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# Automated filtration capture immunoelectrochemical assay of bacteria

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## Abstract

An automated system for filtration capture and immunoelectrochemical detection of bacteria in liquid samples is described. The detector incorporates a porous electrode in contact with the filter, rather than the solid electrode used previously, to allow sample and reagent solutions to be delivered in a flowing stream. This eliminated the need for manual assembly of the electrode and filter for each assay and allowed repetitive assays on a single filter/electrode. The electrochemical response of the novel gold grid electrode under static and flow conditions was found to be consistent with theory for a planar electrode operating in laminar flow conditions. A computer-controlled fluid handling system was coupled to the detector for delivery of samples and reagents at controlled flow rates and times. The combination of flow detector and fluid handling system allows for automation of the previous assay protocol as well as providing new operating modes with enhanced background rejection and improved sensitivity. The use of these operating modes is demonstrated by a simple assay for Escherichia coli O157:H7 with virtually no background current.

Key words: Filtration; Immunoelectrochemistry; Bacteria; Detection

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Abbreviations: AP, alkaline phosphatase; p-APP, p-aminophenylphosphate; CBS, Carbonate-buffered saline

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## 1. Introduction

Culture methods for detection of bacteria are simple and extremely sensitive, but require days or weeks to obtain results. For many applications, such as testing fresh foods for pathogens, this assay time is prohibitive, and there is great interest in rapid assays which provide results in hours or minutes (Feng, 1992, 1996). Many rapid assays involve two basic steps - a capture step in which the target organism is isolated from the sample matrix and/or concentrated in a relatively small volume, followed by a detection step in which a measurable signal is generated from the captured bacteria. 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.

Work in our laboratory has focused on immunoelectrochemical (IEC) detection combined with a number of capture approaches. IEC methods utilize antibody-enzyme conjugates to label bacteria, followed by incubation of the enzyme labeled bacteria with substrate to form a product which is detected electrochemically. Detection limits of ~10000 bacteria/mL were reported when IEC detection was used in an ELISA with the electrode measuring the bulk concentration of product formed by cells captured on the walls of a microtiter plate well (Brooks et al., 1992). Detection limits were lowered dramatically to ~100 bacteria/mL when the bacteria were captured directly on antibody-coated electrodes (Rishpon et al., 1992; Hadas et al., 1994, Brewster et al., 1996). Electrochemical detectors respond only to molecules at the surface or able to diffuse to the surface during the course of the current measurement. A high concentration of product was produced in this surface region by capturing the enzyme-labeled cells directly on the electrode, leading to much higher signal than obtained. In our hands, antibody coated glassy carbon electrodes exhibited low background signal (due to non-specific adsorption of antibody-enzyme conjugate) and excellent sensitivity for captured cells. However, the capture efficiency was very low (<0.1% of cells present were captured), and electrodes were subject to fouling by exposure to raw samples. These drawbacks stimulated investigations of other capture methods such as

immunomagnetic capture (Skjerve and Olsvik, 1991; Fratamico et al., 1992; Yu and Bruno, 1996; Gehring et al., 1996) and filtration which would allow the inherent sensitivity of IEC detection to be fully utilized.

Capture of bacteria by filtration is efficient, fast, simple, inexpensive, and readily scaled up to volumes of tens to hundreds of mL in order to increase assay sensitivity (provided the sample matrix does not clog the filters). It is not surprising that filtration capture has been used extensively for microbial analysis of water and food samples. Detection of microbes captured on filters can be achieved by a variety of methods, including traditional microbiological techniques such as culturing and microscopy. Electrochemical detection of electroactive compounds released by bacteria captured on filters was investigated as a method for urine analysis (Matsunaga and Nakajima, 1985). This approach was capable of non-specific detection of bacteria, but the detection limit was  $>10^6$  cells/mL. A colorimetric immunofiltration assay in which filtered cells were labeled with antibody-enzyme conjugate after capture has been used to produce a rapid and sensitive ( $\sim 10^4$  cells/mL) high throughput analysis system (Paffard et al., 1996). Immunofluorescent labeling of filtered cells 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. Importantly, these authors developed and validated methodology for processing samples from a complex food matrix (ground beef) to produce extracts suitable for filtration capture in less than 30 minutes. This result (and work of others on filtration/microscopy detection of bacteria in foods) indicates that filtration capture is a viable approach for practical assays in food matrices, and can give sensitivity and speed limited primarily by the detection technology.

Recently we reported IEC detection of bacteria captured on filter membranes (Brewster and Mazenko, 1997). Solutions containing bacteria and antibody-alkaline phosphatase conjugate were filtered, and the filters (with bacteria) then placed directly on a solid carbon disk electrode. Using the same protocols for substrate incubation and product detection used previously for antibody-

coated electrodes, a similar response of  $\sim 1$  nA/bacteria (*E. coli*) was obtained. This result indicated that the high sensitivity achieved by localizing bacteria on the electrode using surface-attached antibody could also be attained by mechanically holding bacteria in contact with the electrode. In experiments designed to minimize background by using very small volumes (10  $\mu$ L) of concentrated bacteria solutions (and consequently exposing the filter to very small amounts of conjugate) it was shown that this approach was capable of detecting fewer than 100 bacteria in less than 10 min (detection only). However, with sample sizes of 100-1000  $\mu$ L, the relatively high background current arising from conjugate non-specifically adsorbed to the filter membrane resulted in a detection limit of  $\sim 5000$  bacteria/mL. In addition, a great deal of skill was required to reproducibly bring the filter, the electrode, and the substrate solution in intimate contact without introducing air bubbles or disturbing the captured bacteria. To address these problems and further develop the filtration capture/immunochemical detection assay, we needed to eliminate the need for manual coupling of filter and electrode, and provide a means for delivering samples and reagents to the detector in a reproducible and convenient manner. This has been accomplished by the design of a new integrated electrode/filter detector cell for performing filtration capture immunochemical assays in flowing streams and a computer-controlled sample and reagent delivery system for conducting experiments with the detector cell. The electrochemical and flow properties of the detector cell are characterized, and new operating modes that are made possible by combined flow detector and fluidics system are discussed.

## **2. Materials and Methods**

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 & Perry Laboratories, Inc. (Gaithersburg, MD). Polycarbonate track etched membrane filters (0.2  $\mu$ m pore size) were from Poretics, Inc. (Livermore, CA). Tris(hydroxymethyl)aminomethane (Tris), Tween-20, and gold grids for transmission electron

microscopy were from Sigma (St. Louis, MO). Disodium p-nitrophenyl phosphate (p-NPP) was from Pierce (Rockford, IL). 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

TBS: 25 mM tris(hydroxymethyl)-aminomethane (Tris), 150 mM sodium chloride, pH 7.6. TTBS: 25 mM Tris, 150 mM sodium chloride, 1.0% Tween-20, pH 7.6. CBS: 100 mM carbonate-bicarbonate buffer, 100 mM sodium chloride, pH 9.5. TCBS: 100 mM carbonate-bicarbonate buffer, 100 mM sodium chloride, 0.1% Tween-20, pH 9.5. Fe(II): 1 mM  $K_4Fe(CN)_6$  in CBS. Substrate: 1 mg/mL p-APP in CBS containing 1 mM  $MgCl_2$ . Conjugate: lyophilized goat alkaline phosphatase conjugated to anti-*E. coli* O157:H7 was rehydrated in 50% glycerol to give a 0.1  $\mu$ g/mL solution and stored at  $-10^\circ C$ . Aliquots of this solution were diluted 1:450 in TBS shortly before use to give working AP-conjugate solution. *E. coli* O157:H7 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  $\sim 4 \times 10^9$  cells/mL in phosphate-buffered saline (0.1 M sodium phosphate, 0.15 M NaCl, pH 7.4) and stored at  $-10^\circ C$ . Working solutions were prepared from thawed cells by washing twice with TBS by centrifugation at 3400g for 5 minutes, removal of the supernate, and resuspension of the pellet in TBS. The concentration of washed cells was determined microscopically using a Petroff-Hauser chamber. Sodium hydroxide solutions were filtered through 0.45  $\mu$ m PVDF syringe filters, all other solutions (other than bacteria samples) were filtered through 0.2  $\mu$ m nylon or polycarbonate filters before use.

### 2.3. Apparatus

A BAS 100B/W Electrochemical Analyzer, gold disk electrode, Ag/AgCl reference electrode, and Vycor rod were from Bioanalytical Systems, Inc. (West Lafayette, IN). The tip of a 6 mm x 70 mm Ag/AgCl reference electrode was wrapped with a platinum wire to form a reference/counter electrode unit and stored in 3 M NaCl when not in use. All potentials refer to this reference electrode system.

### 2.4 Detector

The electrode/filter detector was built from a Kel-F cross coupling for 1.5 mm outside diameter tubing (Valco Instruments, Houston, TX), as shown in Figure 1. Two arms of the cross had an inside diameter of 1.1 mm, and the other arms had an inside diameter of 0.78 mm. A 0.25 mm gold wire was bent over at the end and inserted about 0.2 mm inside one arm of the cross to act as a contact to the working electrode. The wire was sealed into an adjacent arm with a rubber septum and threaded fitting (not shown). The electrode/filter assembly consisted of a polyethylene washer (5 mm OD, 2.3 mm ID, 0.100 mm thick), a 400 mesh gold electron microscopy grid (Sigma) working electrode (3.05 mm OD, 0.020 mm thick), a polycarbonate membrane filter (5 mm OD, 0.010 mm thick), and a PEEK frit (5 mm OD, 3.2 mm active diameter, 1.58 mm thick) (Upchurch Scientific, Oak Harbor, WA). The frit was smoothed on both sides by wet sanding with 600 grit and 1500 grit silicon carbide abrasive paper. The assembly was sealed together and held against the gold wire with a stainless steel washer and a flangeless fitting (Upchurch). The third arm of the cross was sealed with a fitting consisting of a plug of porous Vycor pressed into a 1/4-28 Leur adapter (Upchurch) and sealed with an o-ring. This fitting provided solution contact for the reference and counter electrodes which were placed in 3 M NaCl in the chamber above the Vycor plug.

## *2.5 Sample/reagent delivery system*

The computer-controlled fluid handling system was assembled in-house and consisted of a host computer, an autosampler, a syringe module, and a valve module. A schematic diagram of the system is shown in Figure 2. The host computer (Macintosh, Apple Computer Inc., Cupertino, CA) communicated with the modules via RS-232 signals routed by an intelligent switch (Mini Smart Switch, B&B Electronics, Ottawa, IL). The autosampler, a Model M12677005 from Scientific Measurement Systems (Grand Junction, CO), moved a probe (a 130 mm length of 1.5 mm OD PEEK tubing) on an XYZ arm within a 177 mm x 238 mm x 115 mm volume. The sampler had four rack positions and racks for 15 mL test tubes, microcentrifuge tubes, and microtiter plates. The syringe module consisted of a power supply and two XL3000 Modular Digital Pump units from Cervo Scientific Instruments (Sunnyvale, CA). The pumps had a maximum resolution of 12000 steps, a maximum speed of 10000 steps/s, and a minimum speed of 20 steps/s. Slower flows were possible by executing single steps at a controlled rate using internal "looping" commands. One unit was equipped with a 1 mL glass/Teflon® syringe; the other unit was equipped with a 10 mL glass/Teflon® syringe. Each syringe also had a motorized 3 position valve (inlet, outlet, bypass). The valve module consisted of six 3-way and two 2-way all-Teflon® selenoid valves activated by a microcontroller with a high current driver module, along with appropriate power supplies. The valves (3-way, 100T3MP24-32 and 2-way, 100T2NC24-32) were from Bio-Chem Valve Corp. (Boonton, NJ). The microcontroller (SPCL-0001-X22) and high current driver (NMIS-3020) were from New Micros Inc (Dallas, TX). For the experiments described here, the valves were connected as shown in Figure 2, using 1.5 mm outside diameter fluoropolymer tubing, a PEEK cross, and flange-free end fittings from Upchurch Scientific (Oak Harbor, WA). The internal diameter of the sample loop, transfer line to the autosampler, and the waste lines was 0.76 mm; the remaining tubing had an internal diameter of 0.25 mm.

Software written in-house provided control of the modules via hierarchical tables of commands. Lists of elementary commands (e.g. valve on, syringe motion, XYZ sampler position) along with execution times were stored in tables referred to as macros. A typical macro described the sequence of commands to perform a sample injection and would include variables for such parameters as syringe speed, sample volume, and sample position. Lists of macros along with execution times were stored in tables referred to as methods. A typical method described the sequence of macros required to perform an analysis, as well as values for any variable parameters defined in the macro.

### *2.7 Electrochemical Measurements*

All electrochemical measurements employed the software-driven BAS 100W analyzer. The electrode cleaning and activation step performed each time a new electrode was installed in the system consisted of a linear sweep voltammogram in 1M NaOH or KOH starting at 0 mV and cycling anodically to +1000 mV, cathodically to -500 mV, and anodically back to 0 mV at 100 mV/s. Electrode performance was then tested by cyclic voltammetry from 0 to 500 mV at 50 mV/s in Fe II solution. For characterization of the gold grid electrode a length of 0.25 mm diameter gold wire was pushed through the mesh at the edge and wrapped around the rim to provide mechanical support and electrical contact. The grid was completely immersed during testing so that 2-3 mm of the gold wire was also exposed to solution and contributing to the observed current. The parameters used in cyclic voltammetry, timebase amperometry, and Osteryoung square wave voltammetry are given in the figure captions.

### *2.7 Filtration capture immunoelectrochemical assay*

Bacteria stocks were prepared by serial 10x dilution from the  $10^9$  bacteria/mL working solution with TBS in polypropylene microcentrifuge tubes. Samples for analysis were prepared by

mixing 100  $\mu\text{L}$  of the appropriate bacteria stock (10x the final concentration) with 900  $\mu\text{L}$  of conjugate solution, incubated 20 minutes at 40°C, and kept on ice until analysis. Blank solutions (no cells) were prepared using 100  $\mu\text{L}$  of TBS and 900  $\mu\text{L}$  of conjugate solution, following the above protocol.

Referring to Figure 2, the positions of the 3-way valves (V0, V1, and V2) are indicated by a 0 or 1. The state of the 2-way valves (V6 and V7) is either closed (0) or open (1). Unless otherwise noted, the valve states are as shown in the figure. Samples and reagents were introduced by connecting the injection loop to the autosampler with valve V0 = 1, aspirating the required volume into the injection loop at 1000  $\mu\text{L}/\text{min}$  with the 1 mL syringe, connecting the injection loop to the remainder of the system with V0 = 0, and dispensing a specified volume of carrier (CBS) at a specified flow rate from the 10 mL syringe. Prior to aspirating any fluids the injection loop and transfer line were flushed by dispensing 1200  $\mu\text{L}$  of CBS at 5000  $\mu\text{L}/\text{min}$  into a waste tube on the autosampler.

The direct analysis method consisted of the following steps: a) aspirate 250  $\mu\text{L}$  of TTBS, dispense 125  $\mu\text{L}$  at 250  $\mu\text{L}/\text{min}$ ; b) aspirate 100  $\mu\text{L}$  of sample, dispense 500  $\mu\text{L}$  at 500  $\mu\text{L}/\text{min}$ ; c) dispense 1200  $\mu\text{L}$  at 5000  $\mu\text{L}/\text{min}$  to a waste tube with V0=1 (flush sample loop and connecting tubing); d) dispense 2000  $\mu\text{L}$  at 2000  $\mu\text{L}/\text{min}$  with V6=1 and V7=0; e) dispense 250  $\mu\text{L}$  at 500  $\mu\text{L}/\text{min}$  with V7 = 1; f) immerse the sample probe in 1 M NaOH for 10 s; g) immerse the sample probe in CBS for 5 s; h) aspirate 100  $\mu\text{L}$  of substrate, dispense 140  $\mu\text{L}$  at 125  $\mu\text{L}/\text{min}$ ; i) record Osteryoung Square Wave Voltammograms (OSWV) 0, 2, 4, and 6 minutes after conclusion of substrate flow with V7=0; j) dispense 250  $\mu\text{L}$  at 500  $\mu\text{L}/\text{min}$ ; k) aspirate 50  $\mu\text{L}$  of 1M KOH, dispense 500  $\mu\text{L}$  at 500  $\mu\text{L}/\text{min}$  to destroy AP in the detector. OSWVs were run under the following conditions: initial potential: -200 mV; final potential: 200 mV; step amplitude: 4 mV; square wave amplitude: 50 mV; frequency: 10 Hz. The initial OSWV was subtracted from the final OSWV to remove current from solvent oxidation and yield a background-subtracted voltammogram. The background-subtracted peak current was measured by applying the Analysis

function of the BAS 100W to the background-subtracted data to generate a baseline across the shoulders of the peak and measure the net peak current from that baseline.

The transfer analysis method consisted of the following steps: a) aspirate 250  $\mu\text{L}$  of TTBS, dispense 125  $\mu\text{L}$  at 250  $\mu\text{L}/\text{min}$ , dispense 125  $\mu\text{L}$  at 250  $\mu\text{L}/\text{min}$  with  $V_2=0$  and  $V_7=0$ ; b) aspirate 100  $\mu\text{L}$  of sample, dispense 500  $\mu\text{L}$  at 250  $\mu\text{L}/\text{min}$  with  $V_2 = 0$  and  $V_7=0$ ; c) dispense 1200  $\mu\text{L}$  at 5000  $\mu\text{L}/\text{min}$  to a waste tube with  $V_0=1$ (flush sample loop and connecting tubing); d) dispense 2000  $\mu\text{L}$  at 2000  $\mu\text{L}/\text{min}$  with  $V_6=1$  and  $V_7=0$ ; e) dispense 250  $\mu\text{L}$  at 250  $\mu\text{L}/\text{min}$  with  $V_2 = 0$  and  $V_7=0$ ; f) dispense 500  $\mu\text{L}$  at 500  $\mu\text{L}/\text{min}$  with  $V_1 = 1$ ; g) immerse the sample probe in 1 M NaOH for 10 s; h) immerse the sample probe in CBS for 5 s; i) aspirate 100  $\mu\text{L}$  of substrate, dispense 140  $\mu\text{L}$  at 125  $\mu\text{L}/\text{min}$  with  $V_7=1$ ; j) record Osteryoung Square Wave Voltammograms (OSWV) 0, 2, 4, and 6 minutes after conclusion of substrate flow with and  $V_7=0$ ; k) dispense 250  $\mu\text{L}$  at 500  $\mu\text{L}/\text{min}$ ; l) aspirate 50  $\mu\text{L}$  of 1M KOH, dispense 500  $\mu\text{L}$  at 500  $\mu\text{L}/\text{min}$  to destroy AP in the detector. The background-subtracted peak current was determined as above.

### 3. Results and Discussion

#### 3.1 Flow detector

A significant challenge in developing a detector for flow filtration IEC was locating an electrode material with the appropriate mechanical, chemical, and electrochemical properties. The electrode needed to be porous to permit flow of sample and reagent to the filter, have low surface area to minimize adsorption of conjugate and permit the use of pulsed electrochemical methods, be chemically compatible with samples and reagents, and provide stable, facile electron transfer to the enzyme product with minimal background current. Metal grids used as sample supports for transmission electron microscopy are available at low cost in a variety of hole sizes and materials (copper, nickel, and gold). Grids used here were 20  $\mu\text{m}$  thick, 3.05 mm in diameter, with a circular rim and an array of 20  $\mu\text{m}$  wide bars and 42  $\mu\text{m}$  holes in the center. Gold grids had 55%

open area and therefore virtually no effect on flow, very low surface area, were chemically inert, of the appropriate size and shape to fit standard 1/4-28 low pressure tubing fittings, and exhibited good electrochemical characteristics. Reliable electrical contact to the electrode was established by using a gold wire contacting the front surface of the grid. Although the contact wire is exposed to solution and therefore contributed to the background current, this appeared to have a negligible effect and did not warrant attempts to insulate the wire. Close membrane/electrode contact and mechanical support was provided by a polymer frit which was smoothed to prevent tearing of the membrane when the electrode/filter assembly was compressed.

### *3.2 Electrode properties*

As we were not aware of any reports on the use of electron microscopy grids as electrodes, experiments were conducted to characterize their electron transfer and flow response properties. Previous work used planar (flat) macroscopic electrodes which were mechanically polished before use to remove surface oxides and adsorbed contaminants which inhibited electron transfer. Since mechanical polishing of the grids was not feasible, an electrochemical cleaning/activation process was devised. Because of the unusual geometry of the grids, including features with dimensions on the order of 10  $\mu\text{m}$ , it was important to determine whether the grids exhibited the complex behaviour of microelectrodes or porous electrodes, or could be treated as planar electrodes (with well understood current vs. time and current vs. flow behavior). As shown in Table 1, the grid was compared to a planar disk electrode and a cylindrical helix electrode using cyclic voltammetry as a diagnostic tool (Bard and Faulkner, 1980). Peak separation provided a measure of the electron transfer rate,  $k$ , while peak current provided a measure of effective electrode area (current is also dependent on  $k$ ). Uncleaned electrodes gave poorly defined peaks with peak current density  $<0.3 \mu\text{A}/\text{mm}^2$  and peak separation  $>400 \text{ mV}$ , indicative of sluggish electron transfer (data not shown). Electrochemically cleaning the electrodes by cycling between large anodic and cathodic potentials in 1 M NaOH resulted in well defined peaks with peak current density  $\sim 1$

$\mu\text{A}/\text{mm}^2$  and peak separation  $<200$  mV. No evidence of microelectrode behavior (enhanced current density, sigmoidal voltammograms) was observed. The flow response of the detector was assessed by injecting 600  $\mu\text{L}$  (the maximum volume) of Fe II through the detector at various flow rates and recording current as a function of time (data not shown). Plateau current was proportional to the square root of flow rate over the limited range of flows (125 - 500  $\mu\text{L}/\text{min}$ ) accessible with the system, as expected for a planar, non-porous electrode in laminar flow. From these results it was concluded that gold grids behaved essentially as planar electrodes with effective surface area equal to the calculated geometric area. The grids exhibited stable electrochemical characteristics even after exposure to relatively large concentrations of proteins, surfactants, substrate, and products, and electrode to electrode reproducibility was excellent (for the single lot of grids examined).

### *3.3 Dispersion*

The fluid handling system shown in Figure 3 utilized a 1 mL motorized syringe, an autosampler, and a valve to aspirate samples and reagents into the injection loop. The material in the loop was then carried into the system by pumping carrier buffer through the loop and into the system with a 10 mL syringe. During aspiration and pumping solutions were dispersed (mixed with and diluted by the carrier buffer). It was necessary to study the extent of dispersion in order to determine the appropriate volumes and flow rates to use in various steps of the assay. For example, it was necessary to determine the volume of buffer required to quantitatively carry a 100  $\mu\text{L}$  sample through the detector during the capture step. Dispersion was evaluated by measuring the concentration of Fe(II) electrochemically as function of time for different injection volumes and flowrates. Data from an experiment to determine the the concentration of substrate reaching the detector for various injected volumes are shown in Figure 3. Fe (II) was aspirated into the injection loop at 1000  $\mu\text{L}/\text{min}$ , and then pumped to the detector at 125  $\mu\text{L}/\text{min}$  while recording current as a function of time. For large injections ( $>200$   $\mu\text{L}$ ) dispersion affected only the

brown material similar to that which appeared in aged pAPP solutions. Several approaches to solution of this problem, including use of a different substrate, are currently under investigation.

### *3.5 Transfer assay*

A significant limitation of the static assay was the high background current from conjugate adsorbed to the membrane, which obscured the signal from low levels of bacteria. The detector electrode and filter were also exposed to other components of the sample, which may result in fouling or otherwise interfere with the assay. We are currently investigating a new mode of operation which may reduce or eliminate these problems. It can be seen in Figure 2 that by switching valves V2 and V7, flow could be directed to either filter F1, or to the Detector. Flow through F1 could also be reversed by switching valves V1, V6, and V7 to (potentially) transfer material captured on the filter surface to the detector (or to waste). An assay was implemented which utilized this scheme to capture bacteria in a sample on the primary filter F1, while the conjugate and other soluble sample constituents were washed through to waste. Flow through F1 was then reversed to transfer the captured bacteria (but not material adsorbed to F1) to the detector, where they were captured and detected as in the direct assay. In a preliminary experiment, solutions containing conjugate and either 0 (blank) or  $10^5$  *E. coli* O157:H7/mL were analyzed by the direct and transfer protocols. In transfer mode the blank gave no detectable signal (limit of detection  $\sim 10$  nA), while the response for the bacteria ( $10^4$  cells injected) was 430 nA after a 6 minute incubation. In direct mode, the blank response was 530 nA after 3 minutes, and was off scale by 6 minutes, clogging the membrane and preventing analysis of the bacteria sample. The reduction of the large blank response to a nondetectable level with this simple and rapid protocol could improve the sensitivity of the assay by one or more orders of magnitude. Additional experiments have confirmed that background current can be virtually eliminated with this approach, but further study is required to be show that transfer of bacteria from primary filter to detector can be performed reproducibly and quantitatively for a wide range of bacteria concentrations.

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### 3.5 Future plans

In addition to performing automated detection of bacteria in processed samples, the fluid handling system is amenable to incorporation of some or all of the sample preparation steps, including addition of enzymes and surfactants to improve filterability, prefiltration, and addition of conjugate. Particularly for large sample volumes, it could be more effective to capture cells on a filter and then label them using a small volume (~50  $\mu$ L) of conjugate, rather than adding conjugate to the raw sample. Studies of this approach and the transfer method are underway, and will be reported in the near future.

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Table 1. Characteristics of gold electrodes				
Electrode	Area (mm <sup>2</sup> )	Cathodic Peak Current (μA)	Peak current density (μA/mm <sup>2</sup> )	Peak Separation mV
Planar Disk	2.01	2.49	1.24	84
Cylindrical Helix	47.1	38.9	0.83	137
Grid	12.5	11.8*	0.94	171

The planar disk electrode was a 1.6 mm diameter gold rod embedded in a Kel-F cylinder and polished to a mirror finish before storage. The cylindrical helix consisted of a 7 turn helix (5 cm) and a 1 cm straight section of 0.25 mm diameter gold wire. The grid was a 3.05 mm diameter 0.02 mm thick disk with an array of 42 μm square openings separated by 20 μm bars in the center surrounded by a solid rim 300 μm wide. \* Grid current corrected for 3 mm gold wire lead.

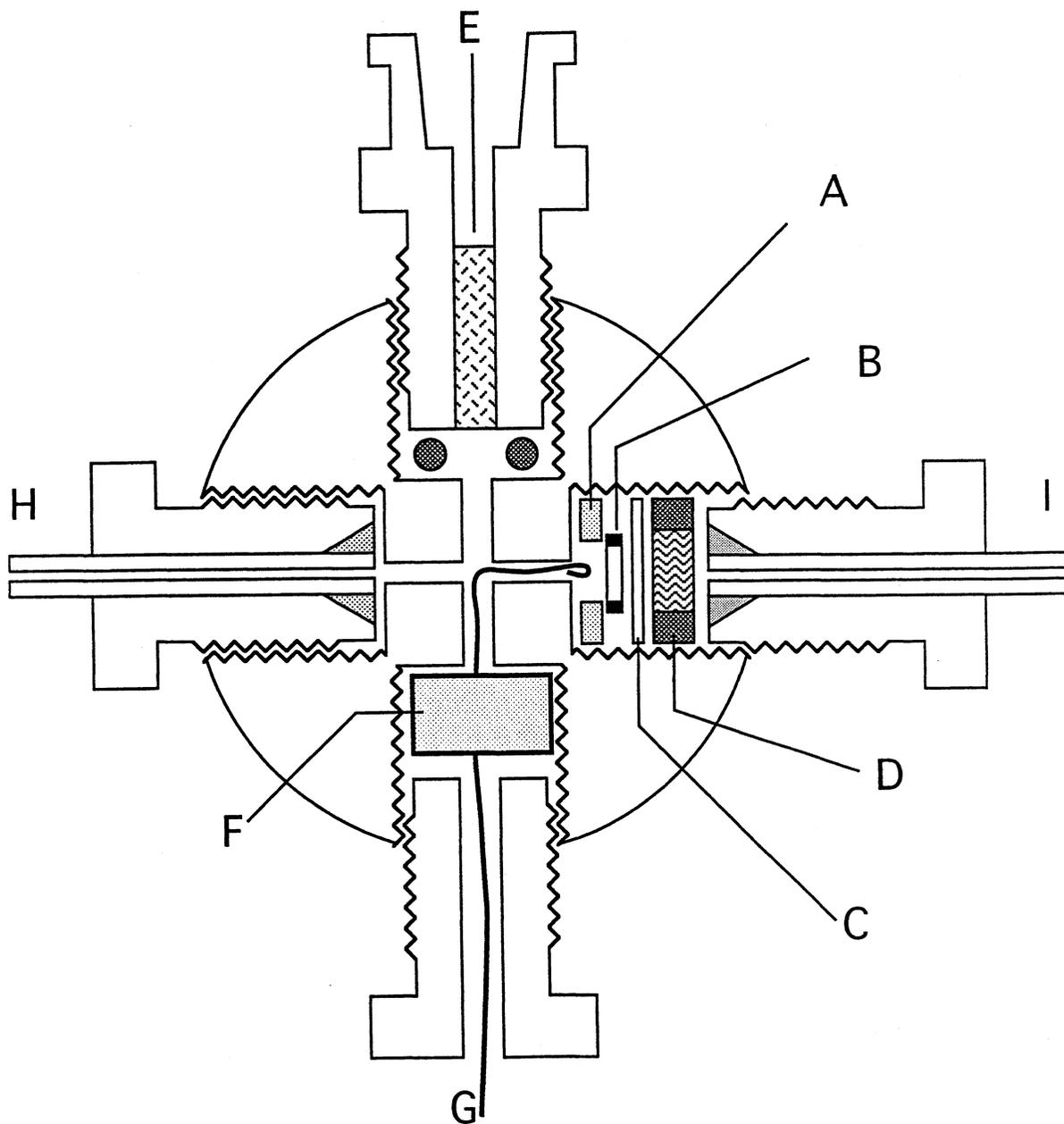


Figure 1. Diagram of the filter/electrode detector. A: polymer washer; B: gold grid electrode; C: filter membrane; D: frit; E: porous Vycor plug; F: rubber septum; G: gold contact wire; H: inlet connection; I: outlet connection.

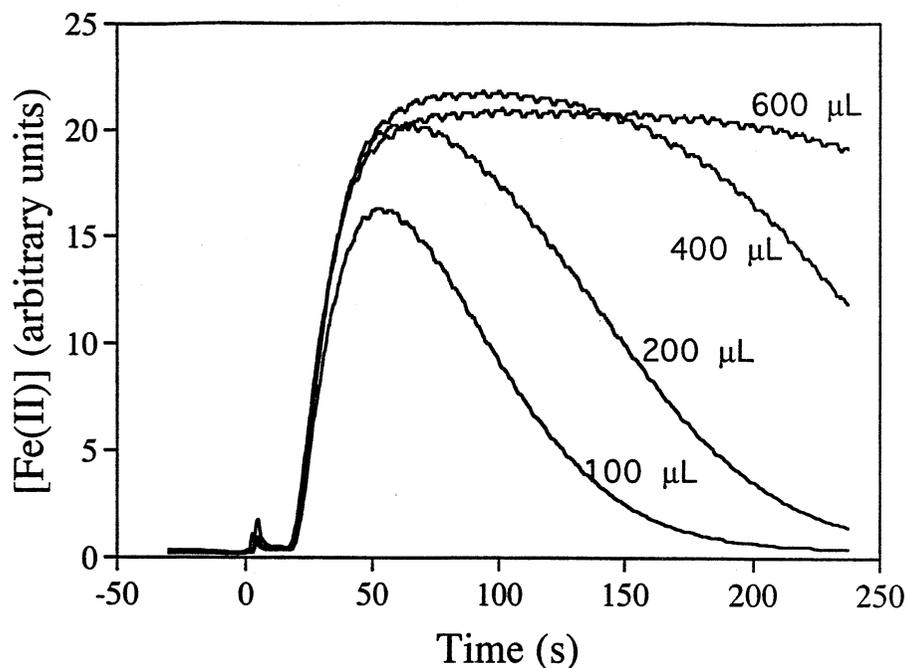


Figure 2. Schematic diagram of the fluid handling system indicating syringes, valves, and tubing connections. V0, V1, V2: 3 way selenoid valves. V6 and V7: 2-way selenoid valves. 3 way valves V3, V4, and V5 available but not used in this configuration. Not to scale. See text for details.

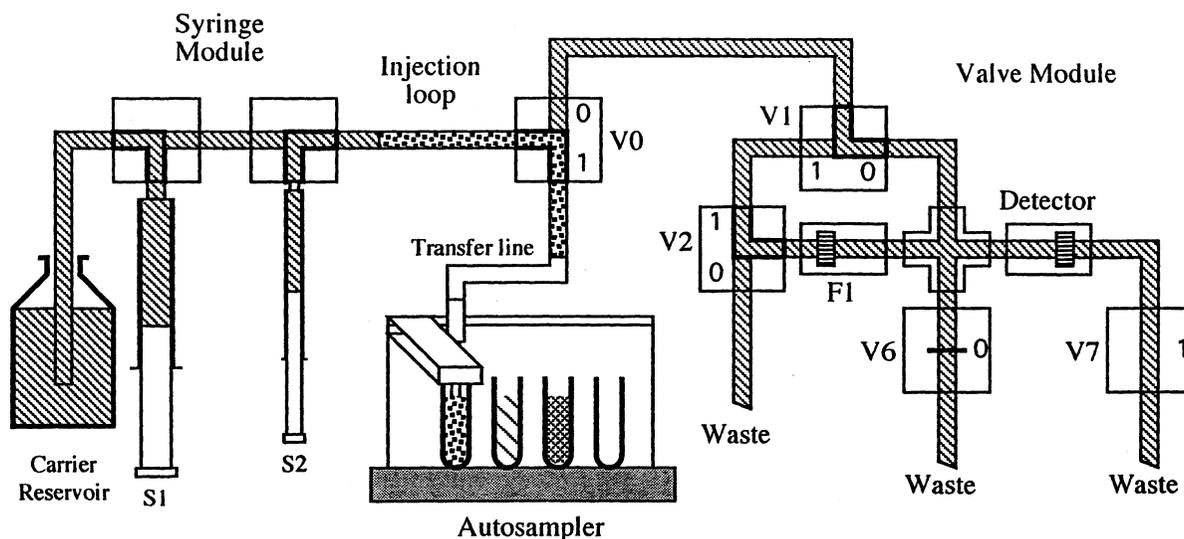


Figure 3. Effect of dispersion and injection volume on the concentration of sample reaching the detector. Samples of 1 mM  $K_4Fe(CN)_6$  in CBS were injected at time 0 at a flow rate of 125  $\mu\text{L}/\text{min}$ .