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SPECIAL ISSUE PAPER

David L. Stokes · Guy D. Griffin · Tuan Vo-Dinh

Detection of *E. coli* using a microfluidics-based antibody biochip detection system

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Abstract This work demonstrates the detection of *E. coli* using a 2-dimensional photosensor array biochip which is efficiently equipped with a microfluidics sample/reagent delivery system for on-chip monitoring of bioassays. The biochip features a 4×4 array of independently operating photodiodes that are integrated along with amplifiers, discriminators and logic circuitry on a single platform. The microfluidics system includes a single 0.4 mL reaction chamber which houses a sampling platform that selectively captures detection probes from a sample through the use of immobilized bioreceptors. The independently operating photodiodes allow simultaneous monitoring of multiple samples. In this study the sampling platform is a cellulosic membrane that is exposed to *E. coli* organisms and subsequently analyzed using a sandwich immunoassay involving a Cy5-labeled antibody probe. The combined effectiveness of the integrated circuit (IC) biochip and the immunoassay is evaluated for assays performed both by conventional laboratory means followed by detection with the IC biochip, and through the use of the microfluidics system for on-chip detection. Highlights of the studies show that the biochip has a linear dynamic range of three orders of magnitude observed for conventional assays, and can detect 20 *E. coli* organisms. Selective detection of *E. coli* in a complex medium, milk diluent, is also reported for both off-chip and on-chip assays.

Introduction

It is estimated that food borne pathogens account for up to 80 million illnesses in the United States each year, with approximately 9,000 cases resulting in death. Among such pathogens, *Escherichia coli 0157:H7* is becoming an increasingly significant factor in these occurrences. *E coli* has generally been used as an indicator of fecal contamination in water, as well as a valuable tool in genomic research. However, *E. coli 0157:H7* has specifically become a bacteria of rising concern in the past decade. It is a facultative anaerobe which attacks the intestines of its hosts, leading to abdominal cramps, fever, diarrhea, bloody diarrhea and dehydration. In extreme cases, the poison can lead to hemolytic uremic syndrome (HUS) which is manifested by kidney failure, stroke, seizures, and even death for victims with depressed immune function (especially the young and the elderly). Incubation period in the intestines is typically 3–10 days before the onset of toxin poisoning, with the ensuing illness running its course in 5–10 days. Antibiotics are rarely effective and can often aggravate the illness through the possible accelerated toxin release resulting from cell lysis. While most commonly found in food of animal origin, *E. coli 0157:H7* can taint vegetables through the use of contaminated fertilizers. Furthermore, run-off from farms can contaminate water supplies which can serve as far reaching vectors for the pathogen. Adequate cooking and proper hygiene can prevent the incidence of human infection. Nevertheless, significant *E. coli 0157:H7* outbreaks have become more common, and more far reaching in the last decade, despite America's record of having the safest food supply in the world. One of the worst outbreaks in history was traced to a tainted well at the 1999 Washington County Fair, NY. Approximately 1000 cases were reported, with 2 fatalities. Other recent, high profile incidents have involved tainted Jack-in-the-Box hamburgers, which affected approximately 700 people (4 deaths) in the Pacific Northwest in 1993; and contaminated Odwalla fruit juices, which infected approximately 70 widely scattered people in 1996. Hudson Foods recently implemented a recall of 25 million pounds of meat, the largest meat recall in history. The expanding problem of food borne illnesses may be attributed to an increase in international commerce, overextended food regulatory agencies, and the evolution of antibiotic-resistant organisms. The need for more expedient, accurate, and cost-effective

D. L. Stokes · G. D. Griffin · Tuan Vo-Dinh (✉)
Advanced Monitoring Development Group,
Life Sciences Division, Oak Ridge National Laboratory,
Oak Ridge, TN 37831-6101, USA
e-mail: vodinht@ornl.gov

screening processes is obvious for food products, drinking water supplies, and recreational waters. Traditional analyses have involved such time-consuming methods as membrane filtration, culturing and immunodiffusion assays. Turn around times for off-site evaluations could be as high as 30–48 h for such techniques.

In response to the rising incidence of food borne illnesses, increased efforts have been devoted towards the development of more efficient pathogen detection schemes. For *E. coli* analysis, the EPA has recently approved a new method, "MI Agar Membrane Filter Method for Total Coliforms and Escherichia coli" [1–3]. Extensive studies have been directed towards evaluations and comparisons of various emergent trademark technologies, such as Colilert, Quantitray, Coliplate, Enterolert, BAX E. coli 0157, Reveal 8 E. coli 0157-H7 screening test, VIP EHEC, VIDAS E. coli 0157 (ECO), EHEC-Tek, Tecra E. coli 0157 visual immunoassay, Petrifilm and the Difco EZ coli rapid detection system [4–11]. Such studies encompassed analyses of drinking water [4], watersheds [5, 6], and various food products [7–11]. In some cases, exceptional sensitivity (10–15 organisms) was demonstrated [8]. Selectivity for *E. coli 0157:H7* detection was also demonstrated, even for potentially injured *E. coli* in the presence of outnumbering competing bacteria [7], and in the presence of non-*0157:H7 E. coli* [11]. Some studies were also highlighted by analysis times as low 15–24 h [8, 11]. In other work, selective detection of *E. coli 0157:H7* was observed for chicken carcass washes when performing conductance measurements of samples incubated in Coliform Medium [12]. Robertson et al. reported an analysis time span of less than a workday via measurement of *E. coli* glucoronidase [13]. Some very innovative technologies have also been recently reported. Multiplex PCR systems have been developed for analysis of drinking water [14] and rural streams [15]. Seo et al. have reported 4-cell detection with a 6-h analysis time when combining immunomagnetic bead separation with flow cytometry (IMFC) for the investigation of various inoculated food samples [16]. Furthermore, single-cell detection in less than 3 h has been reported via laser scanning of peptide nucleic acid (PNA) probes [17].

This paper describes an antibody-based biochip having a 2-dimensional photosensor array detector that is directly coupled to a microfluidics sample/reagent delivery system for rapid, on-chip detection of *E. coli*. This compact system features a 4×4 array of independently operating photodiodes (0.9 mm diameter each) which are integrated along with amplifiers, discriminators and logic circuitry on a single platform via the complementary metal oxide semiconductor (CMOS) process. The potential for implementing the CMOS technology for combining photosensors and associated microelectronics into a compact, inexpensive integrated circuit (IC) chip is the principal advantage of this sensor over other 2-dimensional detectors, such as charge-coupled devices (CCDs) and charge injection devices (CIDs). The fluidics system includes a single 0.4 mL reaction chamber which houses a sampling platform that selectively captures detection probes from a delivered complex sample through the use of immobilized bioreceptors. Any device which incorporates bioreceptors, such as antibodies, DNA, enzymes and cellular components of living systems, is referred to as a biosensor. Moreover, any biosensor that involves the use of a microchip system for detection is considered a biochip. Before the incorporation of microchip detection devices, biosensors were typically based on fiberoptic or planar probe substrates from which analytical signals were directed to separate detection systems consisting of appropriate wavelength selection devices and conventional photodetectors, including photomultiplier tubes (PMTs) and CCDs [18–28]. More recently in our laboratory, an IC microchip detector-based biochip using DNA bioreceptors has been developed for detection of sequence-specific genetic constituents in complex samples [28, 29]. In addition, we have exploited an advantage of the independently operating photodiodes to enable the simultaneous measurement of multiple sample screening tasks [30]. Both antibody-based probes for the detection of *p53* protein and DNA probes for the detection of *Micobacterium tuberculosis* were incorporated on a single sampling platform.

This study extends the capability of the biochip developed in our laboratory via the incorporation of a microfluidics module in the previously described biochip system [28–30] for the first time. Such an improvement can minimize sample handling, expedite analyses, and allow real-time monitoring of screening processes (thereby facilitating sampling platform design, optimization of reaction parameters, and identification of anomalous system behavior). In this work the sampling platform is a cellulosic membrane which is exposed to *E. coli* organisms and subsequently analyzed using a sandwich immunoassay involving a Cy5-labeled antibody probe. Antibody probes have provided excellent selectivity with previously reported immunosensors for chemical and biological analyses [24, 31, 32]. This selectivity arises from a required complementary relationship between three-dimensional biochemical conformations of an antigen and an antibody. As this paper will demonstrate, the combined effectiveness of the IC biochip and the antibody probe-based assay yields exceptional quantitative ability, sensitivity and selectivity. Furthermore, when using a pretreated sample platform format, the microfluidics-based biochip system described herein may allow *E. coli* detection in less than 1 h. All of these attributes are compiled in a compact, user-friendly, inexpensive device which may be amenable to on-site use at food processing plants, sewage treatment facilities, doctors' offices, and various flowstream monitoring points of environmental or industrial interests.

Experimental

Solid-phase immunoassays. A sandwich immunoassay method using Cy5-labeled antibody probes was implemented in this study, as depicted in Fig. 1. Ninety-six-well plates with a membrane composed of mixed cellulose esters forming the bottom of each well (Multi Screen- HA clear plates, Cat. No. MAHA N45610, Milli-

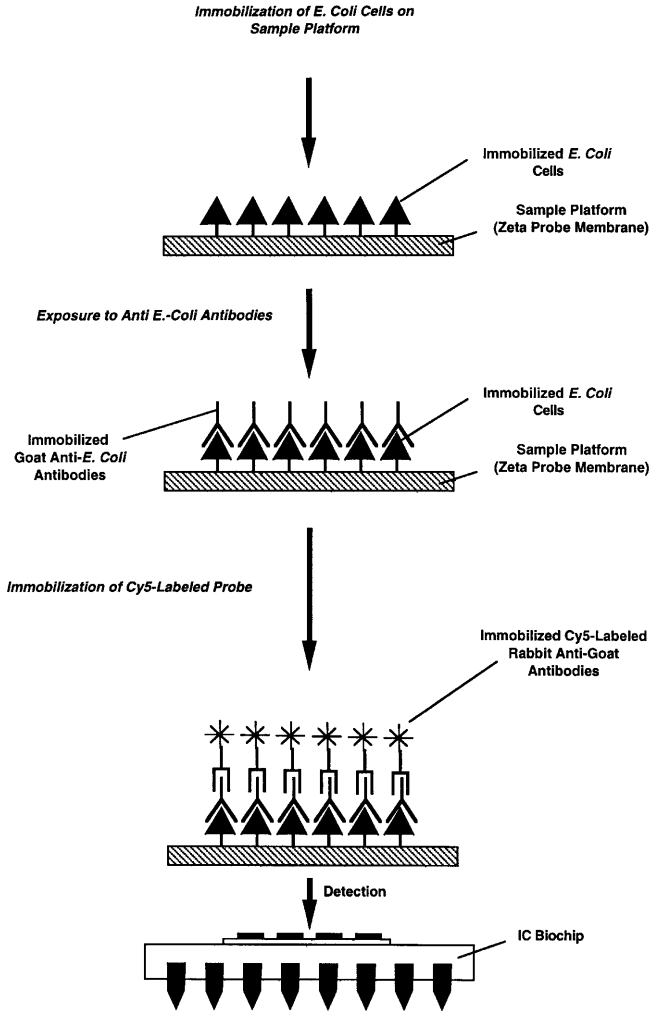

Fig. 1 Graphic depiction of the sandwich immunoassay method for *E. coli 0157:H7* detection using Cy5-labeled antibody probes

pore Corp., Bedford, MA 01730) were used for the assays. The membranes in the wells were pre-wetted for 1 min with 200 μL of phosphate buffered saline (PBS), followed by removal using the Multi Screen vacuum manifold (Cat. No. MAVM 09601, Millipore Corp., Bedford, MA 01730). Various dilutions of the antigen were then pipetted into the wells. The antigen used was a preparation of heat-killed *Escherichia coli 0157:H7* organisms (Cat. No. 50–95–90, Kirkeguard and Perry Laboratories, Inc., Gaithersburg, MD 20879), containing $\approx 7.0 \times 10^9$ organisms/mL. The cell suspension was usually diluted in 0.1 M sodium carbonate buffer, pH 9.6, although for certain experiments, a milk diluent/blocking solution concentrate (diluted 1 : 20 with distilled water- final milk concentration was 0.1% non-fat dry milk, Kirkeguard and Perry Laboratories, Cat. No. 50–82–01, Gaithersburg, MD 20879) or a bovine serum albumin (BSA) diluent/blocking solution concentrate (diluted 1 : 15 with distilled water- final BSA concentration was 0.7% BSA, Kirkeguard and Perry Laboratories, Cat. No. 50–61–00, Gaithersburg, MD 20879) was used. Aliquots of 80 μL of varying dilutions of *E. coli* cell suspension were pipetted into separate wells of the Multi Screen plates. Following 1 h of incubation at room temperature (24 °C), the antigen suspension was removed using the vacuum manifold. 200-μL aliquots of a solution of 0.25% BSA, 0.05% Tween 20 in PBS was added to each well to block any unoccupied binding sites, and the plate was incubated for 1 h at room temperature, followed by removal of the blocking solution. Dilutions of a goat affinity-purified antibody to *E. coli 0157:H7* (Kirkeguard and Perry Laboratories, Cat. No. 01–95–90, Gaithersburg, MD 20879) were prepared in the same buffer used for the blocking step. Usual dilutions were 1 : 25 or 1 : 50. All wells received 80-μL aliquots of this primary antibody. After incubation for 1–1.5 h at room temperature, the antibody solution was removed using the vacuum manifold. Each well was then washed 4 × using 0.5% Tween 20 in PBS, by filling the well by means of a pasteur pipette and removing the wash solution by vacuum filtration. A secondary anti-species antibody with covalently attached dye molecules was used to complete the immunoassay sandwich. This antibody was a purified rabbit anti-goat IgG(H+L) antibody (ZyMax Grade) conjugated with Cy5 (Cat. No. 81–1616, Zymed Laboratories, San Francisco, CA 94080). This antibody was diluted (usually 1 : 25 or 1 : 50) in the same buffer as was used for the primary antibody, and 80 μL aliquots were dispensed into appropriate wells. After incubation for 1–1.5 h at room temperature in the dark (i.e. plate wrapped in aluminum foil), the wells were emptied by vacuum filtration, and all wells were washed 5–6 × with 0.5% Tween in PBS. Wells were subsequently assayed as described below. Negative controls were prepared by treating other wells with blocking solution, primary antibody and secondary antibody, but no *E. coli* antigen.

Instrumentation. A schematic diagram of the integrated circuit (IC) biochip detection system with associated excitation and signal collection optics is illustrated in Fig. 2. The 632.8-nm line of a HeNe laser (Model 106–1, Spectra-Physics, Inc., Eugene, OR) was selected for excitation of the Cy5 label. The laser beam was filtered with a 632.8-nm bandpass filter (Cat. No. P3–633-A-X516, Corion, Franklin, MA) and directed through a diffractive optic device, which split the laser beam into a 4 × 4 array of equally-intense laser beams. The laser beam array was focused at the plane of the sampling platform to form sharp laser excitation spots with approximately 1-mm spacing. The intensity of each laser spot was estimated to be ≈ 0.2 mW. A 1 : 1 image of the laser spot array was projected onto the corresponding 4 × 4 array of photosensors of the IC biochip via a gradient index microlens array (Cat. No. 024–5680, OptoSigma®, Santa Ana, CA). A combination of a 633-nm holographic notch filter (Cat. No. HNPF-633–1.0, Kaiser Optical Systems, Inc., Ann Arbor, MI) and a thin-film dielectric filter with a high-pass at 645 nm (Visionex, Atlanta, GA) was used to isolate the Cy5 emission signal from the laser line. Voltage output from the IC biochip was recorded with a strip chart recorder (Model BD40, Kipp and Zonen, Delft, The Netherlands) or from a digital multimeter (Model 506, Protek). The integrated microchip detector described above is not yet commercially available and was designed at Oak Ridge National Laboratory. The individual photodiodes of the 4 × 4 array are square with 900-μm edges. They are arranged with 1-mm center-to-center spacing. The photodiodes

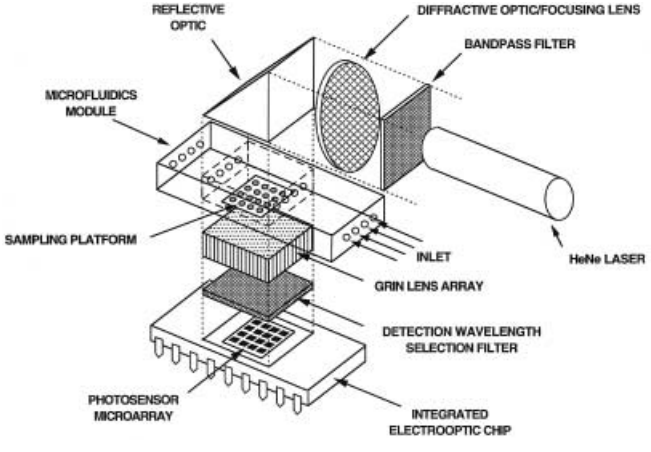

Fig. 2 Schematic diagram of the IC biochip detection system

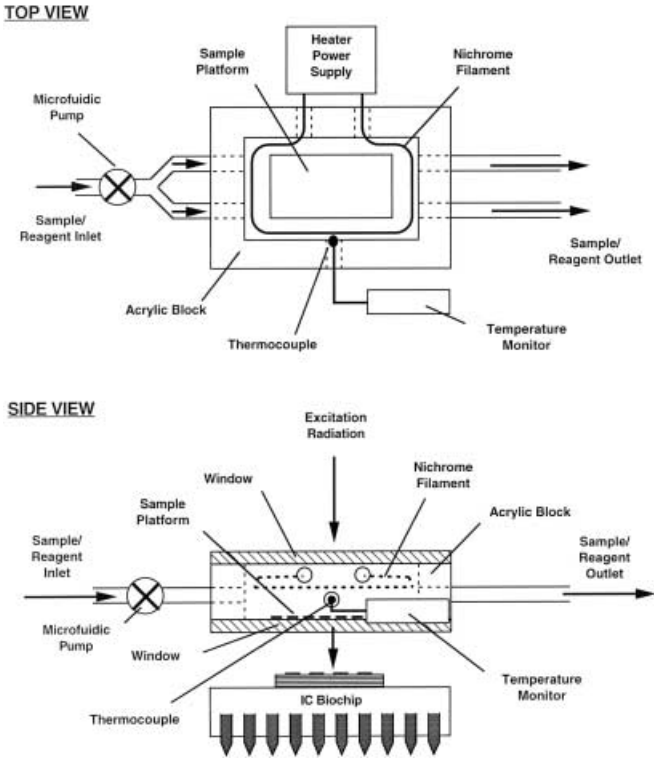

Fig. 3 Schematic diagram of a microfluidics system designed for use with the IC biochip

and the accompanying electronic circuitry were fabricated using a standard 1.2 μm n-well CMOS process.

For initial immunoassay and IC Biochip sensitivity studies, membranes were cut from the bottoms of the reaction wells of the 96-well plates and placed at the focus of the 4×4 laser beam array. For studies involving on-chip immunoassays, membranes were placed in a microfluidics sampling chamber, also at the excitation focal point.

The microfluidics sampling chamber. A schematic diagram of the microfluidics sampling chamber for on-chip immunoassays is illustrated in Fig. 3. The sampling chamber and sample delivery channels were drilled into a $1'' \times 1'' \times 1/8''$ Plexiglas slab (OPTIX®, Plaskolite, Inc., Columbus, OH) by conventional means. A 1/2″ diameter hole was drilled through the center of the block, resulting in a 0.4 mL total sampling volume. A series of 1/16″ diameter holes were also drilled from the edges of the Plexiglas slab to the central sampling chamber. These channels housed the plumbing used for reagent or sample delivery or purging. For these studies, the plumbing was 1/16″ o.d. × 1/50″ i.d. Peek tubing (Cat. No. 1532, Upchurch Scientific, Oak Harbor, WA). Inlet plumbing was also attached to a peristaltic pump capable of a flow rate of ≈ 1 mL/min. Some channels from the edge of the Plexiglas block were used as ports for various accessories, such as a nichrome heating filament and a thermocouple temperature probe (Aeropak). Once all plumbing and other accessories were seated in the Plexiglas block, the channels were all sealed with 5 min epoxy. The sampling chamber was sealed with a pair of glass windows cut from microscope slides. The bottom window was held permanently in place by 5-minute epoxy. The top window was intended to be removable, allowing for easy replacement of the sampling platform (membrane). High vacuum grease (Dow Corning Corp., Midland, MI) was used to temporarily hold the top window in place during on-chip assays. The laser excitation was introduced through the top window. Cy5 fluorescence signals from immobilized antibody probes on the sampling platform were collected through the bottom window of the sampling chamber.

Results and discussion

Evaluation of the IC biochip. For each new application investigated for the new IC biochip technology, success depends on the combination of both the efficiency of the specific immobilization reaction (e.g immunoassay or DNA hybridization) as well as the efficiency of signal transduction. A calibration curve was therefore generated for an initial evaluation of the feasibility of *E. coli 0157:H7* screening using the IC Biochip technology. The results of these calibration measurements are illustrated in Fig. 4. Each data point corresponds to blank-subtracted signal to correct for signal derived from nonspecific binding. This preliminary evaluation did not involve the use of the microfluidics module. Instead, the complete sandwich immunoassay was performed using conventional laboratory practice, independent from the IC biochip system. Separate membranes were prepared for each different dilution of immobilized *E. coli* organisms. Each membrane was then placed at the focal point of the excitation laser beam array for measurement with the IC biochip. As demonstrated by the calibration curve, a linear response was observed for the entire region investigated (approximately 90–20,000 organisms). The estimated number of organisms probed for each measurement was based on the ratio of the area of laser spot to the total area of the membrane, assuming even distribution of organisms on the membrane. For surface-based analytical techniques such as the sandwich immunoassay, linear dynamic range can be limited more by saturation of the sampling membrane surface than by the efficiency of the signal transducer. Because the area of the surface probed for each measurement is so small (≈ 0.16 mm^2), the linear dynamic range exhibited in this study is excellent. Furthermore, no saturation of the surface was evident for this practical range.

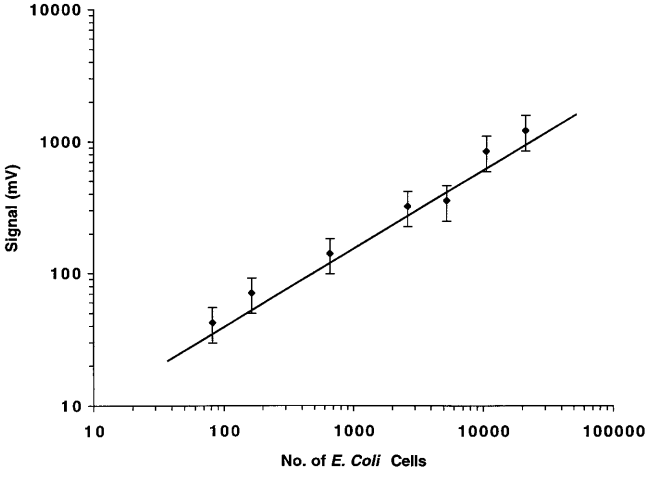

Fig. 4 Calibration curve for *E. coli 0157:H7* detection using Cy5-labeled antibody probes in the sandwich immunoassay technique

The detection of less than 100 organisms achieved with the biochip is excellent, especially when considering that no sample amplification was performed. Sample amplification techniques such as culturing in selective enrichment broths can be a limiting factor in analysis time, often extending to several days. Another approach is enzyme amplification. The sandwich immunoassay technique employed in this study was performed in 4–5 h. This assay time could be shortened by using a direct, single-step assay. This modification would require the use of a fluorescently-labeled, *E. coli 0157:H7*-specific antibody. Due to the expense and availability of labeled pathogen-specific antibodies, however, we have opted to use the sandwich assay format with a species-specific labeled antibody for detection. With this method, a single type of labeled antibody probe can be used in a multitude of immunoassays, making the technique more cost-effective and versatile.

A critical aspect to the effectiveness of a biosensor is selectivity. This attribute can greatly simplify and expedite an analysis by minimizing sample pretreatment steps. Indeed, minimized sample handling and the potential for *in-situ* analysis are principal motivations for the development of biosensors. For immunoassays, the selectivity can be exceptional because it is based on the complementary interaction between three-dimensional biochemical conformations of an antigen and an antibody. Nevertheless, the incidence of competitive, nonspecific binding can be significant in a sample matrix composed of large and varied biomolecules. As an evaluation of selectivity, we used the IC biochip for the detection of *E. coli 0157:H7* in milk diluent. Milk is a common source of *E. coli* infection in humans, often arising from insufficient pasteurization processes. In this evaluation, various concentrations of *E. coli 0157:H7* organisms were prepared in 0.1% milk and distributed on individual membranes, as described previously. Sandwich immunoassays were performed for each membrane, independently from the IC biochip system. Figure 5 illustrates the calibration curve for *E. coli* detection from the milk matrix as measured with the IC biochip. For comparison, a similar calibration curve for the detection of *E. coli* from a simple, standard sodium carbonate/bicarbonate buffer matrix (generated on the same day) is also included in the figure. More than three orders of magnitude of linearity was observed for both curves. As would be expected, some attenuation of the *E. coli* signal was observed for the milk matrix relative to the carbonate buffer. Slightly different calibration curve slopes were also observed for the two media. These features suggest evidence of some nonspecific binding between *E. coli* cell surface receptors and various biomolecules of the milk matrix. Such interactions can not only impede the immobilization of the *E. coli* organisms on the membrane platform, but also block interaction between the organisms and the *E. coli*-specific antibodies. Nevertheless, Fig. 5 illustrates that such effects are not dramatic for the 0.1% milk matrix. Some additional calibration may be required for quantitative analyses involving matrices of higher milk concentration.

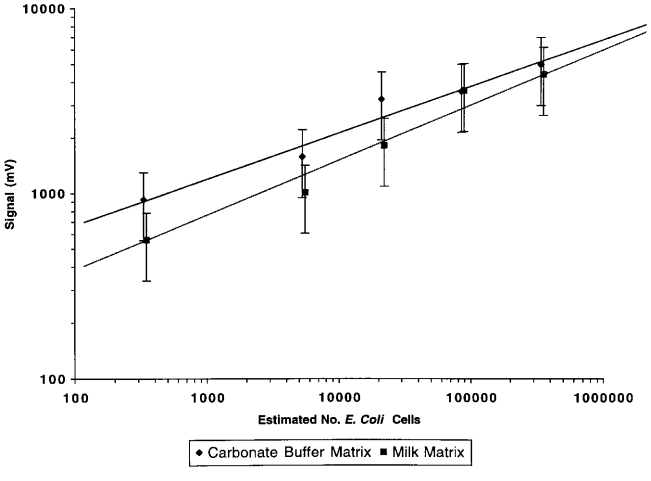

Fig. 5 Calibration curves for *E. coli 0157:H7* detection in both 0.1% milk-based sample matrix, and standard sodium carbonate/bicarbonate buffer matrix

Evaluation of the microfluidics-based biochip system for E. coli detection. Potential applications of the IC biochip system are far reaching. Whether involving direct sample injection or on-line monitoring of liquid or gaseous flow streams, the ultimate bioassay would be based in a liquid medium. We have therefore developed a microfluidics system for sample and reagent delivery to the sensing area of the biochip for on-chip, real-time monitoring of bioassays. Besides providing the obvious potential for on-line monitoring, such a system can also aid in fundamental studies which can facilitate optimization of sample platform design and various bioassay reaction parameters (e.g. temperature, reagent concentrations, incubation periods, reagent flow rates, etc.). As an initial demonstration of feasibility, the sandwich immunoassay method was repeated for detection of *E. coli 0157:H7* in the microfluidics module. Our intended approach to microfluidics-based detection is to generally pretreat sampling platforms with bioreceptors prior to installation in the reaction chamber of the microfluidics system. Sample and sensing probe introduction steps would subsequently be performed with the microfluidics system. In this study, we closely mimicked this approach by pretreating a membrane with *E. coli* organisms and *E. coli*-specific antibodies prior to placement in the reaction chamber of the microfluidics module (the first two steps of the sandwich immunoassay). The pretreated membrane was subsequently incubated with the Cy5-labeled anti-species antibody and rinsed on-chip via the microfluidics system.

Figure 6 a illustrates the Cy5-fluorescence profile as a function of incubation and rinsing times. This profile is a real-time stripchart recording corresponding to the signal output from a single detection element of the biochip. The initial baseline region of the profile corresponds to signal yielded by the pretreated membrane, which included approximately 20,000 organisms per probed area (as defined

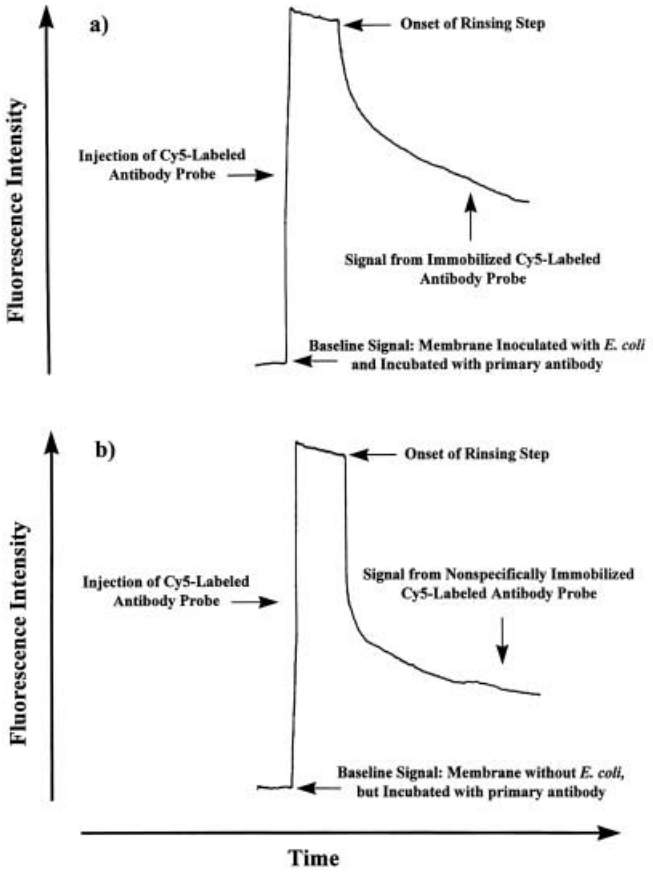

Fig. 6 Real-time fluorescence profiles for on-chip incubation with Cy5-labeled antibody probes and subsequent rinsing for a) signal from *E. coli*-innoculated membrane (≈ 20,000 organisms) (solid curve); signal from blank membrane (dashed curve), and b) signal from blank membrane, obtained via the microfluidics system

by the laser spot size). The reaction chamber was primed with PBS buffer at this point. The sharp increase in signal corresponds to the inflow of Cy5-labeled antibody solution (≈ 0.04 μg/mL) in the reaction chamber, while the ensuing high plateau region corresponds to a stop-flow incubation period, which was approximately thirty min. The sharp decrease in signal likewise corresponds to the onset of rinsing with the Tween/PBS buffer solution. The intensity of Cy5 fluorescence signal is thereafter recorded for 15 min of rinsing with a flow rate of ≈ 1 mL/min (note the time scale is different for the incubation and rinsing regions of the profile). The residual fluorescence signal level observed after rinsing arises from a possible combination of *E. coli*-specific immobilization of the Cy5-labeled antibody probe plus various nonspecific interactions between the antibody probe and non-*E. coli*-derived features of the membrane surface. As an evaluation of the contribution of nonspecific binding to fluorescence signal, a similar reaction profile, illustrated in Fig. 6b, was recorded for a blank membrane. The blank membrane was pretreated with blocking reagent and the *E. coli*-specific antibody, but no *E. coli* organisms. The fluorescence profile for the blank membrane illustrates some significant signal from residual nonspecific interactions. Nevertheless, a clear difference in stabilized signal levels is observed, particularly after 15 min of rinsing, thus illustrating the potential for on-chip *E. coli* detection with the microfluidics system.

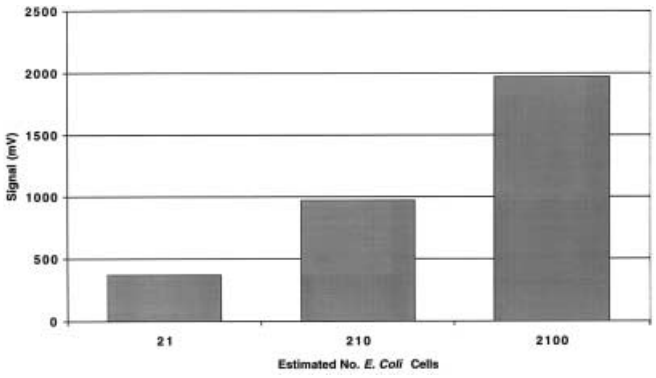

Fig. 7 Blank-subtracted signals for various numbers of *E. coli* organisms after on-chip incubation with Cy5-labeled antibody probe and 15-min rinse time

An additional demonstration of the feasibility of on-chip monitoring of the immunoassay is shown in Fig. 7. Using the procedure described above, the microfluidics system was used to measure signals for various numbers of probed *E. coli* organisms. The signal levels depicted by the figure correspond to blank-subtracted signals obtained after 15 min of rinsing. Repeated measurements performed with the biochip have produced a relative standard deviation of approximately 15%. While a proportional response of signal level to the number of probed organisms is observed, the true highlight of this experiment was the detection of as little as 21 *E. coli* organisms.

The selective detection of *E. coli 0157:H7* from a milk-doped sample matrix was repeated with the microfluidics-based IC biochip, as well. As before, a membrane was pretreated with a cell suspension prepared in 0.1% milk. Following incubation with the *E. coli*-specific antibody, the membrane was placed in the reaction chamber for on-chip incubation with the labeled species-specific antibody. The estimated number of probed organisms was 200. Fig. 8 illustrates blank-subtracted signals at various times during the rinsing step. For comparison, corresponding signals for an equal number of *E. coli* organisms suspended in the standard carbonate buffer is also included in the figure. As was demonstrated in the previous selectivity study, the signals observed for the milk-doped matrix sample were uniformly lower than those for the carbonate buffer matrix sample. This figure indicates additional care should be taken with the use of the microfluidics system. Relative to the carbonate buffer-based sample, the milk-doped sample exhibits a decrease in the Cy5 fluorescence signal as a function of rinse time. It was previously mentioned that large biomolecules associated with a complex matrix such as milk can interfere with both the immobilization of *E. coli* organisms on the membrane surface and the subsequent interaction between the organisms and the diagnostic antibodies. As Fig. 8 illustrates, such effects can be more pronounced with the mi-

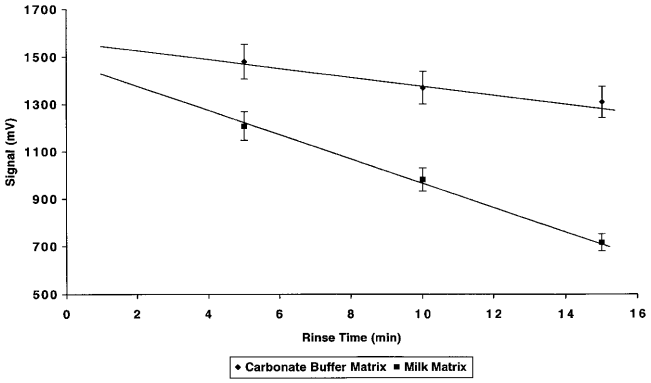

Fig. 8 Blank-subtracted signals for ≈ 200 *E. coli* organisms acquired at various rinse times after on-chip incubation with Cy5-labeled antibody probe, for both 0.1% milk-based sample matrix, and standard sodium carbonate/bicarbonate buffer matrix

crofluidics-based technique because they can promote flow-induced erosion of the *E. coli* organisms, or diagnostic antibodies, or both, during the final rinsing step. In spite of these conditions, the feasibility of absolute detection of less than 100 organisms of *E. coli 0157:H7* in complex media has been demonstrated.

Conclusion

An IC biochip device has been developed at Oak Ridge National Laboratory which efficiently couples bioreceptor-based sample platforms with a microchip-based photosensor. The photosensor is composed of a 4×4 array of microscale photodiodes which are integrated with associated microelectronic circuitry onto a single platform via CMOS technology. In this study, we have applied the IC biochip to the detection of *E. coli 0157:H7* via a sandwich immunoassay involving Cy5-labeled antibody probes. Immunoassays were based on cellulosic sampling platforms. As an extension of previous developments, this work also describes a microfluidics system developed for on-chip monitoring of bioassays. Quantitative detection capability for *E. coli* has been demonstrated through routinely observed linear dynamic ranges of up to three orders of magnitude. Furthermore, *E. coli* limits of detection of as low as 20 organisms have been observed for non-amplified samples. Finally, selective detection has been demonstrated for *E. coli* samples doped with 0.1% milk. These analytical features can combine to yield relatively short analysis times. For example, this study indicates that analysis times of less than 1 h are possible when using a prefabricated sampling platform in the microfluidics-equipped IC biochip system. In addition, the system is very compact, user friendly, and cost-effective, with the potential for high throughput and simultaneous multiple assays. In summary, this new device may be amenable to use in doctors' offices, food processing plants, sewage treatment facilities, and various process monitoring points of environmental and industrial interest.

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References

1. Brenner KP, Rankin CC, Sivaganesan M, Scarpino PV (1996) Appl Environ Microbiol 62:203
2. Brenner KP, Rankin CC, Sivaganesan M (1996) J Microbiol Methods 27:111
3. Brenner KP, Rankin CC, Roybal YR, Stelma GN, Scarpino PV, Dufour AP (1993) Appl Environ Microbiol 59:3534
4. Fricker EJ, Illingworth KS, Fricker CR (1997) Water Res 31: 2495
5. Lifshitz R, Joshni R (1998) Environ Toxicol Water Qual 13: 157
6. Eckner K (1998) Appl Environ Microbiol 64:3079
7. Blackburn CD, McCarthy JD (2000) Intern J Food Microbiol 55:285
8. Woody JM, Stevenson JA, Wilson RA, Knabel SJ (1998) J Food Protection 61:110
9. Johnson JL, Brooke CL, Fritschel SJ (1998) Appl Environ Microbiol 64:4390
10. Bulte M, Heckotter S, Schott W, Kirschfeld R, Kockel J (1998) Fleischwirtschaft 78:690
11. FristenbergEden R, Sullivan NM (1997) J Food Protection 60:219
12. Edmiston AL, Russell SM (2000) J Food Protection 63:264
13. Robertson W, Palmateer G, Aldom J, Van Bakel D (1998) Water Sci Technol 38:87
14. Tsen H-Y, Jian L-Z (1998) J Appl Microbiol 84:585
15. Turner SJ, Lewes GD, Bellamy AR (1997) Water Sci Technol 35:337
16. Seo KH, Brackett RE, Frank JF (1998) Intern J Food Microbiol 44:115
17. Prescott AM, Fricker CR (1999) Mol Cell Probes 13:261
18. Fodor SPA, Read JL, Pirrung MC, Stryer LT, Lu A, Solas D (1991) Science 251:767
19. Anderson RC, McGall G, Lipshutz RJ, In: Manz A, Becker H (eds) (1998) Microsystems Technology in Chemistry and Life Science, Springer Verlag, Berlin, pp 117–129
20. Schena M, Shalon D, Davis RW, Brown PO (1995) Science 270:467
21. Piunno PAE, Krull UJ, Hudson, RHE, Damha MJ, Cohen H (1995) Anal Chim Acta 228:205
22. Kumar P, Wilson RC, Valdes JJ, Chambers JP (1994) Material Sci Eng C1:187
23. Eggers M, Hogan M, Reich RK, Lamture J, Ehrlich D, Hollis M, Kosicki B, Powdrill T, Beattie K, Smith S, Varma R, Gangadharam R, Mallik A, Burke R, Wallace D (1994) Biotechniques 17:516
24. Vo-Dinh T, Tromberg BJ, Griffin GD, Ambrose KR, Speaniak MJ, Gardenhire EM (1987) Appl Spectrosc 41:735
25. Vo-Dinh T, Griffin GD, Sepaniak MJ, In: Wolfbeis OS (ed) (1991) Fiber Optic Chemical Sensors and Biosensors, CRC Press, Boca Raton, FL
26. Vo-Dinh T, Houck K, Stokes DL (1994) Anal Chem 66:3379
27. Vo-Dinh T, Isola NR, Alarie JP, Landis D, Griffin GD, Allison S (1998) Instrum Sci Technol 26:503
28. Vo-Dinh T (1998) Sensors and Actuators B 51:52
29. Vo-Dinh T, Alarie JP, Isola NR, Landis D, Wintenberg AL, Ericson MN (1999) Anal Chem 71:358
30. Vo-Dinh T (1999) Proc 6th Annnual Biochip Technologies Conference: Chips for Hits '99, Berkeley, CA
31. Vo-Dinh T, Alarie JP, Johnson RW, Sepaniak MJ, Santella, RM (1991) Clin Chem 37:531
32. Rowe CR, Scriggs SB, Feldstein MF, Golden JP, Ligler FS (1999) Anal Chem 71:433