Comparison of Radioimmunoassay with a New Immunofluorescent Method (FIAX) for Measuring Tobramycin in Serum

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A new immunofluorescent method (FIAX) was compared with a radioimmunoassay procedure for the determination of tobramycin serum concentrations. When assaying three tobramycin control sera repeatedly, within-run and run-to-run variations by the FIAX method were all within acceptable limits, and no statistically significant differences were found. Results of 155 sera from patients who had been treated with tobramycin showed a correlation coefficient of 0.93 between the two methods. The FIAX method represents another practical alternative for determining tobramycin serum levels.

The aminoglycoside group of antibiotics possesses a broad spectrum of activity against gram-negative organisms, and tobramycin represents one of the aminoglycosides currently in use in treating life-threatening infections. As with other aminoglycosides, there is a relatively narrow range between toxic and therapeutic concentrations of tobramycin. To insure adequate patient treatment, frequent monitoring of aminoglycoside serum drug levels has been advocated to avoid either inadequate or excessively high serum concentrations of the antibiotic (3, 9, 11, 14). Numerous methods have been described for assaying tobramycin serum concentrations including: bioassay (8, 10, 12), enzymatic assays (13), gas-liquid chromatography (6, 7), high-pressure liquid chromatography (1), and radioimmunooassay (RIA) (2).

In this study, the FIAX immunofluorescent assay method was compared with RIA to determine the following: (i) within-run precision; (ii) run-to-run reproducibility; (iii) accuracy of determinations by comparison with expected values; and (iv) the reliability of the FIAX method in determining tobramycin levels in patient sera.

MATERIALS AND METHODS

This study employed 155 selected serum samples from patients who had been under treatment with tobramycin. Specimens that could not be assayed on the day they were obtained were frozen and stored at −20°C until tested.

RIA. Tobramycin RIA kits were obtained from Diagnostic Products Corp., Los Angeles, Calif. All samples were assayed according to their recommend protocol.

FIAX. All reagents used for the FIAX assay procedure were supplied by International Diagnostic Technology, Santa Clara, Calif.

The solid-phase immunofluorescence method primarily involves competition between a fluorescein-labeled and an unlabeled antigen for a fixed amount of antibody immobilized on a reactive surface. In this case, specific anti-tobramycin antibody was immobilized on a defined polymeric surface such as that which is present on the StIQ sampler. This sampler was immersed sequentially into a mixture of fluorescein-labeled tobramycin and a sample (standards, controls, or unknown) containing unlabeled tobramycin. Thus, the amount of fluorescein-labeled tobramycin that attaches to the specific anti-tobramycin antibody is inversely proportional to the concentration of unlabeled tobramycin in the sample.

The following procedure outlines the assay protocol used in this study: 700 μl of fluorescein-labeled tobramycin was dispensed into 12- by 75-mm glass tubes to which 10 μl of sample (standard, control, or unknown) was added; each sample was tested in duplicate. All tubes were shaken briefly by hand. StIQ samplers were placed into these tubes and then mixed on a mechanical shaker for 25 min at room temperature. The tubes and StIQ samplers were removed from the shaker and allowed to equilibrate for an additional 5 min before reading. Fluorescence is measured on a fluorometer set at 995 nm (excitation) and 530 nm (emission). The degree of fluorescence was expressed in fluorescence signal units. The fluorometer was adjusted to read 190 fluorescence signal units with a reference solution containing 1 μg of tobramycin per ml.

A standard curve was constructed with tobramycin standards (included in the FIAX kit) representing 1, 2, 4, 8, and 16 μg/ml. The concentration of tobramycin in both the controls and unknowns was determined by
interpolation. It is recommended that a standard curve be established for every run (testing up to 20 samples in addition to the standards).

In comparing the two systems, three tobramycin controls representing high (12.0-μg/ml), midrange (5.5-μg/ml), and low (2.5-μg/ml) serum concentrations of tobramycin were included in 14 separate runs to assess run-to-run reproducibility. In addition, these same controls were used to assess within-run precision by assaying each control 22 times in a single run. Controls were prepared in our laboratory by diluting tobramycin stock solution (Eli Lilly & Co., Indianapolis, Ind.; lot no. S1-36 8T, 1,000 μg/ml) in normal human serum (Microbiological Associates, Bethesda, Md.; lot no. 95389) and then stored at −70°C. For daily usage, they were thawed and kept at 4°C for up to 1 month. These controls are used routinely in our laboratory as part of the standard quality control procedure.

RESULTS

The precision of the FIAx system when compared with the RIA method is shown in Table 1. When either method was compared for within-run variations using three different tobramycin controls, no statistically significant differences were found by the chi-square method (P > 0.05) (5). The respective coefficient of variations were greater for the FIAx method than for the RIA method.

The reproducibility of run-to-run values were within acceptable limits for both methods, and no statistically significant differences were found (Table 2). However, it should be pointed out that the values obtained by the FIAx method consistently overestimated the actual tobramycin levels.

When sera from 155 patients who had received tobramycin were analyzed by both methods (Fig. 1), the majority (148) of these sera contained tobramycin levels of less than 7 μg/ml (as determined by RIA). Applying statistical correlation analysis, a slope of 0.99, an intercept of −0.16, and a correlation coefficient of 0.93 were obtained. Using the related measures t test (5), it was shown that the difference between the respective means was not statistically significant (P > 0.05).

DISCUSSION

This evaluation has demonstrated that the FIAx method for performing tobramycin assays correlated well with the RIA method. In analyzing our control samples, the FIAx method overestimated the tobramycin concentrations; however, these differences were not statistically significant. On the other hand, when individual patient sera were assayed for tobramycin, the FIAx method showed a trend toward slightly underestimating the tobramycin concentrations when compared with the RIA method. These differences also were not statistically significant. The reasons for these two observations remain unknown.

The direct immunofluorescent assay described in this study is both a rapid and a simple assay procedure from which results can be obtained in less than 1 h. In contrast to the RIA method, the FIAx method takes considerably less time and eliminates the problems dealing with radioactive waste disposal. The entire assay can be performed in a single test tube, and the reaction product is bound to a cellulose filter and assayed directly in a fluorospectrophotometer. Both methods require the use of a standard curve for each run. Thus, tests are usually run once or twice a day and “stat” assays of individual specimens are less convenient and less efficient.

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**TABLE 1. Comparison of FIAx and RIA methods for within-run precision**

<table>
<thead>
<tr>
<th>System</th>
<th>Tobramycin controls (μg/ml)*</th>
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<tbody>
<tr>
<td></td>
<td>12.0</td>
<td>5.5</td>
<td>2.5</td>
</tr>
<tr>
<td>FIAx</td>
<td>14.89 ± 0.74 (4.95)c</td>
<td>6.77 ± 0.79 (11.67)</td>
<td>3.28 ± 0.43 (13.16)</td>
</tr>
<tr>
<td>RIA</td>
<td>11.73 ± 0.61 (5.17)</td>
<td>5.17 ± 0.20 (3.93)</td>
<td>2.56 ± 0.07 (2.83)</td>
</tr>
</tbody>
</table>

* Number of replicates: 22.
* Results expressed as mean ± standard deviation.
* Coefficient of variation.

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**FIG. 1. Tobramycin levels in patient sera as assayed by FIAx and RIA methods.**
TABLE 2. Comparison of FIAX and RIA methods for run-to-run reproducibility

<table>
<thead>
<tr>
<th>System</th>
<th>12.0</th>
<th>5.5</th>
<th>2.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIAX</td>
<td>13.71 ± 0.66 (4.83)c</td>
<td>6.93 ± 0.85 (12.24)</td>
<td>3.24 ± 0.41 (12.52)</td>
</tr>
<tr>
<td>RIA</td>
<td>12.48 ± 0.89 (7.11)</td>
<td>5.27 ± 0.43 (8.10)</td>
<td>2.56 ± 0.28 (10.78)</td>
</tr>
</tbody>
</table>

a Number of replicates: 14.
b Results expressed as mean ± standard deviation.
c Coefficient of variation.

Of interest is that the imprecision of both methods requires that samples be run in duplicate to obtain optimal accuracy. Both the FIAX and RIA methods are specific in terms of not cross-reacting with other antimicrobial agents (4). The FIAX method requires 10 μl of sample, which could be important in cases where a limited amount of specimen may be obtained, e.g., neonates. Reagents used for the FIAX and RIA systems are comparably expensive, and instruments required to perform these analyses are quite sophisticated. Thus, neither system is considered practical for small laboratories unless other applications can be used for the respective instruments.

In summary, the FIAX system compared favorably to the RIA system for performing tobramycin assays and appears to be an acceptable alternative method for performing tobramycin assays.

LITERATURE CITED