Comparison of Enzyme-Multiplied Immunoassay Technique with Fluorescence Polarization Immunoassay for Determination of Gentamicin and Tobramycin Levels in Serum

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We assayed serum gentamicin and tobramycin specimens by the enzyme multiplied immunoassay technique (Syva EMIT) and the fluorescence polarization immunoassay (Abbott TDx). When interassay and intraassay control samples were evaluated, both methods gave an overall coefficient of variation of less than ±10%. Using patient serum samples, we obtained excellent correlation with both methods in the assay of gentamicin (correlation coefficient, 0.985) and tobramycin (correlation coefficient, 0.982).

MATERIALS AND METHODS

EMIT. The Syva EMIT method for gentamicin and tobramycin assay utilizes a 100-test kit containing reagents and calibrators (Syva, Palo Alto, Calif.). Kit controls are purchased separately from Syva. Three modules comprise the fully automated instrumentation: the autocarousel, which dilutes samples and sequentially adds reagents; the Gilford Stasar III spectrophotometer, which aspires the contents of the autocarousel reaction cup into a spectrophotometric flow cell; and the LP-6000 microprocessor, which reduces data input originating from the Gilford Stasar (Syva).

FPIA. The Abbott TDx assay method for gentamicin and tobramycin utilizes a 100-test reagent kit, a calibration kit, and a control kit. The fully automated instrumentation is provided in one self-contained unit which dilutes specimens, adds reagents reads reactions by means of a fluorescence polarization analyzer, and reduces data. Abbott Laboratories, North Chicago, Ill., manufactures the TDx kit and instrumentation.

Specimens. We obtained clinical serum specimens from patients who were receiving either gentamicin or tobramycin. Serum samples were assayed by both methods on the day they were drawn from the patient. All clinical specimens, calibrators, and controls were run in duplicate. All data represent means of duplicate tests.

Statistical analysis. Data were analyzed by the Radio Shack Advanced Statistical Analysis package, which includes descriptive statistics, correlation, and linear regression.

RESULTS

We assessed the accuracy and precision of both methods by using spiked serum controls containing 1.0, 5.0, or 10.0 μg of either gentamicin or tobramycin per ml. Tables 1 and 2 illustrate the high degree of precision and accuracy we obtained with the EMIT and FPIA for both aminoglycosides.

Interassay controls were assayed over a period of 30 days. Syva and Abbott claim a day-to-day coefficient of variation (CV) of ±10% or less. Our interassay results were within these guidelines for all EMIT and FPIA controls except for the 1.0-μg/ml gentamicin control, which had coefficients of variation of 13.2 (EMIT) and 13.7 (FPIA).

We assayed intraassay controls within a single gentamicin or tobramycin run. The intraassay CVs were within the manufacturers’ guidelines (CV, ±10%) except for the 1.0-μg/ml tobramycin control, which had an EMIT CV of 10.3%.

The correlation between the EMIT and the FPIA was excellent (Fig. 1 and 2). We assayed 77 serum specimens obtained from patients receiving tobramycin. These serum specimens ranged from ca. 1.0 to 12.0 μg/ml in tobramycin content. Comparing the two methods, we found a correlation coefficient of 0.982, a standard deviation of 2.67 (EMIT) and 2.37 (FPIA), and a regression line slope of 0.873. The mean tobramycin serum values were 5.75 (EMIT) and 5.24 (FPIA) μg/ml. We assayed 71 serum specimens obtained from patients receiving gentamicin. The serum samples ranged from ca. 1.0 to 13.0 μg/ml in gentamicin content. Comparing the two methods, we found a correlation coefficient of 0.985, a standard deviation of 2.76 (EMIT) and 2.44 (FPIA), and a regression line slope of 0.870. The mean gentamicin serum values were 5.75 (EMIT) and 5.24 (FPIA) μg/ml.

DISCUSSION

Between 1969 and 1983, the aminoglycoside specimen load in our laboratory increased from 350 to 11,000 requests per year. The bioassay, RIA, and HPLC techniques have provided our laboratory with accurate serum concentration.

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Each instrument and corresponding aminoglycoside method had acceptable standard curve stability. With either method, curve recalibration was necessary once every 2 to 4 weeks. Rarely, we encountered calibration curves that lasted under 24 h on each instrument.

With the exception of the 1.0-μg/ml gentamicin and tobramycin control samples, our CVs were acceptable for each method and aminoglycoside. Witebsky et al. (8) also observed elevated coefficients of variation with low-concentration gentamicin samples (mean, 1.3 μg/ml). We offer no explanation for this phenomenon. However, a coefficient of variation of 13 to 14% at the 1.0-μg/ml level is not considered clinically significant.

We could not assess the effect of additional antimicrobial agents on the precision and accuracy of assaying for gentamicin or tobramycin in patient serum samples. The product literature of Syva and Abbott states that the presence of

data. However, as our specimen loan increased yearly, each of these methods became too labor intensive to handle a large daily specimen volume efficiently. In addition, each method had its own disadvantages. The bioassay had lengthy preparation and turnaround times. RIA required strict iso-
tope handling regulations and time-consuming waste dispos-
al, whereas HPLC required lengthy technical time as well as handling of toxic volatile sample preparation reagents.

The Syva EMIT and the Abbott TDx methods are precise, reproducible, and fully automated. Each has a standard curve range that encompasses most of the serum concentrations found clinically. The concentration range for EMIT standard curve calibrators is 1.0 to 16.0 μg/ml for both gentamicin and tobramycin; the FPIA range is 1.0 to 10.0 μg/ml. Clinical specimens whose concentrations exceed upper curve limits are diluted in pooled human serum, and final results are multiplied by the dilution factor.

<table>
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<tr>
<th>Assay</th>
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<th>Target value</th>
<th>Sample statistics</th>
<th>Unbiased estimate of population parameters</th>
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* Abbreviations are the same as in Table 1.
additional antimicrobial agents such as amikacin, carbenicillin, cephalothin, chloramphenicol, clindamycin, erythromycin, kanamycin, neomycin B, penicillin G, streptomycin, sulfanilamide, and tetracycline will not interfere with the assay. Results from the study of Ngui-Yen et al. Agree with these findings (5).

Initial capital outlay for both instruments is high. The Abbott TDx analyzer retails for ca. $44,750.00, and the Syva AutoLab 6000 is ca. $19,850.00. The price of a TDx gentamicin or tobramycin 100-test reagent pack (includes disposable cartridges and cuvettes) is $272.00. The calibrators are $50.00 per kit. The Syva 100-test reagent pack (includes calibrators) costs $200.00. Syva disposables cost $2.20 per 100 tests. The average technical time to set up one calibration curve, one control, and one patient serum sample is approximately the same for both systems.

The Syva EMIT and Abbott TDx methods provide accurate, precise, and fully automated aminoglycoside measurements. Both instruments are particularly well suited to high-volume laboratories, but low-volume laboratories may also find them acceptable.

LITERATURE CITED