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NOTES

Serum Aminoglycoside Assay by Enzyme-Mediated Immunoassay (EMIT): Correlation with Radioimmunoassay, Fluoroimmunoassay, and Acetyltransferase and Microbiological Assays

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Enzyme-mediated immunoassay (EMIT) serum aminoglycoside assay results were accurate and precise and correlated well with radioimmunoassay, fluoroimmunoassay, and acetyltransferase and microbiological assay determinations.

The aminoglycosides gentamicin and tobramycin are commonly used for therapy of serious bacterial infections. However, since there is only a narrow margin between effective and toxic blood concentrations (8), it is necessary to monitor serum levels. Assay methods include microbiological assays (2), enzymatic assays (3, 11), radioimmunoassay (RIA) (4), fluoroimmunoassay (5, 13), enzyme-mediated immunoassay (6, 12, 14), hemagglutination inhibition (7), and chromatographic (1, 9) methods.

Homogeneous EMIT tests (Syva Corp., Palo Alto, Calif.) are used extensively for drug monitoring, having the advantages of extreme speed, technical simplicity, and high specificity, and EMIT gentamicin and tobramycin assays are now available. In EMIT, enzyme-labeled drug competes with free drug in the test sample for antidrug antibodies. Typically, when the enzyme-labeled drug binds to antibody, the enzyme's activity is reduced; thus, enzyme activity correlates with the concentration of drug in the test sample. The enzyme label is usually glucose 6-phosphate dehydrogenase from *Leuconostoc mesenteroides* since its activity may be simply determined spectrophotometrically. We have compared EMIT with the microbiological assay, acetyltransferase assay, RIA, and fluoroimmunoassay.

All assays were done with five or six calibrators (0, 1, 2, 4, 8, and 16 μg/ml) prepared in pooled human serum, except for the quenching fluoroimmunoassay, for which the top calibrator was 12 μg/ml, and most EMIT assays, for which the 16-μg/ml calibrator was excluded (see below). The microbiological assay was a large-plate method, with *Klebsiella edwardsii* subsp. *atlantae* (NCTC 10896) as the indicator organism (13). The acetyltransferase assay was performed as described by Broughall and Reeves (3). [^{125}I]gentamicin RIA kits (Diagnostic Products Corp., Los Angeles, Calif.), quenching fluoroimmunoassay kits (Technia Diagnostics Limited, London, England), and substrate-labeled fluoroimmunoassay kits (Ames Laboratories, Elkhart, Ind.) were used according to the manufacturers' instructions. EMIT-AMD kits (lot J03; Syva) were used. Manual assays were performed according to the manufacturers' instructions in a Gilford Stasar III linked to a CP5000 clinical processor (Syva). Samples and reagents were simultaneously diluted and dispensed into autoanalyzer cups (Syva) with a pipettor diluter (Syva model 1500). Standard curves were plotted manually on the graph paper supplied with the kits.

Preliminary studies with EMIT indicated that some autoanalyzer cups (from suppliers other than Syva) gave poor precision, as did any protocol which allowed insufficient time for the first dilutions of sample to equilibrate. All EMIT results reported here were obtained by using Syva cups and a protocol allowing first dilutions of at least 1-min equilibration. The 16-μg/ml calibrator, both neat and diluted 1:6, and the 2-μg/ml calibrator were assayed 12 times (six duplicates). Coefficients of variation were 11.28, 5.4, and 3.5%, respectively. The results for the 2-μg/ml and 16-μg/ml (assayed diluted) calibrators were accurate (2 ± 0.07 and 15.7 ± 0.85 μg/ml). In contrast, the mean result for the 16-μg/ml standard, assayed undiluted, was only 12.4 ± 1.4

μg/ml. At between 10 and 16 μg/ml a large increase in aminoglycoside concentration produces only a small increase in enzyme activity; we therefore decided to exclude the 16-μg/ml calibrator. When a sample containing >8 μg/ml was encountered, it was immediately diluted to bring it within the range of 2 to 8 μg/ml and reassayed.

To check accuracy, six samples from the United Kingdom gentamicin assay quality control circulation and three gentamicin quality control samples from the United States were assayed. EMIT results showed a mean error of only −3.02% (standard deviation, 3.92).

Sera spiked with tobramycin, amikacin, streptomycin, spectinomycin, and netilmicin (1 to 40 μg/ml) were assayed to check EMIT specificity. Only netilmicin-containing sera cross-reacted and gave between 70 and 40% of the response of gentamicin.

Fifty-two clinical serum samples were assayed for gentamicin (when possible) by EMIT, microbiological assay, RIA, fluoroimmunoassay, and acetyltransferase assay: 40 of these were assayed by all methods. Correlation coefficients and regression curves are shown in Fig. 1. The results of all assays correlated well with EMIT results. The means (micrograms per milliliter) of the 40 samples assayed by all methods were as follows: EMIT, 4.38; microbiological assay, 4.39; radioimmunoassay, 4.40; fluoroimmunoassay, 3.91; and acetyltransferase, 4.73.

In a more limited study of the tobramycin assay kit, we found that there was no cross-reaction with gentamicin, and with 10 clinical specimens containing tobramycin we obtained a correlation coefficient of 0.95 between EMIT and plate assay results and one of 0.99 between EMIT and substrate-labeled fluoroimmunoassay results.

Standard curves for gentamicin or tobramycin generated over a period of 20 days from single kits showed excellent reproducibility; the only value which changed significantly was the absorbance reading of the zero calibrator(i.e., the blank), which gradually increased over the period.

Aminoglycoside assay by EMIT proved to be rapid; to construct a standard curve and assay a pair of samples in duplicate took <15 min. This compared favorably with up to 18 h for microbiological assays (2) and 45 to 90 min for fluoroimmunoassay (13), RIA and acetyltransferase assay (3). Such speed has distinct advantages. It makes possible the rapid assay of samples arriving at any time of the day or night, and it allows

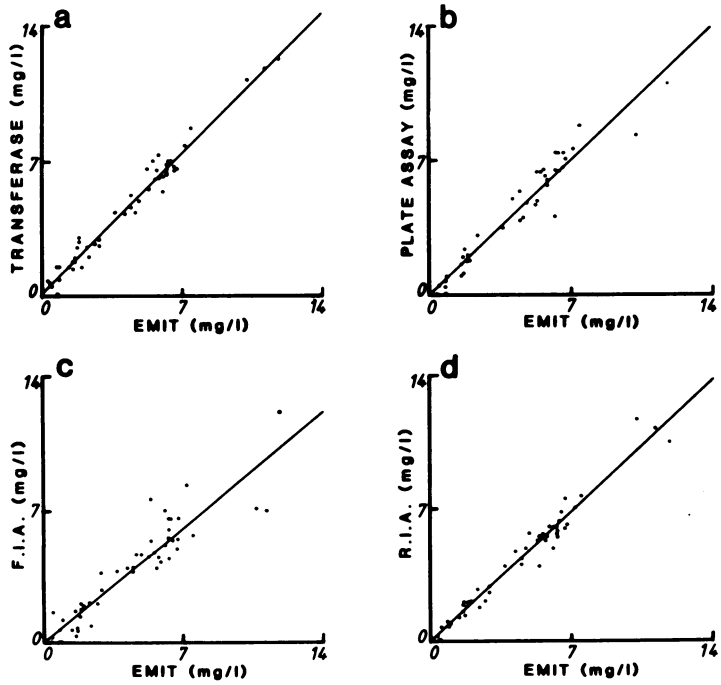

FIG. 1. *Comparison of gentamicin determination in serum by EMIT and by (a) acetyltransferase assay, (b) microbiological plate assay, (c) quenching fluoroimmunoassay (F.I.A.), and (d) RIA. Correlation coefficients: (a) 0.99; (b) 0.98; (c) 0.93; (d) 0.96.*

more time for the samples to travel from patient to laboratory; thus, samples from other hospitals can be assayed and reported within minutes of receipt, and skilled personnel have more time to devote to other tasks.

EMIT, as performed in this study, is technically very simple, but our results indicate that a protocol allowing first dilutions to equilibrate before second dilutions are made should be used and the precision-performance of autoanalyzer cups other than those recommended by Syva should be checked. With optimal protocol and cups we obtained coefficients of variation of around 5%. The makers claim coefficients of variation of <10%. Because dilution and reassay are rapid by EMIT, we limited our top calibrator to 8 μg/ml and diluted and reassayed all samples of >8 μg/ml. The extra precision thus obtained has less importance clinically than with lower values, but it is necessary when standard solutions are assayed or pharmacokinetic data are being obtained.

When British and American gentamicin quality control samples were assayed, EMIT discrepancies were always slight underestimates, but the significance of this is unclear since the mean results from other laboratories assaying these samples by other methods were similarly slight underestimates of the assigned values (unpublished data).

We found good correlation between the results of EMIT and those of microbiological assay, acetyltransferase assay, RIA, and fluoroimmunoassay. The mean acetyltransferase result was about 10% higher and the mean fluoroimmunoassay result was about 10% lower, but these discrepancies are probably of little significance for clinical assays. Sanders et al. (10) found a similar good correlation between EMIT and RIA but a poorer correlation between EMIT and a microbiological assay when a gram-positive indicator organism was used.

With the gentamicin assay reagents there was significant cross-reaction with netilmicin. Since netilmicin and gentamicin are almost never coprescribed, this should cause no problems in practice; in fact, netilmicin can be assayed with EMIT gentamicin reagents and netilmicin calibrators (unpublished data).

We conclude that, when technical simplicity, speed, versatility, and high specificity are required, EMIT seems to be an excellent choice for gentamicin or tobramycin assay.

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