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# Filtration capture and immunoelectrochemical detection for rapid assay of *Escherichia coli* O157:H7<sup>1</sup>

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Received 6 May 1997; revised 22 August 1997; accepted 9 September 1997

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## Abstract

A new approach for rapid assay of bacteria in liquid samples is described. Cells were labeled by incubation with an enzyme-antibody conjugate and captured by filtration of the sample/conjugate mixture through a 0.2  $\mu\text{m}$  filter. The enzyme-labeled cells were detected by placing the filter on the surface of an electrode, incubating with enzyme substrate, and measuring the current produced by oxidation of the electroactive enzyme product. Assay time was 25 min and a detection limit of  $\sim 5000$  cells/ml was obtained for *E. coli* O157:H7. Background current due to non-specific binding of conjugate to the filter was the primary factor controlling the detection limit, and fewer than 50 cells could be detected when very small sample volumes (10  $\mu\text{l}$ ) were used to minimize background current. © 1998 Elsevier Science B.V.

**Keywords:** Filtration; Immunoelectrochemistry; *E. coli* O157:H7; Bacteria; Detection

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## 1. Introduction

Conventional microbial culture methods exploit the unique metabolic characteristics and rapid reproductive cycles of microorganisms to provide simple, yet highly selective and sensitive assays. Cells are grown on selective media, or a series of media, until sufficient organisms are present ( $\sim 10^9$  cells) to form visible colonies. The price for this simplicity

and sensitivity is time — typical assays require more than a week to complete. For many applications, such as testing fresh foods and water supplies for pathogen contamination, there is a need for results on a much shorter time scale. Numerous rapid methods have been devised to meet this need (Feng, 1992, 1996), and new approaches continue to be introduced. These may be broadly classified into methods which require growth of the target organism (either pre-enrichment to increase the number of target organisms to a detectable level, or cell growth which is a requirement of the detection process), and no-growth methods. The former require a minimum of several hours to complete, while the latter methods, in principle, are capable of analysis times of minutes.

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Abbreviations: AP, alkaline phosphatase; *p*-APP, *p*-aminophenylphosphate; TBS, Tris-buffered saline

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<sup>1</sup> Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

No-growth methods include colorimetric and fluorescent immunoassays (Ibrahim, 1986; Wyatt et al., 1993), immuno-fluorescence microscopy (Tortorello and Stewart, 1994), DNA-based tests (Curiale et al., 1990; Bej et al., 1994; Fratamico et al., 1995), immuno-optical (Watts et al., 1994) and immuno-piezoelectric (Minunni et al., 1995) sensors, immunomagnetic electrochemiluminescence (Yu and Bruno, 1996), and immunoelectrochemistry (Brooks et al., 1992; Rishpon et al., 1992; Hadas et al., 1992). A common strategy in these methods is the use of separate capture and detection steps. In the capture step the target organism (or a component such as DNA) is isolated from the sample matrix and concentrated in a relatively small volume. The detection step results in generation of a measurable signal from the captured material with amplitude proportional to the number of cells present in the sample. The detection limit is determined by the inherent sensitivity of the detection step, the efficiency and degree of concentration achieved in the capture step, and the rejection of interferences and background responses by both steps.

In our laboratory, immunoelectrochemical (IEC) detection has shown considerable promise as the basis for rapid bacteria detection schemes. Fewer than 100 cells labeled with antibody-AP conjugate could be detected within 5 min after capture on an antibody-coated electrode (Brewster et al., 1996). The very low detection limit was due to the inherent sensitivity of electrochemical detection and the localization and concentration of the cells achieved by immunocapture at the electrode surface. However, the efficiency of this capture approach was very low (< 0.1% of cells present were captured), and electrodes were subject to fouling by exposure to raw samples.

Alternative capture methods which retained the advantages of surface immunocapture while providing high capture efficiency and limited electrode fouling were therefore sought. Immunomagnetic capture has been widely used in microbial assays (Luk and Lindberg, 1991; Skjerve and Olsvik, 1991; Fratamico et al., 1992), and was recently coupled with immunoelectrochemical detection in an assay for *Salmonella typhimurium* (Gehring et al., 1996). This approach is especially attractive when target bacteria must be isolated and concentrated from

complex matrices, since samples with high viscosity and high levels of particulates can be processed directly. Potential disadvantages are the time required for capture (30–60 min), the limited capture efficiency (~ 50%), and the limited ability to scale up the method to large (> 2 ml) sample volumes. Efficient capture requires magnetic particle concentrations on the order of  $10^7$ /ml, and the sheer mass of a large volume of particles can make detection of low numbers of tightly-bound bacteria problematical. Cost may also be an issue as the price of several mls of commercial particles is not insignificant.

Filtration capture can be very rapid (~ 1 min), efficient (~ 100%), inexpensive, and readily scaled up to volumes of hundreds of ml. Filtration capture coupled with microscopy and culturing has long been used for microbial analysis of water samples. The primary limitation of the method is plugging of the filter by samples with high viscosity or high levels of particulates. Several studies (Pettipher and Rodrigues, 1982; Tortorello and Gendel, 1993; Tortorello and Stewart, 1994) have shown that milk, juices, and food extracts prepared by 'stomaching' solid foods can be processed for filtration capture by a combination of heat, detergent, trypsin, and coarse (~ 5  $\mu$ m) pre-filters. High recoveries (> 90%) have been attained in 15 min using this approach, making filtration a viable capture method for food analysis. Several rapid methods based on filtration capture have been reported. Direct electrochemical detection of cells was investigated as a method for urine analysis (Matsunaga and Nakajima, 1985), but the detection limit was >  $10^6$  cells/ml and detection was non-specific. Immunofluorescent labeling followed by microscopic enumeration has recently been used to detect low levels of specific pathogens in foods (Tortorello and Stewart, 1994). The sensitivity of this filtration-microscopy approach was very good (< 100 cells/ml), but detection of low bacteria levels required tedious examination of hundreds of microscope fields. A colorimetric immunofiltration assay in which filtered cells were labeled with antibody-enzyme conjugate has been used to produce a rapid and sensitive (~  $10^4$  cells/ml) high throughput analysis system (Paffard et al., 1996). However, lower detection limits (in the range of 1–100 cells/ml) are needed for many pathogen detection applications. Coupling of filtration capture with im-

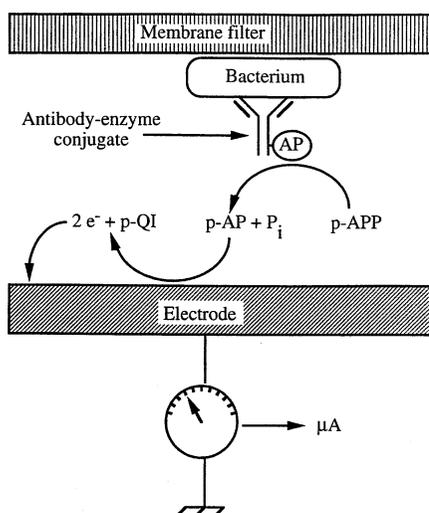


Fig. 1. Detection scheme for filtration immunoelectrochemical assay. *p*-APP: *para*-aminophenyl phosphate; *p*-AP: *para*-aminophenol; P<sub>i</sub>: inorganic phosphate; *p*-QI: *para*-quinone imine (oxidation product of *p*-AP); e<sup>-</sup>: electron.

munoelectrochemical detection, as outlined in Fig. 1, could provide a rapid, selective, and sensitive analysis method. We report here initial studies of such an approach and its use in a presumptive assay for the pathogen *E. coli* O157:H7.

## 2. Materials and methods

### 2.1. Materials

Water was deionized in-house with a Nanopure water treatment system (Barnstead, Dubuque, IA). Alkaline phosphatase (AP)-conjugated goat anti-*E. coli* O157:H7 antibody was from Kirkegaard and Perry (Gaithersburg, MD). Membrane filters of cellulose acetate (0.2 μm pore size) and nitrocellulose (0.45 μm pore size) were from Schleicher and Schuell (Keene, NH); native and blackened polycarbonate track etched filters (0.2 μm pore size) were from Poretics (Livermore, CA). Tris(hydroxymethyl)aminomethane (Tris), Sigma-FAST *p*-NPP Substrate tablets (containing *p*-nitrophenyl phosphate and Tris buffer), bovine serum albumin (BSA) fraction V, and Tween-20 were from Sigma (St. Louis, MO). Disodium *p*-nitrophenyl phosphate (*p*-

NPP) and the following blocking buffers in Tris buffered saline were from Pierce (Rockford, IL): 1% Blocker™ casein, 10% bovine serum albumin (BSA), and 1% SuperBlock™ Blocking Buffer. Flat bottomed polystyrene microwell plates and Tygon™ tubing were from Fisher Scientific (Philadelphia, PA). The substrate for electrochemical detection, *p*-aminophenyl phosphate (*p*-APP), was prepared by catalytic reduction of *p*-nitrophenyl phosphate as described by Gehring et al. (1996). All other chemicals used were of reagent grade.

### 2.2. Solutions

Fe(II) solution was prepared by dissolving K<sub>4</sub>Fe(CN)<sub>6</sub> at 1 mM in carbonate buffer. *p*-NPP solutions were prepared by dissolving Sigma-FAST *p*-NPP and buffer tablets in water following the manufacturers directions. Substrate solution was prepared by dissolving *p*-APP in carbonate buffer to give a 5 mM solution and used within 4 h of preparation. Conjugate solution was prepared by rehydrating lyophilized goat anti-*E. coli* O157:H7 AP conjugate in 50% glycerol to give a 0.1 μg/ml solution and stored at -10°C. Aliquots of this solution were diluted 1:450 in TBS (25 mM tris(hydroxymethyl)aminomethane (Tris), 150 mM sodium chloride, pH 7.6) shortly before use to give working AP-conjugate solution. *E. coli* O157:H7 (ATCC 43895) were grown in Brain Heart Infusion Broth (BHI, Difco, Detroit, MI) and irradiated following the protocols previously described (Gehring et al., 1996). Cells were aliquoted at a concentration of 1 × 10<sup>9</sup> cells/ml in phosphate-buffered saline (0.1 M sodium phosphate, 0.15 M NaCl, pH 7.4) and stored at -10°C. Working solutions were prepared from thawed cells by dilution to 5 × 10<sup>8</sup> cells/ml with TBS, and washing twice with TBS by centrifugation at 2000 *g* for 5 min, removal of the supernate, and resuspension of the pellet in TBS. The cell suspension was diluted with TBS to 10<sup>7</sup> cells/ml and kept on ice until use.

### 2.3. Apparatus

A BAS 100B/W Electrochemical Analyzer equipped with a C-2 Cell stand, glassy carbon disk

electrodes, Ag/AgCl reference electrodes, and polishing materials were from Bioanalytical Systems (West Lafayette, IN). The tip of a 6 mm  $\times$  70 mm Ag/AgCl reference electrode was wrapped with a platinum wire to form a reference/counter electrode unit and stored in 3 M KCl when not in use. All potentials refer to this reference electrode system. The working electrode was polished before each experiment using 0.05  $\mu$ m alumina, cleaned by sonicating for 30 s in water, and its performance verified by cyclic voltammetry of Fe(II). An EL 311s Microplate reader (Bio-Tek Instruments, Winooski, VT) controlled by a Macintosh Plus computer (Apple Computer, Cupertino, CA) running  $\Delta$ Soft software (BioMetallics, Princeton, NJ) was used to read absorbance of microwell plate samples at 405 nm.

#### 2.4. Detection unit

The detection unit is shown in schematic form in Fig. 2. It consisted of three major components: a working electrode, a membrane filter and support, and a stainless steel housing. The working electrode was a 3 mm diameter glassy carbon disk embedded in the end of a 6.3  $\times$  75 mm Kel-F cylinder which fit tightly into one opening of the housing (a 1/4" swage to 1/4" flared tubing adapter, The SwageLok Companies, Solon, OH). The filter was a 6 mm diameter disc cut from the membrane material. A 6 mm diameter disc of Whatman #1 filter paper (Fisher Scientific) and a flexible polymer ring 2 mm high, 3 mm inner diameter, and 6 mm outer diameter (cut from a section of Tygon<sup>TM</sup> tubing) were used to

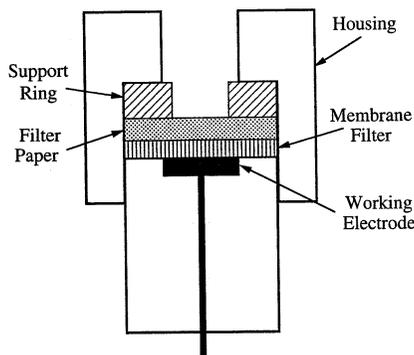


Fig. 2. Schematic diagram of the detection unit. Components are not drawn to scale.

support the membrane and hold it tightly against the electrode surface. In some early experiments the polymer ring was omitted. The components were assembled in the order shown, then pressed tightly together by forcing the electrode into the housing. The space above the filter paper was filled with substrate solution and the reference and auxiliary electrodes were placed in contact with the liquid. The friction fit between the electrode body and the housing held the components of the assembly together and prevented leakage of the substrate solution.

#### 2.5. Filtration capture

A small polymer funnel (7 mm inside diameter) from a disassembled Microcon<sup>TM</sup> centrifugal micro-concentrator (0.5 ml sample size unit, Amicon, Beverly, MA) was used for filtration capture. A disk of polyethylene sheet with a 3 mm hole in the center was cut to fit closely inside the bottom of the funnel. The filter membrane was placed on top of the polyethylene disk so that all solution flow was through the central 3 mm area of the filter (matching the electrode size). Filtration was conducted by adding solution dropwise from a pipette while vacuum ( $\sim -0.5$  atm) was applied. Typical flow rates were  $\sim 5$   $\mu$ l/s.

#### 2.6. Colorimetric evaluation of blocking agents and membranes

For initial evaluation, filter membranes were cut into 3 mm discs and placed in microwells along with 100  $\mu$ l of TBS (control) or one of the blocking agents (as a 1% solution in TBS) casein, bovine serum albumin (BSA), Tween-20, or Super Block<sup>TM</sup>. The wells were covered with plastic film and the plate incubated overnight at 4°C ( $n = 2$  for each treatment). The liquid was removed from the wells, and the filters were washed 3  $\times$  with 200  $\mu$ l TBS for 10 min with slow shaking (covered) at room temperature. Conjugate solution was then added to each well (100  $\mu$ l of 1:500 dilution of conjugate) and incubated for 30 min at room temperature. The filters were rinsed 3  $\times$  with TBS as above, and transferred to new microwells. *p*-NPP (200  $\mu$ l) was added to each well and allowed to react for 30 min (10 min for control nitrocellulose) at room tempera-

ture with slow shaking on a vortexer. A 100  $\mu\text{l}$  aliquot from each well was transferred to a new microwell, mixed with 25  $\mu\text{l}$  3 N NaOH to quench enzyme activity, and the absorbance at 405 nm was determined. The blank absorbance for *p*-NPP treated as above was subtracted from all readings to give the net absorbance. The absorbance of control nitrocellulose was multiplied by 3 to account for the shorter incubation time.

For evaluation of filtration blocking of polycarbonate membranes, 100  $\mu\text{l}$  of TBS (control) or one of the blocking agents (as a 1% solution in TBS) Tween-20, ovalbumin, or BSA was filtered through the membrane under vacuum as described below, followed by 100  $\mu\text{l}$  of conjugate, and 3 drops of TTBS (1% Tween-20 in TBS). The filter membrane was transferred to a 1.5 ml microcentrifuge tube and reacted with 200  $\mu\text{l}$  of *p*-NPP for 30 min at room temperature with gentle vortexing. A 100  $\mu\text{l}$  aliquot of the solution was transferred to a microwell and the absorbance at 405 nm was determined.

### 2.7. Filtration immunoelectrochemical assay

Cells were serially diluted from the working solution with TBS to  $10 \times$  the final concentration, diluted 1:10 with conjugate solution, and incubated for 15 min at 40°C. The mixture of cells and conjugate was filtered through a 6 mm filter membrane disk which had been wetted with 1 drop ( $\sim 40 \mu\text{l}$ ) of TTBS. The filter was washed by applying 3 drops of TTBS under vacuum, and transferred with forceps to the surface of the electrode. One drop of substrate solution was applied to the membrane, the filter paper disk and polymer ring were placed on top of the membrane, and the detection unit was assembled. The housing was filled with substrate solution, the reference/auxiliary electrode unit was inserted into the top of the housing, and the 'IR test' function of the electrochemical workstation was used to ensure that the solution resistance was less than 500  $\Omega$ , indicating that no air bubbles were present in the system. Five minutes after initial exposure of the filter membrane to substrate solution, an Osteryoung Square Wave Voltammogram was recorded under the following conditions: initial potential:  $-200 \text{ mV}$ ; final potential:  $200 \text{ mV}$ ; step amplitude:  $4 \text{ mV}$ ; square wave amplitude:  $25 \text{ mV}$ ; frequency:  $6 \text{ Hz}$ . A

baseline was generated across the shoulders of the peak and the peak current measured using the BAS 100 W software.

## 3. Results

### 3.1. Evaluation of filter material and blocking protocol

Initial evaluation of membranes and blocking agents was performed using a colorimetric assay for alkaline phosphatase enzyme activity remaining in membranes after exposure to blocking agents and conjugate. As shown in Table 1, nitrocellulose exhibited the highest levels of non-specific binding, followed by cellulose acetate and polycarbonate. Blocking with BSA, Casein, and TTBS significantly reduced protein binding for all materials, while SuperBlock was much less effective. Based on these results, polycarbonate was selected as the optimum membrane material. Experiments were next conducted to determine the optimum blocking agent when blocking was performed by filtration rather than soaking. Casein clogged the filters and was therefore replaced with ovalbumin in these experiments. Ovalbumin, BSA, and Tween 20 all produced similar low levels of binding (data not shown).

### 3.2. Effect of sample volume on detection limit

In the assay protocol used here, larger sample volumes were expected to yield a larger signal (as greater numbers of cells are captured), but also a

Table 1  
Colorimetric evaluation of non-specific binding of conjugate to filter membranes

Membrane	Blocking agent				
	none	BSA	casein	Tween 20	Super Block
Nitrocellulose	4.968	0.107	0.032	0.052	0.593
Cellulose acetate	0.123	0.035	0.043	0.038	0.109
Polycarbonate #1	0.019	0.000	-0.002	0.011	0.025
Polycarbonate #2	0.010	0.001	0.000	0.011	0.003

Values shown are the absorbance at 405 nm corrected for the *p*-NPP blank. 1% solutions of blocking agent in TBS were used. See text for details.

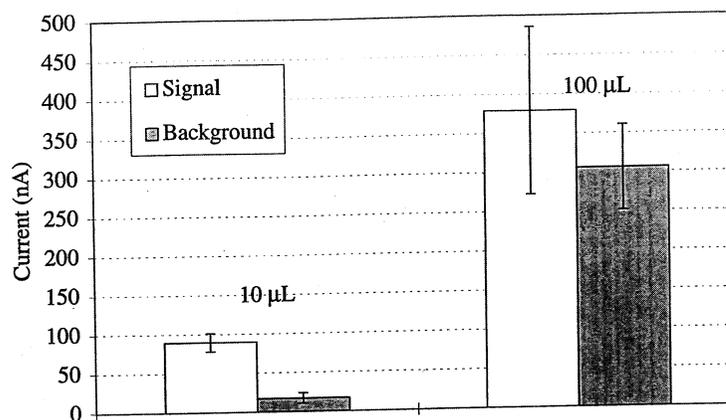


Fig. 3. Effect of sample volume on detection limit and background immunoelectrochemical response. Error bars represent one standard deviation about the mean of four replicates. Sample volume indicated. See text for details.

larger background response since the filter membrane was exposed to more conjugate during filtration. To assess the impact of sample volume on background, replicate samples ( $n = 4$ ) containing 100 cells in volumes of 10  $\mu\text{l}$  ( $10^4$  cells/ml) and 100  $\mu\text{l}$  ( $10^3$  cells/ml) were assayed. As shown in Fig. 3, increasing the sample size from 10 to 100  $\mu\text{L}$  (while

keeping the number of cells constant) resulted in more than ten-fold increase in background current which precluded detection of 100 cells.

### 3.3. Filtration immunoelectrochemical response

The assay response for a range of *E. coli* O157:H7 concentrations is shown in Fig. 4. Data points for the lower bacteria concentrations are replotted in the inset to more clearly show the magnitude and variance of the response. Six replicates were run at each concentration, except at the  $10^5$  cells level where only 3 replicates were run. The data showed very high variance at concentrations of bacteria which gave currents above 2–3000 nA. This phenomenon was consistently observed in this and other immunoelectrochemical systems, although its cause is unknown. The response for 100 cells was less than two standard deviations above the blank response (0 cells) and therefore was not considered detectable. The response for 500 cells was more than two standard deviations above the blank, and we therefore estimate the detection limit as 5000 cells/ml (500 cells filtered). Similar results were obtained over a period of months using the same lots of cells and conjugate (data not shown).

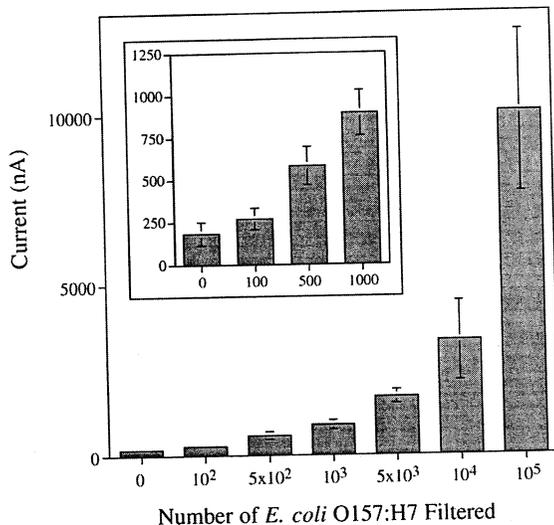


Fig. 4. Filtration immunoelectrochemical assay response for *E. coli* O157:H7. Inset shows the data for low bacteria concentrations on an expanded scale. Sample volume: 100  $\mu\text{l}$ . Number of cells per sample as shown. Concentration in cells/ml was ten times the number of cells per sample. Error bars represent one standard deviation about the mean of six replicates, except at the  $10^5$  cells level where only 3 replicates were run.

## 4. Discussion

Successful coupling of filtration capture with immunoelectrochemical detection required a means

reproducibly bringing captured cells into close contact with the electrode, and a means of minimizing the background current arising from conjugate non-specifically bound to the membrane. After trials with a variety of filtration devices and electrode configurations, the apparatus and procedures described above were adopted. Filtration was carried out with a simple vacuum apparatus, and the filters were then manually transferred to the detection unit. Overall analysis time was 25 min, including 15 min for incubation of bacteria with conjugate, 2 min for filtration and washing of the bacteria, 3 min for assembly of the detection unit, and 5 min for incubation with substrate and detection. Non-specific binding of antibody-AP conjugate to filters in the presence of various blocking agents was assessed using colorimetric methods to screen commonly available commercial materials. Polycarbonate filters blocked with TTBS exhibited the lowest levels of conjugate binding and were used for further work.

Even with polycarbonate filters and TTBS blocking, background current due to conjugate bound to the filter was significant. The dependence of the background current on sample volume and its effect on the detection limit are shown in Fig. 3. Samples containing a fixed concentration of conjugate and 0,  $10^3$ , and  $10^4$  bacteria/ml were prepared and incubated. The left side of Fig. 3 shows results obtained when 10  $\mu$ l of the 0 and  $10^4$  bacteria/ml solutions were filtered, washed, and analyzed in quadruplicate. The background current from 10  $\mu$ l of the blank (0 bacteria/ml) was  $17 \pm 7$  nA and the current for 10  $\mu$ l of the  $10^4$  bacteria/ml sample (100 bacteria in total) was  $89 \pm 12$  nA, giving a net current due to the bacteria alone of 62 nA. Under these conditions the calculated (Currie, 1968; Gehring et al., 1996) detection limit was 35 bacteria (3500/ml). The right side of Fig. 3 shows results for a similar set of measurements using 100  $\mu$ l volumes of the 0 and  $10^3$  bacteria/ml samples. In this case the background current from the blank was  $305 \pm 55$  nA and the current for 100  $\mu$ l of the  $10^3$  bacteria/ml sample (100 bacteria in total) was  $379 \pm 107$  nA, giving a calculated detection limit of 440 bacteria (4400/ml). This increase in the magnitude and variance of the background current with increased sample volume was also observed for 250 and 500  $\mu$ L samples (data not shown).

It had been expected that by increasing sample volume we could lower the detection limit by concentrating more cells on the filter. However, the increase in background current with increased sample volume actually led to the opposite result, i.e. somewhat higher detection limits (in terms of bacteria/ml) for larger sample volumes. A sample volume of 100  $\mu$ l was selected for further work in order to: (1) avoid evaporative and adsorptive losses associated with smaller sample volumes, and (2) avoid the high variance in response observed for currents above 2000 nA (see above) associated with larger sample volumes. The response of the assay was characterized over several orders of magnitude using samples of *E. coli* O157:H7 in TBS. As shown in Fig. 4, the response was approximately linear for concentrations up to  $10^4$  cells/ml (1000 cells), and the estimated detection limit was approximately 5000 cells/ml (500 cells).

These results indicate that filtration immunoelectrochemical assays can provide high speed and sensitivity, and that further development and optimization is justified. It was shown that mechanically holding bacteria in close contact with the electrode provided the same high detection sensitivity ( $\sim 1$  nA/bacterium) observed for bacteria captured on antibody-coated electrodes. Under conditions where background current was minimized (10  $\mu$ l sample volume), fewer than 50 *E. coli* O157:H7 could be detected in a 25 min assay. However, under typical assay conditions using sample volumes of 100  $\mu$ l, the detection limit was approximately 500 bacteria or 5000 bacteria/ml. The primary factor controlling detection was non-specific binding of conjugate to the filter membrane, and improved detection limits will require a significant reduction in non-specific binding. New membrane materials (e.g. polysulfone) and blocking procedures, as well as alternative assay protocols, are under investigation toward this end.

## References

- Bej, A.K., Mahbubani, M.H., Boyce, M.J., Atlas, R.M., 1994. Detection of *Salmonella* spp. in oysters by PCR. *Appl. Environ. Microbiol.* 60, 368-373.
- Brewster, J.D., Gehring, A.G., Mazenko, R.S., Van Houten, L.J., Crawford, C.J., 1996. Immunoelectrochemical assays for bac-

- teria: use of epifluorescence microscopy and rapid-scan electrochemical techniques in development of an assay for salmonella. *Anal. Chem.*, submitted.
- Brooks, J., Mirhabibollahi, B., Kroll, R.G., 1992. Experimental enzyme-linked amperometric immunosensors for the detection of *Salmonellas* in foods. *J. Appl. Bacteriol.* 73, 189–196.
- Curiale, M.S., Klatt, M.J., Mozola, M.A., 1990. Colorimetric deoxyribonucleic acid hybridization assay for rapid screening of *Salmonella* in foods: collaborative study. *J. Assoc. Off. Anal. Chem.* 73, 248–251.
- Currie, L.A., 1968. Limits for qualitative detection and quantitative determination. *Anal. Chem.* 40, 586–593.
- Feng, P., 1992. Commercial assay systems for detecting food-borne *Salmonella*: a review. *J. Food Prot.* 55, 927–934.
- Feng, P., 1996. Emergence of rapid methods for identifying microbial pathogens in foods. *J. AOAC Int.* 79, 809–812.
- Fratamico, P.M., Schultz, F.J., Buchanan, R.L., 1992. Rapid isolation of *Escherichia coli* O157:H7 from enrichment cultures of foods using an immunomagnetic separation method. *Food Microbiol.* 9, 105–113.
- Fratamico, P.M., Sackitey, S.K., Wiedmann, M., Deng, M.Y., 1995. Detection of *Escherichia coli* O157:H7 by multiplex PCR. *J. Clin. Microbiol.* 33, 2188–2191.
- Gehring, A.G., Crawford, C.G., Mazonko, R.S., Van Houten, L.J., Brewster, J.D., 1996. Enzyme-linked immunomagnetic electrochemical detection of *Salmonella typhimurium*. *J. Immunol. Methods* 195, 15–25.
- Hadas, E., Soussan, L., Rosen-Margalit, E., Farkash, A., Rishpon, J., 1992. A rapid and sensitive heterogeneous immunoelectrochemical assay using disposable electrodes. *J. Immunoassay* 13, 231–252.
- Ibrahim, G.F., 1986. A review of immunoassays and their application to *Salmonellae* detection in foods. *J. Food Prot.* 49, 299–310.
- Luk, J.M.C., Lindberg, A.A., 1991. Rapid and sensitive detection of *Salmonella* (O:6,7) by immunomagnetic monoclonal antibody-based assays. *J. Immunol. Methods* 137, 1–8.
- Matsunaga, T., Nakajima, T., 1985. Electrochemical classification of gram-negative and gram-positive bacteria. *Appl. Environ. Microbiol.* 50, 238–242.
- Minunni, M., Mascini, M., Guilbault, G.G., Hock, B., 1995. The quartz crystal microbalance as biosensor, a status report on its future. *Anal. Lett.* 28, 749–764.
- Paffard, S.M., Miles, R.J., Clark, C.R., Price, R.G., 1996. A rapid and sensitive enzyme linked immunofilter assay (ELIFA) for whole bacterial cells. *J. Immunol. Methods* 192, 133–136.
- Pettipher, G.L., Rodrigues, U.M., 1982. Rapid enumeration of microorganisms in foods by the direct epifluorescence technique. *Appl. Environ. Microbiol.* 44, 809–813.
- Rishpon, J., Gezundhajt, Y., Soussan, L., Rosen-Margalit, E., Hadas, E., 1992. Immuno-electrodes for the detection of bacteria. In: *Biosensor, Design and Application*, ch. 6. ACS Symposium Series No. 511, pp. 59–72.
- Skjerve, E., Olsvik, Ø., 1991. Immunomagnetic separation of *Salmonella* from foods. *Int. J. Food Microbiol.* 14, 11–18.
- Tortorello, M.L., Gendel, S.M., 1993. Fluorescent antibodies applied to direct epifluorescent filter technique for microscopic enumeration of *Escherichia coli* O157:H7 in milk and juice. *Food Prot.* 56, 672–677.
- Tortorello, M.L., Stewart, D.S., 1994. Antibody-direct epifluorescent filter technique for rapid direct enumeration of *Escherichia coli* O157:H7 in beef. *Appl. Environ. Microbiol.* 60, 3553–3559.
- Watts, H.J., Lowe, C.R., Pollard-Knight, D.V., 1994. Optical biosensor for monitoring microbial cells. *Anal. Chem.* 66, 2465–2470.
- Wyatt, G.M., Langley, M.N., Lee, H.A., Morgan, M.R.A., 1991. Further studies on the feasibility of one-day *Salmonella* detection by enzyme linked immunosorbent assay. *Appl. Environ. Microbiol.* 59, 1383–1390.
- Yu, H., Bruno, J.G., 1996. Immunomagnetic-electrochemiluminescent detection of *Escherichia coli* O157 and *Salmonella typhimurium* in foods and environmental water samples. *Appl. Environ. Microbiol.* 62, 587–592.