Evaluation of a Commercial Counterimmunoelectrophoresis Kit for Detection of Staphylococcus aureus Teichoic Acid Antibodies

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A commercial kit from Diagnostica, Inc., Miami, Fla., was studied for its ability to detect antibodies to the teichoic acids of Staphylococcus aureus. A comparative study of the Diagnostica counterimmunoelectrophoresis (CIE) system and our gel double-diffusion method was undertaken with 156 serum samples from 142 patients. Included were 25 cases of staphylococcal and non-staphylococcal endocarditis, 30 cases of S. aureus bacteremia, 19 cases of nonbacteremic S. aureus infection, 39 cases of hospitalized patients without a staphylococcal infection, and 29 normal controls. Agreement between methodologies was attained in 138 (88.5%) of the 156 samples tested and in 127 (89.4%) of the 142 patients. Of 13 patients with culture-proven S. aureus endocarditis, significant antibody titers were found in all patients (100%) by CIE and in 12 patients (92.3%) by double diffusion. No significant titers were found in normal sera by CIE, but four sera were positive by double diffusion. Of 80 sera from patients with no evidence of S. aureus infection, 4 (5.0%) were positive by CIE and 7 (8.8%) were positive by double diffusion. The Diagnostica CIE kit appears to provide a suitable means for the detection of deep-seated S. aureus infections.

Assays for elevated or rising titers of teichoic acid antibodies are reported to be of value in the detection and management of deep-seated Staphylococcus aureus infections (1, 3, 5, 8, 9) such as bacterial endocarditis. Unfortunately, differences in antigen preparation and assay systems generate a certain degree of variability among reported data (11) and result in obfuscation of test usefulness. Although recent publications (7, 11) suggest modifications to improve test standardization, a commercially available system may help to reduce the problems associated with teichoic acid antibody assays. Diagnostica, Inc., Miami, Fla., now offers a counterimmunoelectrophoresis (CIE) kit for the detection of teichoic acid antibodies. The purpose of our investigation was to compare the Diagnostica teichoic acid antibody detection kit with the gel double-diffusion system used in our clinical laboratory.

MATERIALS AND METHODS

Specimens. A total of 156 serum samples were collected from 142 hospitalized patients and from a normal control population. Patients had a spectrum of clinical diagnoses most often relating to either possible endocarditis or S. aureus infection. Sera were stored at −70°C until tested by both methods on the same day.

Patient categorization. Patients were divided into five major categories on the basis of a review of their clinical records. The first group included all endocarditis patients, and patients from the four remaining groups showed no evidence of endocarditis.

The endocarditis group (group 1) consisted of 25 cases. Diagnosis of endocarditis was based on the following criteria: bacteremia and pulmonary infiltrates in abusers of intravenous drugs; bacteremia with the appearance of valvular insufficiency, peripheral manifestations, or positive cultures at surgery; and bacteremia with a pathological valve. Patients in this group were further categorized into 13 cases caused by S. aureus and 12 cases caused by other bacteria. Of the 12 cases, 6 were caused by coagulase-negative staphylococci, including 2 cases of prolonged bacteremia with infected ventriculotriatral shunts. The remaining six cases were caused by non-staphylococci and included three cases of viridans group streptococci, two cases of Escherichia coli, and one case of Bacillus sp.

The S. aureus bacteremia group (group 2) consisted of 30 cases. S. aureus bacteremia cases were further classified as complicated or uncomplicated (10). In complicated bacteremias, primary sites of infection were not eradicated within 48 h of bacteremia detection. Uncomplicated bacteremias were associated with eradication of the primary source or absence of persistent bacteremia or both.

The nonbacteremic S. aureus infection group (group 3) consisted of 19 cases.

The non-staphylococcal disease group (group 4) consisted of 39 cases. This group was comprised of
hospitalized patients with a variety of clinical diagnoses but without evidence of staphylococcal infection.

The normal control group (group 5) consisted of 29 individuals.

**Antigen preparation for gel diffusion.** Antigen used in the gel diffusion assay was prepared from the Lafferty strain of *S. aureus*, kindly supplied by Arthur White, Indianapolis University Medical Center, Indianapolis, Ind. Overnight cultures from tryptic soy broth (Difco Laboratories, Detroit, Mich.) were centrifuged, washed with 0.01 M sterile phosphate-buffered saline (pH 7.4), and resuspended in 0.01 M sterile phosphate-buffered saline. This bacterial suspension was treated with 2.0 mg of lysostaphin (Sigma Chemical Co., St. Louis, Mo.) and placed on a rotary shaker at 37°C overnight. The resulting cell debris was separated by centrifugation, and the supernatant was used in the double-diffusion assay. An antigen suspension which proved to be the most sensitive was selected by titration against a known positive control as suggested by Sheagren et al. (7). A precipitin line to teichoic acid was confirmed by a line of identity with purified teichoic acid prepared by the method of Oeding (6).

**Gel double-diffusion assay.** Plates were prepared by adding 8.0 ml of 1.0% agarose (Litex type LSA; Accurate Scientific Co., Hicksville, N.Y.)-4.0% dextran T-70 (Pharmacia Fine Chemicals, Piscatway, N.J.) in a 0.25 M barbital-borate buffer (pH 8.6) onto clean glass slides (50 by 75 mm). All wells were 3 mm in diameter, and the center-to-center interval distance was 8 mm. The antigen suspension was placed in the center well, and dilutions of serum were placed in five of the outer wells. One outer well contained positive serum as a control. Plates were incubated at room temperature overnight in a humidity chamber. The next day, the plates were examined and placed in a refrigerator for another 6 to 8 h before being examined again. The test was considered positive if precipitin lines were present at a serum dilution of 1:2 after refrigeration.

CIE system. CIE was performed with the kit developed by Diagnostica, Inc. Supplied in the kit are antigen, antibody-positive control, CIE 10-test plates, and barbital-acetate buffer (pH 8.6). The teichoic acid sonicate of the Lafferty strain of *S. aureus* was prepared by a modification of the method of Crowder and White (1). The plates for CIE measure 40 by 80 mm with wells 3 mm in diameter, and the center-to-center interval distance is 11 mm. Power was supplied by an electrophoresis system from Hyland Diagnostics, Deerfield, Ill. Each serum was tested undiluted and diluted 1:2 and 1:4, along with a 1:4 dilution of the positive control serum and a negative control serum. After addition of the antigen to the cathodal well and test samples to the anodal well, a constant current of 30 mA was applied for 30 min. After electrophoresis, the plate was incubated at 5°C for 1 h and then examined for precipitin lines. The presence of a precipitin line at a serum dilution of 1:4 was considered a positive test.

## RESULTS

Categorization of subjects and comparison of teichoic acid antibody testing by CIE and double diffusion are summarized in Table 1. Of the 142 cases studied, there was agreement in 127 (89.4%). Agreement between methodologies was obtained in 138 (88.5%) of the 156 samples tested.

In group 1, positive titers were found in 12 of the 13 cases of *S. aureus* endocarditis by both methods, whereas all 13 patients were found positive by CIE. Of 22 serum samples tested from these patients, 19 were in agreement between methods. Neither method detected significant antibody levels in the six cases of endocarditis caused by coagulase-negative staphylococci. In another six cases of non-

| TABLE 1. Comparison of *Staphylococcus aureus* teichoic acid antibody testing by CIE and double diffusion in a selected population |
|-------------------------------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| Group                                           | Patient results                      | Serum results                      |
|                                                 | Total no. | Both positive | CIE positive | Double diffusion positive | Agreement between methods |
|                                                 |           |               |              |                                | Total no. | Agreement between methods |
| Endocarditis                                    |           |               |              |                                |           |                          |
| *S. aureus*                                     | 13        | 12            | 13           | 12                             | 12        | 22                         | 19 |
| Coagulase-negative staphylococci                | 6†        | 0             | 0            | 0                              | 6         | 6                          | 6  |
| Non-staphylococcal                              | 6†        | 0             | 1            | 0                              | 5         | 6                          | 5  |
| *S. aureus* bacteremia                          |           |               |              |                                |           |                           |
| Complicated                                     | 19        | 7             | 8            | 9                              | 16        | 23                         | 20 |
| Uncomplicated                                   | 11        | 1             | 2            | 1                              | 10        | 11                         | 10 |
| Nonbacteremic *S. aureus* infections            | 19        | 3             | 3            | 4                              | 18        | 20                         | 18 |
| Non-staphylococcal disease                      | 39†       | 1             | 3            | 3                              | 35        | 39                         | 35 |
| Normal controls                                 | 29†       | 0             | 0            | 4                              | 25        | 29                         | 25 |

† No evidence of *S. aureus* infection.
staphylococcal endocarditis, one patient, blood culture positive for *E. coli*, was positive by CIE.

Of the 19 complicated bacteremia cases in group 2, seven patients were positive by both techniques. A significant titer was found by CIE in one patient with an infected median sternotomy site. Two other patients, one with pneumonia and one with an epidural abscess, were positive by double diffusion. Both methods detected antibodies in 1 of 11 patients with uncomplicated *S. aureus* bacteremia. CIE detected antibodies in one additional patient with concurrent multiple myeloma.

Both techniques detected antibodies in 3 of 19 patients with nonbacteremic staphylococcal infection (group 3). Two of these patients had osteomyelitis, whereas one had a deep wound infection. Two additional samples from a patient with an infected median sternotomy site were positive by double diffusion.

Group 4, consisting of patients with non-staphylococcal disease, included 39 cases. One patient with a ventriculotrial shunt infection due to microaerophilic streptococci was positive by both assays. Another patient with viral pneumonia and one with an undefined connective tissue disease were positive by CIE, and two patients with atopic dermatitis were positive by double diffusion.

In group 5, of 29 normal controls, 0 were positive by CIE, whereas 4 were positive by double diffusion. Of 80 total patients having no evidence of *S. aureus* infection, 4 (5.0%) were positive by CIE and 7 (8.8%) were positive by double diffusion.

**DISCUSSION**

The detection of antibodies to the cell wall teichoic acids of *S. aureus* correlates with the presence of endocarditis or other deep tissue infection (1, 3, 5, 9). Although the test appears to be of proven value, a lack of test standardization results in some confusing variability (11). Most commonly, the antigen is a bacterial ultrasonic extract, but others report using a lysostaphin preparation (2) or a buffer extraction (8). Other factors that vary among procedures include the *S. aureus* strain used for antigen preparation, antigen concentration, agar matrix, gel diffusion or CIE, and positive control serum. Although two recent publications (7, 11) seek to improve assay standardization by technical modifications, another alternative would be the adoption of a reliable commercial test. For the past several years, a gel double-diffusion assay has been routinely used in our clinical laboratory for the detection of teichoic acid antibodies. The Diagnostica CIE kit was compared with our assay with sera from patients for whom the teichoic acid antibody test was requested, from other selected patients, and from normal controls.

Although both methods use an antigen prepared from the Lafferty strain of *S. aureus*, the tests differ in that a lysostaphin preparation was used in the double diffusion assay, whereas the Diagnostica kit used an ultrasonic extract. Sheagren et al. (7) found fewer false-negative results with an ultrasonic extract than with a lysostaphin preparation. Our investigation found no appreciable differences between the two tests in the detection of antibodies in patients with *S. aureus* endocarditis or complicated bacteremia. Others (1, 9, 10) also reported similar sensitivity in detecting antibodies in 90 to 100% of patients with *S. aureus* endocarditis and in 50% of patients with complicated bacteremia.

Since the Diagnostica kit uses CIE, its results are available within 1 to 2 h, whereas double diffusion in gel requires at least 18 h. Although more rapid to perform, CIE methods are associated with a greater frequency of false-positive reactions. This problem may be lessened by requiring a positive test to demonstrate a higher titer. This was done in both of the methods we used. A positive serum was required to have a titer of 1:2 or greater in the double-diffusion test and 1:4 or greater with the CIE kit. With these criteria, the percentage of significant antibody titers in 80 individuals with no evidence of *S. aureus* infection was slightly lower for CIE (5.0%) than for double diffusion (8.8%). Other studies show similar percentages (3–5).

In general, the double-diffusion test with the lysostaphin preparation and the CIE kit with an ultrasonic extract gave comparable results among our groups of patients. The more widespread adoption of a commercial kit may help to eliminate the problems associated with the variability of teichoic acid antibody assays.

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**LITERATURE CITED**


