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Laboratory Evaluation of Five Assay Methods for Vancomycin: Bioassay, High-Pressure Liquid Chromatography, Fluorescence Polarization Immunoassay, Radioimmunoassay, and Fluorescence Immunoassay

MICHAEL A. PFALLER,[1,2]† DONALD J. KROGSTAD,[1,2] GEORGE G. GRANICH,[2] AND PATRICK R. MURRAY[1,2]*

Divisions of Laboratory Medicine and Infectious Diseases, Departments of Medicine and Pathology, Washington University School of Medicine,[1] and Barnes Hospital Clinical Microbiology Laboratory,[2] St. Louis, Missouri 63110

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We compared the precision and accuracy of five methods used to measure the concentration of vancomycin in serum: bioassay, high-pressure liquid chromatography, fluorescence polarization immunoassay (FPIA [TDX; Abbott Laboratories, North Chicago, Ill.]), radioimmunoassay (RIA), and fluorescence immunoassay. Based on an analysis of seven standards and of 106 patient samples, all five methods were accurate, and four (bioassay, high-pressure liquid chromatography, FPIA, and RIA) were also precise. The FPIA was the most precise and the fluorescence immunoassay was the least precise of the methods tested; intrarun coefficients of variation for these two methods were 0.9 to 3.0% versus 8.9 to 14.5%, and interrun coefficients of variation were 2.8 to 8.1% versus 12.2 to 16.2%, respectively. The RIA was inconvenient because it required an extra dilution of the specimen being tested and an additional (64 μg/ml) vancomycin standard for specimens with 32 to 64 μg of vancomycin per ml. Based on its rapid turnaround time and the stability of its standard curve, we believe that the FPIA is the best method currently available to quantitate vancomycin in the clinical laboratory.

The use of vancomycin for the treatment of gram-positive infections in seriously ill patients has increased rapidly in recent years (3, 9, 13). This change is largely due to the emergence of methicillin-resistant strains of *Staphylococcus aureus* and *Staphylococcus epidermidis* (3, 4, 9, 16), but it also reflects the increasing prevalence of other penicillin-resistant organisms, such as *Corynebacterium* spp. (12, 19). In addition, vancomycin is often the only effective alternative for the treatment of serious gram-positive infections in patients allergic to both the penicillins and cephalosporins (3, 9).

One of the major reasons for the limited usage of vancomycin in the past was its toxicity. Ototoxicity is dose related and is associated with elevated vancomycin levels of ≥80 μg/ml in serum (2, 6, 9, 11, 13), although a recent report suggests that peak serum levels of 45 to 50 μg/ml may also produce ototoxicity (17). The other known side effects of vancomycin are less severe or less frequent: the "red neck syndrome" caused by histamine release is often confused with allergy to vancomycin and can be prevented with antihistamines (24); reversible neutropenia occurs in ca. 2% of patients (6); and nephrotoxicity is rare unless vancomycin is used in conjunction with an aminoglycoside (6). Although current preparations of the drug are purer than those previously available and appear to be less toxic (6), most workers attempt to avoid serum concentrations of ≥50 to 80 μg/ml because of their ototoxicity and potential nephrotoxicity (9, 11, 13). Although the doses required to achieve serum levels of 15 to 20 μg/ml can be determined by using a nomogram in many cases (11), measurement of vancomycin concentrations in serum is essential to optimize therapy in seriously ill patients with changing renal function (14). Thus, there is a need to monitor serum levels of vancomycin to avoid dose-related toxicity and to assure mean serum levels of 15 to 20 μg/ml for optimal therapy.

The assay techniques available to quantitate vancomycin in serum have included the bioassay (5, 11, 20, 21), high-pressure liquid chromatography (HPLC) (18), and radioimmunoassay (RIA) (5). Recently, two new immunoassays have also become available, one with fluorescence polarization (FPIA [TDX; Abbott Laboratories, North Chicago, Ill.]) (10, 15) and the other with a conventional fluorescence immunoassay (FIA) (American Diagnostics, Newport Beach, Calif.). Because these two immunoassays are potentially more convenient to use in laboratories performing large numbers of vancomycin assays, we compared their precision and accuracy with the three established methods (bioassay, HPLC, and RIA).

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MATERIALS AND METHODS

Reference samples. Vancomycin powder obtained from Eli Lilly & Co. (Indianapolis, Ind.) was used to prepare a stock solution containing 1,000 μg of vancomycin per ml in glass-distilled water. Seven standards containing 5, 10, 25, 40, 55, 70, and 85 μg of vancomycin per ml were then prepared by diluting the aqueous stock solution with antibiotic-free pooled human serum. These seven standards were used to perform the intrarun precision and accuracy studies for all five assay systems.

Three lyophilized serum specimens containing vancomycin were provided with the FPIA and were used to evaluate the interrun precision and accuracy of the five assay systems. These controls were reconstituted with glass-distilled water to achieve final vancomycin concentrations of 7, 35, and 75 μg/ml.

Patient specimens. Serum specimens (106) collected from

* Corresponding author.
† Present address: Department of Pathology, University of Iowa Hospitals and Clinics, Iowa City, IA 52242.

patients receiving vancomycin during a 2-week period were analyzed by the bioassay, HPLC, FPIA, RIA, and FIA methods. Specimens were frozen at −20°C until they were tested.

Bioassay. The bioassay was performed with tryptic soy agar medium (Difco Laboratories, Detroit, Mich.) containing 5% NaCl and 10^5 U of penicillinase per ml (BBL Microbiology Systems, Cockeysville, Md.) to inactivate aminoglycosides and β-lactams, respectively (21, 23). After the agar medium was seeded with *Bacillus globigii*, 2.5-mm wells were produced with a punch and filled with portions of either the serum from a patient or a vancomycin standard. After incubation at 35°C for 18 h, the diameters of the zones of inhibited growth were measured to the nearest 0.1 mm with a Zeiss stereomicroscope and plotted (on the abscissa) versus the logarithm of the vancomycin concentration (on the ordinate). This assay was routinely calibrated by using five standards prepared in antibiotic-free pooled human serum (containing 5, 10, 20, 40, and 80 μg of vancomycin per ml).

HPLC assay. The HPLC assay was a modification of the method described by Uhl and Anhalt (18; J. P. Anhalt, personal communication) and utilized ion-exchange gel chromatography followed by elution with sodium borate. A 0.5-ml portion of the sample to be tested was mixed with an equal volume of the internal-standard solution (which contained 16 μg of ristocetin sulfate per ml, 0.02 M acetic acid, and 0.16 M Na_2SO_4) and applied to a polypropylene column (catalog no. 731-1550; Bio-Rad Laboratories, Richmond, Calif.) containing 0.8 ml of carboxymethylethyl-Sephadex (bead size, 40 to 120 μm; Sigma Chemical Co., St. Louis, Mo.) which had been suspended in 0.2 M Na_2SO_4 and permitted to swell overnight. The column was then washed with two 1-ml volumes of Na_2SO_4 (0.2 M, pH 6.8) and 0.5 ml of $Na_2B_4O_7$ (0.5 M, pH 9.45). Vancomycin and ristocetin were eluted from the column by a second 0.5-ml volume of the $Na_2B_4O_7$ buffer, and 40 μl of 8.5% phosphoric acid was added to this eluate before samples of it were injected into the chromatograph.

Chromatography was performed with a Waters 6000A pump, U6K injector, and model 450 variable wavelength detector with a μBondapak C 1830-cm steel column (inside diameter, 3.9 mm; Waters Associates, Milford, Mass.). This procedure used a 50-μl sample volume and a 1.5-ml/min flow rate for the mobile phase, which was a mixture of 0.01 M potassium phosphate buffer (pH 3.8 to 4.0) and acetonitrile (90:10 by volume). A standard curve was then constructed by plotting the ratio of the vancomycin and ristocetin peak heights at 210 nm versus vancomycin concentration for the same five standard specimens used to calibrate the bioassay. The vancomycin concentrations of unknown specimens were determined by calculating their peak height ratios (vancomycin peak height divided by the ristocetin peak height) and extrapolating from the standard curve.

FPIA. The FPIA (TDX) was performed in accordance with the instructions of the manufacturer as described by Schwenzer and co-workers (15) and by Jolley et al. (10). This system (like the FIA) utilizes fluorescein-labeled vancomycin which competes with the unlabeled vancomycin in the sample for antibody-binding sites. Because the polarization of fluorescent light emitted by the fluorescein-labeled vancomycin increases as the vancomycin is bound to the antibody, this assay provides a measure of bound and free labeled vancomycin in a competitive binding assay. The relationship between the concentration of unlabeled vancomycin and polarization is determined from a standard curve derived by testing the six lyophilized standards which are supplied with each kit and which contain 0, 5, 10, 25, 50, and 100 μg of vancomycin per ml in normal human serum.

RIA. The RIA (American Diagnostics) used was a competitive binding assay (8) and was performed in accordance with the instructions of the manufacturer. In this assay, 100 μl of patient serum or vancomycin standard (which had been diluted 1:101 in 0.01 M phosphate-buffered saline, pH 7.5) was mixed with 100 μl of ^{125}I-labeled vancomycin and 100 μl of rabbit anti-vancomycin immunoglobulin G (IgG) antibody. During a 10-min incubation at room temperature, vancomycin in the sample competed with ^{125}I-labeled vancomycin for the rabbit anti-vancomycin IgG antibody. The vancomycin bound to the rabbit anti-vancomycin IgG antibody was then precipitated by adding 100 μl of sheep anti-rabbit IgG antibody and incubating at room temperature for 20 min. After centrifugation (at 1,500 × *g* and room temperature for 10 min), the supernatant was immediately decanted, and the radioactivity in the precipitate was counted with a gamma counter (Micromedic 4/600; Horsham, Pa.). Six vancomycin standards (containing 1, 2, 4, 8, 16, and 32 μg of vancomycin per ml) in normal human serum were provided by the manufacturer and were used to construct standard curves for the RIA by plotting the percentage of the ^{125}I-labeled vancomycin which had been bound by the rabbit anti-vancomycin IgG and precipitated by the sheep anti-rabbit IgG (i.e., the percentage of the ^{125}I counts which were in the pellet) versus the vancomycin concentration on logit-log paper.

FIA. In the FIA (American Diagnostics) a measured amount (100 μl) of diluted patient serum or vancomycin standard (diluted 1:51 in 0.01 M phosphate-buffered saline, pH 7.5) was mixed with 100 μl of fluorescein-labeled vancomycin and 100 μl of rabbit anti-vancomycin IgG antibody. During a 30-min incubation at room temperature, vancomycin in the sample competed with fluorescein-labeled drug for the rabbit anti-vancomycin IgG. The vancomycin bound to the rabbit anti-vancomycin antibody was then precipitated by using a sheep anti-rabbit IgG antibody. After centrifugation (at 1,500 × *g* and room temperature for 10 min), the fluorescence of the supernatant was measured by using a fluorimeter with an excitation wavelength of ca. 490 nm which measured emitted light at wavelengths of ≥520 nm (Amerifluor, American Diagnostics). Six vancomycin standards (containing 0, 8, 16, 32, 64, and 128 μg of vancomycin per ml) in normal human serum were provided by the manufacturer and were used to construct the standard curve for the FIA with the HP 41CV data reduction system (Hewlett-Packard, Palo Alto, Calif.).

Statistical analysis. Statistical calculations were performed with an HP85 personal computer (Hewlett-Packard) and with a PDP-11/24 mini computer (Digital Equipment Corp., Maynard, Mass.). The statistical programs employed used standard methods to compute the mean, standard deviation, and coefficient of variation, and for linear regression analysis with the least-squares technique (2).

RESULTS

Standard curves. The bioassay demonstrated a semilogarithmic relationship between the diameter of the zone of inhibited growth and the logarithm of drug concentration between 5 and 80 μg of vancomycin per ml ($r \geq 0.98$; $P < 0.01$). In contrast, as previously reported by Uhl and Anhalt (18), HPLC revealed a direct linear relationship between the standard vancomycin concentrations tested (from 5 to 80

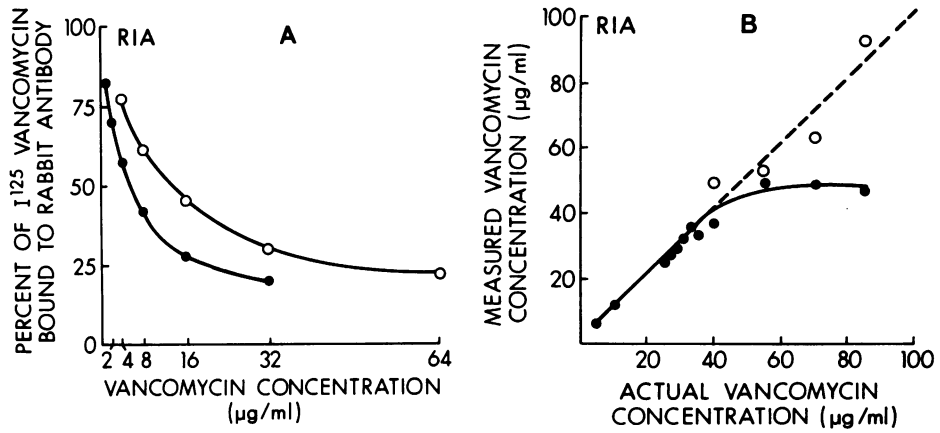

FIG. 1. (A) In the RIA, additional dilution with buffer (1:201 [○] versus 1:101 [●]) and an additional vancomycin standard of 64 μg/ml are necessary to measure vancomycin concentrations of 32 to 64 μg/ml. (B) If specimens with ≥32 μg of vancomycin per ml are not diluted with vancomycin-free serum before they are tested (●), the results of the RIA (using the suggested standard curve of the manufacturer) are misleading. Specimens with 32 to 64 μg of vancomycin per ml were diluted 1:1 with vancomycin-free serum; those with 70 and 85 μg of vancomycin per ml were diluted 1:2 and 1:3, respectively (○).

μg/ml) and the peak height ratios for vancomycin to ristocetin. In the FPIA, the relationship between vancomycin concentration (in μg/ml) and fluorescence (in millipolarization units) was also nonlinear and was best described by a weighted log-logit curve fit as previously reported by Jolley et al. (10). In contrast to the RIA and FIA, this standard curve was stable for at least 3 weeks. In the RIA, the relationship between vancomycin concentration (x, in μg/ml) and the percentage of the total [^{125}I]vancomycin counts in the pellet (y) was well described by a logit-log curve fit (2) of the following form: logit (y) = $\log_e [y/(1 - y)] = [m \log_{10} (x)] + b$. This standard curve (Fig. 1A) was stable for less than 24 h and was repeated with each run. Serum specimens with ≥32 μg of vancomycin per ml were diluted with drug-free serum because the standard curve for the RIA extended only to 32 μg of vancomycin per ml and did not include a vancomycin standard of 64 μg of vancomycin per ml (Fig. 1). In the FIA, the relationship between vancomycin concentration and the fluorescence of the supernatant was nonlinear (Fig. 2A) and was well described by a log-log relationship (r = 0.9992; $P < 0.001$) (Fig. 2B). However, there was sufficient interrun variability in the FIA standard curve that it was necessary to rerun the standards and to calculate a new standard curve for each run. The FIA and RIA standard curves (Fig. 1A and 2A) are the inverse of one another because the FIA tests the supernatant, whereas the RIA counts the precipitate after similar precipitation procedures.

Precision. To define intrarun precision, each of the seven standards containing 5, 10, 25, 40, 55, 70, and 85 μg of vancomycin (which had been prepared in antibiotic-free pooled human serum) per ml was tested 10 times in a single run with each assay method (Table 1). The results of those studies were similar for the bioassay and HPLC (coefficients of variation [CVs] ranged from 3.3 to 6.4% and 2.4 to 5.2%, respectively). The FPIA was more precise (with CVs of 0.9 to 3.0%), and the FIA and RIA were less precise (with CVs of 8.9 to 14.5% and 4.7 to 10.6%, respectively).

To define interrun precision, we tested each of the three control specimens provided with the FPIA (containing 7, 35, and 75 μg of vancomycin per ml in antibiotic-free pooled human serum) on 12 different occasions. The CVs observed were similar with the FPIA and HPLC (ranging from 2.8 to

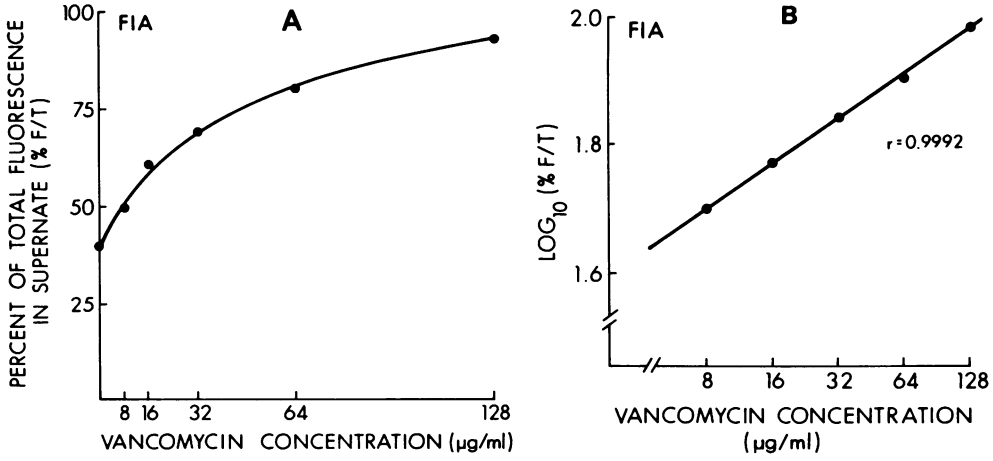

FIG. 2. In the FIA, the relationship between the percentage of the total fluorescence in the supernatant (% F/T) and the vancomycin concentration is nonlinear (A) and is well described by a log-log plot (B) of the equation $\log_{10} y = 0.2167(\log_{10} x + 1.51)$ where y is % F/T and x is the vancomycin concentration in μg/ml (r = 0.9992; $P < 0.001$).

TABLE 1. Comparison of the intrarun precision of five vancomycin assay methods

Reference sample vancomycin concn (μg/ml)	Mean vancomycin determination[a] by:				
	Bioassay	HPLC	FPIA	RIA[b]	FIA
5	5.1 (3.3)	4.8 (2.7)	5.5 (2.2)	6.0 (6.7)	5.5 (14.5)
10	10.6 (5.9)	10.0 (2.4)	9.9 (3.0)	12.3 (10.6)	11.5 (12.2)
25	23.7 (6.4)	25.8 (3.1)	27.0 (1.6)	23.9 (8.4)	25.2 (10.3)
40	40.7 (5.7)	40.9 (3.5)	41.5 (2.0)	49.3 (9.5)	39.1 (9.7)
55	51.9 (4.5)	58.7 (5.1)	57.6 (1.7)	53.1 (7.2)	51.9 (8.9)
70	68.9 (3.6)	71.8 (5.2)	71.2 (1.9)	62.8 (7.0)	72.2 (9.4)
85	91.8 (5.3)	84.7 (3.1)	88.2 (0.9)	93.5 (4.7)	82.9 (10.3)

[a] Results are expressed as the means (μg/ml) of 10 determinations for each standard in a single run. CVs are in parentheses.
[b] Specimens containing more than 35 μg of vancomycin per ml were diluted with drug-free serum for testing with the RIA as follows: 1:1 at 40 and 50 μg/ml, 1:2 at 70 μg/ml, and 1:3 at 85 μg/ml (see text for details).

8.1% and 4.8 to 9.6%, respectively). The bioassay and RIA were less precise (with CVs of 7.3 to 13.4% and 7.3 to 11.0%, respectively), and the FIA was the least precise method (with CVs of 12.2 to 16.2%) (Table 2).

Accuracy. Linear regression was used to define accuracy by comparing the experimental values obtained for the seven vancomycin standards with their theoretical (expected) values, by using the slope and *y* intercept to estimate the proportional and constant errors, respectively (22). With the mean value for each standard obtained from the intrarun precision studies described above for the regression analysis, all five methods correlated well with the expected theoretical values (Table 3).

Potential causes of imprecision in the FIA. Because these studies indicated that the FIA was less precise than (but similar in accuracy to) the other assays, we examined several potential causes of this variability, including (i) inadequate centrifugation to remove vancomycin bound to rabbit anti-vancomycin IgG and the sheep anti-rabbit IgG, (ii) manual pipetting errors, (iii) decay or loss of fluorescence with time, and (iv) variability in the tube-fluorimeter system used to read the results.

Early in the FIA studies, it became apparent that even slight agitation of the borosilicate-glass test tubes (12 by 75 mm) used to perform this assay disturbed the precipitate at the bottom of the tubes and altered the fluorescence measurements obtained in the fluorimeter. Therefore, the procedure recommended by the manufacturer was modified to centrifuge the reaction mixture at 13,000 × *g* for 5 min (rather than 1,500 × *g*) in 1.5-ml microcentrifuge tubes before examining the supernatant in the fluorimeter. This modification produced a firm pellet and obviated clouding of the supernatant (from agitation of the pellet) but did not eliminate the variability observed in this assay system (CVs remained between 8 and 14%) (Tables 1 and 2).

To test for variability due to manual pipetting errors, we examined the accuracy of the MLA micropipettes (Medical Laboratory Automation, Mt. Vernon, N.Y.) which were used for the FIA and tested whether an automatic pipetting device would decrease the variability (CV) of the assay. Based on 0.1-ml water samples, the manual pipettes were both accurate and precise (10 0.1-ml samples weighed a mean 0.09936 ± 0.0005 g with a CV of 0.5%). The use of an automatic pipetter (Fisher model 240; Fisher Scientific Co., Pittsburgh, Pa.) also did not affect the variability of the FIA. The CVs observed remained between 8.9% and 15.7%.

To test whether the fluorescence observed in the FIA decreased with time, the six standards provided with the FIA and the three controls provided with the FPIA were tested and repetitively examined for their fluorescence over a 5-h period. Although there was a slow decrease in fluorescence with time (~1% in 1 h and 5 to 6% in 5 h), its magnitude was insufficient to explain the variability (imprecision) of the FIA, which was routinely read within 30 min of performing the assay.

To determine whether the variability observed in the FIA resulted from an insufficient sample volume or from problems with the fluorimeter, we retested the seven standards by using a larger volume (achieved by a twofold dilution of the reaction mixture in assay buffer) and a different fluorimeter (Aminco SPF-500). Neither of these modifications significantly improved the precision of the FIA.

Patient specimens. A total of 106 patient specimens were analyzed by all five methods. Correlation coefficients for the FPIA versus the HPLC, bioassay, and RIA were 0.977, 0.973, and 0.967, respectively (Table 4). Although the correlations between the FPIA and the other methods for patient samples were similar to those seen with the reference samples (0.967 to 0.977 versus 0.983 to 0.999) (Fig. 3A), the correlations between the FIA and the other methods exam-

TABLE 2. Comparison of the interrun precision of five vancomycin assays

Reference sample vancomycin concn (μg/ml)	Mean vancomycin determination[a] by:				
	Bioassay	HPLC	FPIA	RIA[b]	FIA
7	6.9 (10.1)	6.2 (9.6)	7.4 (8.1)	7.0 (8.6)	6.8 (16.2)
35	33.5 (13.4)	31.2 (6.1)	35.9 (3.9)	32.7 (11.0)	33.0 (16.1)
75	74.1 (7.3)	71.3 (4.8)	75.7 (2.8)	81.8 (7.3)	77.2 (12.2)

[a] Results are expressed as the means (μg/ml) of 12 determinations on 12 separate runs. CVs are in parentheses.
[b] Specimens containing ≥35 μg of vancomycin per ml were diluted with drug-free serum for testing with the RIA as follows: 1:1 at 35 μg/ml and 1:3 at 75 μg/ml.

TABLE 3. Accuracy of five vancomycin assays: least-squares analysis for seven standards[a]

Assays compared	Slope	y Intercept	Correlation coefficient
Bioassay vs theoretical	1.040	−1.281	0.996
HPLC vs theoretical	1.015	+0.342	0.999
FPIA vs theoretical	1.029	+0.357	0.999
RIA vs theoretical	1.006	+1.293	0.982
FIA vs theoretical	0.975	+0.800	0.998

[a] The seven vancomycin concentrations tested ranged from 5 to 85 μg/ml (see Table 1). Each sample was assayed 10 times, and the mean values were used for the regression analysis.

TABLE 4. Comparison of five vancomycin assay methods: correlation coefficients for 106 patient specimens

Assay method	Correlation coefficient[a] for the following assay:			
	HPLC	FPIA	RIA	FIA
Bioassay	0.975	0.973	0.962	0.901
HPLC	—[b]	0.977	0.964	0.919
FPIA	—	—	0.967	0.918
RIA	—	—	—	0.899

[a] Correlation coefficients were calculated by least-squares linear regression analysis (2), using the values obtained for the same specimen by different methods as matched pairs (see text for details).

[b] —, Correlation coefficients specified elsewhere in the table (e.g., the FPIA row has no entry for HPLC because the HPLC row [above] contains the correlation coefficient for HPLC versus FPIA).

ined were considerably lower for patient samples than for the reference samples (0.899 to 0.929 versus 0.975 to 0.997) (Fig. 3B). For each method tested, the weakest correlations were observed with the FIA.

DISCUSSION

The increased use of vancomycin in patients with serious infections due to antibiotic-resistant gram-positive organisms has resulted in an increased demand for rapid, accurate quantitation of serum concentrations of vancomycin (9, 14). Three of the established methods for vancomycin quantitation (bioassay, HPLC, and RIA) have been shown in this study and others to be both precise and accurate (Tables 1 to 3) (5, 11, 18). However, each of these techniques has disadvantages which make it unacceptable. Although the bioassay is inexpensive and does not require large sample sizes (three ~5-μl portions of serum), its disadvantages include interference from other antimicrobial agents used in combination with vancomycin (21, 23) and a turnaround time of ≥6 h. Although some of the potentially interfering antibiotics can be inactivated (21), this often cannot be done when the patient is receiving multiple antimicrobial agents, including agents such as erythromycin, trimethoprim-sulfamethoxazole, and β-lactamase-resistant penicillins and cephalosporins. In our experience, virtually all specimens submitted for the measurement of vancomycin levels (>95%) contain other antimicrobial agents; ca. 40% of those specimens contain antimicrobial agents which cannot be inactivated. In addition, the theoretical 6-h turnaround time is rarely achieved in our laboratory because our large volume makes it necessary to batch specimens. As a result, bioassay determinations are generally not available until the next day and are thus less clinically useful.

Although HPLC is specific, precise, and accurate, it is impractical for laboratories performing large numbers of assays. The extensive sample preparation (including the elution of the drug from the column) and the relatively long analysis time (ca. 15 min per sample) preclude a rapid turnaround time and thus make this technique less desirable for routine clinical purposes. In addition, the relatively expensive instrumentation and the technical expertise required effectively restrict HPLC assays to large clinical or reference laboratories.

Although the technology to perform RIAs with ^{125}I is readily available at major medical centers, it is often not available in smaller institutions. Use of this technique is further compromised by the need to dispose of γ-emitting waste, by the relatively short shelf life of the reagents, by the need for a new standard curve at least daily, and by

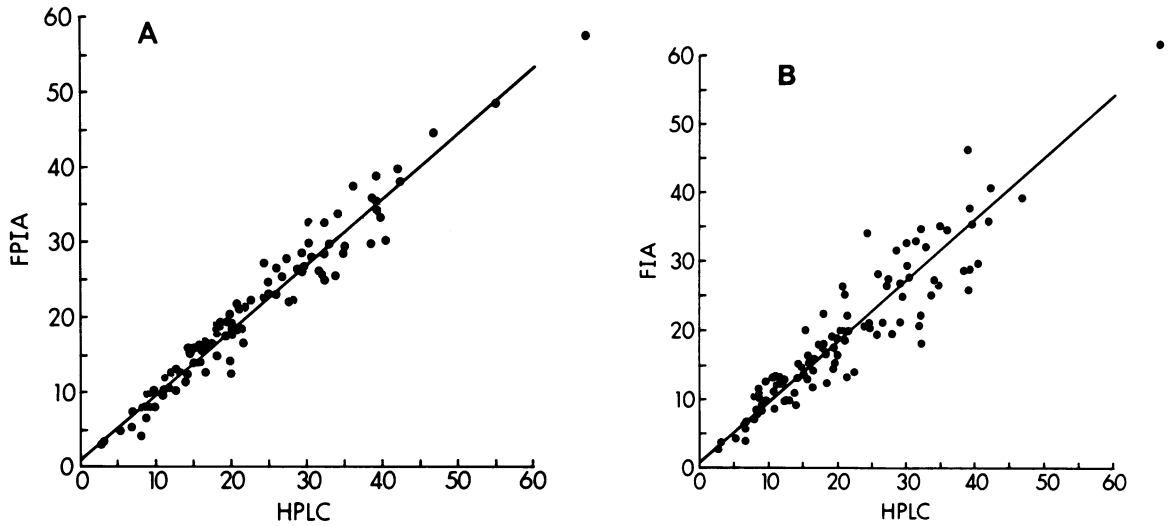

FIG. 3. With the 106 patient specimens, the strongest correlations (with HPLC) were observed with the FPIA (A) and the weakest were observed with the FIA (B). Similar results were also observed with the bioassay and the RIA (Table 4). (A) y, $0.877x + 0.819$; r, 0.977. (B) y, $0.899x + 0.539$; r, 0.919.

interference from other γ-emitting isotopes in specimens obtained from patients who are having ^{67}Ga or ^{99}Tc studies (1).

In contrast to the three established methods described above, the FPIA is both rapid and automated, although it does require a significant investment if the machine is purchased. A single assay can be completed in 5 min and up to 80 tests can be performed per hour. Because sample dilution, timing, reading, and calculation of the results are all automated, the technologist time required to perform the assay is significantly less than that required for bioassay, HPLC, or RIA. In this study, the FPIA proved to be precise and accurate (Tables 1 to 3) and correlated well with the bioassay, HPLC, and RIA in the analysis of patient specimens (Table 4). These findings confirm and extend those recently reported by Schwenzer et al. (15) and by Filburn et al. (7), who demonstrated that the stored standard curve was stable for at least 1 month and that the FPIA was not significantly affected by icteric, lipemic, or hemolyzed samples.

Although the FIA was accurate and correlated well with the bioassay in the analysis of reference samples (Tables 1 and 2), its precision was inferior to that of the FPIA and the three established methods (bioassay, HPLC, and RIA) (Tables 1 and 2). The CVs observed with the FIA were unacceptably high, whereas the CVs for the FPIA were quite low and were comparable to those observed with HPLC and the bioassay.

In conclusion, this study demonstrates four important points. First, the FPIA vancomycin assay is an excellent alternative to both the bioassay and HPLC. The stability of its stored standard curve and the ease of performing the assay make it useful for both stat and batch analyses. Second, in its present state the FIA is too imprecise for clinical use. In addition, the requirement for a new standard curve for each run (of the FIA) both increases its cost and seriously limits its use for emergency determinations. Third, the need to dilute all specimens with ≥ 32 μg of vancomycin per ml seriously compromises the value of the RIA, despite its precision, which is better than that of the FIA (15% of the specimens received in our laboratory have vancomycin concentrations of >32 μg/ml; <2% have vancomycin concentrations of <4 μg/ml). Finally, although the bioassay and HPLC are accurate and precise, the prolonged turnaround times of both methods and the complexity of HPLC make them less useful than the FPIA for most clinical laboratories.

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