Comparison of Fluorescence Polarization Immunoassay and Bioassay of Vancomycin

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Human serum samples were analyzed for vancomycin concentrations by two different methods: the fluorescence polarization immunoassay and the disk plate bioassay. Each assay method offered acceptable precision. The correlation between both assay methods was excellent (correlation coefficient = 0.985). Excluding technical time, the bioassay was the least expensive method to perform but was more labor intensive than the fluorescence polarization immunoassay.

Serious methicillin-resistant Staphylococcus aureus infections are an increasing problem in major medical centers (2, 4, 17, 19). Staphylococcus epidermidis infections present a growing problem in patients with prosthetic devices or indwelling catheters (5, 13). In addition to staphylococci, the prevalence of other gram-positive bacteria resistant to penicillins and cephalosporins has been documented (3, 11). Concurrently, vancomycin has become an increasingly popular treatment either of these beta-lactam-resistant organisms or for the penicillin-allergic patient. As a consequence, vancomycin has become the largest dollar-volume antimicrobial agent purchased in our pharmacy department.

The incidence of adverse reactions to vancomycin therapy has been documented to include rash, phlebitis, ototoxicity, neutropenia, and nephrotoxicity (7). It has been suggested that serum levels of >80 μg/ml should be avoided to prevent ototoxicity (14). Maintenance of vancomycin concentrations of ≥4.0 μg/ml of body fluid has been correlated with prevention (17) and treatment (1, 6, 8, 15) of staphylococcal infections. Thus, to assure therapeutic efficacy and to minimize toxicity, monitoring vancomycin serum levels is necessary. The assay of vancomycin may be accomplished by bioassay (21), high-pressure liquid chromatography (16), radioimmunoassay (10), and fluorescence polarization immunoassay (FPIA) (9). This paper compares the bioassay with the FPIA.

MATERIALS AND METHODS

Bioassay. The disk plate bioassay was performed by the method of Sabath et al. (18). Antibiotic medium no. 5 (Difco Laboratories, Detroit, Mich.) was used as the seed agar. Agar was inoculated with a uniform spore suspension of Bacillus subtilis ATCC 6633 (BBL Microbiology Systems, Cockeysville, Md.) at a ratio of 2 ml of spore suspension to 1,000 ml of molten seed agar. A standard curve was prepared with pooled human serum (GIBCO Diagnostics, Madison, Wis.) and included concentrations of 10, 20, 30, and 40 μg of vancomycin per ml. In addition, the interassay and intraassay control of 30 μg of vancomycin per ml was prepared. All standards, controls, and unknowns (patient serum samples) were run in quadruplicate. Assays were incubated at 37°C for 6 h. Zones of inhibition were read on a Fisher-Lilly antibiotic zone reader (Fisher Scientific Co., Pittsburgh, Pa.). To eliminate bioassay outliers, peak serum specimens were run undiluted and diluted (1:2). Diluted sample results were multiplied by a factor of two.

FPIA. The principles of FPIA have been described previously (12). The FPIA was performed with an automated fluorescence polarization analyzer (TDx) manufactured by Abbott Laboratories, North Chicago, Ill. Commercial reagents, calibrators, and controls were also obtained from Abbott. Calibrators used to standardize the TDx were at concentrations of 0, 5.0, 10.0, 25.0, 50.0, and 100.0 μg/ml. The controls were 7.0, 35.0, and 75.0 μg/ml. All calibrators, controls, and patient sera were run in duplicate.

Specimens. All patient serum samples were obtained from patients receiving a single antibiotic regimen of vancomycin. Samples were assayed by both methods on the day they were drawn from the patient.

Data analysis. All data were computer analyzed with the Radio Shack advanced statistical analysis package which includes both descriptive statistics and correlation and linear regression.

RESULTS

Intrabioassay precision was determined by running the 30-μg/ml control 15 times within a single assay. Interbioassay precision was determined by running the 30-μg/ml control 15 times during 1 month.

TDx intraassay precision was determined by running high-, medium-, and low-concentration patient serum samples 5 times each within a single assay. TDx interassay precision was determined by running the high, medium, and low kit controls 5 times each during 1 month.

Descriptive precision statistics are given in Table 1. With the bioassay, the intraassay precision had a variance of 1.1 and a coefficient of variation (CV) of 3.34. Bioassay interassay precision gave a variance of 0.79 and a CV of 3.1.

FPIA intraassay precision gave a variance of 0.04 and a CV of 1.9 for the low-concentration sample, a variance of 0.03 and a CV of 0.9 for the medium-concentration sample, and a variance of 0.47 and a CV of 1.8 for the high-concentration sample.

Besides the assay controls detailed in Table 1, additional controls were run within each assay. The bioassay control of 30 μg/ml was consistently within an acceptable CV of ±15% of the stated concentration. The FPIA high, medium, and low controls did not consistently fall within an expected CV of ±14% for the 7.0-μg/ml control and a CV of ±10% for the 35.0- and 75.0-μg/ml controls. Unacceptable control values necessitated curve recalibration.

On each day of bioassay, a new standard curve was run.

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TABLE 1. Precision of the bioassay and the FPIA in measuring control sera

<table>
<thead>
<tr>
<th>Assay</th>
<th>No. of runs</th>
<th>Target value</th>
<th>Sample statistics</th>
<th>Unbiased estimate of population parameters</th>
<th>Data distribution coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>M</td>
<td>V</td>
<td>SD</td>
</tr>
<tr>
<td>FPIA intra assay</td>
<td>5</td>
<td>Low</td>
<td>11.2</td>
<td>0.04</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Medium</td>
<td>21.6</td>
<td>0.03</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>High</td>
<td>38.4</td>
<td>0.47</td>
<td>0.69</td>
</tr>
<tr>
<td>FPIA inter assay</td>
<td>5</td>
<td>Low</td>
<td>7.0</td>
<td>0.08</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Medium</td>
<td>34.1</td>
<td>0.90</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>High</td>
<td>73.8</td>
<td>6.4</td>
<td>2.5</td>
</tr>
<tr>
<td>Bioassay intra assay</td>
<td>15</td>
<td>30</td>
<td>30.1</td>
<td>1.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Bioassay inter assay</td>
<td>15</td>
<td>30</td>
<td>29.2</td>
<td>0.79</td>
<td>0.89</td>
</tr>
</tbody>
</table>

M. Mean; V. variance; SD. standard deviation; CV. coefficient of variation.
S. Skewness; K. kurtosis.

On day 1, a new TDx curve was calibrated. When TDx control values were unacceptable, a new standard curve was calibrated. TDx curve re calibration was necessary from at least once every 24 h to once every 28 days, with an average calibration curve lasting 14 days.

**Assay correlation.** The patient serum sample concentrations were used to determine correlation, linear regression, and descriptive statistics (Fig. 1). Sixty-three serum samples were assayed in both the bioassay and the FPIA. The two methods correlated well (correlation coefficient = 0.985). The mean and standard deviation of the FPIA method were, respectively, 20.09 and 14.88. The mean and standard deviation of the bioassay were, respectively, 20.14 and 14.90. The slope of the regression line is 0.933. Our patient sampling did not yield any samples > 80 µg/ml. If a laboratory expects serum vancomycin values > 80 µg/ml, a 1:4 rather than a 1:2 dilution should be employed with the bioassay to maintain the excellent correlation our studies provided.

**DISCUSSION**

Because of the increased use of vancomycin in the treatment of severe gram-positive bacteria infections, the assay of vancomycin is a mandatory measure to ensure not only clinically efficacious serum levels but also avoidance of toxic serum levels. Also, in light of the new government guidelines on diagnostic related groups, hospitals are increasingly aware of the necessity of a satisfactory balance between clinical efficacy and cost-effective procedures.

Bioassay results may be obtained in as little as 4 h. However, our results are based on a 6-h incubation, which resulted in zones that were easier to read. Incubation time may be extended up to 18 h, if necessary. From a clinical standpoint, the difference between readings at 4 or 18 h is probably not significant.

The initial capital outlay for the bioassay is minimal (agar, spore suspension, paper disks, petri dishes, a 20-lambda pipette, and a zone reader). Agar plates may be prepared and then stored at 4 to 8°C for up to 6 weeks. Standard curve samples and controls may also be prepared and then stored at -70°C for up to 6 months. The average technical time to set up one standard curve, one control, and one patient serum sample is ca. 20 min, with an additional 20 min to read and calculate the results. Each additional patient sample requires ca. 3 min of technical time.

The FPIA TDx assay is a completely automated system. Results may be obtained within 15 min after standard curve calibration. The initial capital outlay is high. The TDx analyzer retails for ca. $44,700. The cost for additional supplies (sample and reagent cups, a 50-lambda pipette, and buffer) is minimal; however, the full retail price of the vancomycin reagent pack is $550.00 (100 tests per pack), the calibrators are $15.00 per kit, and the controls are $7.50 per kit. The average technical time to set up one calibration curve, one control, and one patient serum sample is ca. 5 min. Each additional patient sample requires <1 min of technical set-up time.

FIG. 1. Correlation of bioassay and the FPIA.
To a small, low-volume laboratory on a limited budget, the bioassay offers acceptable precision, minimal financial outlay, and minimal daily operating costs. Same-day results may be obtained if the specimen is processed early in the working day. One disadvantage with the vancomycin bioassay is the technical time required for each assay. Although capital outlay for the bioassay is small, the additional time required to make the agar plates and read the assay constitutes an additional cost. There is no doubt that the cost of the TDx assay in terms of material is greater, but there are additional hidden costs in the bioassay which may make the discrepancy less significant. A second disadvantage concerns the serum sample which contains multiple antimicrobial agents; however, with minor assay modifications (18, 20, 22), the presence of penicillins, cephalosporins, aminoglycosides, and rifampin will not prevent the accurate bioassay of vancomycin.

Besides vancomycin, the TDx analyzes a wide range of tests. Large-volume laboratories or laboratories which may share or amortize TDx analyzer expense (or both) may find the FPIA financially acceptable. However, the kit cost per test remains high. Besides full automation, the major advantage with the FPIA is rapid turnaround time and the ability to assay vancomycin in the presence of other antimicrobial agents.

The bioassay and the FPIA offer accurate and precise vancomycin determinations. Initial capital outlay, daily cost analysis, and technical time are also factors which should be examined and personalized for each individual laboratory.

LITERATURE CITED