Evaluation of the Macro-Vue Latex Agglutination Test for Quantitation of Gentamicin in Human Serum

J. E. JOHNSON, S. CRAWFORD, AND J. H. JORGENSEN

Department of Pathology, The University of Texas Health Science Center at San Antonio, and Department of Laboratory Services, Audie Murphy Memorial Veterans Administration Hospital, San Antonio, Texas

Received 29 March 1982/Accepted 10 May 1982

The Macro-Vue Card Test (Hynson, Wescott, and Dunning, Baltimore, Md.) for rapid quantitation of gentamicin in serum was compared with bioassay and radioimmunoassay procedures on sera from 100 patients. Regression analysis of paired results from the bioassay (using Klebsiella pneumoniae ATCC 27799) and the Macro-Vue Card Test indicated a correlation coefficient of 0.89, whereas comparing radioimmunoassay and Card Test results yielded a correlation coefficient of 0.83. The bioassay-radioimmunoassay correlation coefficient was 0.88. The data were further examined by grouping the sera of the patients into three categories based on therapeutic concentration ranges as follows: <2 µg/ml, subtherapeutic range; 2 to 8 µg/ml, therapeutic range; >8 µg/ml, potentially toxic range. Of 100 specimen values, 81 fell into the same concentration range when bioassay and Card Test values were compared. Of the 19 disagreements, 12 were considered minor. Of 100 specimen values compared by radioimmunoassay and the Card Test, 88 fell into the same concentration range. Of the 12 disagreements, 6 were considered minor. Therefore, the Macro-Vue Card Test for gentamicin compared favorably with both the bioassay and radioimmunoassay procedures. The minimal time required to perform the Card Test (12 to 15 min) makes it attractive for situations in which immediate results are needed.

Several different procedures are available to monitor gentamicin levels, including bioassay (4, 5), radioimmunoassay (RIA) (2, 4, 5), radioenzymatic assay (2, 4), and enzyme immunoassays (4, 5). These methods are all acceptable procedures for the measurement of gentamicin. However, they require relatively expensive equipment for performance of the assays.

The Macro-Vue Card Test (Hynson, Wescott, and Dunning, Baltimore, Md.) is designed to provide reproducible results without the necessity for sophisticated equipment and to enable most laboratories to monitor gentamicin serum levels quickly.

The purpose of this study was to evaluate the Macro-Vue Card Test for accuracy and technical facility by comparison with the bioassay and radioimmunoassay procedures on sera from patients.

(This study was presented in part at the 1980 Annual Meeting of the American Society for Microbiology [J. E. Johnson, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, C43, p. 281]).

MATERIALS AND METHODS

Sera. A total of 100 sera were obtained for the purpose of routine therapeutic monitoring from patients receiving gentamicin and hospitalized in either the Audie Murphy Veterans Administration Hospital or the Medical Center Hospital. The sera were either tested immediately or frozen at −20°C until tested.

Bioassay procedure. Antibiotic medium number 11 (Difco Laboratories, Detroit, Mich.) was prepared according to the instructions of the manufacturer, adjusted to pH 8.0 with 5 N sodium hydroxide, dispensed in 10-ml amounts in sterile tubes, and stored in a refrigerator until use. Agar plates were made by melting the required number of tubes in a boiling-water bath, cooling to 50°C, and adding 0.1 ml of an overnight Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) culture of Klebsiella pneumoniae ATCC 27799 to each tube (3). The culture and agar were mixed thoroughly, and plates were poured and allowed to solidify for at least 15 min. Five-millimeter wells were cut into the agar with a cork borer and aspirated. Duplicate wells were filled with 20-µl amounts of each of three drug standards and patient samples. Two assay plates were used for each set of samples. After incubation for 4 to 6 h, the average zone diameter of inhibition was determined for each standard and sample. A standard curve was derived from the average inhibition zone diameters of the three gentamicin standards versus their known concentrations. The concentration of each sample was then read directly from the standard curve.

RIA. Gentamicin levels were determined with gentamicin 125I-iodine RIA kits (Diagnostic Products Corp., Los Angeles, Calif.). Six calibrators (standards) and all patient samples were diluted 1:201 in reconstituted
Tris buffer. Duplicate 100-μl samples were processed according to the directions of the manufacturer and counted for 1 min each with a Gamma 310 radioisotope counter (Beckman Instruments, Inc., Fullerton, Calif.). A logit-log calibration curve was constructed from data obtained with the standards, using an RIA computer program (Hewlett-Packard Co., Palo Alto, Calif.). The gentamicin values (micrograms per milliliter) of sera from patients were calculated directly through the use of the computer program.

**Macro-Vue Card Test.** The Macro-Vue Card Test for gentamicin is a latex agglutination inhibition procedure. Sera from patients were diluted on the card according to the directions of the manufacturer, resulting in 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 dilutions. Four standards consisting of 0.3, 0.4, 0.5, and 0.6 μg of gentamicin per ml were also placed on the test card. Antigentamicin antiserum was added to each well, enabling the gentamicin present in the serum dilutions and in the standards to react with the antibody. Gentamicin-coated latex particles were then added to all of the wells on the card, which was put on a rotator at 100 rpm for 8 min. After mechanical mixing, the card was held under a high-intensity lamp, and the presence or absence of agglutination (clumping) was determined for the standards and the sera from patients. The lowest concentration of the standard showing complete inhibition of agglutination was multiplied by the reciprocal of the highest dilution of the test specimen demonstrating complete inhibition to determine the concentration of gentamicin in the test specimen. One card containing 12 wells for specimen dilutions and 4 wells for standards was needed per serum sample tested.

**Statistical analysis.** Bioassay, RIA, and the Macro-Vue Card Test were each performed by different individuals without knowledge of the other test results. Linear regression analysis was used to determine the coefficients of correlation for RIA-bioassay, bioassay-Macro-Vue Test, and RIA-Macro-Vue Test comparisons.

**RESULTS**

A total of 100 sera from patients were tested with the Macro-Vue Card Test, the *K. pneumoniae* ATCC 27799 agar diffusion bioassay, and a commercially available RIA procedure. Linear regression analysis of paired results from the Card Test and bioassay indicated a correlation coefficient of 0.89 (Fig. 1). Similar results were noted in comparing the paired values for bioassay and RIA, in which a correlation coefficient of 0.88 was noted (Fig. 2). The correlation coefficient obtained with the Macro-Vue Card Test versus the RIA procedure was slightly lower (0.83 [Fig. 3]). Upon comparison of the
DISCUSSION

Numerous studies have demonstrated the value of the RIA method for determination of gentamicin levels (2, 4-6). In a study by Standiford et al. (6) in which the standard error of the estimate was examined, a comparison of the Card Test with RIA indicated that the latex agglutination inhibition procedure agreed within 1.5 μg/ml for 95% of the samples tested. The authors concluded that this level was quite acceptable for routine clinical practice. Similarly, in our study, 88 to 94% agreement was observed by placing the paired results into various concentration ranges, indicating the potential usefulness of the Macro-Vue Card Test in clinical laboratories. The correlation coefficient noted with the Card-RIA comparison in this study of 0.83 (0.87 corrected) compares with the coefficients of 0.84 to 0.97 noted in other studies (5, 6).

The Card Test-bioassay correlation coefficients reported in the literature were 0.82 when a Staphylococcus epidermidis test strain was used (5) and 0.94 when a Bacillus subtilis strain was used (6). Our study, in which a K. pneumoniae strain was used, gave a correlation coefficient of 0.89 (0.85 corrected). The range of the correlation coefficients may be due to the variation normally observed with a bioassay procedure. This variation could be due to the presence of other antibiotics in the test sera. Our study utilized a highly resistant organism (K. pneumoniae) to prevent changes in zone size owing to the influence of other antibiotics. Although the other studies mentioned incorporated a beta-lactamase in their bioassay test systems, falsely elevated results were still noted in the study of Standiford et al. (6). In our study, the presence of various antibiotics, including ampicillin, carbenicillin, cephalothin, chloramphenicol, clindamycin, erythromycin, penicillin, oxacillin, ticar-
penicillin, and vancomycin, did not affect the results obtained by either the bioassay or the Macro-Vue Card Test.

A recent study by Doern et al. (1) in which the RIA, bioassay, and Macro-Vue Card Test were compared concluded that the Card Test was highly variable and not sufficiently precise for use in the clinical laboratory. Sera from patients were not examined in their study. A total of 20 different concentrations of gentamicin were prepared in pooled human sera without antibiotics. These samples were divided into triplicate and subsequently analyzed in two different laboratories. The correlation coefficient (average of the two laboratories) for the Card Test was 0.877, which was quite low in comparison with that observed for bioassay (0.989) and RIA (0.990). The coefficients of variation were 22.5% for the Card Test, 7% for bioassay, and 6% for RIA. However, in examining their data, the greatest variation was noted with either very low or very high gentamicin concentrations. In our study, in which the various concentration ranges were examined (Fig. 4 and 5), only 6 to 7% of the specimens did not fall into the appropriate therapeutic concentration range, indicating that the Card Test results are still of clinical value the vast majority of the time.

The Macro-Vue Card Test was most difficult to interpret with serum specimens containing less than 2 μg of gentamicin per ml, an observation which was also noted in a previous study (6). At the lower dilutions, it became quite difficult to interpret complete inhibition of agglutination. In specimens containing >2 μg of gentamicin per ml, the endpoint was much easier to see.

Overall, the Macro-Vue Card Test is simple to perform and rapid (15 min). The test compares favorably with the RIA and bioassay procedures. Therefore, we feel the Macro-Vue Gentamicin Card Test is useful for determination of gentamicin levels in human serum for laboratories in which small numbers of specimens are run and in those situations where immediate results are needed.

LITERATURE CITED