type of food	EIA positive, culture negative	EIA negative, culture positive	Positive by both methods	Negative by both methods	EIA positive, culture negative	EIA negative, culture positive
Poultry	5	—	1	1	3	—
Sausages	1	—	—	—	1	—
Pasta	1	—	1	—	—	—
Shrimps	3	—	—	2	1	—
Ham	2	—	—	2	—	—
Animal feeds and pet food	10	—	2	6	2	—
Herbal tea	—	1	1	—	—	—
Coconut	—	1	—	1	—	—
Total	22	2	5	12	7	0

Table 3. *Summary of results after retesting*

	Positive by both methods	Negative by both methods	EIA positive, culture negative	EIA negative, culture positive	Total
Original results	134	39	35	3	211
Results after repeat tests on 24 samples	139	51	20*	1†	211

* Thirteen samples with insufficient material to retest, seven samples still gave EIA positive, culture negative upon retest.

† Insufficient material to retest this sample.

Table 4. *Average $OD_{405\ nm}$ readings for different foods known not to contain salmonella*

Type of food	Average $OD_{405\ nm}$ ±standard deviation	$E+(3\times$S.D.)	Number of samples tested
Raw egg	0·151±0·052	0·307	3
Sausage	0·096±0·010	0·126	3
Poultry	0·175±0·106	0·493	3
Shrimps	0·230±0·036	0·338	3
Tea	0·162±0·036	0·279	3
Dried milk	0·133±0·024	0·205	3
Coconut	0·210±0·040	0·330	3
Pasta	0·317±0·057	0·488	3
Feeding meals	0·227±0·048	0·371	8

the number of samples which are EIA positive, culture negative. There is, however, an increase in the number of samples which are EIA negative, culture positive.

Overall, adjusting the cut-off point to suit the type of food appears to increase slightly the number of samples which are EIA negative, culture positive but lowers considerably the number of samples which are EIA positive, culture negative.

DISCUSSION

The initial data presented shows an 82% agreement between the EIA and the cultural methods. However, there are a number of samples where one method gave a positive result and the other a negative result.

Some samples were EIA positive, culture negative and were initially classified as being false-positives by the EIA. It may be that the EIA is more sensitive than culture, as salmonellas may have been missed because they were present in very low numbers or were masked by the growth of other organisms.

The EIA kit will detect most salmonella serotypes at levels as low as 10^5 organisms/ml in the M broth (Mattingly *et al.* 1985). At these levels salmonellas are easily detected by culture so there is little difference in the lower threshold of detection between the two methods.

Reports on the effects of other *Enterobacteriaceae* on the EIA are contradictory. Using the MOPC 467 antibody Robison, Pretzman & Mattingly (1983) and

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Table 5. *Effect of adjusting the base-line for each food*

Type of food	No. tested	No. serotypes present	Positive by both methods		Negative by both methods		EIA positive, culture negative		EIA negative, culture positive	
			0·200*	Adjusted†	0·200	Adjusted	0·200	Adjusted	0·200	Adjusted
Egg	36	10	30	28	3	4	3	2	0	2
Poultry	29	9	17	14	5	8	7	4	0	3
Sausage	11	6	5	5	4	1	2	5	0	0
Herbal tea	8	5	6	6	1	1	0	0	1	1
Dried milk	6	1	3	3	3	3	0	0	0	0
Pasta	5	3	4	4	0	1	1	0	0	0
Shrimps	4	2	1	1	0	0	3	3	0	0
Coconut	1	1	0	0	0	0	0	0	1	1
Animal feeds	91	> 34	56	55	21	30	14	5	0	1
Total	191‡		122	116	37	48	30	19	2	8

* All samples with $OD_{405\ nm} \geqslant 0{\cdot}200$ are classed as positive.
† The base-line is adjusted for each different food.
‡ Only types of food for which a base-line had been assessed were included in thes table.

Mattingly & Gehle (1984) tested a wide range of organisms and found cross-reactivity only with *Arizona hinshawii*, which is not surprising as this organism is a subgenus III salmonella. D'Aoust & Sewell (1986) showed that some strains of *Morganella morganii*, *Citrobacter freundii* and *Escherichia coli* gave positive EIA readings and the presence of such *Enterobacteriaceae* may account for some of the false-positive results.

There have been no reports in the literature on the effects of different foods on the EIA test. However, the original food sample is considerably diluted by the enrichment steps used in the preparation of the EIA test sample. Our data (Table 4) suggest that different foods do have different base-line values and that strict application of the 0·200 cut-off for all foods, as suggested by the manufacturer, may have produced some of the false-positive results. Although adjusting the cut-off point reduces the number of false-positive results in some cases it can increase the number of false-negatives, an even less acceptable situation. In the case of sausages, where the adjusted base-line value is very low (OD = 0·126), there is a substantial increase in the number of samples which are EIA positive, culture negative.

The group of three samples which were EIA negative, culture positive are more difficult to explain. Possibly the strains concerned failed to grow in the M broth to sufficiently high numbers to be detected, or perhaps the base-line used was too high and weak positives appeared as negatives. Two samples from this group were retested; one became positive by both methods and one negative by both methods. Clearly in the first case the EIA did not detect the salmonella which was present. In the second no real conclusions can be drawn.

To conclude, the screening kit is a simple and convenient method for the detection of salmonella in foods and feeds and a presumptive positive can be reported within 48 h.

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