Evaluation of an Enzyme Immunoassay for Serum Gentamicin

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The EMIT (Enzyme Multiplied Immunoassay Technique) for serum gentamicin determination was evaluated by a standard procedure. The precision, accuracy, and specificity were assessed and proved satisfactory. Comparison with a bioassay was done, and results for patient samples showed a correlation coefficient of 0.95 between the two methods. Advantages of the enzyme immunoassay system were the provision of results within 15 min of receipt of blood in the laboratory, a requirement of minimal technical expertise, and an applicability to both large and small workloads. The EMIT proved during evaluation to be a practical alternative to current bioassays for the determination of serum gentamicin concentrations.

Gentamicin is an important broad-spectrum antibiotic, but it possesses a significant potential for toxicity. Due to the relatively small difference between therapeutic and toxic levels, serum assays are necessary for good patient management (9).

Microbiological assays (4), although shown to be slow, inaccurate, and lacking in specificity, have been the most frequently used assay systems for the determination of serum gentamicin concentrations (9, 10).

Several alternative methods, viz. adenylation and acetylation (3, 12), radioimmunoassay (7), fluorescence quenching (1), and high-pressure liquid chromatography, have been described (2). Although these assays lead to increased specificity and accuracy, several drawbacks, such as the problem of the disposal of radioisotopes and the requirement for special technical skills, detract from their advantages (8).

The EMIT (Enzyme Multiplied Immunoassay Technique) methodology first introduced by Syva in 1974 has been used for several years for the determination of the antiepileptic drugs phenytoin, carbamazepine, and phenobarbitone (11). In brief, the technique depends upon the direct competition between a drug in the patient's serum and the same drug covalently bound to an enzyme for sites on an antibody directed against the drug. When enzyme-labeled drug is bound to the antibody, its activity is greatly reduced, and the amount so bound is a function of the concentration of drug in the patient's serum. Thus, the enzyme activity remaining in the test system is likewise related to the serum drug concentration. The latter is determined by comparing the enzyme activity with that produced by a series of standard solutions of the same drug.

In this evaluation the accuracy, reproducibility, and specificity of an enzyme immunoassay for gentamicin were studied. Results for patient samples were compared with those obtained for a microbiological assay. The stability of gentamicin in serum in 4 and −20°C was also assessed.

MATERIALS AND METHODS

Enzyme immunoassay. Each kit of gentamicin enzyme immunoassay reagents (Syva) consists of three vials. The first vial contains a buffer solution containing 55 mmol of Tris-hydrochloride buffer per liter at pH 7.6 and surfactant. The second vial (reagent A) contains lyophilized antibody and the enzyme substrates glucose-6-phosphate and nicotinamide adenine dinucleotide. The third vial (reagent B) contains lyophilized glucose-6-phosphate dehydrogenase coupled to the gentamicin molecule. The reagents and calibrators were prepared by the manufacturer's instructions. The test procedure requires the initial dilution of 50 µl of serum with 250 µl of the buffer and a second dilution of 50 µl of this solution with a further 250 µl of buffer. All dilutions were carried out with a pipettor dilutor (Cavro Scientific Instruments, Sunnyvale, Calif.) supplied under the brand name Syva.

Reagent A (250 µl) and reagent B (250 µl) are then added. The mixture is aspirated into a spectrophotometer with the flow cell maintained at 30°C (Gilford Stasar III; Gilford Instrument Laboratories, Inc., Oberlin, Ohio). The rate of change in optical density at 340 nm is determined by measurements taken at 15 and 45 s after aspiration. A standard curve is prepared by subtracting the change in optical density obtained for the blank from that obtained for the standards, 1, 2, 4, 8, and 16 mg of gentamicin per liter, with the absorbance differences being plotted on log-linear paper. All standards and samples were assayed in duplicate. Values for patient specimens were obtained by reading corrected optical densities from the standard curve.

Bioassay. The microbiological assay is standard

large-plate bioassay, the susceptible organism being *Bacillus subtilus*. Beta-lactamase (300 levy U/ml) is added to the agar. Samples and five standards are loaded in quadruplicate with random distribution.

Incubation time is either 4 or 16 h. The assay used is affected by the presence of erythromycin, clindamycin, co-trimoxazole, and cefoxitin. Patient samples for this study were randomly selected from routine samples submitted for assay. Quality control samples were prepared by adding gentamicin to drug-free serum.

RESULTS

Reproducibility. Between-batch reproducibility was assessed by analyzing control materials containing gentamicin at three different concentrations 15 times over a period of two months. The control materials were also analyzed 10 times within the same run to provide within-batch reproducibility data. The results are presented in Table 1 and 2. During the evaluation, 15 standard curves were generated. The reproducibility of the standard curve was assessed by plotting a mean standard curve from the data and deriving apparent values that would have been obtained for the 2-, 4-, and 8-mg/liter standards. The coefficient of variation for the standard values was 3.7% at 2 mg/liter, 2.8% at 4 mg/liter, and 3.5% at 8 mg/liter.

Accuracy. Gentamicin was added to drug-free serum to obtain concentrations of 1, 2.5, 5, and 10 mg/liter and was analyzed on three different occasions. There was no significant variation among any of the concentrations.

Specificity. Interference in the assay was assessed by adding to serum known amounts of a wide range of antibiotics and determining if any apparent "gentamicin" levels were obtained. The antibiotics kanamycin, streptomycin, ampicillin, clindamycin, tobramycin, sulfamethoxazole/trimethoprim, neomycin, and amikacin were added to the serum at concentrations at least double the normal therapeutic concentrations. No apparent "gentamicin" was detected.

For investigating whether carbenicillin and ticarcillin have any degradative effect on the gentamicin molecule that is detectable by this method, carbenicillin and ticarcillin were added separately at a concentration of 250 mg/liter to serum containing 5.5 mg of gentamicin per liter and assayed at 15 min and 48 h. Both antibiotics caused detectable degradation (20% for carbenicillin and 25% for ticarcillin) between the two times.

Stability. A total of 10 serum samples were analyzed as soon as possible after collection. Five of these were stored at 4°C and analyzed on the following 4 days. Samples of the other five were stored at −20°C and analyzed on three separate occasions. The results obtained under both storage conditions demonstrate that no significant difference was found.

Patient comparison. A total of 80 patient specimens were assayed by both the bioassay method and the enzyme immunoassay technique, with the results and statistical analyses being shown in Fig. 1.
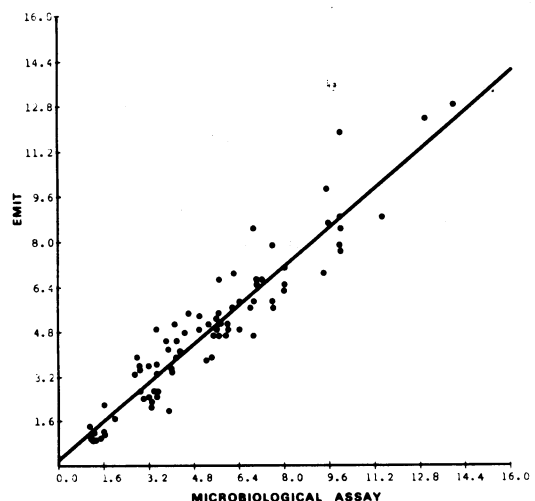
TABLE 1. *Results for between-batch reproducibility experiments*

Gentamicin concn	Parameter[a]			
	n	$\bar{x}$ (mg/liter)	SD	CV
Low	16	2.6	0.09	3.4
Medium	14	5.7	0.22	3.9
High	15	10.2	0.27	2.7

[a] $\bar{x}$, Mean; SD, standard deviation; CV, coefficient of variation.

TABLE 2. *Results for within-batch reproducibility experiments*

Gentamicin concn	Parameter[a]		
	n	$\bar{x}$ (mg/liter)	SD	CV
Low	10	2.6	0.08	3.14
Medium	10	5.7	0.13	2.23
High	10	10.2	0.17	1.7

[a] See footnote a, Table 1.

FIG. 1. *Patient comparison between levels (mg/liter) of gentamicin obtained with the enzyme immunoassay (EMIT) and the microbiological bioassay, with a correlation coefficient of 0.95, a slope of 0.87, and an intercept of 0.35.*

DISCUSSION

The evaluation demonstrates that the reproducibility, accuracy, and specificity of the enzyme immunoassay technique are good. The precision of the standard curve is such that, once prepared in the morning, patient results can be calculated from it throughout the day, provided that the same lot of reagents is used. The specificity studies indicate that the assay system is free from interference from other antibiotics likely to be administered concomitantly with gentamicin. The manufacturers state that the antibiotics netilmicin and sisomicin can cross react. However, neither of these antibiotics is likely to be administered concurrently with gentamicin. The results for the experiments with the antibiotics carbenicillin and ticarcillin confirm those described previously (5), in which it was shown that, in vitro, both antibiotics caused a decrease in bioactive gentamicin concentrations. Because the enzyme immunoassay uses an antibody specific to the intact gentamicin molecule, the results indicate that any degradation products of gentamicin produced by carbenicillin and ticarcillin do not appear to interact with the antibody in the assay system. The stability studies at 4 and −20°C demonstrate that the effects of storage, such as the release of serum components, e.g., free fatty acids (6), do not affect the measurement of serum gentamicin concentrations. The patient comparison data with statistical analysis between the bioassay and the EMIT is acceptable.

Over the period of the evaluation, several advantages of the EMIT became apparent. These are the low degree of technical expertise required, the suitability for both large and small work loads, the freedom from interference by antibiotics most likely to be administered concomitantly with gentamicin, the rapidity of results (i.e., within 15 min), and finally, the expediency of this method to the performance of urgent assays.

In conclusion, the enzyme immunoassay technique for gentamicin performed satisfactorily during the period of evaluation. The acceptable precision and accuracy of the assay, combined with the rapid generation of results, make the EMIT a significant improvement over existing microbiological assays.

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