Assay of Netilmicin, Using Enzyme Immunoassay for Gentamicin

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A homogenous enzyme immunoassay for the quantitative determination of netilmicin in serum was developed. The procedure utilizes a commercially available assay for gentamicin (EMIT; Syva Co., Palo Alto, Calif.). The method was adapted to a microcentrifugal analyzer, and log-log regression analysis was performed with a computer. The results of samples assayed by this method correlate well with microbioassay ($r^2 = 0.985$) and radioimmunoassay ($r^2 = 0.986$). This method is not only precise and accurate, but also very rapid and economical and compares favorably to other available methods of netilmicin assay.

Netilmicin is a new semisynthetic derivative of sisomicin. In vitro studies have shown netilmicin to have a spectrum of activity similar to that of related aminoglycosides. Although netilmicin has been shown to be slightly less active against Pseudomonas aeruginosa in vitro than gentamicin or tobramycin, some strains of Enterobacteriaceae which are resistant to gentamicin and tobramycin are susceptible to netilmicin (3, 8, 10, 12, 16). In laboratory animals, netilmicin has demonstrated a lower potential for nephrotoxicity than has gentamicin (4, 9). Although less variability in serum netilmicin concentration per dose has been suggested, judicious monitoring of blood concentration of netilmicin is likely to be important clinically (14).

Utilization of cross-reacting antibodies to assay multiple antibiotics has been previously reported by Broughton et al. (1). We report an adaptation of a homogenous enzyme immunoassay (EIA) for gentamicin for the determination of netilmicin concentrations in human serum. Although the commercial EIA was developed to assay gentamicin, sufficient netilmicin cross-reactivity occurs to allow its use for the assay of netilmicin. When adapted to the microcentrifugal analyzer (Instrumentation Laboratory, Spokane, Wash.), this assay has proven to be rapid, accurate, and precise and compares favorably with microbioassay and radioimmunoassay.

MATERIALS AND METHODS

Netilmicin sulfate powder and injectable netilmicin sulfate were obtained by Schering Corp. (Bloomfield, N.J.), EMIT-amd gentamicin kits were obtained from Syva Co. (Palo Alto, Calif.), antibiotic medium no. 11 was from Difco Laboratories (Detroit, Mich.), and the netilmicin radioimmunoassay kit was from the American Diagnostics Corp. (Newport Beach, Calif.).

Standards and sample preparation. Serum was collected and pooled from 10 healthy volunteers receiving no drugs. Netilmicin standards for the microbioassay and the EIA were prepared by the addition of netilmicin sulfate powder to pooled serum. Standard concentrations used were 0.0, 0.5, 1.0, 2.0, 4.0, and 8.0 μg/ml. For the radioimmunoassay, the manufacturers’ standards were employed. Serum samples for determination of accuracy were prepared from 1-ml samples of pooled serum spiked with netilmicin sulfate powder. Twenty-one serum samples for intermethod comparison were obtained by venipuncture from 10 patients receiving netilmicin for infection. Patient serum was stored at −80°C until assayed. All patient samples were assayed on the same day by microbioassay, radioimmunoassay, and EIA. Serum samples for between-day and within-day precision determinations were spiked with netilmicin sulfate powder to yield concentrations of approximately 1.5, 3.4, and 8.0 μg/ml.

Microbioassay and radioimmunoassay. Biological assays of netilmicin were performed by the cup plate technique (6) with Klebsiella pneumoniae American Type Culture Collection (ATCC) 1296 as the test organism in antibiotic medium no. 11. Each sample was assayed in triplicate. The radioimmunoassay was performed with the American Diagnostics kit as described by the manufacturer. Each sample was run in duplicate.

EIA procedure. The EIA for netilmicin was adapted from a previously described EMIT-amd assay for gentamicin on the Multitstat III microcentrifugal analyzer (7). Reagent A, reagent B, and buffer were prepared as described by the manufacturer. Working reagents A and B were prepared by diluting 1 ml of A with 9.4 ml of buffer and 1 ml of B with 4.3 ml of buffer. The sample volume was 6 μl, and the working reagent A and B volumes used were 187 and 93 μl, respectively. Working reagent and sample volumes

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samples of different concentrations were run 10 times 1 day and in duplicate on 10 consecutive days. The mean concentration ± standard deviation and coefficient of variation of 10 samples in the low, medium, and high therapeutic ranges for between-day and within-day precision is shown in Table 1.

The EIA was found to accurately predict netilmicin concentrations up to 8 µg/ml. The line of regression, comparing known concentrations to EIA values, was described by: \( y = 1.05x + 0.1 \). The correlation coefficient \( (r) \) was 0.988.

The comparison of our method with the bioassay, as determined by linear regression analysis, is depicted in Fig. 2. The line of regression is described by: \( y = 1.01x - 0.02 \), with an \( r^2 \) value of 0.985. We found no significant difference between these two methods.

Likewise, Fig. 3 depicts the comparison of our method to the radioimmunoassay. The line of regression is described by: \( y = 0.78x + 0.42 \) with an \( r^2 \) value of 0.986. A slight tendency toward overestimation of serum netilmicin concentrations was observed for the radioimmunoassay when netilmicin concentrations were above 2 µg/ml. This tendency accounts for the low slope of the regression line (\( m = 0.78 \)).

**RESULTS**

As expected, preliminary work with this method indicated that the EIA antibody has lower affinity for netilmicin than for gentamicin. Therefore, a given netilmicin concentration will produce less glucose 6-phosphate dehydrogenase activity than would an equivalent concentration of gentamicin. Figure 1 depicts just how this problem was overcome. The manufacturer suggests taking absorbance readings at 15 and 45 s. The bottom curve of Fig. 1 shows that the difference in \( \Delta A \) for different netilmicin concentrations is small, which results in poor precision. However, when the second reading is taken at 120 s, as in the top curve of Fig. 1, \( \Delta A \) differences are greater and precision is improved. Precision is poor when concentrations are greater than 8 µg/ml (coefficient of variation, \( \geq 10\% \)). Because of this, samples with concentrations above 8 µg/ml were diluted and reassayed. To assess reproducibility and precision, three

![Absorbance Curve](http://aac.asm.org/Downloaded from http://aac.asm.org)
short period of time. Recent studies comparing five methods used for gentamicin assay found the EIA to perform well. Correlation between methods was satisfactory; however, when simplicity, speed, cost, and ease of automation were reviewed, the EIA was found to be the preferred method (13, 15).

Although designed to assay gentamicin, our method can be used for netilmicin, which allows purchase of one assay for two antibiotics and thus reduces cost to medical laboratories. When the EIA is run on the microcentrifugal analyzer only one-third of the reagents used in the manual method are consumed, further reducing the cost. This allows 330 assays to be run from one kit as compared with the 100 suggested by the manufacturer. The efficiency of this method becomes apparent when the work load is heavy. One person can comfortably run up to 100 assays per day.

h. Netilmicin assay as performed with adaptation of the gentamicin EIA in this study was not only accurate and precise, but very economical in cost and time. For laboratories and hospitals possessing a microcentrifugal analyzer, we believe this method is preferable to other available assay methods for monitoring patient netilmicin concentrations.

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