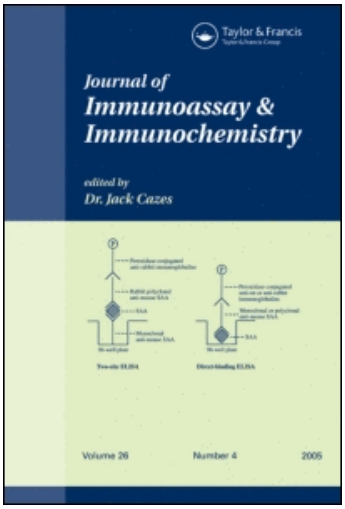

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MEASUREMENT OF HUMAN ANTIBODY ACTIVITY AGAINST ESCHERICHIA COLI AND PSEUDOMONAS AERUGINOSA USING FORMALIN TREATED WHOLE ORGANISMS IN AN ELISA TECHNIQUE

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ABSTRACT

Using an ELISA technique, specific IgG and specific IgM antibodies to several strains of Escherichia coli and Pseudomonas aeruginosa were measured in 100 normal adults. The distribution of antibody activity to E. coli was narrow, with mean values less than 0.50 OD units. The one exception was in IgG activity to E. coli O·. Mean values for activity against P. aeruginosa ranged from 0.35 to 0.79 OD units. Significant rank order correlations were found for IgM activity among all E. coli and P. aeruginosa strains. The correlations were less consistent for the IgG activity. This baseline data will be used to monitor antibody activity to these common microbes along with several other parameters in a group of ill surgical patients.
(KEY WORDS: ELISA, Escherichia coli, Pseudomonas aeruginosa, antibody)

INTRODUCTION

The enzyme-linked immunosorbent assay (ELISA) has recently gained acceptance in the clinical setting as a sensitive method for the determination of antibodies to a wide variety of antigens. Its

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advantages include relative simplicity, high sensitivity, and the ability to detect antibodies of specific immunoglobulin classes. We describe an ELISA technique to measure IgG specific and IgM specific antibodies to several strains of two common gram-negative organisms, Escherichia coli and Pseudomonas aeruginosa. Formalin-killed whole bacteria were used as antigen and a method of standardization was employed that permitted comparison of results from assay to assay. Information from these standard assays was used to define expected ranges of these antibodies in a population of normal individuals. In subsequent studies to be conducted in surgical and burn trauma patients, these ranges will be used as guidelines to anticipate or monitor infections from these organisms.

MATERIALS AND METHODS

Antigens

Four strains of each organism, E. coli and P. aeruginosa, were prepared for the ELISA. Three of the E. coli strains were selected according to biochemical characteristics out of several clinical isolates. Their serotyping, done by Dr. R.A. Wilson of the E. coli Reference Center, Pennsylvania State University, were as follows: E. coli 076;K·;H1, E. coli O⁻;K·:NM (did not react with standard O types) and E. coli O·;K·:NM (weak multiple reaction of anti-sera). The fourth strain was American Type Culture Collection Strain number 25922, hereafter designated E-ATCC. The Pseudomonas organisms were typed for their "O" antigens as recommended by the serotyping

committee of the Japan Pseudomonas Aeruginosa Society (1). The typing was kindly performed by Dr. I. Allen Holder of the Shriners' Burn Institute of Cincinnati, Ohio. The Pseudomonas organisms were M, E and EG, according to O serotype. The fourth was ATCC 27853, hereafter designated P-ATCC. Each organism was cultured in trypticase soy broth, harvested by centrifugation and washed 3 times in phosphate buffered saline (0.05M, pH 7.2) containing 0.001M EDTA (PBS-EDTA). The organisms were killed by resuspension in 0.5% buffered formalin saline for 24 hr at room temperature. Each organism was washed 3 times with PBS-EDTA and stored at 4°C in PBS-EDTA.

Sera

One hundred samples of sera were obtained from the Louisville Blood Center of the American Red Cross. Serum to be used as a reference was obtained from a healthy laboratory worker. All sera were stored at -70°C until testing.

Conjugates

Alkaline phosphatase-labeled goat anti-human IgG (gamma chain specific) and anti-human IgM (mu chain specific) were purchased from Sigma Chemical Company (St. Louis, MO).

Buffers

Antigens were diluted in carbonate buffer (0.05M, pH 9.6) to enhance absorption to the polystyrene. Phosphate buffered saline (0.05M, pH 7.2) with 0.5% bovine serum albumin (PBS-BSA) was used as a blocking buffer. PBS with 0.05% Tween 20 and 0.02% sodium

azide, designated PTA, was used as a plate washer. PTA with 0.5% BSA (PTA-BSA) was used for diluting. Substrate was diluted in carbonate buffer with 0.001M $MgCl_2$.

ELISA Procedure

The ELISA was modified from the method of Engvall and Perlmann (2). The assay was performed in flat-bottomed 96-well polystyrene microtest plates (Flow Laboratories, Inc., Hamden, CT). Each plate was preincubated (37°C) in a humidified chamber for 30 min (to achieve more uniformity in electrostatic activity of the polystyrene). Two hundred microliters (μL) of a suspension of bacteria (10^8 organisms/well) in carbonate buffer were added to each well and incubated for 3 hr at 37°C. The contents of the plates were shaken out and the wells rinsed with PBS. Non-specific absorption of serum proteins was minimized by incubating the plates for 90 min at 37°C with 200 μL aliquots of PBS-BSA. The plates were then washed 3 times with PTA on a microplate washer (Biomedical Research and Development Laboratory, Gaithersburg, MD). Two hundred μL aliquots of diluted human serum (1:200 in PTA-BSA) were added and the plates were incubated for 2 hr at room temperature. The alkaline phosphatase conjugates (each 1:1000 in PTA-BSA) were added (200 μL aliquots) to the washed plates and incubated overnight (15-18 hr) at room temperature. The plates were washed in the plate washer and 200 μL nitrophenol phosphate substrate (Sigma) 1 mg/mL in carbonate buffer with 0.001M $MgCl_2$ was added to each well. The plates were incubated for 90 min at 27°C. The enzyme reaction was stopped by the addition of 50 μL of 1N NaOH to each well. Optical density

(OD) was measured photometrically at 410 nm with a rapid microplate reader (Minireader, Dynatech Laboratories, Inc., Alexandria, VA). Controls for each plate included wells with antigen but no serum and wells with serum but no antigen. Each serum sample was tested in triplicate and the results were averaged.

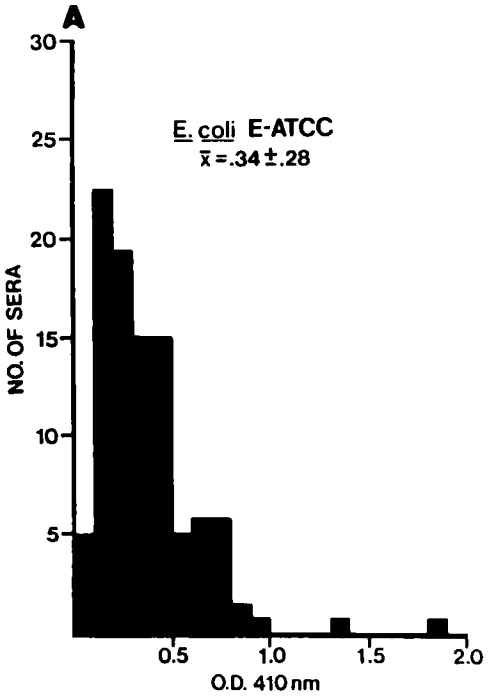
Standardization

A standardization procedure patterned after Manschiem and Kasper (3) was devised to compensate for inherent variation within the ELISA. The serum used as the reference was found to be within normal limits for total immunoglobulins (IgG-1442 mg% and IgM-385 mg%) as measured by radial immunodiffusion (Kallested Laboratories, Chaska, MN). The reference serum (1:200) was included on each plate. The OD that the reference serum yielded in the first particular assay performed (day 1) was designated as a standard and was used to correct data from any subsequent corresponding ELISA (day X) according to the formula:

$$\text{Corrected OD test serum} = \frac{\text{Standard OD day 1}}{\text{Standard OD day X}} \times \text{OD test serum day X}$$

Statistics

The rank order of IgM and IgG antibody activity of the 100 sera was determined for each bacterial strain. Various correlations among these rank orders were assessed using the Pearson product-moment correlation coefficient (r). A stringent criterion for significance was set at 0.001 because of the large number of correlation coefficients that were calculated. At this probability level, a correlation coefficient of .321 or greater is significant.



FIGURES 1 A-D IgM activity of 100 serum samples against four strains of E. coli.

RESULTS

One hundred samples of sera (1:200 in PTA-BSA) were tested for specific IgM and IgG activity against four strains of E. coli and P. aeruginosa. The E. coli data are presented in Figures 1 (IgM) and 2 (IgG) and the P. aeruginosa data are presented in Figures 3 (IgM) and 4 (IgG). These data represent an approximation of the distribution of antibody activities of these strains in a normal population.

The distribution of E. coli antibody activity was narrow, with mean values less than 0.50 OD units (Figs. 1 and 2). The exception

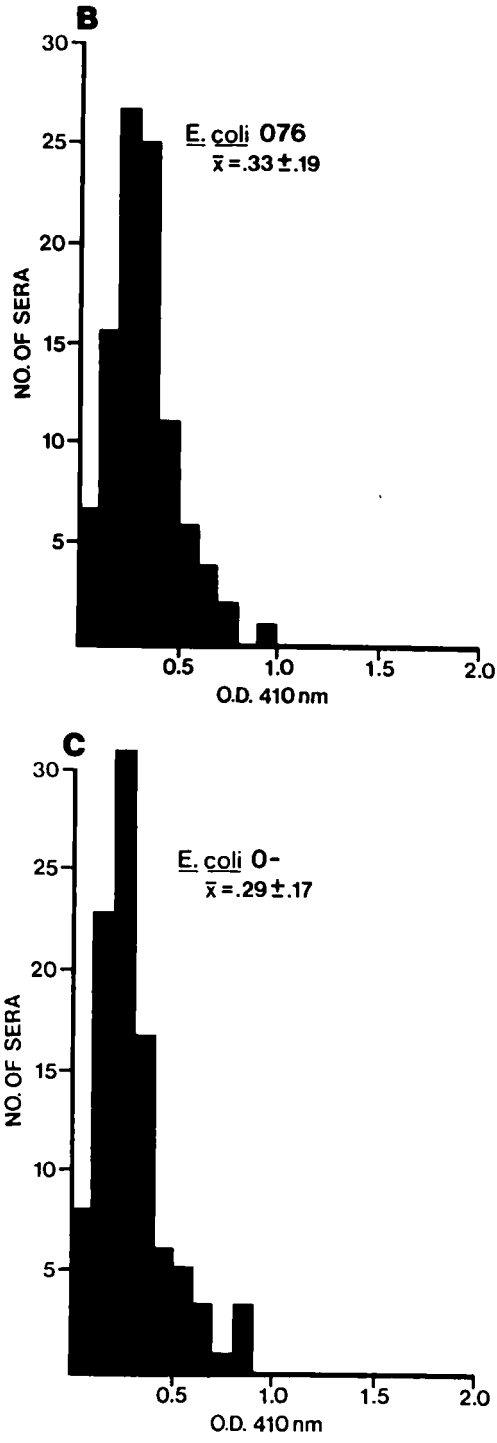


Figure 1 (continued)

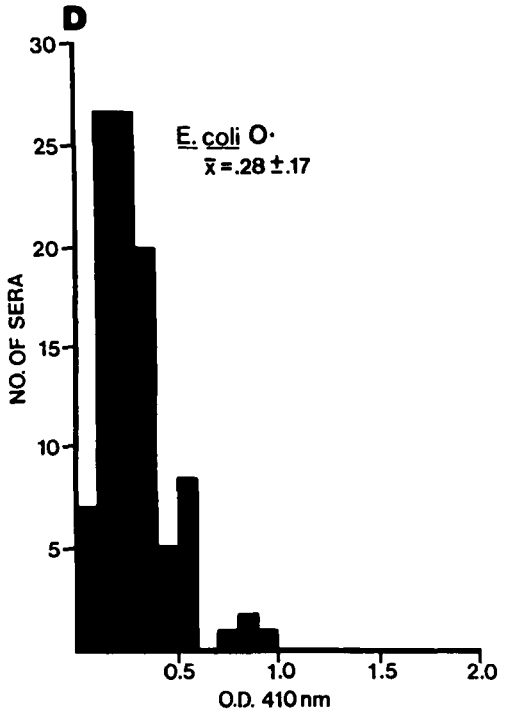
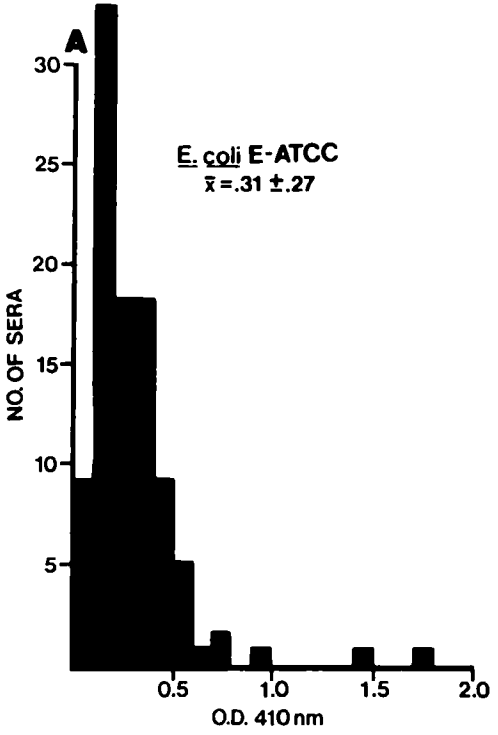


Figure 1 (continued)

to this tendency was seen in the IgG activity against E. coli O· (Fig. 2D), with a mean and standard deviation of 0.72 ± 0.52 OD units, and greater than one-half of the values above 0.5 OD units. The distribution of antibody activity against P. aeruginosa was similar for both IgM and IgG with the mean values ranging from 0.35 to 0.79 OD units (Figs. 3 and 4).

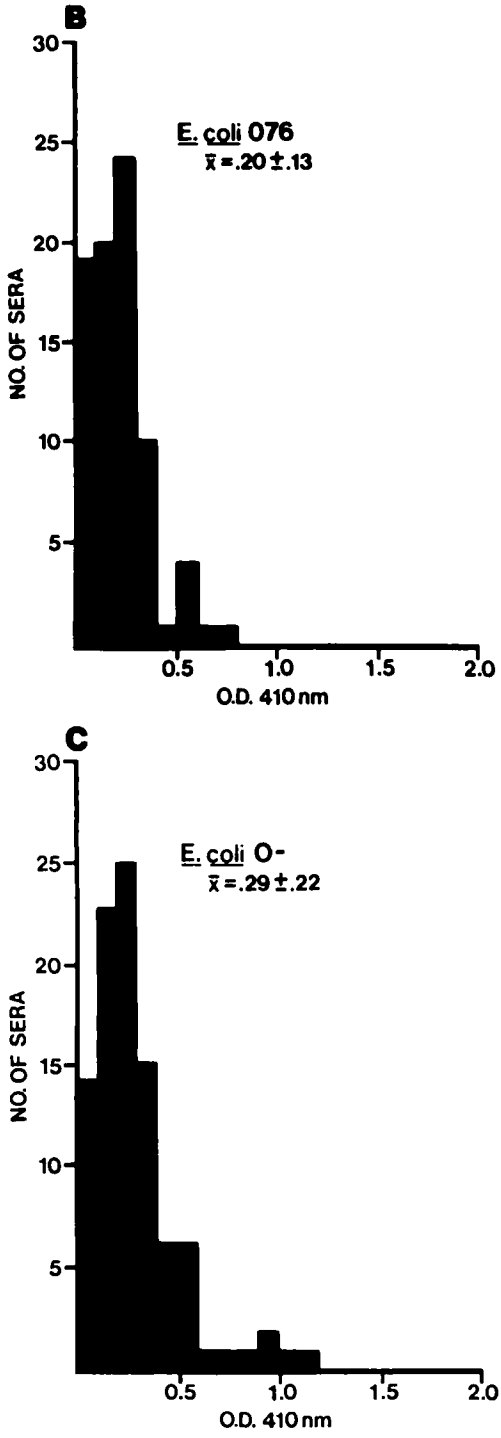
The data were analyzed to seek correlations with each individual's rank order of serum reactivity between strains of the same organism. Significant correlations were found for the IgM activity



FIGURES 2 A-D IgG activity of 100 serum samples against four strains of E. coli.

among all E. coli and P. aeruginosa strains (Table 1). The correlations among the IgG activities (Table 2) were less consistent, although a moderate degree of correlation was seen for the Pseudomonas strains. The high correlation (r = .902) between the rank order of serum IgG activities against Pseudomonas strain E and strain EG (Table 2) is likely due to their shared O serotype.

The rank order correlations for IgM activity when compared between E. coli and P. aeruginosa were also significant for all strains (Table 3). However, there was no correlation between sera



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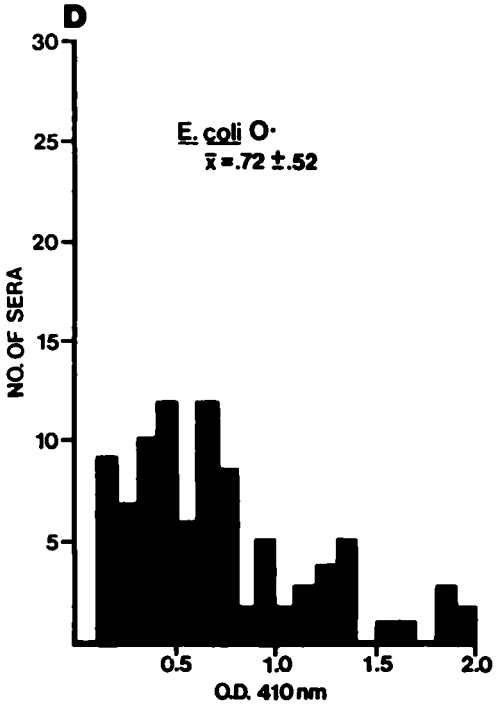
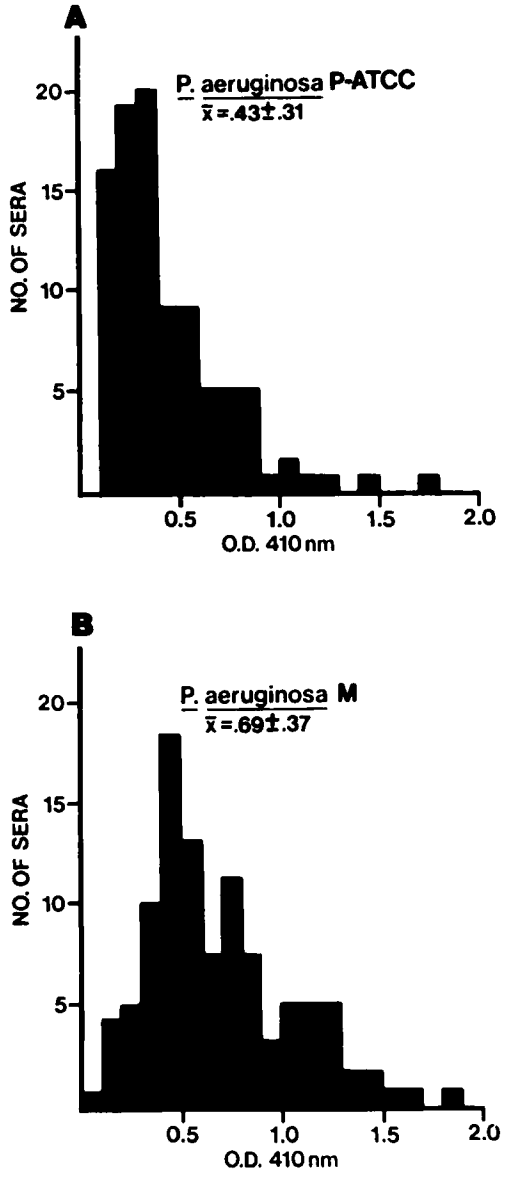


Figure 2 (continued)

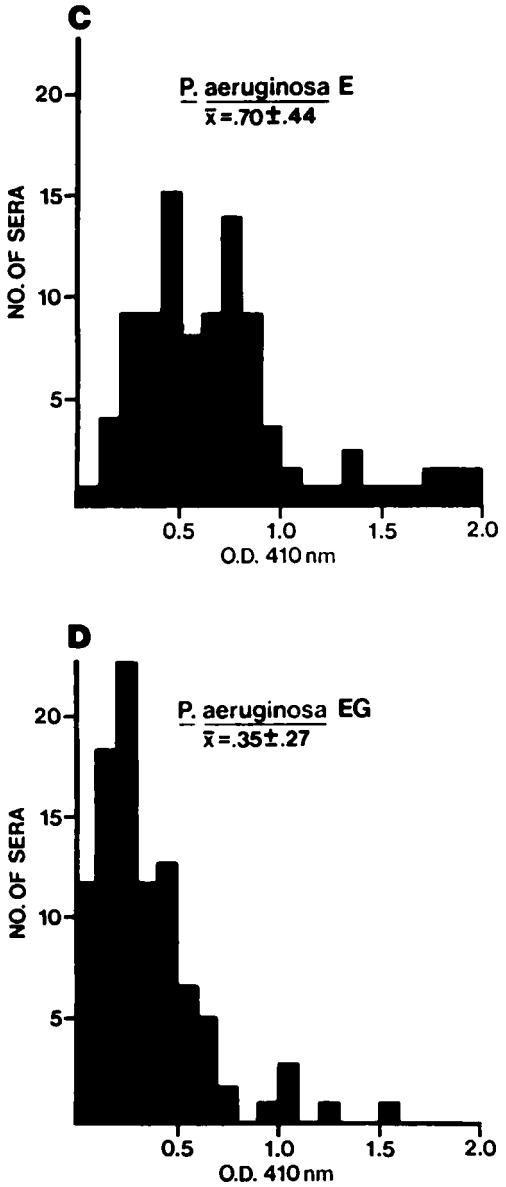
with respect to IgG activity between E. coli and P. aeruginosa. When rank orders of IgM and IgG reactivity within the same strain of an organism were compared, little correlation was seen with only E. coli 076 and P. aeruginosa showing mild correlation (r = .333 and .371, respectively) between IgM and IgG.

DISCUSSION

ELISA techniques are finding widespread application in both research and clinical laboratories. The ELISA being developed for use in this laboratory is part of a long-range study to measure



FIGURES 3 A-D IgM activity of 100 serum samples against four strains of P. aeruginosa.



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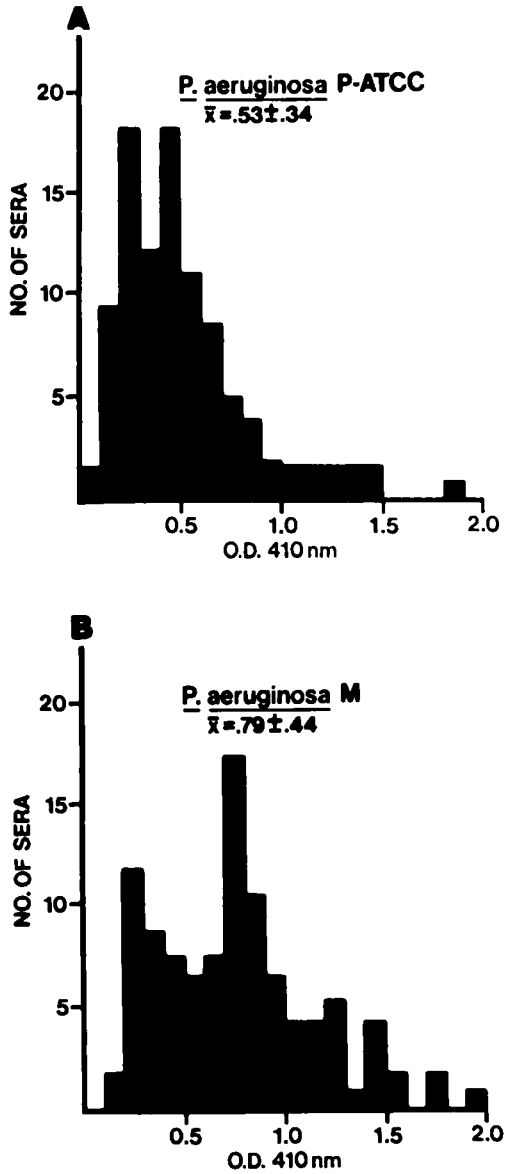

FIGURES 4 A-D IgG activity of 100 serum samples against four strains of P. aeruginosa.

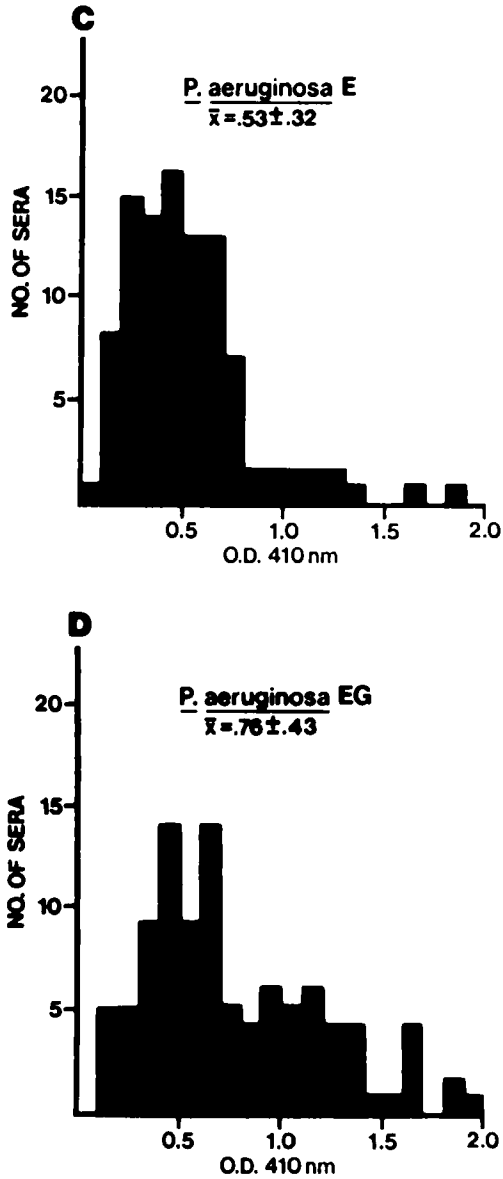

Figure 4 (continued)

several parameters of the host immune response in surgical and burn trauma patients. One of those parameters is pre-existing, as well as subsequent, levels of specific antibodies to common hospital pathogens. The ELISA described herein measures serum antibody activity to two common pathogens, E. coli and P. aeruginosa. In this report, several strains of each organism were selected from recent hospital isolates in addition to a well-defined ATCC strain; they were used to define expected values of antibody activity using sera from volunteer adult blood donors. Whole organisms were used as the antigen in the system, since the whole cell technique has the advantage of an antigen preparation that is both simple to prepare and offers an array of surface type-specific antigens that are known to elicit antibody production (4, 5).

In the sample population of 100 individuals, we found a range of antibody activity from very low (less than 0.10 OD units) to extremely high (approaching 2.0 OD units). This variation may be a reflection of differing degrees of exposure to the bacteria, individual genetic ability to respond to a specific antigenic challenge (6), or different phases of an ongoing immune response.

The overall appearance of the histograms constructed from the data suggests some similar patterns within the strains of the same organism. With one exception, both IgM and IgG antibody activities to E. coli were confined within relatively narrow limits, whereas the distribution of the P. aeruginosa antibody activity was more expanded. The narrow confines of E. coli data were consistent with the ubiquitous nature of the organism as a constituent of intesti-

TABLE 1.

Rank Order Correlation Coefficients (r) for IgM Activity Against Various E. coli and P. aeruginosa Strains (n = 100)

E. coli Strains	r^a	P. aeruginosa Strains	r^a
E-ATCC/076	.614	P-ATCC/M	.771
E-ATCC/O⁻	.715	P-ATCC/E	.748
E-ATCC/O·	.345	P-ATCC/EG	.975
076/O⁻	.861	M/E	.835
076/O·	.802	M/EG	.791

a: $p < 0.001$

TABLE 2.

Rank Order Correlation Coefficients (r) for IgG Activity Against Various E. coli and P. aeruginosa Strains (n = 100)

E. coli Strains	r^a	P. aeruginosa Strains	r^a
E-ATCC/076	.500	P-ATCC/M	.602
		P-ATCC/E	.423
		M/E	.698
		M/EG	.529
		E/EG	.902

a: $p < 0.001$

TABLE 3.

Rank Order Correlation Coefficients (r) for IgM Activity Compared Between E. coli and P. aeruginosa Strains (n = 100)

E. coli Strain/P. aeruginosa Strain	r[a]
E-ATCC/P-ATCC	.804
E-ATCC/M	.703
E-ATCC/E	.704
E-ATCC/EG	.591
076/P-ATCC	.719
076/M	.698
076/E	.612
076/EG	.691
0-/P-ATCC	.762
0-/M	.769
0-/E	.676
0·/P-ATCC	.468
0·/M	.674
0·/E	.621
0·/EG	.524

a: $p < 0.001$

nal flora and perhaps indicated a uniform antigenic exposure to E. coli in this population. The more uneven distribution of antibody activity of P. aeruginosa may be related to the tendency of this organism to be a less frequent component of normal enteric flora.

The specific IgM activity of the sera when compared on an individual basis was found to be similar between strains of the same organism and even between different organisms. This may reflect surface enterobacterial antigens shared between strains of the same

organism and between members of the same family (7-10). Fewer numbers of correlations were found when the IgG activity was examined and may reflect the tendency for lipopolysaccharides to induce primarily the IgM response (11, 12). This fact may also explain the lack of correlation when both the IgM and IgG responses to the same organism were compared.

The intent of this inquiry was to establish a working ELISA for descriptive use with ill surgical and burn trauma patients using volunteer blood donors as a reference group. We expected that typical hospital isolates of common microbes would elicit a normally distributed sequence of antibody activities. While we recognize the potential liability of using whole organisms derived from single strains, we cautiously conclude that there is sufficient overlap or commonality of antigens between strains as expressed by the IgM activities to allow further testing of this hypothesis in ill patients in a retrospective fashion. We are also considering alternate antigen preparations, such as extracts, which may provide broader representation without compromising relevance of the antibodies detected.

For the patient who does become infected, previously stored sera can be tested against the subsequently isolated pathogen itself. That, however, is only a part of the process, and we shall continue to seek a panel of antigens providing immunologic "fingerprinting" of capabilities a patient may bring to meet the challenge of acquired infection. This information becomes important since it has been shown that antibody to purified Pseudomonas aeruginosa

lipopolysaccharide and high-molecular-weight polysaccharide conferred protection against sepsis in a mouse burn wound model (13).

At the time of admission, we propose to perform antibody assays on a battery of antigens that will serve as baseline values and as a current antibody profile for each individual. This data may be clinically applicable in the following situations. If admission values fall significantly outside our previously defined expected range, we could anticipate infection with E. coli and P. aeruginosa and subsequently institute antimicrobial therapy. Similarly, serial changes from admission levels could be useful in cases of occult infection and could lead to the prompt initiation of appropriate antibiotic therapy. We have preliminary evidence in a limited series of patients to support the above applications (unpublished observations).

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