JOURNAL OF CLINICAL MICROBIOLOGY, May 1984, p. 680–686
0095-1137/84/050680-07$02.00/0

Vol. 19, No. 5

Methodological Aspects of *Staphylococcus aureus* Peptidoglycan Serology: Comparisons Between Solid-Phase Radioimmunoassay and Enzyme-Linked Immunosorbent Assay

BERTIL CHRISTENSSON,[1]* FRANK ESPERSEN,[2] SVEN ÅKE HEDSTRÖM,[1] AND GÖRAN KRONVALL[3]

Department of Infectious Diseases, University Hospital, S-221 85 Lund[1] and Department of Medical Microbiology, University of Lund, S-223 62 Lund,[3] Sweden, and Statens Seruminstitut, Department of Clinical Microbiology, Hvidovre Hospital, DK-2650 Hvidovre, Denmark[2]

Received 28 November 1983/Accepted 31 January 1984

In the present studies we compared the ability of two commonly used assays, solid-phase radioimmunoassay and enzyme-linked immunosorbent assay (ELISA), to detect human antibodies to *Staphylococcus aureus* peptidoglycan. ELISA was superior, with a reproducibility of 12.0%, as compared with 18.1% in solid-phase radioimmunoassay. Much lower serum dilutions could be used in ELISA. We also studied the effects of solubilizing the antigen by lysostaphin, lysozyme, or ultrasonication. Lysostaphin-treated peptidoglycan cannot be recommended since solid-phase radioimmunoassay could not distinguish positive from negative serum samples with this preparation. On the other hand, the sensitivity in both assays was high when peptidoglycan treated with lysozyme for 240 min or with ultrasonication for 30 min was used as antigen. The interassay correlation between solid-phase radioimmunoassay and ELISA was slightly better with sonicated peptidoglycan (correlation coefficient = 0.94, $P < 0.01$), as compared with lysozyme-treated peptidoglycan (correlation coefficient = 0.76, $P < 0.01$). We recommend the ELISA with sonicated peptidoglycan as antigen for use in routine serology.

Sensitive methods measuring the antibody response to *Staphylococcus aureus* peptidoglycan (PG) have been recommended as valuable aids in the serological diagnosis of serious staphylococcal infections (1, 11, 13). Different assays (radioimmunoassay and enzyme-linked immunoassay [ELISA]) and different methods for antigen preparation (lysostaphin treatment, lysozyme treatment, and ultrasonication) have been used in studies based on different patient materials. Thus, the various results from these studies are difficult to compare. If the anti-PG assay shall be used in the future as a routine diagnostic method in serious staphylococcal infections, a standardization of the assay will be required.

The aim of this study was to compare different antigen preparations by using solid-phase radioimmunoassay (SPRIA) and ELISA to define optimal conditions when measuring human immunoglobulin G (IgG) antibodies to *S. aureus* PG.

MATERIALS AND METHODS

Preparation of *S. aureus* cell walls. Two *S. aureus* strains, E2371, rich in protein A (2), and E1369, protein A deficient (9), were used in the present studies. The culture medium was Truche medium consisting of 40 g of peptone per liter (Orthana, Copenhagen, Denmark) with NaCl and glucose added to final concentrations of 5 and 2 g/liter, respectively. The pH of this medium was 7.5. Truche agar plates contained in addition 1.5% (wt/vol) Noble agar (Difco Laboratories, Detroit, Mich.). The bacteria were grown on these plates for 18 h at 37°C, scraped off the plates, and washed three times in saline. Then the bacteria were suspended in distilled water (2.5 ml/g) and disintegrated by three passages through an X-press (D-25; AB Biox, Nacka, Sweden) according to the method of Yoshida et al. (14). Whole, unbroken bacteria were removed twice by centrifugation at 3,000 × *g* for 15 min at 4°C. Cell walls were harvested from the supernatant by centrifugation at 10,000 × *g* for 25 min at 4°C, washed six times in distilled water, and lyophilized. From 20 g of whole bacteria, 40 and 72 mg of cell walls were obtained from strains E2371 and E1369, respectively. These cell walls were used for immunizing rabbits.

Preparation of *S. aureus* PG. PG from strains E2371 and E1369 was prepared by a modification of the method of Park and Hancock (8). Whole, washed bacterial cells grown as described above were extracted in 10% trichloracetic acid for 72 h at 4°C. After centrifugation at 4,000 × *g* for 10 min at 4°C, the pellet was further extracted with 5% trichloracetic acid for 10 min at 90°C. The pellet was centrifuged at 4,000 × *g* for another 10 min at 4°C. Then it was digested in 0.05 M NH_4HCO_3 buffer, pH 8.2, containing 0.005 M NH_4OH with 1 mg of crystalline trypsin per ml (Difco) for 18 h at 37°C. Insoluble PG was harvested by centrifugation at 4,000 × *g* for 10 min at 4°C, washed three times in distilled water, and dialyzed two times against distilled water for 24 h before lyophilization. From 50 g of whole bacteria, 4.2 and 5.7 g of insoluble PG were obtained from strains E2371 and E1369, respectively.

Solubilization of *S. aureus* PG. Insoluble PG from both *S. aureus* strains was solubilized by sonication or by treatment with lysozyme or lysostaphin. Ultrasonicated PG (SO-PG) was prepared by suspending PG in distilled water to a final concentration of 0.2 g/ml and sonicating at 20,000 Hz/s. The sonication continued for 30 min according to the method of Helgeland et al. (5). Thereafter, the supernatants were centrifugated at 48,000 × *g* for 1 h at 4°C, dialyzed against distilled water, and lyophilized. From 1 g of insoluble PG, 19 and 12 mg of SO-PG were obtained from strains E2371 and E1369, respectively. PG solubilized by lysozyme (LZ-PG)

* Corresponding author.

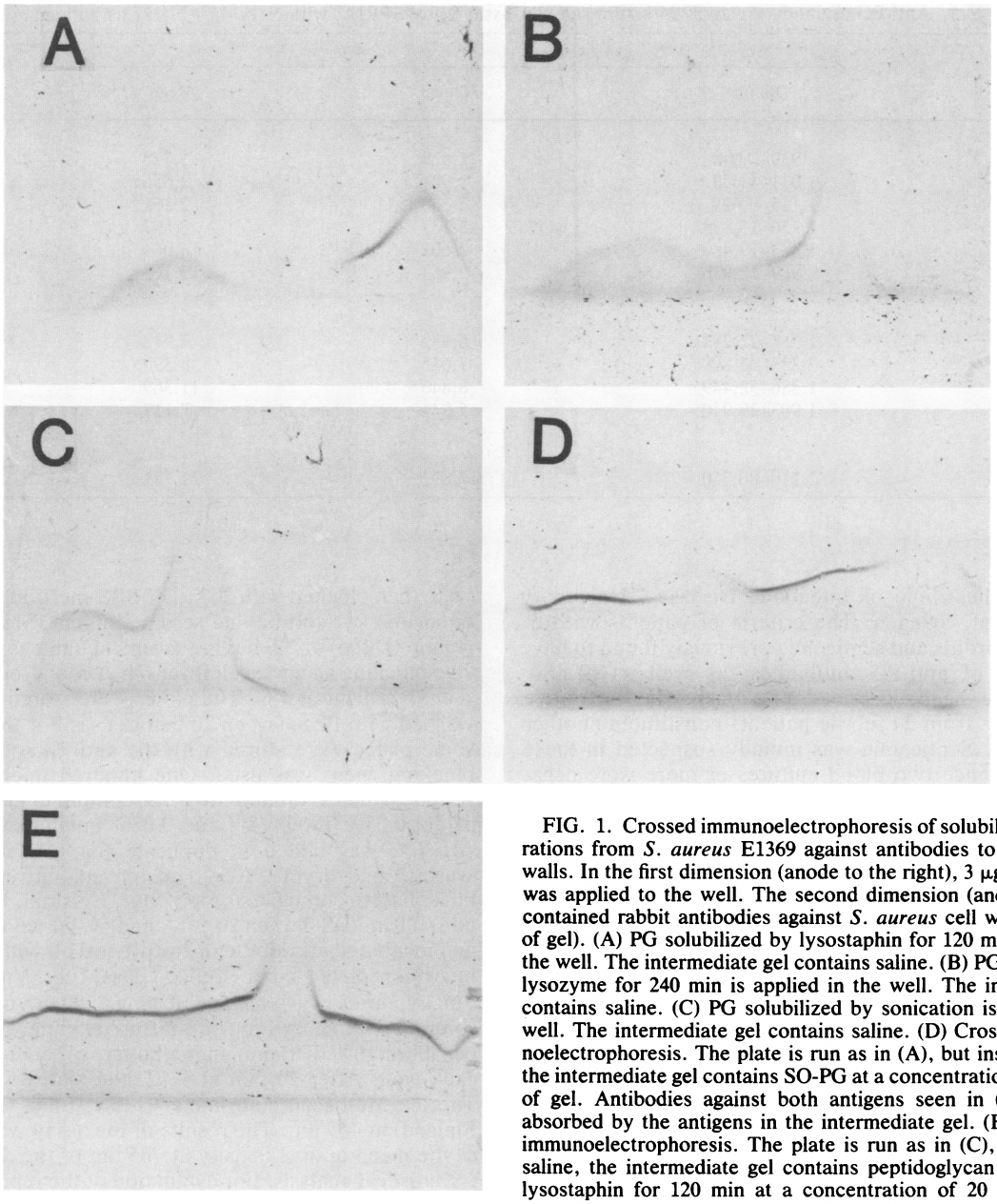

FIG. 1. Crossed immunoelectrophoresis of solubilized PG preparations from *S. aureus* E1369 against antibodies to *S. aureus* cell walls. In the first dimension (anode to the right), 3 μg of the antigen was applied to the well. The second dimension (anode at the top) contained rabbit antibodies against *S. aureus* cell walls (10 μl/cm^2 of gel). (A) PG solubilized by lysostaphin for 120 min is applied in the well. The intermediate gel contains saline. (B) PG solubilized by lysozyme for 240 min is applied in the well. The intermediate gel contains saline. (C) PG solubilized by sonication is applied in the well. The intermediate gel contains saline. (D) Crossed-line immunoelectrophoresis. The plate is run as in (A), but instead of saline, the intermediate gel contains SO-PG at a concentration of 20 μg/cm^2 of gel. Antibodies against both antigens seen in (A) have been absorbed by the antigens in the intermediate gel. (E) Crossed-line immunoelectrophoresis. The plate is run as in (C), but instead of saline, the intermediate gel contains peptidoglycan solubilized by lysostaphin for 120 min at a concentration of 20 μg/cm^2 of gel. Antibodies against both antigens have been absorbed.

was obtained by incubating PG at a final concentration of 5 mg/ml in 0.01 M phosphate buffer, pH 6.2, containing 0.2 mg of egg white lysozyme per ml (Sigma Chemical Co., St. Louis, Mo.) at 37°C for 120, 180, 240, and 300 min. After centrifugation at 48,000 × *g* for 1 h at 4°C, the supernatants were heated at 90°C for 10 min to destroy the enzyme. They were then dialyzed against distilled water and lyophilized. From 1 g of insoluble PG the yield of LZ-PG was 20 mg for strain E2371 and 15 mg for strain E1369. PG solubilized by lysostaphin (LS-PG) was prepared by incubation of PG at a final concentration of 5 mg/ml in 0.1 M phosphate buffer, pH 7.5, containing 0.1 mg of lysostaphin per ml (Sigma) at 37°C for 30, 60, 120, 180, 240, and 300 min. The remaining procedures were as described for LZ-PG. The yield of LS-PG from 1 g of insoluble PG was 10 and 18 mg for strains E2371 and E1369, respectively. All solubilized PG preparations were dissolved in distilled water to a final concentration of 0.1 g/liter.

Polyspecific rabbit antibodies against *S. aureus* cell walls. Five rabbits were immunized intracutaneously with *S. aureus* cell walls for 12 months according to the method of Harboe and Ingild (4). Each rabbit received at each injection 100 μl of an antigen preparation containing cell walls in saline (15 g/liter) from both strains E2371 and E1369, together with 100 μl of incomplete Freund adjuvant. Immunoglobulins from the pooled antisera were isolated and concentrated (4). The final preparation contained a colloid concentration of 28 g/liter measured by refractometry with human IgG as standard.

Patient material. Patient and control serum samples were

TABLE 1. Anti-PG antibody levels of 24 serum samples assayed by SPRIA, with *S. aureus* E2371 as antigen source

PG treatment and time (min)	cpm			
	Range	Median	Mean	Background
LS-PG				
30	939–3,048	1,529	1,728	557
60	1,031–4,453	1,924	2,056	549
120	1,115–4,629	2,208	2,161	610
180	1,236–15,838	3,187	3,597	557
240	1,483–19,480	3,601	4,064	546
300	989–12,036	2,963	3,165	575
LZ-PG				
120	1,588–22,281	6,989	7,948	424
180	2,333–43,899	7,035	8,390	374
240	1,286–52,449	8,840	11,369	391
300	1,903–29,940	5,841	7,117	412
SO-PG				
30	2,110–80,750	17,832	21,731	438

collected at the Clinic of Infectious Diseases, University Hospital, Lund, Sweden. The criteria of patients with *S. aureus* endocarditis and septicemia previously found to have raised levels of anti-PG antibodies, as well as healthy controls, have been described previously (1). Forty-five serum samples from 24 febrile patients constituted another control group. Septicemia was initially suspected in these patients, but since two blood cultures or more were negative, these patients were considered nonsepticemic. The male/female ratio of this control group was 15:9, and their ages ranged from 20 to 80 years, with a median age of 56 years.

Immunoelectrophoretic methods. All PG preparations were evaluated by means of crossed immunoelectrophoresis (3, 12). The different preparations (0.5 to 3.0 μg) were applied to the wells, and the first-dimension electrophoresis was carried out for 70 min at 10 V/cm. The second-dimension electrophoresis was performed overnight (18 h) at 2 V/cm in gel containing rabbit antibodies against *S. aureus* cell walls (10 to 20 μl/cm^2 of gel). Different preparations of PG were compared by crossed-line immunoelectrophoresis (7). Electrophoresis was performed using 1% agarose (Litex, Copenhagen, Denmark) and Tris-barbital buffer (pH 8.6; ionic strength, 0.02). The immunoprecipitates were stained with Coomassie brilliant blue (10).

SPRIA. A SPRIA measuring IgG antibodies against the different PG preparations was used as described previously (1). Briefly, solubilized PG was coated onto plastic surfaces which then were incubated with the serum samples. IgG antibodies bound to PG were then detected with radiolabeled protein A and expressed as counts per minute.

ELISA. An ELISA modified from that of Engvall and Perlmann (1a) was developed to quantitate IgG antibodies against PG. Two different types of 96-well microtiter plates were compared: Nunc Immunoplate I (Nunc AS, Roskilde, Denmark) and Dynatech M 129B (Dynatech AG, Kloten, Switzerland). One hundred microliters of antigen solution in phosphate-buffered saline (PBS) (0.12 M NaCl, 0.03 M phosphate, pH 7.2) with merthiolate was added to the microtiter wells and left to adsorb for 1 h at 37°C and then overnight at 4°C. The plates were then washed (Flow Multiwash, Solna, Sweden) in PBS containing 0.05% Tween 20 and dried. Remaining binding sites on the plastic surface were then blocked with 200 μl of PBS-merthiolate containing either 4% (wt/vol) bovine serum albumin (Sigma) and 0.1% gelatin (Difco) or 1% bovine serum albumin as in the SPRIA. The blocking agent was left for 6 h at room temperature and then overnight at 4°C. The plates were stored at −20°C and washed with PBS-Tween 20 before use. For the comparison some plates were stored with the antigen solution, and no blocking agent was used. One hundred microliters of the serum samples diluted in PBS containing 0.02% sodium azide, 0.05% Tween 20, and 0.02% human serum albumin, pH 7.4, was added in duplicate for 1 h at 37°C. After washing and drying, 100 μl of an anti-human IgG–alkaline phosphatase conjugate (Orion, Oy., Helsinki, Finland) diluted 1/100 in PBS-Tween 20 containing 0.1% gelatin was added and incubated at room temperature for 1 h with shaking on a microtiter plate shaker (Bellco Glass, Inc., Vineland, N.J.). Finally, after washing and drying, 100 μl of a substrate solution containing 1 mg of *p*-nitrophenyl phosphate (Sigma) per ml in 10% diethanolamine buffer (pH 9.8) was added to each well. After 30 min at 37°C, the plates were read in a Titertek Multiscan colorimeter (Flow, Eflab Oy., Helsinki, Finland) at 405 nm. The results of the assay were expressed as the mean optical density at 405 nm of the duplicates.

Statistical analysis. For evaluation of the reproducibility of an assay, the analytical error of the method was calculated. The error was expressed as the standard deviation of the single determination $s = \pm\sqrt{(Sd^2/2n)}$, where Sd^2 equals the squared sum of differences within pairs and n equals the number of pairs. Since the absolute differences within paired observations rose with higher counts-per-minute or optical density values, the standard deviations of the single determinations were calculated as percentage values. The significance of the correlation coefficient (r) was determined by the Spearman rank correlation coefficient (r_s).

RESULTS

Evaluation of PG preparations by crossed immunoelectrophoresis. When insoluble PG from both *S. aureus* strains was investigated in crossed immunoelectrophoresis against antibodies to *S. aureus* cell walls, no visible immunoprecipitates were detected. All solubilized preparations, however, showed two visible precipitates (Fig. 1), but the antigens

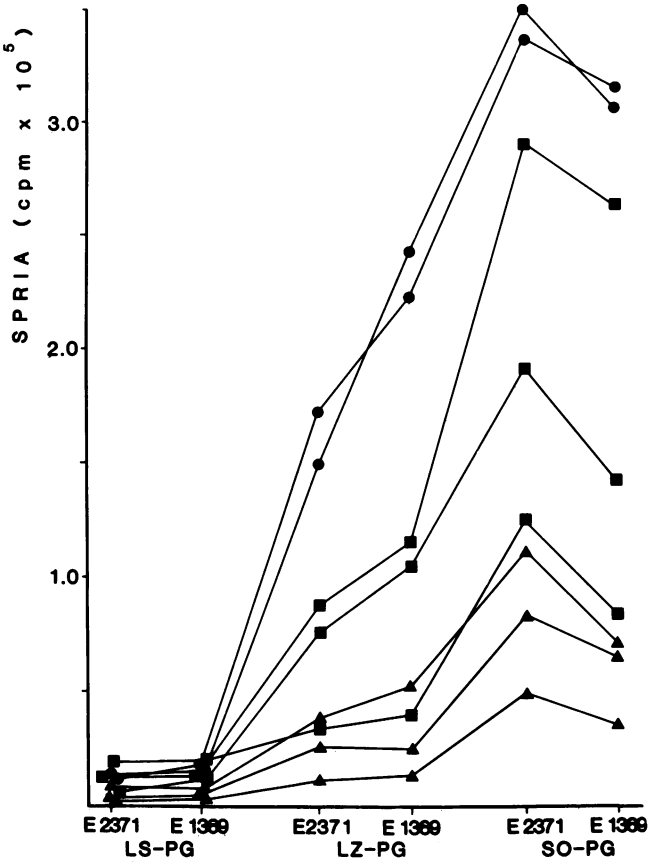

FIG. 2. Anti-PG antibodies determined by SPRIA with LS-PG, LZ-PG, and SO-PG from *S. aureus* E2371 and E1369. Serum samples at 1/10 dilutions from two patients with *S. aureus* endocarditis (●), three patients with *S. aureus* septicemia (■), and three healthy controls (▲) were investigated.

obtained by different treatments of PG from the same *S. aureus* strains showed some variations in electrophoretic mobility (Fig. 1A, B, and C). In cases of enzyme treatment, the optimal incubation time was roughly estimated by comparing the height of the precipitates, and optimal time for both strains was 120 and 240 min for lysostaphin and lysozyme, respectively (data not shown). Comparison of the different preparations by means of crossed-line immunoelectrophoresis (Fig. 1D and E) gave identical results. By inclusion of another preparation of PG in the intermediate gel, it was possible to absorb all antibodies against the visible immunoprecipitates both when preparations from the two different strains were compared and when different preparations from the same strain were compared.

Evaluation of different PG preparations as antigens in SPRIA. PG preparations obtained by sonication and enzyme treatment at various periods of time were used in SPRIA at antigen concentrations of 1 μg/ml. Serum samples at 1/40 dilutions from 24 febrile nonsepticemic control patients were assayed, and median, mean, and range values are expressed in Table 1. Both lysostaphin treatment and lysozyme treatment for 240 min gave peak counts-per-minute values. Since the background levels differed only slightly, the two 240-min preparations and the SO-PG were used in the following experiment.

By using the three selected PG preparations from both *S. aureus* strains, eight serum samples at a 1/10 dilution were investigated. Two patients with *S. aureus* endocarditis and two patients with *S. aureus* septicemia have previously been reported to have high anti-PG antibody levels in SPRIA with SO-PG from strain E1369, whereas one *S. aureus* septicemia patient together with three healthy controls previously showed low antibody levels (1). The same relations between antibody levels could now be obtained by using both LZ-PG and SO-PG from either *S. aureus* E1369 or E2371 (Fig. 2). Since the background levels were equal, the assay with SO-PG gave the highest counts-per-minute values, followed by the LZ-PG assay. On the other hand, when LS-PG from the two strains was used, much lower counts-per-minute values, not distinguishing positive serum samples from negative, were obtained (Fig. 2). There were no differences between the two *S. aureus* strains when LS-PG was used as antigen. LZ-PG from strain E1369 gave slightly higher counts-per-minute values than that from E2371, whereas the opposite was true with SO-PG (Fig. 2).

The influence of different antigen and antibody concentrations in SPRIA was also investigated. One positive and one negative serum sample diluted 1/40 were assayed with LZ-PG from strain E2371 at six different antigen dilutions (Fig. 3). Antigen concentrations from 0.1 to 1 μg/ml gave relatively similar counts-per-minute values for both serum samples tested, whereas rapidly decreasing counts-per-minute values were obtained when the antigen concentration was raised. A concentration of 1.0 μg/ml was used in the following experiment.

The same two serum samples were then serially diluted from 1/10 to 1/5,120 with LS-, LZ-, and SO-PG from strain E1369 at 1 μg/ml (Fig. 4). Again, LZ- and SO-PG gave far higher counts-per-minute values than LS-PG. The differences were most extreme at low serum dilutions and were equalized at higher dilutions. A serum dilution of 1/40 was selected to be used in the following experiment for differentiating positive from negative serum samples.

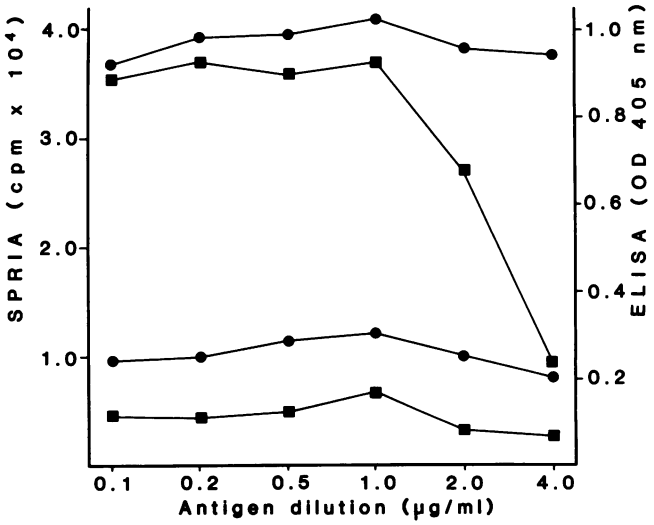

FIG. 3. Anti-PG antibodies determined by SPRIA (■) and ELISA (●) with LZ-PG from *S. aureus* E2371 at six different antigen concentrations (range, 0.1 to 4.0 μg/ml). One positive serum sample (top) and one negative serum sample (bottom) were investigated at 1/40 dilutions.

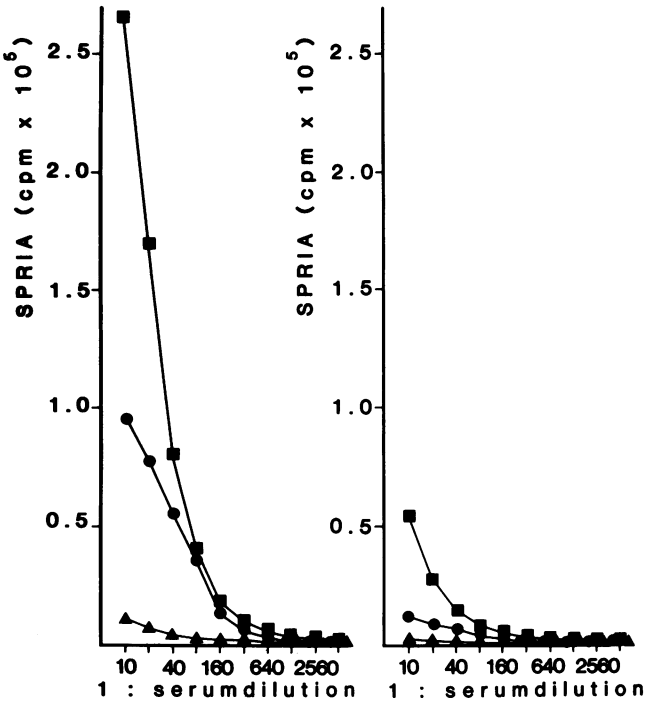

FIG. 4. One positive (left) and one negative (right) serum sample were serially diluted (1/10 to 1/5,120). Anti-PG antibodies were determined by SPRIA with LS-PG (▲), LZ-PG (●), and SO-PG (■) from *S. aureus* E1369.

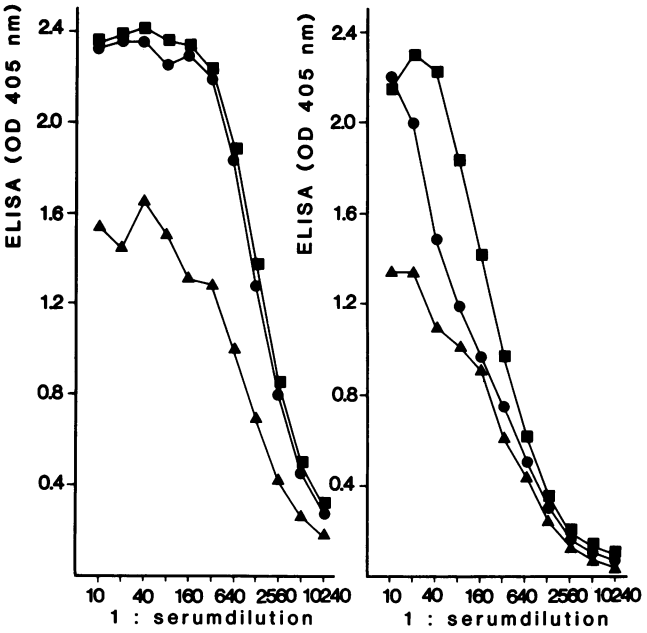

FIG. 5. One positive (left) and one negative (right) serum sample were serially diluted (1/10 to 1/10,240). Anti-PG antibodies were determined by ELISA with LS-PG (▲), LZ-PG (●), and SO-PG (■) from *S. aureus* E2371.

The analytical error of the test was ±4.5% between duplicates at the same occasion and ±18.1% when the serum samples were diluted and tested at different occasions.

Evaluation of different PG preparations as antigens in ELISA. No obvious differences between the two types of microtiter plates were seen (data not shown), and Nunc Immunoplate I was used in the ELISA. Neither did the choice of blocking agent influence the results (data not shown), which is why the same one as in SPRIA was chosen (PBS + 1% bovine serum albumin). Microtiter plates with the blocking solutions were stored without impairment at −20°C for at least 3 months. When no blocking agent at all was used, a considerable nonspecific binding to the plastic was obtained. This uptake varied from serum to serum even at the lower serum dilutions (data not shown).

Since only small differences between PG preparations from the two *S. aureus* strains were seen in SPRIA, only strain E2371 was used in ELISA.

The LS-, LZ-, and SO-PG were compared in ELISA. Optical density values registered when the LZ-PG and SO-PG constituted the antigen were generally higher and more discriminating than those obtained with the LS-PG. The differences were, however, almost equalized at the highest serum dilutions. One example is shown in Fig. 5 in investigation of the same positive and negative serum samples as in Fig. 3, here serially diluted from 1/10 to 1/10,240. Suspected prozone phenomena were seen in some ELISA systems at the four lowest serum dilutions (data not shown). A serum dilution of 1/1,500 used in the following correlation experiment could differentiate previously known positive serum samples from negative (data not shown).

Only small differences in optical density values were seen when the effects of different antigen concentrations were investigated in ELISA (Fig. 3). The same antigen dilution as in SPRIA (1.0 μg/ml) was used in the following experiment.

A comparison was made between the LZ-PG and SO-PG with 45 serum samples from 24 febrile nonsepticemic control patients (Fig. 6). The value for r was 0.90, and r_s was 0.93 ($P < 0.01$). The analytical error of the method was ±2.9%

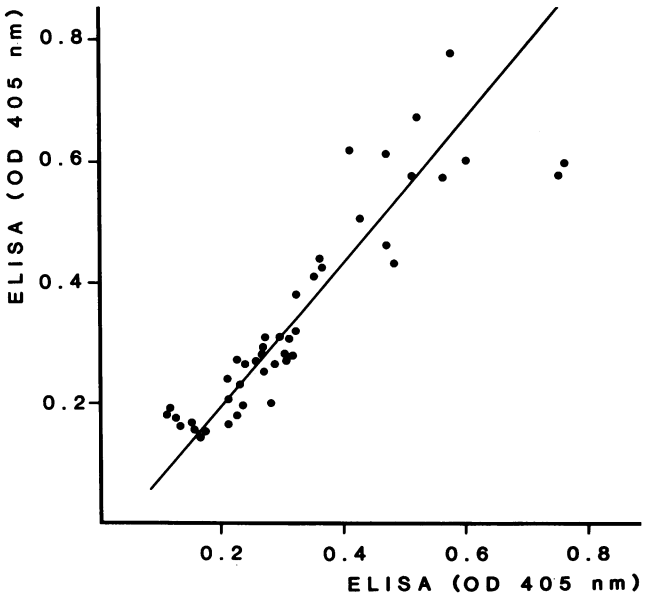

FIG. 6. Correlation between anti-PG antibody levels of 45 serum samples assayed by ELISA with LZ-PG (ordinate) and SO-PG (abscissa). r = 0.90 by regression analysis.

between duplicates on the same plate and ±12.0% between different plates, occasions, and dilution events.

Comparison between SPRIA and ELISA. Twenty-three serum samples from the febrile nonsepticemic control group were assayed in both SPRIA and ELISA. With LZ-PG, r was 0.76 (Fig. 7), whereas it was 0.94 when SO-PG was used (Fig. 8). Corresponding values for r_s were 0.85 and 0.93, respectively, which in both cases were statistically significant ($P < 0.01$).

DISCUSSION

The *S. aureus* PG cell wall skeleton is a complex molecule known to have at least three different major antigenic structures (6). When this part of the cell wall is solubilized by enzyme digestion or ultrasonication, probably different mixtures of immunogenic and nonimmunogenic fragments of both carbohydrate and protein origin are obtained. It is therefore not surprising that various results can be obtained when different antigen preparations are used in different assay systems.

In the comparison of antigen preparations from two different *S. aureus* strains, E2371 and E1369, no obvious differences could be found. On the other hand, the way of solubilizing the PG seemed to be important. In crossed immunoelectrophoresis, some variations in antigen-antibody precipitates could be seen when comparing LS-, LZ-, and SO-PG (Fig. 1). However, in crossed-line immunoelectrophoresis all PG preparations cross-reacted serologically when polyspecific rabbit antibodies against *S. aureus* cell wall preparations were used (Fig. 1). When human anti-PG antibodies were detected in SPRIA and ELISA, it was apparent that much lower values were obtained when LS-PG was used as compared with LZ-PG and SO-PG (Fig. 2, 4, and 5). Furthermore, the duration of the enzyme digestion seemed to be important, since treatment of the PG for 240 min yielded peak counts-per-minute values in SPRIA (Table 1). These results are in agreement with those of Wheat et al. (13) concerning the LZ-PG, but they reported a complete loss of activity after treating the antigen with lysostaphin for 240 min. These differences in optimizing the duration of the enzyme treatment are difficult to explain. Differences in enzyme activities from different batches and in relations between enzyme and substrate concentrations may be some reasons. Sonication seems to be easier to reproduce in different laboratories, and since our assays with SO-PG were at least as sensitive as the lysozyme assays, ultrasonication of the PG should be the recommended procedure.

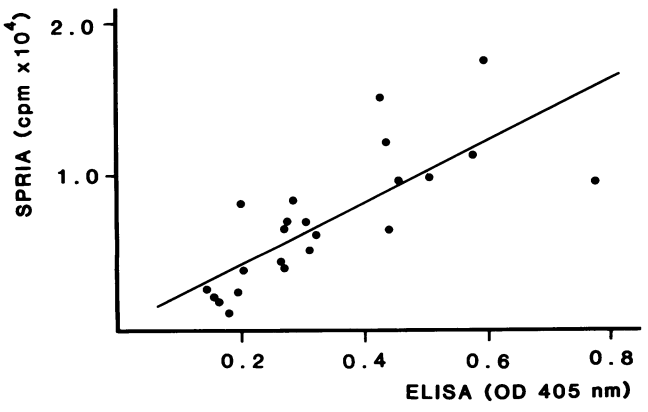

FIG. 7. Correlation between anti-PG antibody levels of 23 serum samples assayed by SPRIA (ordinate) and ELISA (abscissa) with LZ-PG. $r = 0.76$ by regression analysis.

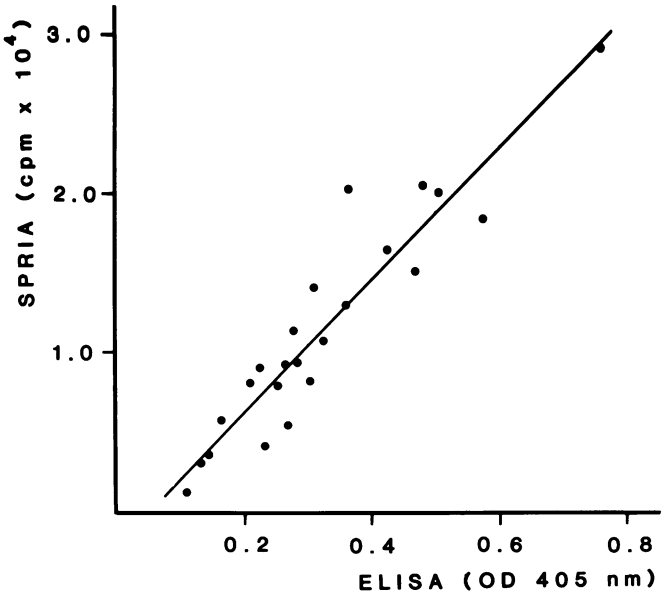

FIG. 8. Correlation between anti-PG antibody levels of 23 serum samples assayed by SPRIA (ordinate) and ELISA (abscissa) with SO-PG. $r = 0.94$ by regression analysis.

It is of great advantage to a routine laboratory assay if a single serum dilution can be used. Therefore, titration curves with various antigen and serum dilutions were determined for each assay. In our assays we chose antigen concentrations of 1.0 μg/ml (Fig. 3), which differ only slightly from those of Wheat et al. (13) and Verbrugh et al. (11), which were 2.0 μg/ml. The serum dilution chosen in our SPRIA was 1/40, versus 1/3,000 in the SPRIA of Wheat et al., and corresponding values with our ELISA versus that of Verbrugh et al. were 1/1,500 and 1/512, respectively. The slopes of our serum titration curves in comparison with various antigen preparations were not parallel (Fig. 4). This might indicate differences in antibody avidity of the serum specimens investigated. These data further strengthen the need for standardizing not only the antigen preparations but also the serum dilutions when comparing results from different laboratories.

The reproducibility of the ELISA expressed as the analytical error of the method was better (±2.9 to ±12.0%) than that of the SPRIA (±4.5 to ±18.1%). When using the SO-PG as antigen, the interassay correlation was high ($r = 0.94$, $P < 0.01$) (Fig. 8). The interassay correlation was slightly lower ($r = 0.76$) but still statistically significant ($P < 0.01$) when LZ-PG was used (Fig. 7). The lack of absolute correlation can be explained by the differences in reproducibility between the assays. There is, however, another obvious difference betweeen the two assays. In SPRIA, radiolabeled protein A is used to detect the IgG antibodies, and since the subclass IgG3 does not bind to protein A, this might contribute to explain the differences compared with the anti-human IgG used in ELISA. Wheat et al. (13) showed a good correlation ($r = 0.96$) when testing IgG antibodies of 37 serum samples in SPRIA with LZ-PG and SO-PG. Our results with ELISA (45 serum samples; $r = 0.90$, $r_s = 0.93$, $P < 0.01$) are in agreement with those results (Fig. 6).

In summary, both LZ-PG and SO-PG can be used in either

SPRIA or ELISA when detecting human anti-PG IgG antibodies. The LS-PG cannot be recommended. The ELISA has some advantages: the reproducibility is better, and a higher serum dilution can be used with improved specificity. Although only two *S. aureus* strains were investigated, the choice of strain seems to be of less importance, whereas the method of antigen preparation, like the duration of enzyme treatment, is crucial. A standardization of these methodological aspects, together with an evaluation of optimal antigen-antibody relationships, is important if the assays are to be used in the routine serological diagnosis of *S. aureus* infections.

ACKNOWLEDGMENTS

We thank Barbro Hansson and Anni Bethien for skillful technical assistance and Jonas Blomberg for technical advice.

This study was supported by grants from the Medical Faculty, University of Lund, Sweden, and by Astra Pharmaceutical Company, Södertälje, Sweden.

LITERATURE CITED

1. **Christensson, B., F. Espersen, S. Å. Hedström, and G. Kronvall.** 1983. Solid-phase radioimmunoassay of immunoglobulin G antibodies to *Staphylococcus aureus* peptidoglycan in patients with staphylococcal infections. Acta Pathol. Microbiol. Immunol. Scand. Sect. B **91**:401–406.

1a. **Engvall, E., and P. Perlmann.** 1972. Enzyme-linked immunosorbent assay, ELISA. III. Quantitation of specific antibodies by enzyme-labelled anti-immunoglobulin in antigen-coated tubes. J. Immunol. **109**:129–135.

2. **Espersen, F., and I. Clemmensen.** 1982. Isolation of a fibronectin-binding protein from *Staphylococcus aureus*. Infect. Immun. **37**:526–531.

3. **Espersen, F., J. B. Hertz, N. Høiby, and H. H. Mogensen.** 1980. Quantitative immunoelectrophoretic analysis of *Salmonella typhi* antigens and of corresponding antibodies in human sera. Acta Pathol. Microbiol. Scand. Sect. B **88**:237–242.

4. **Harboe, N., and A. Ingild.** 1973. Immunization, isolation of immunoglobulins, and estimation of antibody titre. Scand. J. Immunol. **2**(Suppl. 1):161–164.

5. **Helgeland, S., A. Grov, and K. Schleifer.** 1973. The immunochemistry of *Staphylococcus aureus* mucopeptide. I. Antigenic specificity of the subunits. Acta Pathol. Microbiol. Immunol. Scand. Sect. B **81**:413–418.

6. **Heymer, B., D. Bernstein, K. H. Schleifer, and R. M. Krause.** 1975. Measurement of peptidoglycan antibodies by a radioimmunoassay. Z. Immunitaetsforsch. **149**:168–178.

7. **Krøll, J.** 1973. Crossed-line immunoelectrophoresis. Scand. J. Immunol. **2**(Suppl. 1):79–81.

8. **Park, J. T., and R. Hancock.** 1960. A fractionation procedure for studies of the synthesis of cell-wall mucopeptide and other polymers in cells of *Staphylococcus aureus*. J. Gen. Microbiol. **22**:249–258.

9. **Schiøtz, P. O., N. Høiby, and J. B. Hertz.** 1979. Cross-reactions between *Staphylococcus aureus* and fifteen other bacterial species. Acta Pathol. Microbiol. Scand. Sect. B **87**:329–336.

10. **Uriel, J.** 1964. The characterization reactions of the protein constituents following electrophoresis or immunoelectrophoresis in agar, p. 30–57. *In* P. Grabar and P. Burtin (ed.), Immunoelectrophoretic analysis. Applications to human biological fluids. Elsevier Publishing Co., Amsterdam.

11. **Verbrugh, H. A., R. Peters, M. Rozenberg-Arska, P. K. Peterson, and J. Verhoef.** 1981. Antibodies to cell wall peptidoglycan of *Staphylococcus aureus* in patients with serious staphylococcal infections. J. Infect. Dis. **144**:1–9.

12. **Weeke, B.** 1973. Crossed immunoelectrophoresis. Scand. J. Immunol. **2**(Suppl. 1):47–56.

13. **Wheat, L. J., B. J. Wilkinson, R. B. Kohler, and A. C. White.** 1983. Antibody response to peptidoglycan during staphylococcal infections. J. Infect. Dis. **147**:16–22.

14. **Yoshida, A., C.-G. Heden, B. Cedergren, and L. Edebo.** 1961. A method for the preparation of undigested bacterial cell walls. J. Biochem. Microbiol. Tech. Eng. **3**:151–159.