G. P. Anderson and C. A. Rowe-Taitt, "Water quality monitoring using an automated portable fiber optic biosensor: RAPTOR," SPIE Proceedings, **4206** (February 2001).

This paper was published in February 2001, Volume 4206, Photonic Detection and Intervention Technologies for Safe Food Proceedings New! Yud-Ren Chen, Agricultural Research Service/U.S. Dept. of Agriculture; Shu-I Tu, Agricultural Research Service/ U.S. Department of Agriculture ISBN, and is made available as an electronic reprint with permission of SPIE.

Water quality monitoring using an automated portable fiber optic biosensor: RAPTOR

George P. Anderson* and Chris A. Rowe-Taitt

Naval Research Laboratory, Center for Bio/Molecular Science and Engineering,

Code 6910, Washington, D.C. 20375-5348

ABSTRACT

The RAPTOR is a portable, automated biosensor capable of performing rapid, ten-minute assays on a sample for four target analytes simultaneously. Samples are analyzed using a fluorescent sandwich immunoassay on the surface of short polystyrene optical probes with capture antibody adsorbed to the probe surface. Target analytes bound to the fiber by capture antibodies are detected with fluorescently labeled tracer antibodies, which are held in a separate reservoir. Since target recognition is a two-step process, selectivity is enhanced, and the optical probes can be reused up to forty times, or until a positive result is obtained. This greatly reduces the logistical burden for field operations. Numerous assays for toxins, such as SEB and ricin, and bacteria, such as *Bacillus anthracis* and *Francisella tularensis*, have been developed for the RAPTOR. An assay of particular interest for water quality monitoring and the screening of fruits and vegetables is detection of *Giardia* cysts. *Giardia lamblia* is a parasitic protozoan common in the developing world that causes severe intestinal infections. Thus, a simple field assay for screening water supplies would be highly useful. Such an assay has been developed using the RAPTOR. The detection limit for *Giardia* cysts was $5x10^4$/ml for a 10-minute assay.

Keywords: Biosensor, fiber optic, immunoassay, fluorescence

1. INTRODUCTION

The fiber optic biosensor has developed over the last decade from a single channel laboratory breadboard[1-5] into a portable, automated 4-channel sensor.[6,7] During that time, the fluoroimmunoassay methodology has remained essentially the same. Capture antibodies are immobilized onto the surface of an optical fiber. When sample is flowed over the fiber probe, the immobilized antibody captures analyte. The amount of analyte bound is determined by a subsequent step where the binding of a tracer antibody to the bound analyte forms a fluorescent complex. The biosensor monitors this complex formation by evanescently exciting surface-bound fluorophores with a diode laser. A small portion of the emitted fluorescence is captured by the optical probe and passes back up the fiber to the photodiode detector. Since the excitation intensity and efficiency of fluorescence recovery falls exponentially with distance from the fiber probe surface, the system is highly discriminatory for the surface bound fluorophores.[8]

The RAPTOR (Figure 1) is the only fiber optic biosensor commercially available (Research International, Woodinville, WA) designed to withstand the rigors of field-testing. The system employs a separate 635 nm diode laser to excite each of the four fiber optic probes. To match this excitation wavelength, the fluorescent dye Cy5 is used to tag the tracer antibody. Since the sensor performs a two-step sandwich assay, fluorescent tracer reagent is not contaminated by sample and negligible signals are obtained from optical probes when negative samples are analyzed. Hence, both the fiber probes and fluorescent reagent can be reused until a positive is obtained. This makes the RAPTOR an ideal instrument for continuous monitoring of large numbers of samples. If a positive is detected, the user only needs to replace the "coupon" which holds the four capture antibody-coated optical probes.

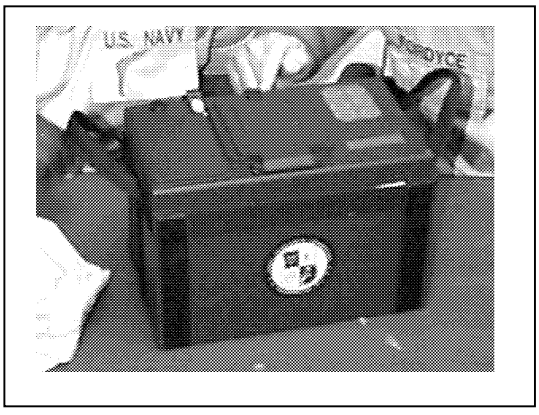

Figure 1. The fiber optic biosensor, RAPTOR.

*Correspondence : Email: ganderson@cbmse.nrl.navy.mil; Telephone: 202 404 6033; Fax 202 767 9594

The optical probes are injection-molded polystyrene, which permits inexpensive mass production. These probes are easily coated with antibody by passive adsorption, over-coated with a stabilizing solution, and dried while being glued into the coupon (Figure 2), thereby providing an extended shelf life. Each coupon is marked with a bar code that tells the sensor what assay steps to perform and the identity of the antibody on the probes. To use, the coupon is inserted into the holder on the RAPTOR, and sample and reagents are introduced through blunt tipped needles, which connect to the coupon upon closure of the top door. Valves in the coupon control the fluid flow; since the sample never passes through any permanent valves, at the worst only the disposable coupon can become clogged. From its initial design, the RAPTOR was made to be field hardened and very simple to use, even for an operator with no technical background. It can be battery operated and accepts samples introduced either manually or automatically from an attached collector.

Data analysis is also completely automated, which removes any subjective bias of the user. After the data are collected and analyzed using preloaded software, with user modifiable settings, the results are displayed on the LCD screen of the instrument in a simple display specifying the assays performed and the assessment (i.e. positive/negative) of the results. If quantitative information is desired, it can be downloaded through a serial port to a computer.

The RAPTOR and the fiber optic biosensors from which it arose have been used to detect and quantify a variety of hazardous substances in numerous sample matrices, such as detection of explosives,[9-11] toxins,[6,7,12-14] bacteria,[6,7,13,15,16] and viruses,[6,7] in ground water,[10] soil extracts,[11] air extracts,[16] meat homogenates,[12,13] and clinical fluids.[15,17]

In this paper, we present data on the detection of *Giardia lamblia* in drinking water. *Giardia* is a protozoan parasite highly resistant to chlorine and difficult to remove from water supplies by filtration due to its small size. This organi sm is highly infectious; ingestion of less that 10 cysts of *Giardia* may result in illness.[18] Furthermore, infection may be asymptomatic and disease transfer among family members is commonly reported. The work described here incorporates an assay for *Giardia* into a pathogen-detecting coupon that can simultaneously test for *B. anthracis* and *F. tularensis.*

2. EXPERIMENTAL

2.1. Buffers and reagents

Anti-*Giardia* antibody (Mab 7D2 ascites) and antigen was provided by Dr. Ted Nash (NIH, Bethesda, MD).[19] Mab 7D2 IgG was purified using MEP-HyperCel (Life Technologies). Mab 7D2 ascites (1 ml) was diluted with phosphate buffered saline pH 7.2 (PBS) and bound onto a 5 ml MEP-HyperCel column equilibrated with PBS. After the UV absorbance returned to baseline the column was washed with 50 mM Tris-HCl (pH 8.0) until baseline absorbance was obtained. The column was equilibrated with PBS, then the purified 7D2 IgG was eluted with 50 mM sodium citrate (pH 4). The purified IgG was dialyzed against 3 changes of PBS and stored at 4°C. Additional anti-*Giardia* antibody (Mab AG1 and Cy5-labeled AG1) was purchased from Waterborne Inc (New Orleans, LA). The anti-ovalbumin, anti-*B. anthracis*, and anti-*F. tularensis* antibodies and antigens were all provided by Naval Medical Research Command, Silver Spring, MD. RAPTOR wash buffer consisted of phosphate buffer (Sigma, 8.3 mM, pH 7.3) containing 0.05% (v/v) Triton X-100 (TX-100) and 0.01% (w/v) sodium azide (PBT). Casein, bovine serum albumin (BSA), and sea salts were also purc hased from Sigma (St. Louis, MO). The river water was taken from the Potomac river near the Naval Research Laboratory. The pond water was collected in Seabrook, MD. The feces were collected from a healthy mix-breed dog.

2.2. Probe and Coupon Preparation

Capture antibodies were immobilized onto fiber optic probes by passive adsorption. Injection-molded, polystyrene fiber optic probes (Research International) were first blackened at their distal ends to prevent reflection of excitation light. Black-tipped fibers were then placed into capillary tubes (100 µl, cut to 4 cm length) pre-filled with 36 µl of the appropriate antibody solution, in general, 100 µg/ml IgG in 0.1 M sodium carbonate buffer (pH 9.6). After overnight incubation at 4 °C, unbound IgG was rinsed off the probes by brief immersion in dH_2O, and then they were incubated in immunoassay stabilizer (Advanced Biotechnologies Inc.). The probes were then ready to be mounted into the disposable coupons (Figure 2). Described in detail previously ,[14] the

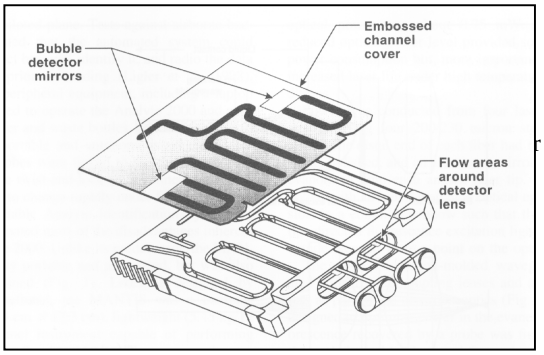

Figure 2. Schematic of assay coupon showing four fiber optic probes, adhesive channel cover and

probes were inserted into the coupon and glued into place using UV-curing epoxy (Norland Optical Adhesive 68). Each coupon was then coded by coloring a series of squares on the back of the coupon with white and black paint pens corresponding to a particular assay recipe that was preprogrammed into the RAPTOR.

2.3. Preparation of Cy5-labeled Antibodies

Cy5-conjugated antibodies were prepared by reacting 3 mg of protein (1 mg/ml) in 50 mM sodium tetraborate, 40 mM NaCl, pH 9.0 with one vial bisfunctional Cy5-reactive dye (λ_{ex}= 649nm, λ_{em}= 670nm; Amersham Life Science Products, Arlington Heights, IL) for 30 minutes at room temperature in the dark. Subsequently, labeled protein was separated from unincorporated dye by size-exclusion chromatography using a BioGel P-10 column (Bio-Rad, Hercules, CA) equilibrated with PBS, pH 7.4. Final protein concentration and dye-to-protein (D/P) molar ratios were calculated based on the A_{280} and A_{650} as described by the manufacturer. D/P ratios ranged from two to 4 Cy5 molecules per IgG. Prior to an assay, the Cy5-reagent was diluted in PBS (pH 7.4) containing 1 mg/ml casein,1 mg/ml BSA, and 0.1% (v/v) TX -100.

2.4. RAPTOR Design and Operation

Inserting a coupon into the RAPTOR aligns all necessary optical paths and engages all required fluidic connections. A pneumatic pump moves buffer, air, fluorescent reagent (from on-board reservoirs), or sample within the system. Serpentine channels in the coupon provide a common path across the probe surfaces. Bubble detectors, which monitor liquid-to-air interfaces, control introduction of sample and fluorescent reagent (Figure 2).

During the two-step sandwich immunoassay, typically 1 ml of an aqueous sample is injected manually into a sample port and flowed over the four fiber optic probes mounted in the assay coupon. Any antigen present in the sample binds to whichever fiber optic probe is coated with capture antibody specific for that antigen; any unbound material is washed away by a brief rinse with PBT. Fluorescently-labeled antibody (the "reagent") is subsequently introduced and binds to the antibody-antigen complexes on the probe surface, completing the sandwich assay. This tracer reagent is maintained at a suitable temperature in an onboard thermal storage module and recovered after each assay cycle, allowing multiple sequential analyses to be performed. Excitation light from four 5 mW Sanyo laser diodes (635 nm) within the RAPTOR is focused into the fiber optic waveguides. An evanescent wave is created along each probe, exciting the fluorescent emission of specifically bound Cy5-labeled antibodies. The portion of the fluorescence captured by the optical probe is collimated by the probe's molded lens and focused onto a photodiode using a ball lens, chosen for its light-gathering power and short focal length. A long -pass dichroic filter (665 nm) rejects reflected laser light. If each fiber optic probe has been coated with capture antibodies with differing specificities, one sample is interrogated for four different analytes simultaneously.

2.5. Assay Procedure

At the beginning of a standard assay, prior to analyte challenge, the RAPTOR automatically initiated a five-minute baseline recipe to establish a background level representing the nonspecific adsorption of the Cy5-reagent to the probes. An initial background rate, the rate of nonspecific binding of the fluorescent antibody to the probe surface, was determined during a 90 second incubation with fluorescent tracer antibody. After tracer reagent recovery, the probes were rinsed with PBT and an initial background wash value was determined. Once background levels were obtained samples could be tested.

Samples were loaded into the sample port using a 1 ml syringe equipped with a blunt-tipped needle. Each assay consisted of multiple steps, all of which were performed automatically by the RAPTOR. First, the coupon was rinsed briefly with PBT wash buffer. Then antigen was allowed to bind to the optical probes during a seven-minute sample incubation period. A subsequent wash with PBT eliminated unbound material. At the same time as the PBT wash, the sample port was also flushed out. Next, the coupon was cleared with air to prevent dilution of the incoming tracer reagent. Then Cy5 -antibody reagent was loaded into the coupon and incubated for 90 s to interrogate the amount of antigen bound to the probes. Following this incubation, the tracer reagent was returned to its reservoir for reuse and a final PBT wash was performed. The entire standard assay cycle, including all wash steps, was completed in ten minutes.

The rate of fluorescence increase during incubation with the Cy5-antibody was calculated ("assay rate"), and a final reading was taken to determine the increase in fluorescence due to Cy5-antibody bound during the entire assay cycle ("wash delta"). While one parameter is sufficient for strongly positive samples, use of both factors greatly reduces the number of false positives obtained.[6,7] All assay data were stored by the RAPTOR and subsequently downloaded through an serial port for

quantitative analysis. Samples were considered positive if the values were greater than the mean of the background values plus three standard deviations.

3. RESULTS

Initial experiments examined the best arrangement of the two monoclonal antibodies directed against *Giardia*. Each was tested as the capture antibody and as the fluorescent reagent. The only arrangement that proved useful was using the combination of Mab AG1 as the capture and Mab 7D2 as the Cy5-labeled reagent (Figure 3). Neither worked well alone, as both capture and labeled reagent, nor did using the Mab 7D2 as the capture and Cy5-labeled AG1 Mab function well.

The optimized response of the biosensor to increasing concentrations of *Giardia* spiked into PBT is shown in Figure 3. Concentrations of *Giardia* from 3 x 10^4 cysts/ml to 3 x 10^5 cysts/ml were tested. Both the assay rates and wash delta values indicated that concentrations of *Giardia* of 3 x 10^4 cysts/ml consistently gave fluorescent values above the mean background plus three standard deviations, a conservative threshold value for detection. There was an increase in signal at 3 x 10^3 cysts/ml, but was not large enough to be reliably detected for all samples. Somewhat unexpectedly both the assay rates and wash deltas leveled off at the higher concentrations; this could be due to saturation of the capture antibody. It is more likely that we did not test antigen at sufficiently high concentrations to drive the binding reaction to completion, since the maximum signal level obtained is low relative to most assays.

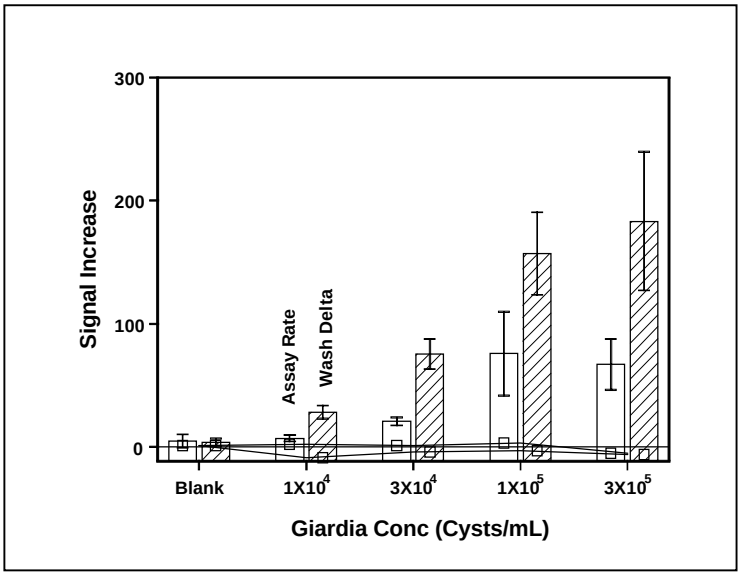

Figure 3. Dose-response curve for *Giardia*. Both the assay rate and wash delta are shown for increasing concentrations of *Giardia.* The mean and SEM is shown for 3 separate probes. The lines show the lack of response observed using Mab 7D2 as the capture antibody instead of Mab AG1.

In order to test for *Giardia* in real samples, cysts were introduced into water from various sources and into dog feces. The water samples were buffered by the addition of 1/10 volume of 10x PBT. The dog feces were suspended using a 2:1 w/w ratio of PBT to feces. After spiking with *Giardia*, the sample was incubated for two hours at 4°C. Then 1 ml of the solution was centrifuged to remove solids, adjusted to back to 1 ml with PBT, diluted with a 1/10 volume of 10x PBT, and then tested. All samples were filtered through a 70 - micron syringe filter during injection into the RAPTOR. Figure 4 shows that neither pond water nor river water caused a decrease in the signal compared to wash buffer for samples spiked with 1 x 10^5 *Giardia* cysts/ml. The percentage of control signal from samples of sea water and dog feces spiked at 3 x 10^5 *Giardia* cysts/ml, the limit of detection for these interferents, is also shown. While sea water and dog feces caused substantial inhibition, detection was still successful.

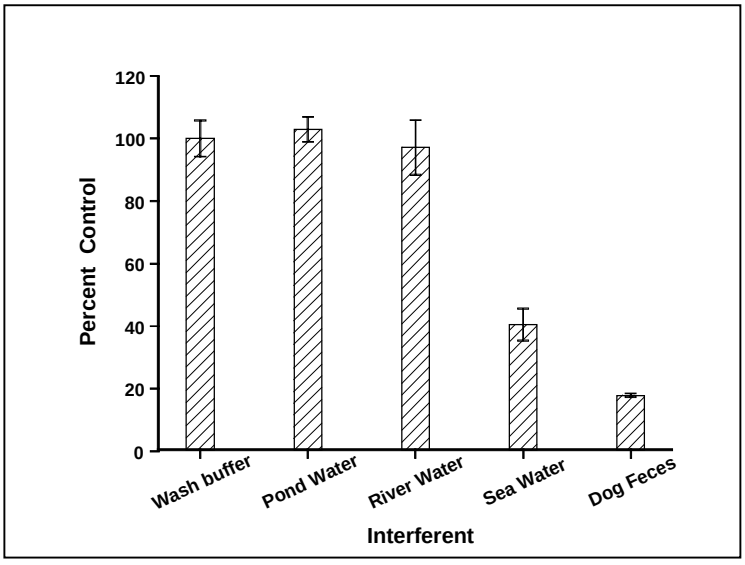

Figure 4. Detection of *Giardia* in complex matrices was compared to control (wash buffer, PBT).

For field testing, a RAPTOR pathogen-specific assay set was developed and tested. The assay for *Giardia* was incorporated into a pathogen coupon, which also contained probes specific for *B. anthracis* and *F. tularensis*. The pathogen-specific assay coupon also included a positive control probe coated with an

anti-ovalbumin antibody. To test that these four assays (ovalbumin, *B. anthracis*, *F. tularensis*, and *Giardia*) were compatible, 10 µg/ml of each Cy-5 labeled tracer antibody (40 µg/ml total) was mixed and placed in the reagent bag. This fluorescent reagent was then used to measure consecutive dose response curves for each of the target analytes. As a control, to ensure that all components of the system were functional, the ovalbumin probe was challenged once at the beginning of each trial with a low concentration of ovalbumin and again at the end of each trial as a final test to gauge coupon and reagent activity. Each of the other analytes was tested sequentially (Figure 5). Once a probe had been challenged with an analyte, it continued to increase for several cycles, even in the absence of additional analyte, until captured antigen was saturated with tracer antibody. Since coupons would normally be used only until the first positive is achieved, the lack of saturation does not present a problem for field-testing.

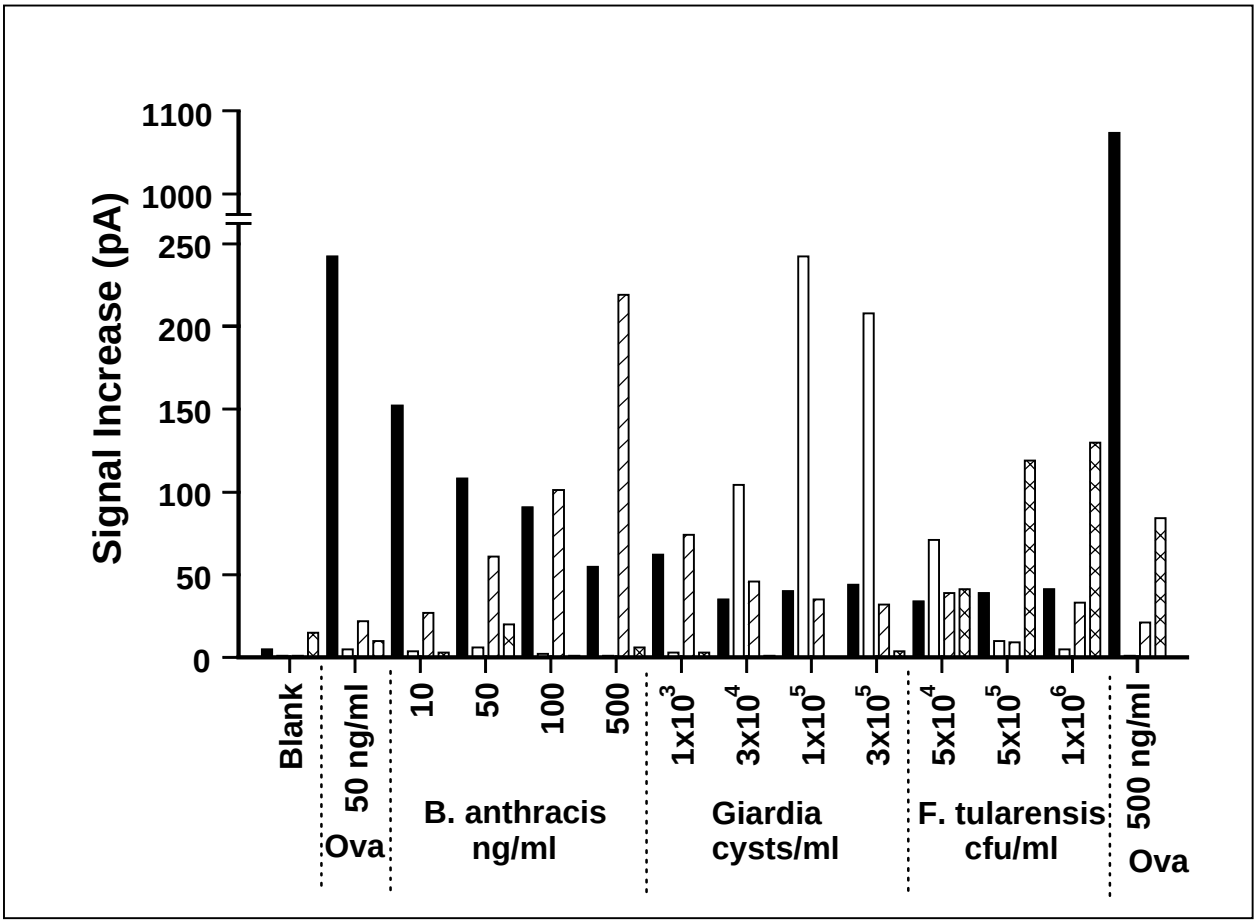

Figure 5. Dose response curve for each analyte on the pathogen coupon assay set. The signal increase obtained after being challenged with increasing amounts of each analyte is shown for each probe. Ovalbumin: solid, *B. anthracis*: hatched, *Giardia*: open, *F. tularensis*: cross-hatched.

4. DISCUSSION

The RAPTOR fiber optic biosensor is well suited for testing food and water supplies for pathogens and toxins and can be used as an in-line monitor or to analyze discrete samples. Only minimal sample preparation (e.g. dilution or coarse filtration) is required. Disposable coupons can be made with optimum assay combinations, allowing multiple samples to be analyzed for multiple pathogens without the necessity of changing probes or reagents. Assay procedures and data analyses are both fully automated, but can be modified by the user to fit the user's specifications. Assay development is straightforward if antibodies are available and antibody-coated probes and fluorescent reagents can be stored for over 1 year. The RAPTOR is portable (12 lb) and can be operated on batteries for use in the field. The assays are fast (3-10 min assays) and sensitive (1-10 ng/ml, 50-5000 cfu/ml, depending on the antibody).

This work demonstrated the RAPTOR's effectiveness for identification of pathogens in environmental samples. The limit of detection was raised in matrices such as sea water and dog feces. In the dog feces this inhibition may in part have been due to the lowered antigen availability, since antigen adsorption to solid matter in the sample matrix could have been considerable. The development of multianalyte coupons, which include a positive control, permit the long term deployment of the RAPTOR, since coupon and reagent activity can be verified. At the conclusion of this work, two RAPTORs with two different assay sets were put into extended field testing to evaluate the long term system and assay endurance.

ACKNOWLEDGEMENTS

We would like to thank Dr. Ted Nash (NIH, Bethesda, MD) for his generation donation of reagents. The views expressed here are those of the authors and do not represent the opinions of the U.S. Navy, the U.S Department of Defens e, or the U.S. Government.

REFERENCES

1. TB Hirschfeld, and MJ Block, "Fluorescent immunoassay employing optical fiber in capillary tube," US Patent 4,447,546. 1984.
2. J.D Andrade and R Vanwagenen, " Process for conducting fluorescence immunoassays without ad ded labels and employing attenuated internal reflection," US Patent 4,368,047, 1983.
3. BJ Tromberg, MJ Sepaniak, MJT Vo-Dinh, and GD Griffin, "Fiber-optic chemical sensors for competitive binding fluoroimmunoassay," *Anal.Chem.* **59**, pp. 1225-1230, 1987.
4. BI Bluestein, M Craig, R Slovacek, L Stundtner, C Urciuoli, I Walczak, and A Luderer, "Evanescent wave immunosensors for clinical diagnostics. In: Biosensors with Fiberoptics," (DL Wise and LB Wingard, Jr., eds.) pp. 181-223 Humana Press, NJ, 1991.
5. FS Ligler, JP Golden, LC Shriver-Lake, RA Ogert, D Wijesuria and GP Anderson, "Fiber-optic biosensor for the detection of hazardous materials," *Immunomethods,* **3**, pp. 122-127, 1993.
6. GP Anderson, KD King, KL Gaffney, and LH Johnson, "Multianalyte detection using the fiber optic biosensor," *Biosensors & Bioelectronics*, **14**, pp. 771-777, 2000.
7. KD King, JM Vanniere, JL LeBlanc, KE Bullock, and G.P. Anderson , "Automated fiber optic biosensor for multiplexed immunoassays," *Environ. Sci. & Tech.* **34**, pp. 2845-2850, 2000.
8. GP Anderson, JP Golden, and FS Ligler, "An evanescent wave biosensor – fluorescent signal acquisition from step-etched fiber optic probes" *IEEE Transactions on Biomedical Engineering,* **41**, pp 578-584, 1994.
9. IB Bakaltcheva, FS Ligler, CH Patterson, and LC Shriver-Lake, "Multi-analyte explosive detection using a fiber optic biosensor," *Anal. Chim. Acta*, **399**, pp. 13-30, 1999.
10. SK Van Bergen, IB Bakaltcheva, JS Lundgren, and LC Shriver-Lake, "On-site detection of Explosives in groundwater with a fiber optic biosensor," *Environ. Sci. & Tech.* **34**, pp. 704-708. 2000.
11. LC Shriver-Lake, C.H. Patterson, and S.K. van Bergen, "New Horizons: Explosive detection in soils with a fiber optic biosensor," *Field Anal. Chem. Tech.* In press. 2000.
12. LA Tempelman, KD King, GP Anderson, and FS Ligler, "Quantitating staphylococcal enterotoxin B in diverse media using a portable fiber optic biosensor," *Anal. Biochem.* **223**, pp. 50-57, 1996.
13. DR DeMarco, EW Saaski, DA McCrae, and DM Lim, "Rapid detection of Escherichia coli O157:H7 in ground beef using a fiber-optic biosensor," *J. of Food Protection*, **62**, pp. 711-716. 1999.
14. KD King, GP Anderson, KE Bullock, MJ Regina, EW Saaski, and FS Ligler, "Detecting staphylococcal enterotoxin B using an automated fiber optic biosensor," *Biosensors & Bioelectronics*, **14**, pp. 163-170. 1999.
15. LK Cao, GP Anderson, FS Ligler, and J Ezzell, "Detection of *Yersinia pestis* F1 antigen by a fiber optic biosensor," *J. Clin. Micro.* **33**, pp. 336-341, 1995.
16. FS Ligler, GP Anderson, PT Davidson, RJ Foch, JT Ives, KD King, G Page, DA Stenger, and JP Whelan, "Remote sensing using an airborne biosensor," *Environ. Sci. & Tech.* **32**, pp. 2461-2466, 1998.
17. GP Anderson, KD King, LK Cao, M Jacoby, FS Ligler, and J Ezzell, "Quantifying serum anti -plague antibody using a fiber optic biosensor," *Clin. Diag. Lab. Imm.* **5**, pp. 609-612, 1998.
18. RD Adam, "The biology of Giardia spp." *Microbiol. Rev.* **55**, pp. 706-732, 1991.
19. JH Boone, JH, TD Wilkins, TE Nash, JE Brandon, EA Macias, RC Jerris, and DM Lyerly, "TechLab and Alexon Giardia enzyme-linked immunosorbent assay kits detect cyst wall protein" *J. Clin. Micro*. **37**: 611-614. 1999.