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Detection of *Salmonella typhimurium* in Dairy Products with Flow Cytometry and Monoclonal Antibodies

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Flow cytometry, combined with fluorescently labelled monoclonal antibodies, offers advantages of speed and sensitivity for the detection of specific pathogenic bacteria in foods. We investigated the detection of *Salmonella typhimurium* in eggs and milk. Using a sample clearing procedure, we determined that the detection limit was on the order of 10^3 cells per ml after a total analysis time of 40 min. After 6 h of nonselective enrichment, the detection limits were 10 cells per ml for milk and 1 cell per ml for eggs, even in the presence of a 10,000-fold excess of *Escherichia coli* cells.

Salmonella infection is one of the most commonly reported causes of food-borne disease in the Western world. Unlike *Campylobacter* spp. which cause sporadic cases of infection, salmonellosis tends to occur in outbreaks. Infection is often caused by poor food hygiene in homes or eating establishments but can also originate from the site of food manufacture or from naturally contaminated foods, such as chickens and eggs. The severity of salmonellosis, which may result in death for some individuals, means that manufacturers need to detect contamination before food is released for sale. The results of being associated with a *Salmonella* outbreak can be economically disastrous. This fact has resulted in avid interest in the development of more rapid and sensitive techniques for detecting bacteria in foods; flow cytometry (FCM) is one of the most promising of these techniques.

Almost all of the 2,200 *Salmonella* species can infect humans; the most common infectious *Salmonella* species are *Salmonella enteritidis* and *Salmonella typhimurium*, which together account for three-quarters of all salmonellosis cases each year. Salmonellae, particularly *S. typhimurium* and *Salmonella dublin*, are commonly found in cattle and are excreted in the feces. This provides an easy route of contamination during milking and milk processing. The consumption of contaminated, unpasteurized milk was a common cause of infection in England and Wales during the 1950s and in Scotland in the 1980s (3). Products in which unpasteurized milk is used as an ingredient may also become contaminated (14, 30, 33).

In recent years the main source of *Salmonella* infection in the United Kingdom has been eggs contaminated with *S. enteritidis* PT4 (18, 42). These eggs have originated from chickens that are asymptomatically infected by eating feed containing *S. enteritidis*. The bacterium infects the organs of the chickens, including the ovaries, which results in the laying of infected eggs (16). The eggs may subsequently be used for human consumption or, if fertilized, hatched, producing chickens which are infected from birth, thus increasing the possible routes of infection. Despite extensive efforts (32), *Salmonella* infection of eggs remains a problem.

The culture technique is still the most commonly used method for detecting *Salmonella* spp. in food, and it can take as long as 3 to 7 days to confirm negative results (10, 11). This fact had led to the development of many new alternative techniques by workers in a wide range of scientific disciplines (5, 25). The most promising methods currently in use or being actively developed include enzyme-linked immunosorbent assays (15, 47), conductance (34, 41), immunosensors (24), and PCR (43, 45, 46). FCM has been widely used to detect bacteria in pure cultures. Fluorescent DNA stains have been used to analyze bacterial population heterogeneity (22) and to produce DNA and light scatter profiles for individual species (1, 38). The viabilities of bacterial populations have also been determined by using various stains, with various degrees of success depending on the species involved (7, 12, 21). Specific species or groups of bacteria can be detected by using FCM with fluorescently labelled monoclonal antibodies (13, 31, 37) and rRNA probes (2, 44).

In this study we developed a rapid, sensitive FCM technique to detect *S. typhimurium* in eggs and milk. A fluorescently labelled antibody was used to specifically detect *S. typhimurium*, and ethidium bromide was used to analyze the total bacterial population.

MATERIALS AND METHODS

Bacteria. (i) Bacterial strains. The bacterial strains used in this study were a *S. typhimurium* strain obtained from the Public Health Laboratory Service, Norwich, United Kingdom, and an *Escherichia coli* strain which was isolated in our laboratory. The strains of both species were grown in heart infusion (HI) broth at 37°C and were stored at 2°C on HI agar slopes.

(ii) Bacterial sample preparation. Overnight cultures of bacteria were serially diluted in peptone salt diluting fluid (PSDF) (19). Egg and milk samples were then inoculated to give the appropriate cell loads by adding no more than 10 μl of cell suspension per ml or per g of food. For reasons of safety, all samples containing bacteria were heated at 70°C for 15 min prior to FCM analysis to cause cell death.

(iii) Plate counting. Data obtained by the plate count method (20) were compared with concentrations obtained by FCM. Samples were serially diluted in PSDF, and 100-μl portions of the appropriate dilutions were added to plates. Plate count agar (Oxoid) was used to determine total bacterial numbers, and XLD agar (Oxoid) was used to determine *S. typhimurium* concentrations.

Antibodies. (i) Production. A *Salmonella* group B-specific

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monoclonal antibody, IFRN0402, was used in this study to label *S. typhimurium* cells. This monoclonal antibody was developed in our laboratory and was provided by members of the Biorecognition and Immunotechnology Group. Details concerning the production of this antibody have been described elsewhere (27, 28). The tissue culture supernatant was partially purified and concentrated by using the ammonium sulfate precipitation procedure of Harlow and Lane (17).

(ii) FITC labelling. The antibody was fluorescently labelled by direct conjugation to fluorescein isothiocyanate (FITC). Ammonium sulfate-precipitated tissue culture supernatant samples were dialyzed overnight against 0.1 M carbonate buffer (pH 9.2) with three buffer changes. Protein concentrations were determined by the UV absorption method (26). A 30-μl portion of a FITC stock solution (1 mg ml^{-1} in dimethyl sulfoxide) was added for each milligram of protein present. Conjugation was allowed to proceed for 3 h at room temperature, after which gel filtration was used to remove any unreacted FITC. A type PD-10 Sephadex G-25 column (Pharmacia) was equilibrated with PSDF–0.1% (wt/vol) NaN_3, which was also used as the elution buffer. The first colored band to elute from the column was collected and stored at 4°C in the dark to prevent photobleaching.

The level of fluorescent labelling of the antibody was determined by measuring the A_{280} and A_{495} of a labelled sample. The molar ratio of FITC to antibody was determined with the following equations: antibody concentration (molar) = $[A_{280} - (0.299 \times A_{494})]/(P \times L \times 75{,}000)$, where L is the path length of the cuvette used (in centimeters) and P is the absorption coefficient of immunoglobulin M (1.20) (17); and fluorescein concentration (molar) = $A_{495}/(E \times L)$, where E is the extinction coefficient of FITC (76,000) (8).

We found that when antibody and FITC were at a molar ratio of 1:3, there was efficient labelling of the antibody. Overconjugation resulted in a loss in antibody activity and specificity.

Cell staining. (i) Ethidium bromide. Samples were stained for 10 min at room temperature with ethidium bromide (final concentration, 5 μg ml of $sample^{-1}$) obtained from a 1-mg ml^{-1} stock solution in PSDF. Permeabilization of the cells with a detergent, such as benzalkonium chloride (38), was not necessary as heat treatment of the cells reduced the membrane integrity, thereby allowing the dye to enter the cells readily.

(ii) FITC-labelled antibodies. The optimum level of labelling of *S. typhimurium* cells, up to a concentration of 10^7 cells ml^{-1}, was found to be 30 μg of total conjugated protein per ml of sample. The antibody conjugate was added to a sample, and the preparation was kept at room temperature for 20 min so that the reaction could end prior to analysis by FCM.

FCM. The FCM apparatus used in this study was designed and built in our laboratory (38). Samples were added by using 1-ml syringes (Terumo) and a slow-motion syringe pump (model 22; Harvard Apparatus) at rates of 5 to 30 μl min^{-1}. The sheath fluid was double-distilled water which had been prefiltered through a 0.22-μm-pore-size filter (Gelman Sciences). A constant pressure of 1 atm (ca. 101 kPa) was applied to the sheath fluid-sample stream which emerged from the flow chamber through an orifice having a diameter of 60 μm. The stream was interrogated directly beneath the orifice, in air, by a focused argon ion laser (model Innova 90; Coherent) operating at a wavelength of 488 nm and an output power value of 70 mW. Narrow-angle light scatter (angle, <20°) was passed through neutral-density filters and a 488- ± 5-nm band pass filter and was collected with a photomultiplier (model 9798B; EMI). Fluorescence was detected at an angle of 90° to the laser beam by two other photomultipliers. A 560-nm dichroic mirror was used to separate green fluorescence and red fluorescence. A 530- ± 15-nm band-limiting filter was placed in front of model 9524QB photomultiplier (EMI) to detect green fluorescence, and a 610- ± 19-nm filter was placed in front of a model 9798B photomultiplier (EMI) to detect red fluorescence. All optical filters were purchased from Omega. Signals from the detectors were processed and digitized with custom-designed electronics and were further analyzed with a model 68020 computer (Radstone, Towcester, United Kingdom). Logarithmic scaling was used for all data; a full scale of 256 datum points represented five decades. Day-to-day alignment of the FCM apparatus was checked by using Fluoresbrite microspheres that had a diameter of 2.17 μm (Polysciences).

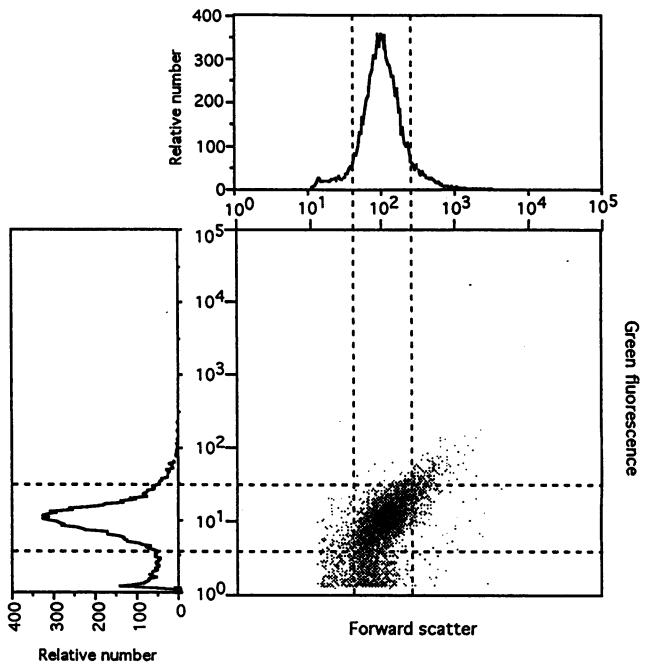

FIG. 1. Single-parameter histograms for forward scatter and green fluorescence data and dual-parameter dot plot for a pure culture of *S. typhimurium* containing 10^6 cells ml^{-1} stained with FITC-labelled IFRN0402. The dotted lines indicate the computer-defined window for cell concentration calculations.

Data analysis. The data were organized as individual histograms showing the spread of forward scatter and fluorescence over the population of cells and also as a correlated, two-parameter histogram (Fig. 1). Computer cursors were set to define a window in the population, from which the concentration of bacteria was derived. The cursors were set at the same distance on both sides of the population peak for each measurement parameter. Coefficients of variation (CVs) were calculated from the single-parameter histograms and were defined as the mean value divided by the standard deviation. Although not strictly mathematically correct, since the data were logarithmic, the CV defined in this way provided a useful indication of the spread of the population.

All of the samples were analyzed in triplicate, and the means were determined for each parameter. Standard deviations were determined, but error bars were generally too small to be shown on the graphs (see below).

For our purposes, background count was defined as the count for a sample which had not been inoculated with bacteria but which had been labelled with antibody and/or DNA stain.

The counts obtained in this way were therefore the background counts attributable to nonbacterial particles present in the samples that had scatter and autofluorescence characteristics similar to those of the target bacteria or exhibited nonspecific binding to the antibody. Obviously, it was not possible to detect levels of bacteria lower than the background count.

Extraction of bacteria from milk. A milk clearing solution was obtained from Promega, Southampton, United Kingdom; this solution is commercially available as part of the Enliten milk assay for total viable organisms. To 1-ml samples of milk, 0.5-ml portions of the clearing solution were added. Samples were mixed by inverting them 10 times before they were centrifuged at approximately 12,000 × *g* in a microcentrifuge for 5 min. The result was a cream pad on top of a clear supernatant; both the pad and the supernatant were carefully removed with an aspirator. The bacterial pellet left at the bottom of the tube was then resuspended in 1 ml of either PSDF (for immediate FCM analysis) or HI broth (if an incubation step was required).

Preparation of egg samples. The surfaces of eggs were scrubbed with ethanol, and the contents were either separated aseptically into yolk and white or left whole. Small volumes of a bacterial suspension were added to egg samples (less than 10 μl of bacterial suspension per g of egg) to give the appropriate bacterial concentration. The samples were then stored at 4°C for 30 min to enable the bacteria to become incorporated in the egg structure. A 1-g sample was placed in a sterile stomacher bag with 9 ml of sterile PSDF, and the preparation was mixed in a Colworth stomacher (capacity, 8 to 80 ml) for 1 min. The resulting mixture was passed through a sterile filter (Millex-SV; pore size, 5 μm; Millipore).

Egg sample analysis. Egg samples (1 ml) were either (i) stained immediately; (ii) stained and, just prior to FCM analysis, added to milk clearing solution at a ratio of egg sample to clearing solution of 2:1; or (iii) diluted 1:10 with HI broth, incubated for 6 h at 37°C, and then stained and cleared.

RESULTS

Preparation of milk samples. The sizes of bacteria generally approach the resolution limit of FCM. Consequently, background light scattering from colloidal and other small particles is of far greater concern in bacterial samples than in mammalian cell samples. Figure 2 shows dot plots obtained with samples of milk containing *S. typhimurium* at a concentration of 1.0×10^6 cells ml^{-1} stained with monoclonal antibody IFRN0402 conjugated to FITC. Figure 2a shows the results obtained with a sample that was treated with phosphate-buffered saline instead of the milk clearing solution and was centrifuged in the usual way. This sample exhibited a high level of background labelling of particulate matter in the milk other than the target bacteria by the antibody; a distinct population could not be defined. Figure 2b shows the results obtained with a cleared sample in which a distinct cell population was observed and analyzed. FCM analysis of different samples revealed that the efficiency of the clearing solution varied depending on the milk type (Table 1). Detection of bacteria in full-fat pasteurized milk revealed a distinct bacterial population with low CVs for both forward scatter and mean green fluorescence data; detection in semiskim milk resulted in similar CVs and similar background levels in negative controls. The clearing solution was less effective with ultrahigh-temperature milk; the results for this milk were similar to the results obtained with an uncleared sample (Fig. 2a).

When present, micelles give a forward scatter signal similar to that of bacteria and bind nonspecifically to large amounts of antibody. Differential interference contrast light microscopic examination revealed that after the clearing solution was added to a sample of milk, there was flocculation and some coalescence of the micelles in the milk (Fig. 3). This effectively increased the micelle size, causing the fat to move more readily to the surface of the milk during centrifugation. The unwanted background signal could therefore be removed. The mechanisms of the clearing process explain why full-fat milk is

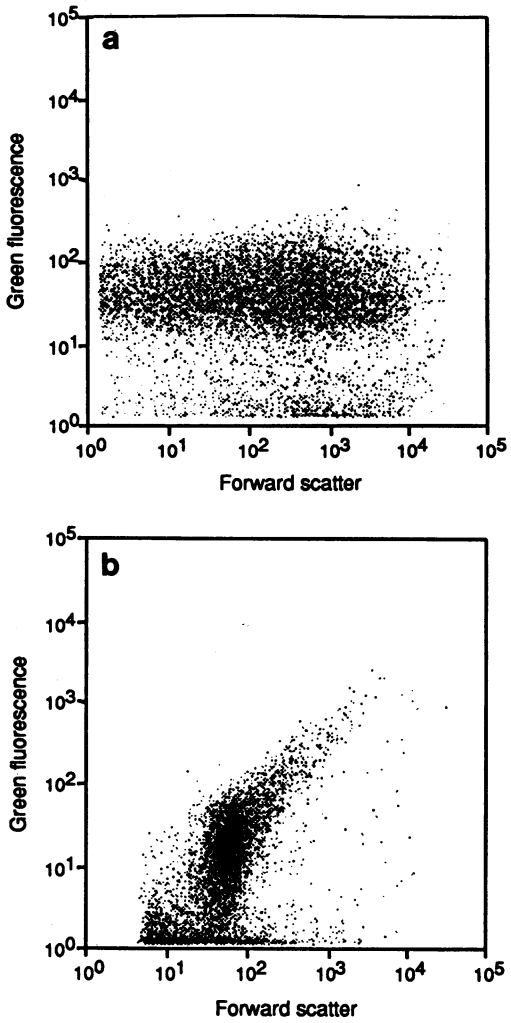

FIG. 2. Scatter plots for milk inoculated with 10^6 *S. typhimurium* cells ml^{-1} and analyzed by FCM. (a) Results obtained with a sample which was not cleared. (b) Results obtained with a sample which was cleared by using the Promega milk clearing solution.

TABLE 1. Population characteristics of bacteria detected by immunofluorescence FCM in various types of milk

Type of milk	CV (%)		Background counts/ml
	Forward scatter	Green fluorescence	
Full fat	26.2	9.6	1.2×10^4
Semiskim	25.0	8.5	1.5×10^4
Ultrahigh temp	28.1	20.1	4.3×10^5

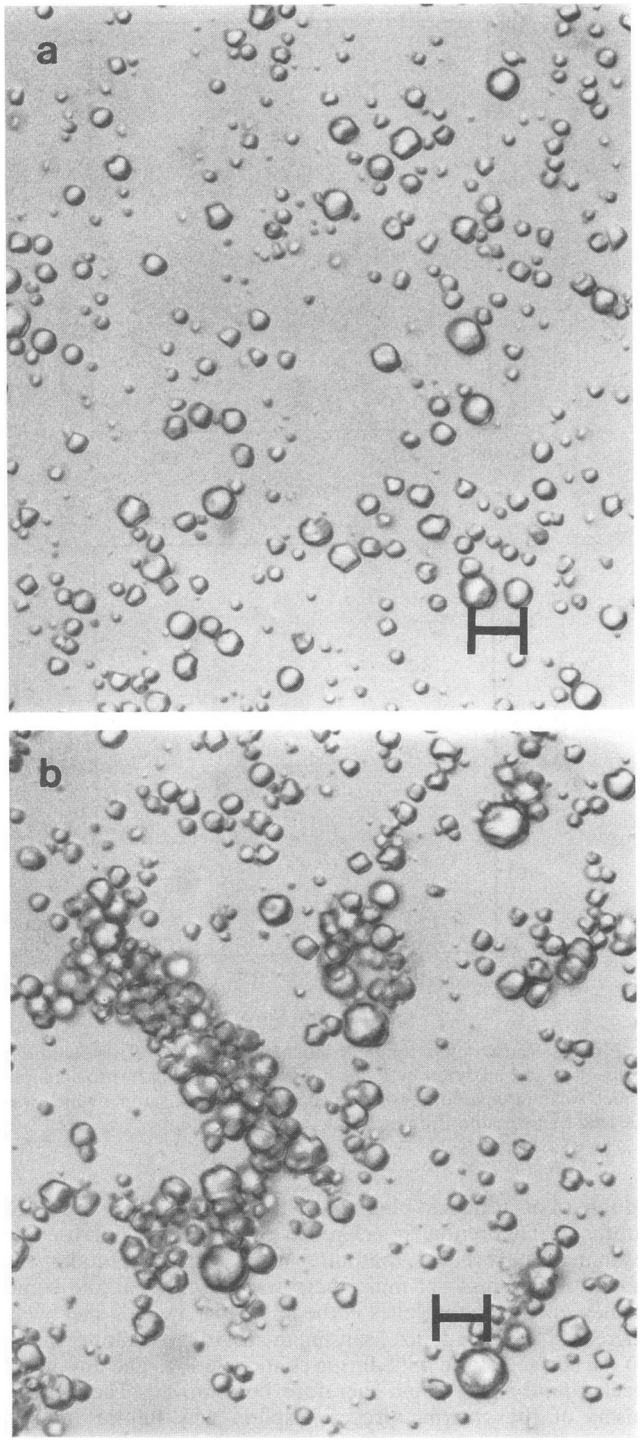

FIG. 3. Differential interference contrast light microscopic examination of untreated full-fat milk (a) and full-fat milk treated with milk clearing solution (b). Bars = 10 μm.

cleared more efficiently than milk treated at ultrahigh temperatures. When milk is subjected to ultrahigh temperatures, protein structural changes occur which cause alterations in micelle behavior. In addition, homogenization causes a reduction in micelle size. These two factors combined make it more difficult for micelles to flocculate and coalesce, and therefore they tend to be left behind in samples during clearing.

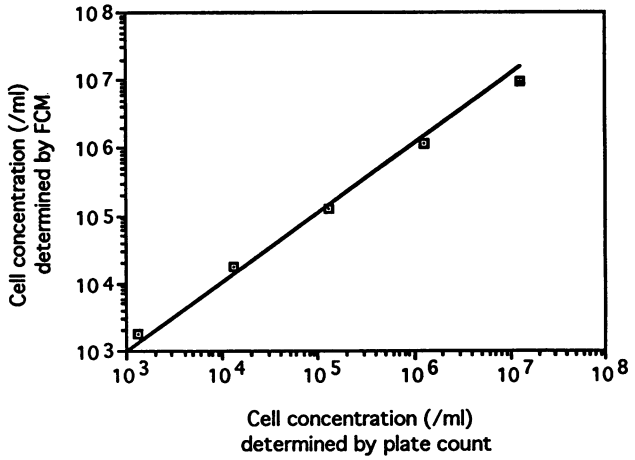

FIG. 4. Relationship between plate counts and FCM counts of *S. typhimurium* in full-fat milk samples. The straight line represents an ideal linear relationship. The correlation coefficient determined by regression analysis was 0.999.

Detection of *S. typhimurium* in milk. Samples of full-fat milk were inoculated with between 1.3×10^3 and 1.3×10^7 *S. typhimurium* cells ml^{-1}. The samples were then cleared, labelled, and analyzed by FCM. Figure 4 shows the expected number of cells, as estimated by plate counting, and the number of cells detected by FCM. There was good agreement between the results obtained with the two methods down to a level of 1.3×10^3 cells ml^{-1}.

Detection of low numbers of *S. typhimurium* cells in the presence of high numbers of *E. coli* cells by using a nonselective incubation step. It was important to evaluate how our system coped with low numbers of *S. typhimurium* cells in the presence of comparatively high numbers of other bacteria; to determine this, we used *E. coli* cells as the other bacteria. Samples of full-fat milk were inoculated with serial dilutions of *S. typhimurium* cells at concentrations ranging from 1.8 to 1.8×10^4 cells ml^{-1}. All samples were also inoculated with 1.6×10^4 *E. coli* cells ml^{-1}; this resulted in ratios of *S. typhimurium* cells to *E. coli* cells ranging from 1:1 to 1:10,000. The samples were subjected to clearing, and each bacterial pellet was suspended in 1 ml of HI broth. (Clearing was performed prior to incubation as we found that if the milk was first incubated in HI broth and then cleared, the process was ineffective and high background levels were obtained.) After incubation, the samples were stained with FITC-labelled IFRN0402 to determine

TABLE 2. Bacterial numbers determined by FCM and plate count methods in milk samples after 6 h of nonselective enrichment

S. typhimurium cells/ml		*E. coli* cells/ml	
Plate count method	FCM method	Plate count method	FCM method
4.8×10^7	1.4×10^7	5.7×10^7	1.5×10^7
8.0×10^6	3.0×10^6	8.4×10^7	4.4×10^7
6.5×10^5	3.7×10^5	4.5×10^7	2.5×10^7
8.8×10^4	4.3×10^4	8.1×10^7	ND[a]
7.1×10^3	2.2×10^4	8.3×10^7	ND

[a] ND, not determined.

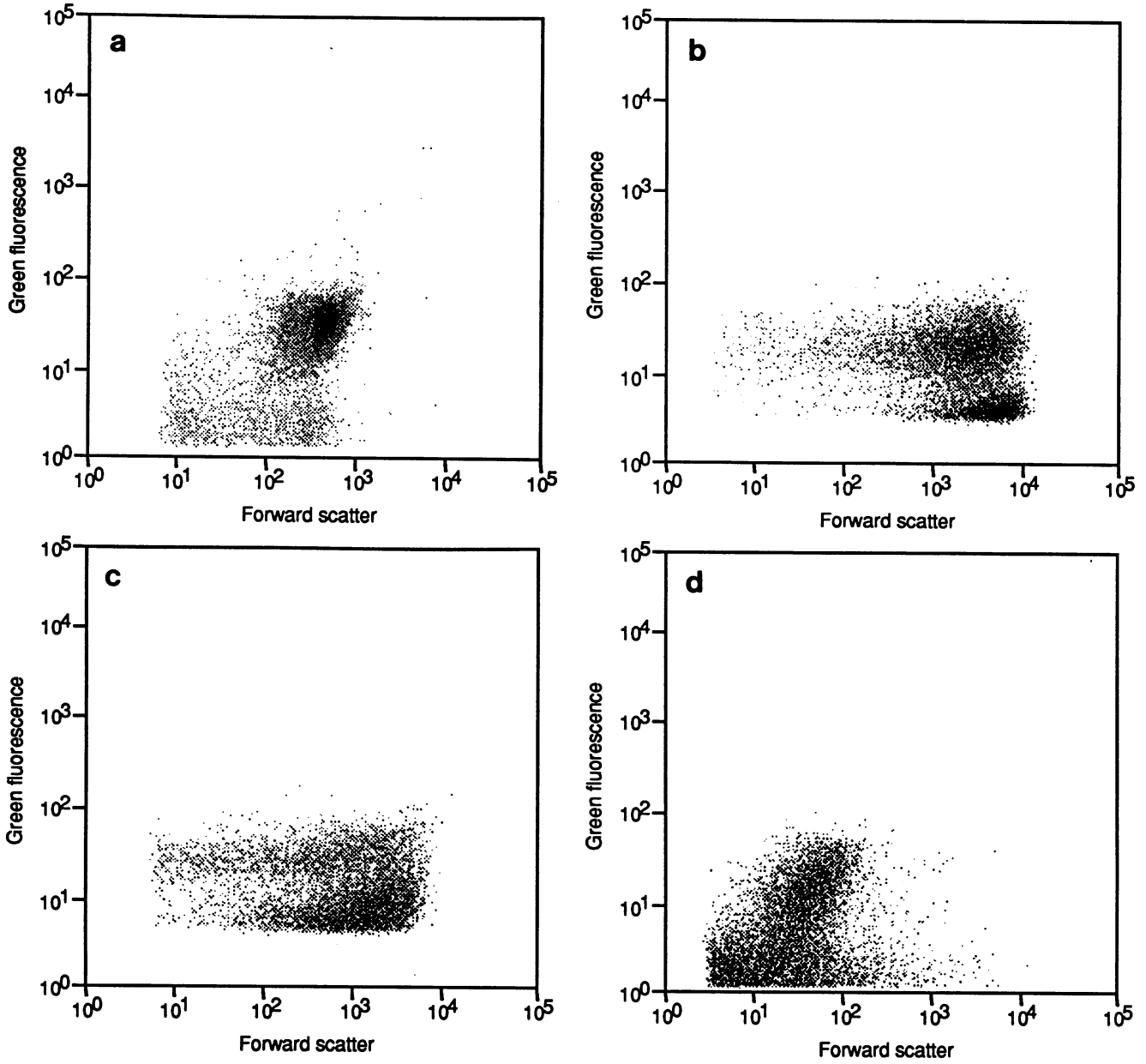

FIG. 5. Detection of *S. typhimurium* in egg samples by FCM. (a) Untreated egg white. (b) Untreated egg yolk. (c) Untreated whole egg. (d) Whole egg treated with milk clearing solution.

S. typhimurium counts and with ethidium bromide to determine total bacterial counts. Table 2 shows the concentrations of *S. typhimurium* and *E. coli* after incubation, as determined by both FCM and plate count methods. (The *E. coli* concentration was determined by subtracting the *S. typhimurium* count on XLD agar from the total count on plate count agar; however, the resulting value included any bacteria naturally present in the milk.) Detection was possible down to an *S. typhimurium* concentration of 10^3 cells ml^{-1}, which corresponded to an initial inoculum of 1.8 cells ml^{-1}.

FCM of stomacher-treated egg samples. Samples of whole eggs and separated whites and yolks were inoculated with *S. typhimurium* at a concentration of 5.6×10^6 cells ml^{-1}, stomached with PSDF, stained, and analyzed. In egg whites (Fig. 5a) a discrete bacterial population was easily distinguished; the CVs for both green fluorescence and forward scatter data were low. Figures 5b and c show the results obtained with egg yolks and whole eggs, respectively. In these cases the populations were less distinct, and the stained matter gave much larger forward scatter signals. Microscopic examinations of the samples revealed that the bacteria had become associated with fat droplets in the egg yolk, causing higher scatter signals, thus making analysis of the bacterial population both difficult and inaccurate. Figure 5d shows that addition of the milk clearing solution to stomached egg samples dispersed the oil droplets, producing a cleaner sample of bacteria that exhibited the expected forward scatter characteristics.

Detection of *S. typhimurium* in eggs. Samples of whole eggs

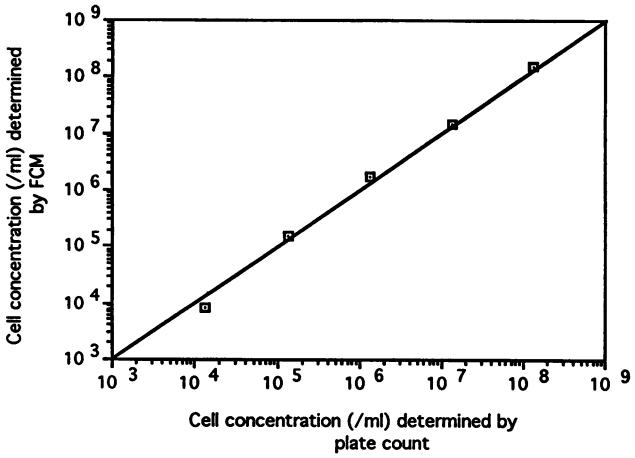

FIG. 6. Relationship between plate counts and FCM counts for *S. typhimurium* in samples of whole eggs. The correlation coefficient determined by regression analysis was 0.998.

TABLE 4. Comparison of assay times and intrinsic detection limits of some commonly used bacterial detection systems[a]

Detection method[b]	Detectable concn (cells ml^{-1})	Assay time (h)	Reference(s)
ELISA	10^5–10^6	2–3	4
Conductance	20	14	34
	<10	12	9
DEFT	10^4	<1	36, 40
Immunosensors			
Amperometric	10^4–10^5	2–3	6
Piezoelectric	10^5	<1	39
ATP assay	10^4–10^5	1	29
Bioluminescence	10^4	1	23
FCM without preenrichment	10^3–10^4	0.5	31
FCM with preenrichment	0.1–1	6.5	31

[a] In all cases, the values are values for detection of bacteria in pure cultures.
[b] ELISA, enzyme-linked immunosorbent assay; DEFT, direct epifluorescent filter technique.

were inoculated with between 1.1×10^3 and 1.1×10^7 *S. typhimurium* cells ml^{-1}, as determined by the plate count method. The stomaching, clearing, and staining procedures were performed, and counts were determined by FCM. Figure 6 shows the FCM counts and the plate counts; there was extremely good agreement between the two types of data down to a concentration of 10^3 cells ml^{-1}. Detection below this concentration was not possible because of high background counts.

Detection of very low numbers of *S. typhimurium* cells in eggs in the presence of high numbers of *E. coli* cells. We performed an experiment with eggs that was similar to the experiment described above for milk. Egg samples were inoculated with between 3.1×10^{-1} and 3.1×10^4 *S. typhimurium* cells ml^{-1}. All samples were also inoculated with 2.5×10^4 *E. coli* cells ml^{-1}; the samples were then incubated for 6 h and analyzed by both FCM and plate count methods. Table 3 shows the *E. coli* and *S. typhimurium* concentrations determined by both methods. We found that detection was possible down to a concentration of 10^3 *S. typhimurium* cells ml^{-1}, which corresponded to an initial inoculum of 0.3 cell ml^{-1}.

DISCUSSION

Detection of *S. typhimurium* in milk. Our results showed that it was possible to detect *S. typhimurium* cells in milk accurately down to a concentration of 1.3×10^3 cells ml^{-1}. Such detection involved a total analysis time of approximately 30 min.

When a 6-h nonselective enrichment step was used, it was possible to detect between 10 and 100 cells ml^{-1}, even in the presence of 10^4 *E. coli* cells. In pure cultures, the corresponding figures were appreciably better, between 0.1 and 1 cell ml^{-1} (31). The efficiency of extraction of the bacteria from the milk before preenrichment was approximately 70 to 80% (as determined by plate counting). We believe that in some way the clearing solution reduced the growth of the bacteria during the preenrichment phase, which accounted for the overall lower detection sensitivity in milk than in pure cultures. The nature of the stress imposed by the clearing solution is not known since its composition has not been revealed by the manufacturer.

Detection of *S. typhimurium* in eggs. The detection limit in eggs, after no enrichment step and a total analysis time of approximately 40 min, was 1.1×10^3 cells ml^{-1}. This value was comparable to the detection limit in pure cultures. However, the procedure used for analysis of egg samples included 1:10 dilution in buffer or broth prior to stomaching. Therefore, the value which we determined corresponded to a concentration of 10^4 cells ml^{-1} in the original egg samples.

The overall level of sensitivity was increased by incorporating a 6-h enrichment step in the procedure, and a preenrichment inoculum of 3.1 cells ml^{-1} in eggs was accurately detected. This result was achieved even in the presence of large numbers of *E. coli* cells, again showing that FCM is capable of detecting low numbers of *S. typhimurium* cells in the presence of large numbers of other bacteria.

Comparison of FCM with other techniques. When detection systems in which an incubation procedure is used are compared, it is important to compare the intrinsic detection limits of the systems and the times for the assays themselves. Although the detection limits of previously described methods may be as low as 1 cell in 25 g of food, a detection system cannot actually intrinsically detect this level; it must rely on an enrichment step to give detectable concentrations. It is obvious then that the lower the intrinsic detection limit of a system, the shorter the incubation step required. Depending on the method, the enrichment step may need to be selective for the target organism. In such cases, longer incubation is required, and some cells (e.g., cells which are viable but are sublethally

TABLE 3. Bacterial numbers determined by FCM and plate count methods in egg samples after 6 h of nonselective enrichment

S. typhimurium cells/ml		*E. coli* cells/ml	
Plate count method	FCM method	Plate count method	FCM method
8.4×10^7	2.2×10^7	8.3×10^8	6.7×10^7
1.2×10^7	6.1×10^6	8.0×10^8	5.2×10^7
1.4×10^6	7.9×10^5	1.1×10^9	8.3×10^7
1.5×10^5	7.5×10^4	1.3×10^9	ND[a]
1.3×10^4	5.5×10^3	1.6×10^9	ND
1.9×10^3	1.5×10^3	1.5×10^9	ND

[a] ND, not determined.

damaged) may not be recovered. The FCM technique described in this paper has immediate detection limits of 10^3 cells ml^{-1} in milk and 10^4 cells ml^{-1} in eggs. If a 6-h nonselective enrichment step is used, the minimum numbers of cells present in the original samples that are amplified by the enrichment and are detectable by FCM are 10 to 100 cells ml^{-1} for milk and 1 to 10 cells ml^{-1} for eggs. Table 4 shows the ultimate sensitivity of our method and the limits of some other commonly used techniques. The FCM method has one of the lowest intrinsic limits of the techniques listed, as well as one of the shortest analysis times.

It should be noted that for the enrichment procedures performed in this study we used cells in the exponential phase of growth. In food samples contaminants were generally stressed during processing, and there is a lag phase before bacterial growth occurs. The length of this phase depends on the degree of cell stress, and prolonged enrichment may be required. However, this is also a problem with other detection techniques, and so the comparison described above remains valid.

A few previous attempts have been made to use FCM to detect bacteria in food. Patchett et al. (35) used DNA stains to detect various bacteria in meat and pate; the detection limits in this study were 10^5 and 10^6 cells ml^{-1}, respectively, and Patchett et al. were not able to detect even 10^7 cells ml^{-1} in milk. Donnelly and Baigent (13) used both a DNA stain and a fluorescently labelled antibody to detect *Listeria monocytogenes* selectively enriched from milk; the intrinsic detection limit was 10^6 cells ml^{-1}. The methods described in this paper can be used for any bacterial species, provided the necessary monoclonal antibodies are available, and improve by far the limits of FCM detection of bacteria in foods.

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