

Comparison of a Radioimmunoassay and a Microbiological Assay for Measurement of Serum Vancomycin Concentrations

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A newly developed radioimmunoassay was used to measure the concentration of vancomycin in 137 specimens of serum from patients being treated with this antibiotic. Of these sera, 84 were also analyzed with a microbiological assay technique for vancomycin. Duplicate determinations were done with each of the techniques. Individual values and averaged values for both methods were used for statistical analyses. The correlation coefficients between all possible combinations of radioimmunoassay and microbiological assay results for the 84 sera were ≥ 0.99 ($P < 0.01$). Values for the regression coefficients of radioimmunoassay results on microbiological assay results ranged from 0.98 ± 0.01 to 1.03 ± 0.01 . The mean percent deviation of radioimmunoassay versus microbiological assay results was -1.56 ± 0.60 . A one-way analysis of variance demonstrated that the use of different standard curves for each batch of specimens assayed by microbiological assay did not significantly influence the results ($P = 0.07$). The microbiological assay and the radioimmunoassay for measurement of serum vancomycin levels yielded essentially identical results.

There has recently been a renewal of interest in using vancomycin in the treatment of staphylococcal infection (3, 5). The increased usage of this drug is probably related to the apparent increased frequency of isolation of antibiotic-resistant strains of *Staphylococcus aureus* from hospitalized patients in the United States (4, 6, 7).

Because vancomycin is potentially ototoxic and nephrotoxic and because serum concentrations after a given dose may vary widely, measurement of levels of this drug in serum is usually appropriate (D. E. Zaske, K. B. Crossley, R. J. Sawchuk, D. L. Uden, and K. E. Mead, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 17th, New York, N.Y., abstr. no. 157, 1977). We have evaluated a newly developed radioimmunoassay (RIA) procedure and have compared it with a microbiological assay (MA) for the measurement of vancomycin serum concentrations. The results are summarized in this report.

MATERIALS AND METHODS

MA. The MA utilized in our studies is a modification of the method described by Sabath et al. (8). Heart infusion agar (Difco Laboratories, Detroit, Mich.) was adjusted to pH 5.5 with 1 N hydrochloric acid (to inactivate aminoglycoside antibiotics). Tubes of agar were melted, inoculated with 0.5 ml of an overnight culture of *Bacillus subtilis* ATCC 6633

grown in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.), and poured into 150-mm petri dishes. Paper disks (no. 740-E, 0.25-in. [ca. 0.64-cm] diameter; Schleicher & Schuell Co., Keene, N.H.) were placed on the agar. Twenty microliters of the patient's serum, arbitrary control sera with two concentrations of vancomycin (42 and 3 $\mu\text{g}/\text{ml}$), and vancomycin standards were pipetted onto individual disks. Vancomycin standards were prepared in a large single lot by repetitive twofold dilutions of a solution containing 2,000 mg of vancomycin per ml with serum from a volunteer and contained 4, 8, 16, and 32 μg of vancomycin per ml. Study specimens, standards, and controls were stored at -70°C until assay.

Each unknown serum (or standard or control) was pipetted onto two disks in each of two petri dishes. The values obtained from each petri dish were averaged; thus, two averaged values (one from each petri dish) were available. These averages (designated MA_a and MA_b) were then treated as the two repetitions used in the analyses. For some statistical comparisons, these values were averaged to yield a value designated as MA_{avg}. After incubation at 37°C for 8 h, the zones of inhibition were read with a vernier caliper. If the unknown sample produced a zone which corresponded to a concentration of vancomycin in excess of 30 $\mu\text{g}/\text{ml}$, it was diluted 1:5 or 1:4 with pooled human serum, and the assay was repeated. Zone diameters were plotted against the standard concentrations of vancomycin on semilog paper, and the concentration of vancomycin in the unknown specimen was interpolated from the graph.

RIA. The RIA procedure used in these studies was developed by Monitor Science Corp., Newport Beach,

Calif. Vancomycin hydrochloride (Eli Lilly & Co., Indianapolis, Ind.) was conjugated to bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) with a modified carbodiimide technique (2). After an appropriate reaction time, the conjugate was dialyzed for 72 h against 0.01 M phosphate-buffered saline at pH 7.8. The vancomycin-albumin conjugate was emulsified in Freund complete adjuvant and administered to New Zealand white rabbits by monthly intramuscular injections. ^{125}I -labeled vancomycin was prepared by a modified chloramine-T method, employing a solution containing 1.5 mg of vancomycin per ml in 0.5 M sodium phosphate buffer (1). After a suitable incubation period, the iodinated vancomycin was purified on a Sephadex G-10 column (Pharmacia, Inc., Piscataway, N.J.) with phosphate-buffered saline eluant.

RIAs were performed as follows. Each assay tube contained 100 μl of standard (or unknown or control) diluted 1:101 in phosphate-buffered saline, 100 μl of antiserum diluted 1:50 in 0.01 M phosphate-buffered saline with 1% normal rabbit serum at pH 7.8, and 100 μl of ^{125}I -labeled vancomycin diluted to yield 50,000 cpm. After Vortex mixing, assay tubes were incubated at ambient temperature for 10 min. After the incubation, 100 μl of sheep anti-rabbit immunoglobulin G was added to each assay tube. The tubes were again Vortex mixed and allowed to incubate for 20 min at ambient temperature. After the second incubation, antibody-bound fractions were separated by centrifugation at 3,400 rpm for 10 min. Supernatants were then aspirated and counted for 1 min in a Packard spectrometer, model 5120 (Packard Instrument Co., Inc., Chicago, Ill.).

Each unknown serum was assayed in duplicate by RIA, and the results were designated as RIA_a and RIA_b. For some analyses, these values were averaged and designated RIA_{avg}.

A total of 137 serum samples from patients being treated with vancomycin and 16 samples of standard solutions (four determinations for each of four samples containing 4, 8, 16, and 32 $\mu\text{g}/\text{ml}$) were assayed by RIA. MA was performed with 84 of these serum samples run in 13 batches. The 53 specimens assayed only by RIA were from patients receiving additional antimicrobial agents which could not be inactivated for MA.

Statistical analyses. Statistical analyses were designed to determine the degree of agreement between results obtained by MA and RIA. The repeatability of each assay technique and the accuracy of the standard solutions used for MA were also studied.

Values obtained by RIA and MA were examined for similarity by calculating the correlation coefficient between RIA and MA results for the 84 samples assayed by both methods. To allow for the prediction of RIA results from MA results, the linear regression equations of RIA results on MA results were estimated for all possible combinations of the dependent variable-independent variable pairs, using both individual determinations and averaged values. Percent deviation of MA results from RIA results (expressed as a percentage of RIA results) was also determined. A frequency distribution of percent deviation was generated, and a 95% confidence range was determined. The effects of using different standard curves in MA were studied by testing for differences in the percent

deviation of MA results from RIA results among the 13 standard groups of data by a one-way analysis of variance.

RESULTS

There was excellent correlation between repeated assays of the same unknown sample by either MA or RIA. The correlation coefficient between two repeated assays of the same sample by RIA was 0.98, and that by MA was 0.99. Both values are highly significantly different from 0 ($P < 0.01$).

RIA was used to measure the concentration of vancomycin in the standard solutions used for MA. The correlation coefficients between individual RIA results (RIA_a and RIA_b) and the assumed concentrations of vancomycin in the standard solutions are 1.00 and 0.96, respectively. The correlation coefficient between the average of the two RIA results and the assumed concentration of the standards is 0.99. All three correlation coefficients are highly significantly different from 0 ($P < 0.01$).

The expected concentrations of the standard vancomycin solutions utilized for MA were consistently lower than the actual concentrations measured by RIA. On the average, the measured concentrations determined by RIA are higher ($P < 0.05$) than the corresponding expected concentrations (with a mean difference of $1.68 \pm 0.73 \mu\text{g}/\text{ml}$). The difference was greater for the standards containing higher concentrations (16 and 32 $\mu\text{g}/\text{ml}$) of vancomycin ($r = 0.65$; $P < 0.01$). Therefore, percent difference should be a better estimate of the bias. The average percent difference (expressed as a percentage of the assumed value) is 9.01 ± 3.64 , which is significantly higher than 0 ($P < 0.05$). The regression coefficient of the assumed concentration of vancomycin in the standard solutions on the average RIA result for the standards is 0.83 ± 0.04 , which is significantly less than 1 ($P < 0.01$). This also illustrates that the standard concentrations are somewhat higher than expected when actually measured by RIA.

The correlation coefficients between the two repetitive measures or the average measure of the 84 samples analyzed by RIA (RIA_a, RIA_b, or RIA_{avg}) and by MA (MA_a, MA_b, or MA_{avg}) are shown in Table 1. All of the nine correlation coefficients are perfect (or nearly perfect) and are highly significantly different from 0. Figure 1 shows the average RIA results versus the average MA results for every sample and again demonstrates a high degree of agreement between the two assay techniques. Thus, RIA results can be predicted with a high degree of accuracy and precision by MA results.

Table 2 lists the regression coefficients and the y -intercepts of the regression equation of

RIA results on MA results for all possible combinations of the dependent variable-independent variable pairs. Only one y -intercept (when $y = \text{RIA}_b$ and $x = \text{MA}_a$) is statistically different from 0; thus, it is highly likely that the regression line goes through the origin. All of the regression coefficients have values very close to 1, although three values are statistically different from 1. Regression of the average RIA result on the average MA result ($y = 0.08 + 1.01x$) demonstrates that the agreement is quite perfect, with RIA results slightly higher than MA results.

The deviation of MA results from RIA results

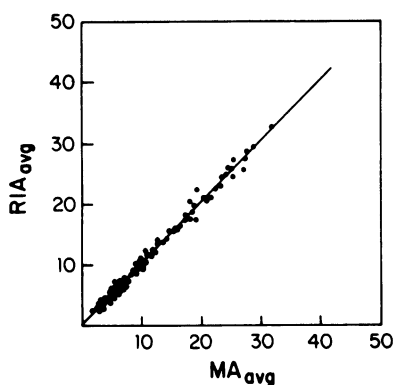


FIG. 1. Correlation between values of RIA_{avg} (y) and MA_{avg} (x) for 84 specimens of serum ($r = 1.00$; $y = 0.08 + 1.01x$).

TABLE 1. Correlation coefficients^a between MA results (MA_a , MA_b , and MA_{avg}) and RIA results (RIA_a , RIA_b , and RIA_{avg})

| | MA_a | MA_b | MA_{avg} |
|---------------------------|---------------|---------------|--------------------------|
| RIA_a | 0.99 | 0.99 | 0.99 |
| RIA_b | 0.99 | 0.99 | 1.00 |
| RIA_{avg} | 1.00 | 1.00 | 1.00 |

^a All values of the correlation coefficients are highly significant ($P < 0.01$).

TABLE 2. Coefficients for the regression equation $y = a + bx$, based on 84 samples

| y | x | $a \pm \text{SE}_a^c$ | $b \pm \text{SE}_b$ |
|---------------------------|--------------------------|-----------------------|---------------------|
| RIA_a | MA_a | 0.03 ± 0.20 | 1.00 ± 0.01 |
| RIA_a | MA_b | -0.09 ± 0.19 | $1.03^b \pm 0.01$ |
| RIA_a | MA_{avg} | -0.06 ± 0.17 | $1.02^b \pm 0.01$ |
| RIA_b | MA_a | $0.30^c \pm 0.17$ | $0.98^b \pm 0.01$ |
| RIA_b | MA_b | 0.20 ± 0.18 | 1.01 ± 0.01 |
| RIA_b | MA_{avg} | 0.22 ± 0.15 | 1.00 ± 0.01 |
| RIA_{avg} | MA_a | 0.16 ± 0.16 | 0.99 ± 0.01 |
| RIA_{avg} | MA_b | 0.05 ± 0.15 | 1.02 ± 0.01 |
| RIA_{avg} | MA_{avg} | 0.08 ± 0.13 | 1.01 ± 0.01 |

^a SE, Standard error.

^b Regression coefficient different from 1 ($P < 0.05$).

^c y -intercept different from 0 ($P < 0.05$).

(expressed as a percentage of RIA results) was further examined to establish an estimate of the range of the deviation. Because MA results were obtained based on 13 standard curves, percent difference for each group of MA results was compared by an analysis of variance. The non-significant F -ratio ($F = 1.76$ at 12 and 71 degrees of freedom; $P = 0.07$) indicates that percent deviation of MA results from RIA results did not change with the use of different standard curves. Percent deviation was also not a function of the observed concentration of vancomycin ($r = 0.06$; $P = 0.31$).

The distribution of percent deviation of MA results from RIA results for the 84 samples assayed by both methods is skewed toward the negative side as expected (Fig. 2). The mean percent deviation, estimated by the medium of the distribution, is -3.7% . The 95% confidence range of percent deviation (estimated by the 2.5th percentile and the 97.5th percentile) is -14.9 to 5.0% . Therefore, 95% of the time, the concentration of vancomycin determined by MA will be within -15 and 5% of the corresponding RIA result.

DISCUSSION

The results of these studies indicate that there is an extremely high correlation between duplicate determinations of serum vancomycin concentrations regardless of whether RIA or MA is used. Because more than 95% of the variability

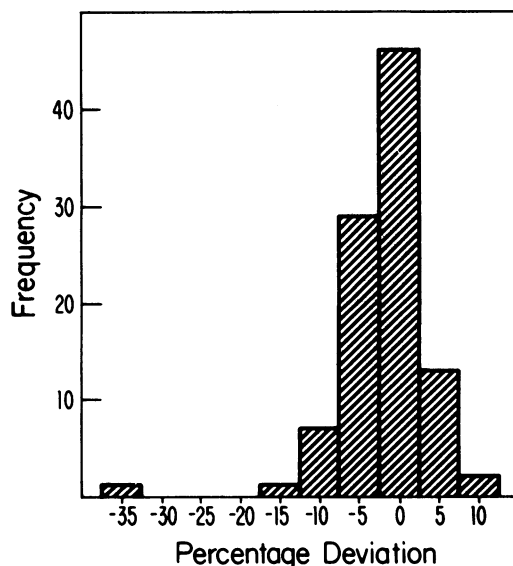


FIG. 2. Frequency distribution of percent deviation of MA results from RIA results (expressed as a percentage of RIA results).

in the duplicate assay can be explained by variation in the initial assay, it is probably unnecessary to assay specimens in duplicate by either technique. The use of averages (RIA_{avg} and MA_{avg}) offered little improvement in the precision of the estimated parameters as compared with the use of single-assay results (RIA_a , RIA_b , MA_a , and MA_b).

Our studies also suggest that the two assay procedures yield essentially identical results. This is supported by the perfect or nearly perfect correlations between individual or average results obtained by the two assay procedures (Table 1) and by the close clustering of points along a 45° line (Table 2 and Fig. 1). Values of the regression coefficients of RIA results on MA results are all very close to 1, indicating an essentially perfect regression situation for all combinations. However, there were three regression coefficients which differed significantly ($P < 0.05$) from 1. Two of these deviations were greater than 1. The lack of a consistent trend for the deviation from 1 for these regression coefficients seems to indicate a true status of a perfect regression.

The two possible sources of variability for MA (the use of locally prepared standard solutions and the need to generate a separate standard curve for each batch of samples) did not appear to have any major effects on results of MA. Based on results from the 84 samples assayed by both MA and RIA, there is no difference in percent deviation from RIA results between different batches of samples assayed with the same lot of standard solutions. The significant mean percent difference between the assumed concentrations in these standard solutions and the corresponding RIA results and the high correlation between the two values suggest that the slope of the standard curve is not altered but may be shifted to a position parallel to the true curve. This effect would not invalidate the assay but would tend to result in lower concentrations by MA when compared with RIA.

As expected from the bias observed in MA standards, RIA results are, in general, higher than MA results. The significant correlations between duplicate RIA results employing the same specimen of serum indicate that RIA for vancomycin is both reliable and repeatable. Because RIA results tended to be higher than MA

values, the distribution of the percent deviation (MA-RIA) is skewed slightly to the left. It seems likely that the higher results obtained with RIA may reflect a reduction in the activity of vancomycin which is known to occur as the pH is lowered (9).

Whereas MA is a useful technique for measuring concentrations of serum vancomycin, it has certain disadvantages. Although aminoglycosides or beta-lactam antibiotics which would interfere with MA may be easily inactivated, there are some other antimicrobial agents (e.g., chloramphenicol and trimethoprim-sulfamethoxazole) which are difficult to eliminate. MA is also considerably slower than RIA. Moreover, unknown antimicrobial agents in the serum of patients receiving vancomycin may yield false results with MA, but this would not be expected to occur with RIA.

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