Evaluation of the Bactec Serum Gentamicin Assay

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A radiometric assay for gentamicin was compared with an established radioimmunoassay protocol. The coefficients of variation for within-run, day-to-day, and overall runs were consistently higher for the radiometric method as compared with the radioimmunoassay. The coefficient of correlation for 84 patient sera tested by the two methods for gentamicin levels was 0.82. Though less precise, the radiometric method was felt to be an acceptable means of determining gentamicin levels in laboratories where the radioimmunoassay is unavailable.

Gentamicin has established itself as an excellent antimicrobial agent for the treatment of life-threatening gram-negative infections. The range between therapeutic efficacy and toxicity for gentamicin, however, is narrow, and serum levels must be carefully monitored. The classical procedures for determining adequate antibiotic therapy require 1 to 2 days for results and are fraught with significant error. During the past several years a variety of methods have been developed to determine gentamicin levels in serum and other body fluids by more rapid and sensitive methods. Such methods include microbiological assays (1, 7, 11, 14, 16, 17, 21), enzymatic assays (4, 5, 13, 19, 20; R. V. Case, Natl. Meet. Am. Assoc. Clin. Chem., 28th, Houston, Tex., abstr no. 000, 1976), and radioimmunoassays (RIAs) (10, 12, 13).

A rapid radiometric technique for determining serum gentamicin levels has been developed by Johnston Laboratories with their Bactec instrument. The system was originally developed for routine monitoring of blood cultures and has been described elsewhere (2, 3, 6, 9, 15, 18). The instrument has also been adapted for definitive identification of *Neisseria gonorrhoeae* and *N. meningitidis* and is currently being developed for rapid identification and antibiotic susceptibility patterns of *Mycobacterium* sp. and the determination of certain aminoglycoside levels in patient sera.

The principle of the Bactec procedure is based on the fact that gentamicin inhibits the synthesis of urease, an inducible enzyme produced by a species of *Proteus*. The amount of urease produced is measured indirectly by the amount of $^{14}\text{CO}_2$ released. The quantity of gentamicin in a sample is inversely proportional to the volume of $^{14}\text{CO}_2$ detected by the instrument. The sensitivity range for this assay, according to the man-

ufacturer, is from <1 to $14\ \mu\text{g}$ of gentamicin per ml.

This study evaluated Johnston Laboratories' gentamicin kit by using the Bactec model 460 to determine gentamicin levels in patient sera as compared with the Monitor Science RIA procedure used routinely in our laboratory.

MATERIALS AND METHODS

Gentamicin test solutions. Two gentamicin test solutions were prepared, containing a low level of gentamicin ($3.5\ \mu\text{g}/\text{ml}$) and a high level of gentamicin ($10.5\ \mu\text{g}/\text{ml}$). The two gentamicin test solutions were prepared by diluting with sterile deionized water a gentamicin ($14.0\ \mu\text{g}/\text{ml}$) standard in water provided by the manufacturer. The two test solutions were then aliquoted into 10 vials each and stored frozen at -20°C till assayed. Both gentamicin test solutions were assayed six times in replicate on 4 separate days by both the Bactec preferred method and the Bactec alternate method according to the manufacturer's instructions.

Bactec. A lyophilized culture of *Proteus* was reconstituted by adding 2 ml of sterile water, and a stock culture was prepared by adding 0.1 ml of the reconstituted culture to a culture medium bottle and incubating it overnight at 37°C with shaking. The stock culture was stored at 4°C for up to 7 days. In the preferred method a working culture was prepared from the stock culture on the day the assays were run. A culture medium bottle was inoculated with 0.2 ml of the stock culture and incubated for 4.5 h at 37°C with shaking. After this incubation, the working culture was immediately used to perform the assays. The alternate method differed from the preferred method only in that the working culture was stored at 4°C and was usable for up to 48 h. Before use the culture was reheated in a 37°C water bath for 15 to 30 min.

A five-point standard curve was run each time an assay was performed, using 0.87, 1.75, 3.5, 7.0, and $14\ \mu\text{g}$ of gentamicin per ml. The assay was performed by adding 0.2 ml of each of the standard gentamicin solutions to base solution bottles followed by 0.2 ml of normal pooled human serum. The low- and high-level

test solutions of gentamicin to be assayed were also added in 0.2-ml quantities to base solution bottles, and 0.2 ml of normal pooled human serum was then added to each of these bottles. After this, 0.2 ml of the *Proteus* working culture was added to each of the base solution bottles, which were shaken and placed in a 37°C water bath for 1 h. At the end of this incubation, 0.5 ml of the reaction stopper (1% defoaming agent in 0.5 N HCl) was added to each of the base solution bottles. The bottles' contents were blended in a Vortex mixer for approximately 10 s and then read on the Bactec 460. The $^{14}\text{CO}_2$ index values were recorded, the standard curve was plotted on semilog paper, and gentamicin levels for the low and high test solutions were determined. Within-run, between-run, and total laboratory coefficients of variation (CVs) were calculated.

RIA. The Monitor Science RIA technique was performed according to the manufacturer's instructions, and CVs were calculated for comparison with the data derived from the Bactec radiometric method. Due to the constant use of the gamma counter for other routinely run tests, the two gentamicin test samples were run when time and space were available and thus were assayed as 2, 2, 8, 8, and 4 replicates on each of 5 days, giving an overall total of 24 individual assays per test solution.

For comparative studies 84 serum samples from patients on gentamicin therapy were assayed by both the Bactec preferred method and the RIA method. The Bactec preferred method was chosen over the Bactec alternate method, since the evaluation of the precision of each of the two methods indicated that the preferred method was more precise than the alternate method. In this study 0.2 ml of patient serum was inoculated into a base solution bottle and 0.2 ml of sterile deionized water was then added.

RESULTS

Table 1 summarizes the ranges of assays and the CVs which were calculated from assays performed on the test samples containing the low and high levels of gentamicin by the Bactec preferred and alternate methods and by RIA for within-run, day-to-day, and total overall laboratory variations. For the low-level gentamicin test solutions, the within-run variations of the Bactec preferred method done on 4 separate days with six replicates run each day ranged from 3.9 to 14.0%, whereas those of the Bactec

alternate method ranged from 8.7 to 20.1%. The RIA procedures run on 5 separate days with two to eight replicates on any given day had CVs which ranged from 0.8 to 5.1%. The day-to-day CV for the Bactec preferred method run on 4 separate days was 9.3%, and for the alternate Bactec method it was 5.0%. The RIA CV for day-to-day variations over 5 days of runs was 4.7%. The overall laboratory CVs for 24 total replicates were 12.5% for the Bactec preferred method, 13.3% for the alternate method, and 5.4% for RIA.

Table 1 also shows similar data for the high-level gentamicin test solutions. The within-run CVs for the Bactec preferred method ranged from 5.8 to 12.0%. The Bactec alternate method CVs ranged from 7.4 to 20.9% and the RIA determinations ranged from 1.1 to 5.2%. The day-to-day CV for the Bactec method run on 4 separate days was 8.8%, and for the alternate Bactec method it was 14.1%. The RIA run on 5 separate days had a CV of 7.7%. Overall total laboratory variation for the Bactec preferred method was 13.5%, that for the Bactec alternate method was 18.6%, and that for RIA was 7.4%.

For comparative studies with the RIA technique, 84 serum samples from patients on gentamicin therapy (Fig. 1) were assayed by both the Bactec preferred method and RIA. These results yielded a coefficient of correlation (r) of 0.82. The linear regression line determined by the method of least squares is $X = 0.75Y + 1.8$.

DISCUSSION

It is apparent from the data that the CVs for the gentamicin assay performed by the radiometric method (Bactec 460) were consistently higher than those done by RIA. The higher CVs were consistently found for the within-run variations, day-to-day variations, and the overall laboratory variation. Also, with one exception, the Bactec preferred method of performing the assays resulted in lower CVs than the Bactec alternate method. In similar studies, reported in their package insert, Johnston Laboratories had CVs of 5.9 and 9.0% for low and high levels of

TABLE 1. CVs of serum gentamicin assays

Gentamicin level	Methodology	Within run				Day to day			Total laboratory	
		No. of runs	No. of replicates	Range of assays ($\mu\text{g/ml}$)	Range of CVs (%)	No. of runs	Range of assays ($\mu\text{g/ml}$)	CV (%)	Total replicates	CV (%)
Low (3.5 $\mu\text{g/ml}$)	Bactec (preferred)	4	6	2.4-4.2	3.9-14.0	4	3.1-3.9	9.3	24	12.5
	Bactec (alternate)	4	6	2.2-4.1	8.7-20.1	4	3.0-3.4	5.0	24	13.3
	RIA	5	2-8	2.8-3.6	0.8-5.1	5	3.0-3.4	4.7	24	5.4
High (10.5 $\mu\text{g/ml}$)	Bactec (preferred)	4	6	6.8-12.5	5.8-12.0	4	8.5-10.9	8.8	24	13.5
	Bactec (alternate)	4	6	6.6-11.2	7.4-20.9	4	7.6-10.3	14.1	24	18.6
	RIA	5	2-8	10.8-14.1	1.1-5.2	5	11.5-13.9	7.7	24	7.4

FIG. 1. Comparison of the Bactec radiometric assay with RIA for gentamicin levels on 84 patient sera. The correlation coefficient is 0.82. The linear regression line determined by the method of least squares is $X = 0.75Y + 1.8$.

gentamicin, respectively, based on a single run of 10 assays for each level. Their CVs for day-to-day variations based on 6 runs were 6.4 and 10.2% for the low and high levels of gentamicin, respectively. Their results were consistent with our findings with the Bactec preferred method. Practically, intra-assay CVs (within-run variations) of 5 to 10% are considered acceptable, whereas inter-assay CVs (day-to-day variations) tend to be higher and fall within a range of 5 to 20% (8).

The data (Table 1) indicate that RIA methodology is significantly more precise than the Bactec radiometric method for determining gentamicin serum levels and that the Bactec preferred method is more precise than the Bactec alternate method. Since the Bactec radiometric method necessitates the use of small syringes for adding the various reagents to a closed system (base solution bottles), whereas the RIA methodology uses precise pipetting, the precision of the former would be expected to be less than the latter, as demonstrated in this study. The overall average assay values of the low-gentamicin test solution were essentially comparable by both the Bactec and the RIA methods; however, at the high level of gentamicin the RIA assay values were consistently higher. Very little, if any, gentamicin is metabolized in vivo (10, 12, 20). Several studies comparing other bioassay methods with RIA have shown good correlation. Thus, although it is not clear at this time why the RIA assay values were higher than the Bactec values, it does not seem to be due to the fact that RIA might be measuring nonbioactive gentamicin present in serum.

Gentamicin assays of 84 patients' sera (Fig. 1) by the Bactec preferred and RIA methodologies show that at the lower levels of gentamicin the Bactec assays were generally lower than the RIA determinations but that at higher levels the Bactec values were higher. Of the 84 sera assayed, 8 determinations were at toxic levels ($>8 \mu\text{g/ml}$) by one or both methods. Of the eight, four were toxic by both methods. Of the remaining four, one was toxic by Bactec and normal by RIA and three were toxic by RIA and normal by Bactec. These are too few in number to establish a trend, but others have reported similar results (12), suggesting that patients on multiple antibiotics may have somewhat increased or decreased gentamicin assay results by bioassay methodologies, whereas RIA assays would not be affected by multiple-drug therapy. The manufacturer points out that most of the antibiotics commonly administered with gentamicin have no effect on the radiometric assay except that carbenicillin at high levels may inactivate gentamicin in serum, giving a false low serum gentamicin level, and that tetracycline may also interfere with the assay, resulting in false high serum gentamicin levels. This could account for some of the discrepancies noted above.

In summary, although RIA methodology is more precise, the precision of the Bactec radiometric method is adequate for those laboratories where RIA equipment is unavailable. It is suggested by the manufacturer that if a serum level is $>14 \mu\text{g/ml}$, the serum should be diluted twofold by adding 0.1 ml of the patient's serum and 0.1 ml of normal pooled human serum to the base solution bottle. The Bactec radiometric method is less expensive, takes no more technical time than the RIA, has longer reagent shelf life, and is an acceptable means of determining gentamicin levels in laboratories without RIA capability.

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