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Detection of *Yersinia pestis* Fraction 1 Antigen with a Fiber Optic Biosensor

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A fiber optic biosensor was used to detect the fraction 1 (F1) antigen from *Yersinia pestis*, the etiologic agent of plague. The instrument employs an argon ion laser (514 nm) to launch light into a long-clad fiber and measures the fluorescence produced by an immunofluorescent complex formed in the evanescent wave region. This sensing area is a short section (12.5 cm) at the end of the optical fiber from which the cladding has been removed and in which the silica core has been tapered. Capture antibodies, which bind to F1 antigen, were immobilized on the core surface to form the basis of the sandwich fluoroimmunoassay. The ability to detect bound F1 antigen was provided by adding tetramethylrhodamine-labeled anti-plague antibody to form fluorescent complexes. The evanescent wave has a limited penetration depth (<1 λ), which restricts detection of the fluorescent complexes bound to the fiber's surface. The direct correlation between the F1 antigen concentration and the signal provided an effective method for sample quantitation. This method achieved a high level of accuracy for determining F1 antigen concentrations from 50 to 400 ng/ml in phosphate-buffered saline, serum, plasma, and whole blood, with a 5-ng/ml limit of detection. Subsequent blind studies, which included serum samples from patients, yielded results in good agreement with measurements by enzyme-linked immunosorbent assay. A major advantage of the fiber optic biosensor is that results can be generated within minutes while isolating the user from hazardous samples. These factors favor development of this biosensor into a facile and rapid diagnostic device.

Yersinia pestis, the etiologic agent of bubonic plague, has afflicted humans for many centuries. Plague continues to be endemic in many parts of the world, including the U.S. Southwest (9). It occurs most frequently as an infection of wild rodents and can be transmitted to humans by the bites of fleas or by handling infected animals. During infection within a warm-blooded host, the fraction 1 (F1) antigen is expressed. This protein-polysaccharide complex represents a major component of the outer membrane capsule. Because detectable concentrations of F1 are obtained in serum during bubonic and pneumonic plague, this antigen has been used for diagnostic testing (5, 19). Plague's rapid progression and its occurrence in locations usually lacking clinical diagnostic laboratories require improved detection methodologies that can be used in the field.

Most immunoassay kits currently used in the field are single-use tests which merely provide a positive or a negative response (7). Biosensors which perform rapid identification of biological agents are being developed to meet the demand for quantitative immunoassays (22). The fiber optic biosensor, described here, fulfills this objective by combining the sensitivity provided by fluoroimmunoassays (8) with advances in fiber optic technology to create a unique biodetection device (2, 6, 11, 16).

The fluoroimmunoassays performed with this fiber optic biosensor are distinctive in that the assay occurs in the evanescent wave region of an optical fiber (Fig. 1). Light propagates in optical fibers by being total internally reflected because of the difference in the indices of refraction between the core and the cladding material (17). However, the electromagnetic field does not fall instantaneously to zero at the core-cladding interface. The total internally reflected light which extends just beyond the core surface into the surrounding cladding or media, typically less than a wavelength, is called the evanescent wave. For the biosensor described here, the sensing region was formed in the area which extends along the final 12.5 cm of the optical fiber from which the cladding has been removed and where direct contact between the fiber core and analyte occurs. Because of the limited penetration depth of the evanescent wave, detection is a surface-sensitive measurement. More importantly, the surface area where binding occurs is increased by 3 orders of magnitude over distal end measurements. However, the difficulties that exist both in providing excitation light and in obtaining a fluorescent signal have limited use of the evanescent wave sensing with clad optical fibers (13, 18). This limitation is caused by the change in the index of refraction between the fiber's cladding and the aqueous sample. Our biosensor overcomes these limitations by use of an optical probe with two distinct tapered sections (2). The first, a short (1-cm) section, tapers rapidly to equalize the mode-carrying capacity of the clad and sensing fiber portions. The remainder of the sensing area (11 cm) tapers gently to permit regeneration of the evanescent wave as well as improve the signal collection efficiency.

The fiber optic biosensor described here has previously been used to detect botulinum toxin, ricin, and pseudexin at low (nanogram-per-milliliter) levels (14, 15). The detection of the *Y. pestis* F1 antigen, described herein, represents a significant advancement in the demonstrated capabilities of the fiber optic biosensor. A quantitative fluoroimmunoassay was developed, and blind tests were conducted with both spiked and patient samples. The excellent agreement with results of enzyme-

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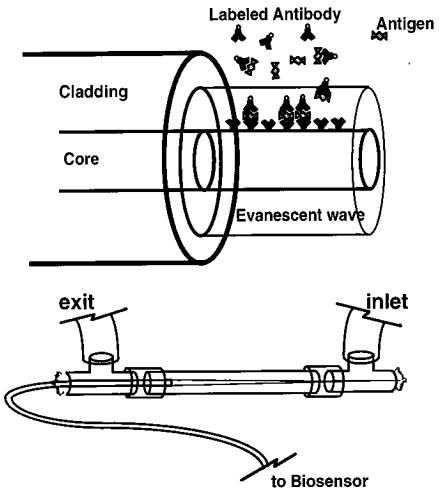

FIG. 1. Schematic representations of the sandwich fluoroimmunoassay and the fiber probe assay chamber.

linked immunosorbent assay (ELISA) is the first proof of this system's worth as a diagnostic tool.

MATERIALS AND METHODS

Reagents. Analytical-grade reagents and distilled water were used to prepare all solutions. The F1 antigen (3), sera from humans with plague, protein G-purified-rabbit anti-plague immunoglobulin G (IgG), and ascitic fluid of the monoclonal antibody YPF1-6H3-1-1-IgG, henceforth referred to as 6H3-IgG, were provided by the U.S. Army Medical Research Institute of Infectious Disease (USAMRIID). The 6H3-IgG monoclonal antibody was developed at US AMRIID by injecting F1 antigen (lot 4; produced by J. E. Williams, Walter Reed Army Institute of Research, Washington, D.C.) into BALB/c mice. This F1 antigen, which was also used in the studies, was coated onto ELISA plates for screening the hybridomas. The monoclonal antibody 3G8-IgA (20, 21) was obtained as a purified protein and was the kind gift of James Burans, Naval Medical Research Institute, Bethesda, Md. The patient sera were obtained by J. E. Williams during a plague outbreak in Namibia from 1976 to 1978. The F1 antigen concentration in the two patient serum samples used in the study was estimated by using twofold dilutions in a capture ELISA (19). The 6H3-IgG was affinity purified by using a 3-ml Avid-Al column, and the bound antibody was eluted with 0.1 M sodium acetate (pH 3.0)–20% glycerol. The purified antibody was then dialyzed against phosphate-buffered saline (PBS)–0.01% sodium azide. The human sera used in making negative controls and the spiked samples that made up the rest of the 15 samples from USAMRIID were tested by ELISA for antibody to or nonspecific reactions with F1 antigen. The sera that were completely negative were pooled.

To prepare the fluorescently labeled antibodies, 1 mg of each antibody (1 mg/ml) was dialyzed against 50 mM borate (pH 9.3)–50 mM NaCl and was then dialyzed overnight in the same buffer containing 0.01 mg of tetramethylrhodamine-5-isothiocyanate, isomer-G (TRITC) (Molecular Probes, Eugene, Oreg.) per ml. Free dye was removed by gel filtration on Bio-Gel P10 (Bio-Rad, Hercules, Calif.) equilibrated with PBS–0.1% sodium azide. The dye-to-protein molar ratio for each antibody preparation was determined to be between 1.0 and 1.5 by the method of Amante et al. (1). Bovine serum albumin (BSA; 2 mg/ml) was added to the labeled antibody prior to storage at 4°C.

Fiber Preparation. The plastic-clad, 200-μm-diameter silica core, optical fiber (Quartz Products, Tuckerton, Del.) was cut into 1-m lengths. A black plastic ferrule connector (Aurora Optics, Blue Bell, Pa.) was attached to the proximal end of each fiber for mounting to the fluorimeter. To form the tapered sensing area, 12.5 cm of cladding was stripped at the distal end to expose the silica core. Residual cladding from the probe area was removed with concentrated hydrofluoric acid (1 min), and the probe area was then washed. The fibers were tapered by computer-controlled immersion into hydrofluoric acid to form tapered fibers (2).

Capture antibodies were immobilized onto the tapered core by the procedure of Bhatia and colleagues (4, 10). Briefly, probes were cleaned with methanol-HCl (1:1) for 30 min, rinsed three times with distilled water, cleaned with concentrated sulfuric acid for 30 min, and rinsed as described above. The probes were then placed in hot distilled water (80 to 100°C) for 20 min. The surface was modified by incubating the probes in 4% thio-terminal silane in toluene (3-mercaptopropyl trimethoxysilane; Fluka, Hauppauge, N.Y.) and then incubating the probes with the heterobifunctional cross-linker 2 mM *N*-succinimidyl 4-maleimidobutyrate (Fluka) in ethanol. Finally, the probes were incubated with the capture antibody at 0.05 mg/ml in PBS. This procedure immobilized the antibody on the fiber surface at approximately 2 ng/mm^2. The fiber probes were placed in storage in PBS–0.1% sodium azide at 4°C.

TABLE 1. Analysis of serum samples[a]

Sample no.	USAMRIID concn (ng/ml)	Biosensor concn (ng/ml)
1	410–820	630
2	410–820	932
3	41–82	74
4	41–82	84
5	3–6	10
6	3–6	12
7	0.3–0.6	12
8	0.3–0.6	0
9	0	0
10	0	0
11	0	0
12	5,000	4,500
13	5,000	7,440
14	500	434
15	500	469

[a] The serum samples prepared at USAMRIID were tested at the Naval Research Laboratory. For the patient samples (samples 1 to 8), the concentration determined by the fiber optic biosensor was compared with that obtained by a capture ELISA. Pooled human serum was used for negative controls (samples 9 to 11). Purified F1 antigen was added to serum for the spiked samples (samples 12 to 15).

Fluorimeter. The laboratory breadboard biosensor (6) uses an Omnichrome (Chino, Calif.) air-cooled argon ion laser, which has a stable, narrow excitation bandwidth at 514.5 nm. This wavelength efficiently excites rhodamine fluorophores but generates very little interfering fluorescence from most clinical samples (8). The laser light is modulated by a chopper to permit phase-sensitive detection.

The laser beam passes through an off-axis parabolic mirror and is launched into the fiber via a biconvex fused-silica lens. The fluorescence generated at the probe returns through the clad fiber and is collimated by the same lens that focused the laser light into the fiber. The fluorescence is reflected by the parabolic mirror and is focused through an emission filter (KV550) onto a photodiode (6).

Immunoassay. The sensing area of the optical probe was held in a 200-μl capillary tube with two T-connectors, and the connectors were sealed with hot glue (Fig. 1). The distal end of the fiber was affixed outside of the capillary tube to prevent excitation of the fluorescence in the bulk solution. The glue's high index of refraction minimized back reflections.

The system's limit of detection of the F1 antigen was determined as follows. The fiber probe was first immersed for 15 min in PBS containing 2 mg of casein per ml, 2 mg of BSA per ml, and 0.1% Triton X-100 to minimize nonspecific absorption. During this time, the fiber probe was continuously exposed to the excitation light in order to bleach the autofluorescent components of the fiber. After a steady baseline was obtained for 2 min, 5 μg of TRITC-labeled 6H3-IgG (T-6H3-IgG) per ml was twice introduced for 5 min to determine the level of nonspecific fluorescence. The fiber was then incubated with successively increasing concentrations of F1 antigen in PBS, whole blood, plasma, or serum (0.5, 5, 10, 50, 100, or 500 ng/ml) for 5 min. For signal generation, T-6H3-IgG was again introduced for 5 min. The fiber was washed with PBS containing 0.1% Triton X-100 for 2 min between incubations of F1 antigen and the T-6H3-IgG. To prevent photobleaching of the fluorescent complexes, the excitation beam was blocked at all times except during signal collection. The protocol described above constituted one test, and a separate fiber was used for each test. Each assay required a 250-μl sample volume, which was transferred into the capillary tube with a syringe. Between use the antibody and antigen solutions were kept on ice.

Sample analysis. The following protocol was used to generate data for the standard curve. Standard F1 antigen solutions (5, 50, 200, or 500 ng/ml) were prepared in PBS. After the determination of the nonspecific fluorescence from the labeled antibody, one of the F1 antigen solutions was introduced for 5 min. After a 2-min wash with PBS–0.1% Triton X-100, the fiber was immersed in T-6H3-IgG (5 μg/ml) for signal generation. To normalize the signal, each fiber tested was immersed in a 500 ng of F1 antigen solution per ml and the T-6H3-IgG solution was reintroduced onto the fiber for signal generation. The fiber was washed between each F1 antigen and T-6H3-IgG incubation with PBS–0.1% Triton X-100 for 2 min. The normalizing F1 antigen and T-6H3-IgG solutions were typically reused for four trials. Three fibers were tested for each F1 antigen concentration. See Fig. 4 for examples of the results obtained with four F1

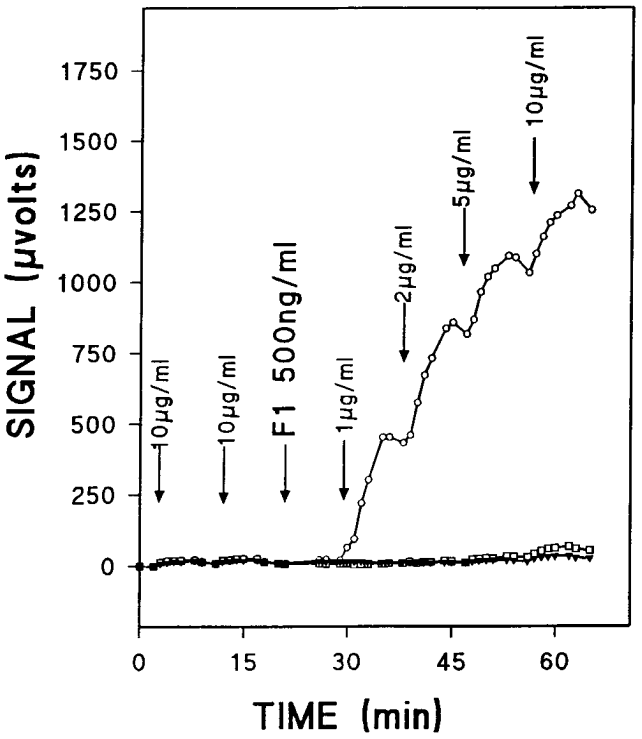

FIG. 2. Comparison of secondary antibodies. Rabbit anti-plague IgG-coated fibers were immersed in F1 antigen (500 ng/ml). Each fiber was then incubated in T-6H3-IgG (○), T-3G8-IgA (□), or T-rabbit anti-plague (▼) at increasing concentrations. Data are representative of at least three separate fibers for each labeled antibody.

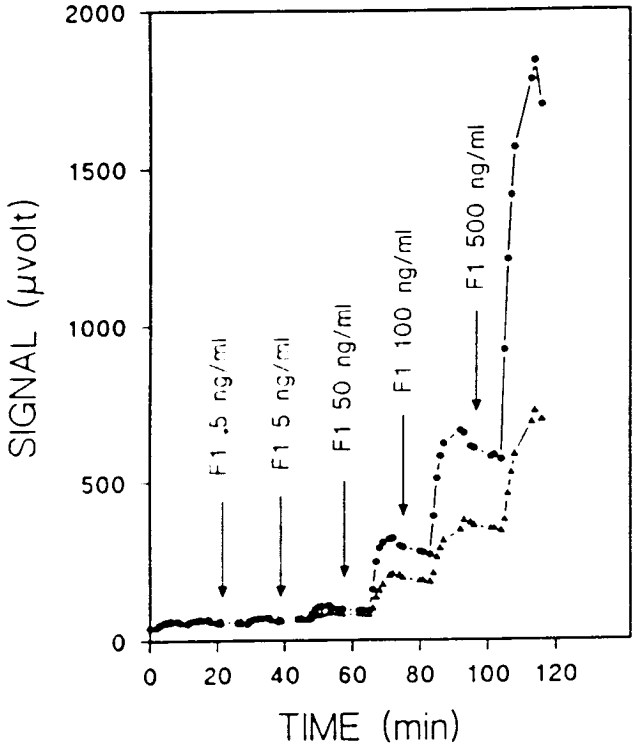

FIG. 3. Selection of secondary antibody concentration. Fibers coated with rabbit anti-plague IgG were incubated with F1 antigen concentrations of 0.5, 5, 50, 100, and 500 ng/ml. T-6H3-IgG was used at concentrations of 2 μg/ml (▲) and 5 μg/ml (●) between the F1 antigen steps. PBS–0.1% Triton X-100 was used to wash the fiber between antigen and antibody additions. This assay format was also used to determine the limit of detection for the F1 antigen.

concentrations. A signal ratio for each fiber was obtained by dividing the fluorescent signal increase owing to the first F1 antigen concentration (5, 50, 200, or 500 ng/ml) by the signal increase owing to the normalizing F1 antigen solution (500 ng/ml). These results made up the standard curve of the F1 antigen concentration-versus-signal ratio (see Fig. 5).

A blind test was performed in which solutions containing F1 antigen spiked into serum, whole blood, or plasma were prepared and coded. Whole blood was diluted 1:1 with PBS, while the serum and plasma solutions contained 10% PBS. The protocol for blind testing followed that used in generating the standard curve, except that the unknown sample replaced the first F1 antigen solution. The signal increase caused by the sample was divided by the signal caused by the normalizing F1 solution (500 ng/ml in PBS). The concentration of F1 antigen in each coded sample was determined by using the calculated signal ratio on the standard curve.

Fifteen additional blind samples were prepared at USAMRIID and were sent to the Naval Research Laboratory to be analyzed by the fiber optic biosensor (Table 1). These samples included different dilutions of two patient serum samples, pooled human serum for negative controls, and spiked samples containing F1 antigen in pooled human serum. All samples were first tested undiluted. Sample concentrations out of the designed test range (exceeding 500 ng/ml) were diluted and retested. The concentration of each sample was determined twice by the signal ratio method described above and was referenced to the standard curve.

RESULTS

Comparison of immobilized antibodies and TRITC-labeled antibodies. Optical fibers were coated with 6H3-IgG, 3G8-IgA, or rabbit anti-plague IgG. By using T-6H3-IgG at a concentration of 2 μg/ml as the secondary antibody, F1 antigen in PBS at concentrations of 5, 50, and 500 ng/ml was tested. The amount of signal increase for each F1 antigen concentration was comparable for the three types of fibers (data not shown).

Each of the three labeled anti-plague antibodies was tested at 1, 2, 5, and 10 μg/ml. The fibers were first coated with rabbit anti-plague IgG and were then incubated with 500 ng of F1 antigen per ml. The amount of signal produced by each labeled antibody was then observed. T-6H3-IgG performed most efficiently as the secondary antibody (Fig. 2). At a 10-μg/ml concentration, T-6H3-IgG generated 1,241 μV of signal, while T-3G8-IgA and T-rabbit anti-plague-IgG generated 13 and 45 μV, respectively. To confirm the failure of T-3G8-IgA and T-rabbit anti-plague IgG to bind to the F1 antigen already introduced to the fiber, T-6H3-IgG (5 μg/ml) was added at the conclusion of each assay. A large signal was observed each time because of the binding of T-6H3-IgG to F1 antigen. Similar assay results were obtained with 6H3-IgG as the capture antibody.

A determination of the optimal secondary antibody concentration was also performed. Fibers coated with rabbit anti-plague antibody were exposed to successively increasing concentrations of F1 antigen (0.5, 5, 50, 100, and 500 ng/ml), and the signal was determined with T-6H3-IgG. Three different concentrations of T-6H3-IgG (2, 5, and 10 μg/ml) were used with each concentration on a separate fiber. At all F1 antigen concentrations, the signal level produced with 5 μg of T-6H3-IgG per ml was higher than that produced with 2 μg of T-6H3-IgG per ml (Fig. 3). When the T-6H3-IgG concentration was further increased to 10 μg/ml, additional signal increase was insignificant (data not shown).

In preliminary studies, the antigen incubation period varied between 1, 5, and 10 min. (data not shown). The signal produced after the 5-min incubation of successively increasing F1 antigen concentrations in PBS (5, 50, 100, and 500 ng/ml) was about three times higher than that produced after the 1-min incubation period. Little signal gain was obtained by increasing

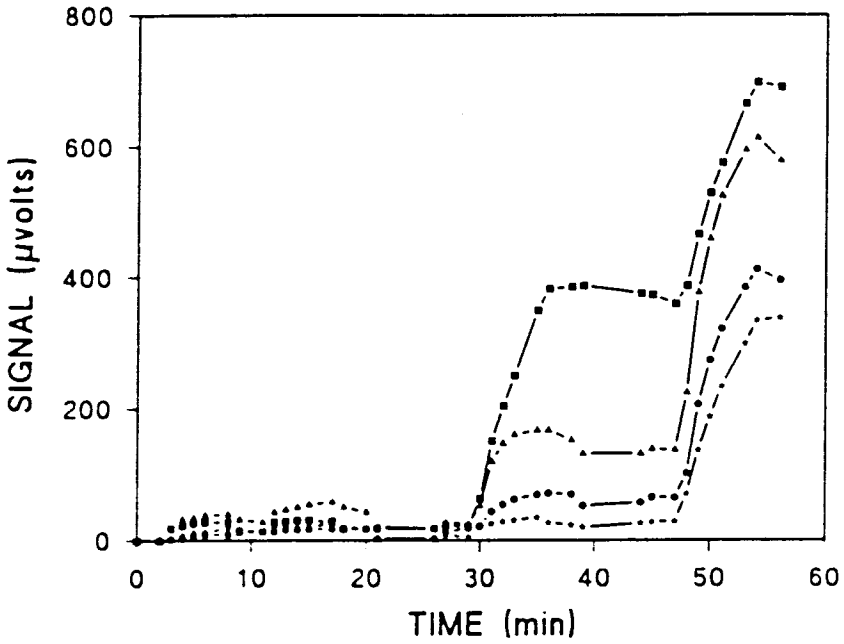

FIG. 4. Assays for standard curve data. Each test for which the results are shown here were generated a point along the standard curve. The fibers were first incubated with 5 ng/ml (★), 50 ng/ml (●), 200 ng/ml (▲), or 500 ng/ml (■); this was followed by exposure to the T-6H3-IgG for signal generation. A second incubation with the normalizing F1 antigen solution (500 ng/ml) followed. Finally, T-6H3-IgG was added to obtain the normalizing signal.

the incubation time to 10 min; thus, 5 min was selected as the standard incubation time.

Limit of detection of the fiber optic biosensor for the F1 antigen. The limit of detection of the system was also investigated over a range of F1 antigen concentrations from 0.5 to 500 ng/ml (Fig. 3). By using fibers coated with rabbit anti-plague IgG and T-6H3-IgG (5 μg/ml) as the labeled antibody, a 5-ng/ml solution of F1 antigen solution produced an average signal of 28 ± 7 μV (standard error of the mean; $n = 13$) over the background. This limit of detection (5 ng/ml) was also observed in fibers with 3G8-IgA or 6H3-IgG as the capture antibody. The range of this assay has been tested at concentrations of F1 antigen as high as 5,000 ng/ml.

Control fibers coated with goat-IgG showed no signal increase upon incubation with F1 antigen (500 ng/ml) and then T-6H3-IgG (5 μg/ml) (data not shown). Nor was a signal increase produced when rabbit anti-plague IgG fibers challenged with an inappropriate antigen, the protective antigen from *Bacillus anthracis* (500 ng/ml). To confirm specificity, a study with sera from patients infected with other gram-negative bacteria would be required. All ensuing experiments used the rabbit anti-plague IgG as the capture antibody immobilized on the fiber.

Standard curve with F1 antigen in PBS. Data for the calibration curve were obtained (Fig. 4). The ratio was calculated by dividing the signal from the initial F1 antigen concentration with the signal produced upon exposure to the normalizing solution of F1 antigen (500 ng/ml). Figure 5 depicts the relationship between concentrations of the first F1 antigen solution and the signal ratios. This relationship was fitted well by the Michaelis-Menton equation, $y = (A \cdot x)/(B + x)$ (Equation 1), where A and B are constants. This nonlinear curve has an r^2 value of 0.96. Testing of F1 antigen concentrations in serum–10% PBS instead of 100% PBS produced a very similar curve.

Analysis of F1 antigen in serum, whole blood, or plasma. Assays were performed on samples in which the F1 antigen was spiked into human serum, whole blood, or plasma. The signal obtained in each medium after 5 min was comparable to that observed for F1 antigen in PBS, for which the limit of detection was 5 ng/ml. This detection limit was maintained in serum and plasma. In whole blood, the 5-ng/ml detection limit was retained with a 10-min incubation period.

The concentrations of the blind samples in which F1 antigen was spiked into serum, whole blood, or plasma were derived by the signal ratio method on the standard curve. The determined concentrations closely matched the spiked concentrations (Fig. 6). The average percent error was 9.4 ± 8 (standard deviation) for the 10 trials. Linear regression of the plot of determined versus spiked concentrations results in a slope of 0.99 with an r^2 value of 0.99.

The concentration determination by the Naval Research Laboratory fiber optic biosensor of patient samples prepared at USAMRIID yielded results highly consistent with those of the capture ELISA (Table 1). The results for the spiked samples were all in good agreement with those of the capture ELISA except for one, which was overestimated because it was not diluted sufficiently to yield a signal ratio on the rising portion of the curve (5 to 400 ng/ml). All negative samples were correctly identified.

DISCUSSION

The detection of *Y. pestis* F1 antigen by the fiber optic biosensor required the formation of fluorescent complexes on the fiber's surface. These complexes were formed when immobilized antibody bound F1 antigen, which was subsequently bound by a fluorescently labeled antibody. These reagents can be added sequentially, as was done for the present study, or simultaneously, as in a one-step sandwich immunoassay. While a one-step assay required less operating time, the two-step sandwich immunoassay was preferred because it permitted re-

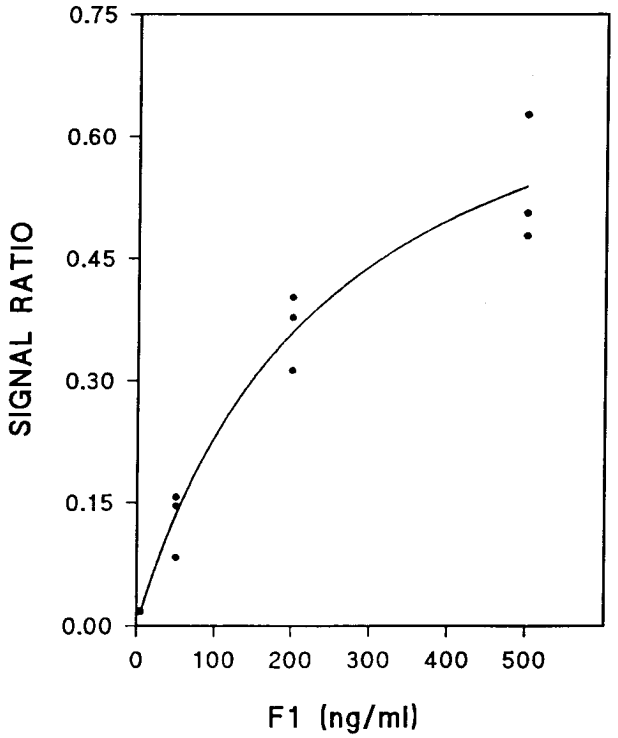

FIG. 5. Standard curve. The signal increase which resulted from the first F1 antigen solution (5, 50, 200, or 500 ng/ml) in PBS was divided by the subsequent increase owing to exposure to 500 ng of F1 antigen per ml. This ratio was plotted against the first F1 antigen concentration. Similar results were obtained in three separate experiments. The assay procedure is described in Materials and Methods.

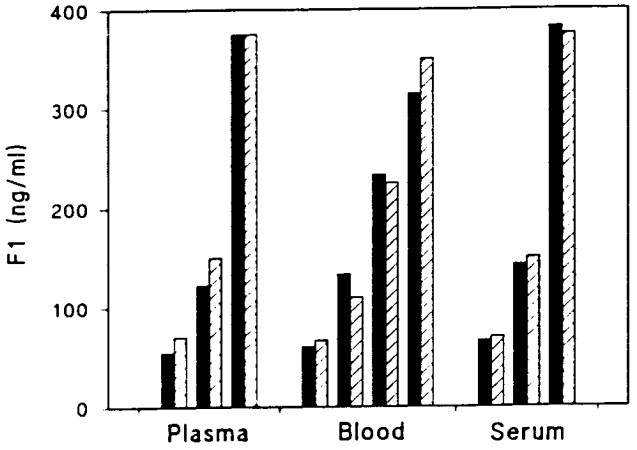

FIG. 6. Determination of F1 antigen concentration in serum, whole blood, and plasma. Three blind concentrations of F1 antigen in serum and plasma and four concentrations of F1 antigen in whole blood were prepared. The signal ratio was calculated for each blind sample, and the corresponding concentration was derived from the standard curve obtained with PBS. The experimental values (■) were compared with the actual F1 antigen concentrations (▨).

use of the normalizing F1 antigen and the T-6H3-IgG solutions. Three different antibodies directed toward the F1 antigen, two murine monoclonal antibodies (6H3-IgG and 3G8-IgA) and a polyclonal rabbit anti-plague IgG, were available to act either as the capture antibody or as the secondary labeled antibody. A comparison of each as the capture antibody yielded no significant difference. Fibers coated with each of these antibodies retained their activities toward F1 antigen during the course of each experiment (up to 4 weeks). Previous experiments have established that antibodies immobilized onto the optical fiber surface retained the majority of their activity in storage for periods of up to 19 months (12).

Each antibody was also tested as the labeled secondary antibody. Of the three, only T-6H3-IgG retained its function (Fig. 2), even though each was labeled at a suitable dye-to-protein molar ratio of 1 to 1.5. The TRITC has a negative impact on the functionalities of the other two antibodies. This was further implied in a subsequent experiment, in which the dye Cy5 was used to label rabbit anti-plague-IgG. The cyanine dye-labeled polyclonal antibody was found to be as effective as T-6H3-IgG. After selecting the T-6H3-IgG as the secondary antibody, its optimal concentration was determined. A concentration that was too low decreased the sensitivity of the assay, while a concentration that was too high resulted in unwanted, nonspecific fluorescence. The optimal concentration for the secondary antibody was 5 μg/ml. This concentration was consistent with that which has been found to be ideal for other assays developed for the biosensor described here (14, 15). These assay conditions yielded a limit of detection of 5 ng/ml. This level was well below that required in clinical testing, in which the F1 antigen concentration in positive serum samples often reaches the microgram-per-milliliter range.

The development of the *Y. pestis* F1 antigen assay demonstrated the potential use of the biosensor for the rapid diagnosis of infection. High levels of consistency between the actual and experimental concentrations determined in spiked samples of whole blood, serum, or plasma indicated that F1 antigen can be directly analyzed in these fluids. Furthermore, these results demonstrated that the use of the normalizing method in determining the F1 antigen concentration in these body fluids is valid. While the fluorescent signal increase correlated with the increasing F1 antigen concentrations, the signal ratio was used to minimize the signal variation produced by individual probes. Signal variation is thought to originate from multiple sources, such as changes in the laser launch angle or differences in points of contact between the probe and the capillary tube. While future biosensor designs aim to minimize fiber variability, the use of this normalizing method provided highly accurate results in the present investigation. In addition, a standard curve could be developed to cover any sample range to meet diagnostic requirements. In these studies, we chose 500 ng of F1 antigen per ml for the concentration of the normalizing solution to maintain accuracy for samples with low antigen concentrations. The fact that concentrations of F1 antigen in blood, serum, or plasma can be accurately derived from the standard curve generated by using F1 antigen in PBS indicates that this method minimizes matrix effects.

Assay of the F1 antigen concentration in 15 blind samples provided by USAMRIID demonstrated that for the patient samples the fiber optic biosensor produced results comparable to those produced by the capture ELISA (Table 1). For the spiked samples, the fiber optic biosensor data correlated well with the actual concentrations, and no false-positive results were reported. However, this success required multiple reagent additions, which lengthened the assay time to nearly an hour. If one were not interested in samples containing concentrations of less than 50 ng/ml and only required approximate concentrations, the assay time could be held to under 15 min. Similarly, if one were screening mostly negative samples, each one could be analyzed fairly quickly with no need to replace the optical probe. In practical application, this would decrease

both the operating time and the expense by separating the negative samples from the positive samples which require further analysis. As the next generation of lightweight, multichannel fiber optic instruments becomes available, this methodology for antigen quantitation will be applicable in a multitude of analytical settings from field testing to bedside monitoring.

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