Latex Agglutination Inhibition Card Test for Gentamicin Assay: Clinical Evaluation and Comparison with Radioimmunoassay and Bioassay

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Received 15 August 1980/Accepted 20 January 1981

Gentamicin levels were determined in 100 serum specimens by a new latex agglutination inhibition card test, a radioimmunoassay (RIA), and a bioassay. Correlation coefficients determined by linear regression analysis demonstrated that the levels obtained by the latex agglutination inhibition card test had a high degree of correlation with the RIA and could be performed much faster and more economically when processing small numbers of specimens. The bioassay had a slightly lower degree of correlation with both the RIA and the latex test and was adversely influenced by concurrently administered antibiotics which could not be eliminated by beta-lactamase. When measuring gentamicin concentrations above 2 μg/ml, the coefficient of variation was less than 14% for the latex agglutination assay compared with 15% for the bioassay and 12% for RIA. The latex agglutination inhibition card test is a rapid, accurate, specific, and reproducible method for monitoring gentamicin levels in patients and is particularly applicable for laboratories processing small numbers of specimens.

MATERIALS AND METHODS

Serum specimens. One hundred serum specimens used in the evaluation were obtained from three sources. A total of 68 clinical specimens were obtained from 38 patients receiving gentamicin, often in combination with other antimicrobial agents at the Baltimore Veterans Administration Medical Center and the Maryland Institute for Emergency Medical Service. Most samples were obtained immediately before and 1 h after antibiotic administration. A total of 24 specimens were obtained from 12 volunteers receiving 1.5 mg of gentamicin per kg in combination with ampicillin, penicillin, or amoxicillin during other antibiotic studies at the Baltimore VA Medical Center. To determine the accuracy of the assays at relatively high levels of gentamicin, eight clinical serum specimens containing an unknown amount of gentamicin, alone or with other antimicrobial agents, were spiked with an additional 10 μg of gentamicin per ml. Antimicrobial agents concurrently administered with gentamicin in the 100 sera included ampicillin (in 16 sera), amoxicillin (8), ticarcillin (12), cephalothin (24), cefoxitin (2), clindamycin (10), chloramphenicol (12), nafcillin (6), dicloxacillin (2), and unspecified (15). Latex agglutination inhibition card test. The latex agglutination inhibition card test (Macrovue
gentamicin card test) was obtained from Hynson, Westcott, and Dunning, Division of Becton, Dickinson Immunodiagnostics and was performed according to instructions provided with the kit. The patient's serum was diluted with buffer directly on the indicated circle of a black plastic-coated card. The serum dilutions were 1:2, 1:3, 1:4, 1:5, 1:6, 1:8, 1:10, 1:12, 1:16, 1:20, 1:24, and 1:32. Four standard concentrations of gentamicin, 0.3, 0.4, 0.5, and 0.6 μg/ml, were also placed on the test card at their indicated positions. A 0.025-ml amount of anti-gentamicin antiserum was added to each serum dilution and to each standard concentration and mixed. One drop of latex antigen suspension containing a specific concentration of a gentamicin latex conjugate was added, and the card containing the mixtures was mechanically rotated for 8 min. The concentration of gentamicin in the serum specimen was determined by multiplying the reciprocal of the highest dilution of serum that inhibited the agglutination reaction by the lowest concentration of gentamicin standard which showed similar inhibition. During much of the study, a needle which delivered 23.2 μl/drop rather than the specified 25 μl/drop was used in the kit to apply buffer diluent to the serum, and the resulting calculations were corrected to reflect this smaller drop size.

**Microbiological assay.** The bioassay kit (Gentasak, BBL Microbiology Systems, Cockeysville, Md.) was used for the evaluation (5). The assay is a paper disk diffusion test which uses as the test organism, Bacillus subtilis spores that are seeded in agar in petri plates. In addition to containing the standard or unknown gentamicin, the paper disks are impregnated with beta-lactamase to eliminate the inhibition observed from concurrently administered penicillins and cephalosporins. Zones for each standard and specimen were measured in quadruplicate.

**RIA.** The RIA (Diagnostic Products Corporation) was performed as described in the product brochure and employed duplicate determinations for each specimen and control.

**Statistical analysis.** The RIA and latex agglutination inhibition tests were performed by different technicians without either having the knowledge of the results obtained by the other method. Linear regression was determined by the method of least squares. Only the 58 samples giving gentamicin values within the range of all three methods were included for the statistical analysis. Scatter was evaluated by the coefficient of correlation (r) and by calculation of the standard error of the estimate (Sy) as suggested by Westgard and Hunt (8). The coefficient of variation (CV) was calculated in the standard manner by dividing the standard deviation by the mean and multiplying by 100.

**RESULTS**

**Linear regression analysis.** Comparison of the serum gentamicin levels obtained by the latex agglutination inhibition card test and RIA for the 100 serum samples is seen in Fig. 1. A linear relationship is observed between the two assays. Correlation coefficients determined by linear regression analysis on 58 samples which contained gentamicin concentrations within the range for all three assay techniques demonstrated a high degree of correlation with a slope of 1.00 and an r value of 0.97. The Y-intercept of the linear regression between the two methods was 0.08. The magnitude of random error indicated by the standard error of the estimate (Sy) was 0.75 μg/ml.

The correlation of serum gentamicin levels obtained by the latex agglutination inhibition card test and the bioassay for the 100 sera is shown in Fig. 2. There were 12 specimens which gave obviously higher gentamicin levels by bioassay than by the latex agglutination inhibition technique due to the interference of concurrently administered antibiotics which could not be eliminated by the beta-lactamase–impregnated disks contained in the bioassay kit. Linear regression analysis using the 58 samples containing gentamicin levels within the range of the three assay techniques excluded all but three of these specimens. Correlation coefficient revealed an r value of 0.94, a slope of 1.23, and a Y-intercept of 1.07 on the 58 samples. The standard error of the estimate (Sy) was 1.04 μg/ml.

The correlation between serum gentamicin levels obtained by RIA compared with those obtained by the bioassay technique was similar to the correlation between the latex agglutination inhibition card test and the bioassay (not shown). The 12 specimens giving high gentamicin levels by bioassay compared with the latex agglutination inhibition assay also were noted in this comparison. The linear regression analysis which excluded all but three of these demonstrated a correlation coefficient of 0.93, similar to the comparison between the latex card test and the bioassay (r = 0.94). The slope was 1.17 and the Y-intercept was −0.75. The standard error of the estimate was 1.20 μg/ml.

**Accuracy and precision.** Table 1 indicates the coefficient of variation, mean, and range obtained for the three assays when gentamicin levels were determined daily on five separate days in 11 serum specimens. The similarity of the means obtained by the three assays is readily apparent. Patient no. 1 had higher levels by the bioassay than by the other two assay techniques, probably due to the interfering effect of clindamycin. The coefficient of variation for samples containing greater than 2 μg/ml was less than 15% (0, 9.1, 13.4, and 11.8) for the latex agglutination inhibition card test compared with less than 12% (4.4, 11.1, 6.7, and 4.4) for the corresponding samples assayed by RIA and less than 15% (11.4, 14.3, 11.1, and 10.3) for the bioassay. For samples containing 2 μg/ml or less, the coefficient was
highly variable for the latex test (0 to 25%), less than 15% for RIA, and could not be determined in most instances by bioassay.

Cost and time analysis. Comparative cost and time analysis for the three assays is indicated in Table 2. The time required to perform the latex agglutination inhibition assay when gentamicin was measured in one specimen was 12 min compared with 70 min for the RIA and 27 min for the bioassay. An additional 4 to 24 h of incubation was required before results could be obtained with the bioassay. For six specimens, the total time required to perform the latex agglutination inhibition assay increased to approximately 48 min (8 min per sample) compared with 75 min (12.5 min per specimen) for the RIA and 144 min (24 min per sample) for the bioassay. For 12 serum specimens, the RIA could be performed faster than the latex agglutination inhibition assay (79 min versus 96 min) and twice as rapidly as the bioassay (156 min).

Analysis of the costs for the purchase of the assay materials as a function of the number of serum samples processed in a single batch indicates that the latex test and bioassay ($4.00 and $4.46, respectively) were much less expensive than RIA ($14.00) when a single specimen is processed. For processing six specimens, the three assays were similar in cost; the latex test ($4.00 per test) was slightly higher than RIA ($3.50) and the bioassay ($3.41). For 12 specimens, RIA was the least expensive of the three assay techniques.
**Table 1.** The mean, range, and coefficient of variation (CV) obtained by the three assays when gentamicin levels were determined for 11 serum specimens assayed on each of five days

<table>
<thead>
<tr>
<th>Patient</th>
<th>Concurrent antibiotic</th>
<th>RIA</th>
<th>Latex</th>
<th>Bioassay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (µg/ml)</td>
<td>Range (µg/ml)</td>
<td>CV (%)</td>
<td>Mean (µg/ml)</td>
</tr>
<tr>
<td>1</td>
<td>Nafcillin and clindamycin</td>
<td>1.64 (1.4-1.8)</td>
<td>10.4</td>
<td>1.62 (1.0-1.9)</td>
</tr>
<tr>
<td>2</td>
<td>Chloramphenicol</td>
<td>1.3 (1.0-1.5)</td>
<td>15.4</td>
<td>1.0 (1.0-1.0)</td>
</tr>
<tr>
<td>3</td>
<td>Ticaricillin</td>
<td>4.02 (3.6-4.2)</td>
<td>8.7</td>
<td>4.48 (3.6-5.3)</td>
</tr>
<tr>
<td>4</td>
<td>Cephalothin</td>
<td>18.5 (17.8-21.0)</td>
<td>8.2</td>
<td>13.2 (13.2-21.2)</td>
</tr>
<tr>
<td>5</td>
<td>Cephalothin</td>
<td>4.08 (3.9-&lt;4.3)</td>
<td>4.4</td>
<td>4.14 (3.6-4.5)</td>
</tr>
<tr>
<td>6</td>
<td>Chloramphenicol</td>
<td>1.40 (1.2-1.5)</td>
<td>10.0</td>
<td>1.16 (1.0-1.4)</td>
</tr>
</tbody>
</table>

*NA, Not applicable.

**Table 2.** Time and cost analysis for each of the three assay procedures when 1, 6, and 12 specimens are processed daily

<table>
<thead>
<tr>
<th>Assay</th>
<th>Cost per sample for (no. of specimens):</th>
<th>Time per sample (min) for (no. of specimens):</th>
<th>Total time (min) for (no. of specimens):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>RIA</td>
<td>$14.00</td>
<td>$3.50</td>
<td>$2.33</td>
</tr>
<tr>
<td>Latex</td>
<td>$4.00</td>
<td>$4.00</td>
<td>$4.00</td>
</tr>
<tr>
<td>Bioassay</td>
<td>$4.46</td>
<td>$3.41</td>
<td>$3.28</td>
</tr>
</tbody>
</table>

*Assuming the number of specimens indicated are performed per day, 5 days per week for 4 weeks. Costs include only the purchase price of the assays and do not include labor and capital equipment, scintillation counters, etc.

*Plus an additional 4 to 12 h of incubation.

**DISCUSSION**

RIA has proved to be a sensitive, accurate method to determine serum levels for gentamicin and other therapeutic agents. It is for this reason that this assay was used in the present study as a reference method. The evaluation indicates that there is very little constant or proportional error between the latex agglutination inhibition assay and the RIA as indicated by a Y-intercept of 0.08 and a slope of 1.0, respectively. The correlation coefficient of 0.97 reflects the minimal random error between the two assays. The degree of random error between the two methods is better demonstrated by the low standard error of the estimate (Sy) of 0.75 µg/ml. Thus, for a given value of the reference method (RIA), the value of the latex agglutination inhibition card test would agree within ±1.5 µg/ml for 95% of the samples (±2 standard deviations) (8). This is well within acceptable limits required for measuring gentamicin levels in clinical specimens.

The reproducibility of the latex agglutination inhibition assay is also very similar to RIA when considering serum concentrations above 2 µg/ml. For serum samples containing less than 2 µg/ml, however, the coefficient of variation was variable for the latex assay and ranged from 0 to 25%. Whereas it is doubtful that this variation at these low concentrations would be of any significance to the clinician in measuring serum concentrations, it may limit the usefulness of the assay for measuring tissue concentrations and levels in body fluids, where more sensitive assays are required.

Time and cost analysis was helpful in placing these two assays in perspective. With the latex inhibition agglutination card test, a sample specimen could be processed within 12 min, and a pair of specimens, "peak" and "valley," could be processed within 20 min, making results of the assay available to the clinician before the time of administration of the next dose of the antibiotic. In contrast, the RIA required 70 min and considerable cost to process one or two specimens. For six serum samples, the latex agglutination assay was still more rapid than the RIA.
nation inhibition card test, the bioassay requires twice as long to set up and read the assay plates for one specimen and three times the time required to process six specimens. An additional 4-h and usually overnight incubation is required for the bioassay before the results are available to the clinician. The amount of serum required to perform the bioassay is also greater than is necessary for the latex agglutination inhibition assay. The latter technique requires less than 0.1 ml of serum, making it readily adaptable for pediatric patients as well as adults.

The latex agglutination inhibition assay is an easy to perform assay which requires minimal specialized equipment. Its speed and relative high degree of accuracy will make the assay particularly valuable for laboratories performing small numbers of gentamicin assays daily.

ACKNOWLEDGMENTS

Our sincere thanks to Merrill Snyder for reviewing the manuscript. This work was supported by the Veterans Administration and Hyson, Westcott & Dunning, Inc.

LITERATURE CITED