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# Comparison of Kinetic and End-Point Diffusion Methods for Quantitating Human Serum Immunoglobulins

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Proficiency testing results were used to compare the kinetic and end-point versions of the single radial immunodiffusion method for quantitating human serum immunoglobulins. Statistical analysis of the results indicated that the results were not normally distributed but that the log normal distribution gives the best fit of any of the well-known frequency distributions. Consequently, statistical analysis of immunoglobulin results must be log transformed before parametric statistical tests can be appropriately applied. In general, there were no significant differences in level, precision, or interlaboratory comparability for these two methods. However, levels were different for participants using Hyland reagents, and better interlaboratory comparability was achieved by the end-point assay for immunoglobulin M. There were no significant differences in reported levels when compared by manufacturer within the same method.

Single radial immunodiffusion is the method most commonly used to quantitate human serum immunoglobulins (IgG, IgA, and IgM). Precipitin rings are measured either while the rings are still expanding (kinetic method) or when they have reached equilibrium (end-point method) (6, 11). The ring size (diameter or area) is then related to the concentration of the immunoglobulin present in the serum tested. Berne's discussion of the difference between the two methods is excellent (2). He concluded that the end-point method is preferable to the kinetic method because the former is not influenced by some of the variables affecting the latter (time, temperature, and diffusion rate, for example), and thereby achieves greater accuracy. Heremans et al. (7) have also reported that kinetic measurements can only be made at the expense of accuracy. The kinetic method is widely used because results are obtained sooner than with the end-point method and because prepared plates are readily available commercially.

The purpose of this study was to examine proficiency testing data to determine the relative accuracy and precision of the kinetic and end-point methods for the quantitation of immunoglobulins.

## MATERIALS AND METHODS

Data for this study were obtained through the proficiency testing program for diagnostic immunology conducted by the Center for Disease Control (CDC), Atlanta, Ga. Results from three separate surveys are included. Detailed descriptions of the methods used for sample preparation have been previously published (13, 15, 16). Briefly, human sera containing immunoglobulins at the desired levels were filtered through sterile membrane filters and dispensed into vials. The adequacy of the samples was confirmed independently by the Diagnostic Immunology Section of the Proficiency Testing Branch, by other CDC laboratories, and by reference laboratories. An ongoing quality control program ensures that all specimens satisfy preestablished criteria for sterility, immunoglobulin level, stability, and between-vial variability. Samples were shipped by first class mail along with the appropriate report forms. Tests were to be performed by the personnel and the methods routinely used by the laboratory, and the results were to be returned within 2 weeks. Details of the methodologies used were supplied by the participants along with their results.

The distributions of immunoglobulin results were analyzed by the Kolmogorov-Smirnov (K-S) test of goodness of fit to determine which transformation of the normal distribution, if any, most accurately described the results (12, 14). Values beyond four standard deviations were considered outliers and were excluded from analysis. As a result of these studies, statistical tests were performed on log-transformed data.

Antisera from the following companies were used by the participating laboratories in this study: Behring Diagnostics, Sommerville, N.J.; Helena Laboratories, Beaumont, Tex.; Hyland, Division of Travenol Laboratories, Inc., Costa Mesa, Calif.; Kallestad Laboratories, Chaska, Minn.; and Meloy Laboratories, Inc., Springfield, Va.

## RESULTS

Table 1 shows the results of the K-S test used to determine which distribution best character-

TABLE 1. *Characterization of distribution of immunoglobulin quantitation results*[a]

| Determination | K-S values for samples | | | | | | |
|---|---|---|---|---|---|---|---|
| | SP6-001 | SP6-002 | SP6-003 | SP6-004 | SP6-005 | BI7-A09 | BI7-A10 |
| IgG | | | | | | | |
| *N* | 307 | 295 | 305 | 307 | 307 | 275 | 273 |
| *ln* | 0.0622 | 0.1517 | 0.1026 | 0.0929 | 0.1224 | 0.1810 | 0.2246 |
| $\sqrt{\ }$ | 0.0876 | 0.0884 | 0.1267 | 0.1013 | 0.0900 | 0.1212 | 0.1705 |
| NT | 0.1071 | 0.0637 | 0.1565 | 0.1319 | 0.0843 | 0.0897 | 0.1520 |
| IgA | | | | | | | |
| *N* | 301 | 299 | 303 | 301 | 301 | 273 | 259 |
| *ln* | 0.0888 | 0.0963 | 0.0832 | 0.0743 | 0.0653 | 0.0845 | 0.1051 |
| $\sqrt{\ }$ | 0.1056 | 0.0799 | 0.1024 | 0.0591 | 0.0843 | 0.0946 | 0.1271 |
| NT | 0.1248 | 0.1002 | 0.1235 | 0.0808 | 0.1063 | 0.1208 | 0.1504 |
| IgM | | | | | | | |
| *N* | 297 | 297 | 297 | 299 | 299 | 268 | 275 |
| *ln* | 0.1220 | 0.0967 | 0.0688 | 0.0848 | 0.1120 | 0.1147 | 0.1109 |
| $\sqrt{\ }$ | 0.2022 | 0.1536 | 0.1223 | 0.1342 | 0.1656 | 0.0997 | 0.1578 |
| NT | 0.3211 | 0.2462 | 0.2214 | 0.2184 | 0.2562 | 0.1291 | 0.2307 |

[a] *N*, Number of test results; *ln*, log transformed values; $\sqrt{\ }$, square root transformed values; NT, non-transformed values (raw results).

izes the immunoglobulin results. In 14 of the 21 distributions (66.7%), the log normal transformation resulted in the best fit. The square root and untransformed distribution resulted in the best fit for 3 (14.3%) and 4 (19.0%) of the sets of immunoglobulin results, respectively. Lower K-S values indicate better fit; therefore, it appears that of the well-known distributions, the log normal most accurately describes the immunoglobulin results. If parametric statistical tests are to be used in analysis of immunoglobulin quantitation data, the results should be log transformed before the analyses are performed. Thus, immunoglobulin results have distributions that are similar to those of other serological tests, and these results can be statistically analyzed by the methods previously described (14).

Table 2 shows the results obtained by the kinetic and end-point methods for IgG, IgA, and IgM for nine samples. When the geometric means of the results were compared, the IgG results reported for the end-point method were consistently about 10% higher than those obtained for the same samples by the kinetic method. No consistent differences were seen between methods for IgA or IgM. The difference seen for the total IgG results may be a reflection of the distribution of reagents used, since the relationship between kinetic and end-point results was not consistent when results were tabulated by method and reagent manufacture (Tables 3–5).

Results were reported for both methods by laboratories using reagents from three manufacturers (Behring, Hyland, and Meloy). When these results were compared, there were no significant differences at the 0.05 probability level. The *t* values were IgG, 1.48; IgA, 0.17; and IgM, 0.0005. Results from laboratories which used Hyland reagents showed a significant difference between the levels obtained by the kinetic method compared with those of the end-point method for all three immunoglobulins. The respective statistical data were as follows: IgG, $t = 2.02$, $P < 0.05$; IgA, $t = 3.67$, $P < 0.0001$; and IgM, $t = 2.86$, $P < 0.01$. The results from laboratories using other manufacturers' reagents did not show any significant differences.

Comparison of levels within methods but between manufacturers revealed that the IgG results of Hyland and Meloy were significantly lower than those of Behring by the *t* test ($P < 0.001$) for both the kinetic and end-point methods.

For IgA, Behring and Hyland results were significantly higher than the Meloy results by the kinetic method ($P < 0.001$), and all were significantly different from each other by the end-point method ($P < 0.001$). For IgM, Hyland and Behring results were significantly lower than the Kallestad results by the kinetic method ($P < 0.05$), and the Hyland results were also significantly lower than the Kallestad results by the end-point method ($P < 0.05$).

Geometric standard deviations of the results obtained by kinetic and end-point methods were compared to assess the relative interlaboratory precision. The difference between the methods was significant for IgM ($t = 3.47$, $P < 0.001$) but was not for IgG ($t = 1.88$) or IgA ($t = 1.00$). On

TABLE 2. *Comparison of kinetic and equilibrium methods of immunoglobulin quantitation*[a]

| Sample | Kinetic method | | | End-point method | | |
|---|---|---|---|---|---|---|
| | *N* | $\bar{x}_G$ | $SD_G$ | *N* | $\bar{x}_G$ | $SD_G$ |
| IgG | | | | | | |
| SP6-001 | 176 | 924.3 | 1.150 | 124 | 987.6 | 1.175 |
| SP6-002 | 175 | 1,346.2 | 1.155 | 123 | 1,442.1 | 1.177 |
| SP6-003 | 174 | 769.1 | 1.142 | 124 | 864.0 | 1.250 |
| SP6-004 | 174 | 691.0 | 1.130 | 124 | 752.9 | 1.159 |
| SP6-005 | 176 | 928.6 | 1.163 | 122 | 981.7 | 1.158 |
| BI7-A09 | 140 | 954.6 | 1.122 | 106 | 1,067.6 | 1.181 |
| BI7-A10 | 145 | 278.6 | 1.245 | 106 | 315.2 | 1.265 |
| BI7-CO5 | 149 | 1,022.6 | 1.113 | 128 | 1,135.1 | 1.160 |
| BI7-CO6 | 150 | 1,023.9 | 1.136 | 126 | 1,149.9 | 1.147 |
| IgA | | | | | | |
| SP6-001 | 171 | 161.6 | 1.257 | 122 | 163.6 | 1.151 |
| SP6-002 | 169 | 268.5 | 1.239 | 122 | 257.2 | 1.172 |
| SP6-003 | 170 | 220.7 | 1.252 | 122 | 212.7 | 1.178 |
| SP6-004 | 170 | 131.0 | 1.261 | 123 | 132.7 | 1.197 |
| SP6-005 | 171 | 163.7 | 1.269 | 123 | 157.6 | 1.296 |
| BI7-A09 | 129 | 171.8 | 1.240 | 95 | 174.0 | 1.187 |
| BI7-A10 | 126 | 33.4 | 1.468 | 100 | 36.9 | 1.528 |
| BI7-CO5 | 145 | 182.2 | 1.228 | 128 | 190.9 | 1.156 |
| BI7-CO6 | 146 | 181.0 | 1.206 | 125 | 189.2 | 1.179 |
| IgM | | | | | | |
| SP6-001 | 164 | 107.2 | 1.230 | 121 | 105.9 | 1.139 |
| SP6-002 | 167 | 155.0 | 1.316 | 123 | 155.0 | 1.167 |
| SP6-003 | 165 | 154.1 | 1.284 | 124 | 138.4 | 1.155 |
| SP6-004 | 167 | 75.9 | 1.295 | 124 | 81.8 | 1.181 |
| SP6-005 | 167 | 105.0 | 1.299 | 124 | 105.4 | 1.158 |
| BI7-A09 | 122 | 97.2 | 1.226 | 89 | 102.9 | 1.113 |
| BI7-A10 | 126 | 37.1 | 1.262 | 85 | 42.9 | 1.325 |
| BI7-CO5 | 145 | 466.3 | 1.293 | 127 | 440.6 | 1.245 |
| BI7-CO6 | 146 | 473.1 | 1.274 | 125 | 442.9 | 1.241 |

[a] Values beyond 4 $SD_G$ are excluded. *N*, Number of results; $\bar{x}_G$, geometric mean; $SD_G$, geometric standard deviation. Results are expressed in milligrams per deciliter.

TABLE 3. *Comparison of single radial immunodiffusion IgG results by antibody source and incubation*[a]

| Test conditions | BI7-CO5 | | | BI7-CO6 | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|
| | *N* | $\bar{x}_G$ | $SD_G$ | *N* | $\bar{x}_G$ | $SD_G$ | *N* | $\bar{x}_G$ | $SD_G$ |
| Less than 25 h of incubation (kinetic) | | | | | | | | | |
| Behring | 4 | 1,220 | 1.11 | 4 | 1,183 | 1.09 | 8 | 1,201 | 1.09 |
| Helena | 10 | 951 | 1.10 | 10 | 1,001 | 1.15 | | | |
| Hyland | 39 | 1,009 | 1.10 | 39 | 1,014 | 1.13 | 78 | 1,011 | 1.11 |
| Kallestad | 50 | 1,011 | 1.09 | 50 | 1,022 | 1.10 | | | |
| Meloy | 37 | 1,034 | 1.09 | 37 | 1,047 | 1.12 | 74 | 1,050 | 1.11 |
| Other | 8 | 1,143 | 1.11 | 8 | 1,103 | 1.15 | | | |
| At least 25 h of incubation (end-point) | | | | | | | | | |
| Behring | 90 | 1,197 | 1.14 | 90 | 1,199 | 1.13 | 180 | 1,198 | 1.13 |
| Hyland | 11 | 1,064 | 1.12 | 11 | 1,073 | 1.15 | 22 | 1,068 | 1.13 |
| Meloy | 19 | 1,002 | 1.09 | 19 | 1,030 | 1.11 | 38 | 1,016 | 1.10 |
| Other | 6 | 1,059 | 1.14 | 6 | 1,009 | 1.14 | | | |

[a] Values beyond 4 $SD_G$ are excluded. *N*, Number of results; $\bar{x}_G$, geometric mean; $SD_G$, geometric standard deviation. Results are expressed in milligrams per deciliter.

TABLE 4. *Comparison of single radial immunodiffusion IgA results by antibody source and incubation*[a]

| Test conditions | BI7-CO5 | | | BI7-CO6 | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|
| | *N* | $\bar{x}_G$ | $SD_G$ | *N* | $\bar{x}_G$ | $SD_G$ | *N* | $\bar{x}_G$ | $SD_G$ |
| Less than 25 h of incubation (kinetic) | | | | | | | | | |
| Behring | 3 | 212 | | 3 | 204 | | 6 | 207 | 1.03 |
| Helena | 9 | 167 | 1.06 | 10 | 166 | 1.13 | | | |
| Hyland | 40 | 227 | 1.28 | 40 | 163 | 1.29 | 80 | 192 | 1.35 |
| Kallestad | 47 | 168 | 1.11 | 48 | 166 | 1.13 | 95 | 167 | 1.12 |
| Meloy | 37 | 165 | 1.16 | 37 | 189 | 1.12 | | | |
| Other | 8 | 179 | 1.17 | 8 | 176 | 1.17 | | | |
| At least 25 h of incubation (endpoint) | | | | | | | | | |
| Behring | 87 | 194 | 1.10 | 86 | 190 | 1.11 | 173 | 192 | 1.11 |
| Hyland | 11 | 241 | 1.08 | 11 | 247 | 1.10 | 22 | 244 | 1.09 |
| Kallestad | 21 | 167 | 1.15 | 20 | 168 | 1.15 | 41 | 167 | 1.15 |
| Other | 8 | 170 | 1.14 | 8 | 165 | 1.26 | | | |

[a] Values beyond 4 $SD_G$ are excluded. *N*, Number of results; $\bar{x}_G$, geometric mean; $SD_G$, geometric standard deviation. Results are expressed in milligrams per deciliter.

TABLE 5. *Comparison of single radial immunodiffusion IgM results by antibody source and incubation*[a]

| Test conditions | BI7-CO5 | | | BI7-CO6 | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|
| | *N* | $\bar{x}_G$ | $SD_G$ | *N* | $\bar{x}_G$ | $SD_G$ | *N* | $\bar{x}_G$ | $SD_G$ |
| Less than 25 h of incubation (kinetic) | | | | | | | | | |
| Behring | 4 | 423 | 1.50 | 4 | 423 | 1.50 | 8 | 423 | 1.50 |
| Helena | 10 | 413 | 1.30 | 10 | 404 | 1.29 | | | |
| Hyland | 40 | 460 | 1.30 | 41 | 459 | 1.26 | 81 | 459 | 1.28 |
| Kallestad | 46 | 505 | 1.25 | 46 | 493 | 1.22 | 92 | 499 | 1.23 |
| Meloy | 40 | 433 | 1.26 | 39 | 460 | 1.24 | | | |
| Other | 5 | 617 | 1.19 | 6 | 543 | 1.31 | | | |
| At least 25 h of incubation (endpoint) | | | | | | | | | |
| Behring | 86 | 440 | 1.22 | 87 | 421 | 1.59 | 173 | 430 | 1.43 |
| Hyland | 11 | 387 | 1.20 | 11 | 395 | 1.22 | 22 | 391 | 1.20 |
| Kallestad | 20 | 472 | 1.31 | 20 | 476 | 1.29 | 40 | 474 | 1.30 |
| Other | 7 | 409 | 1.25 | 7 | 423 | 1.25 | | | |

[a] Values beyond 4 $SD_G$ are excluded. *N*, Number of results; $\bar{x}_G$, geometric mean; $SD_G$, geometric standard deviation. Results are expressed in milligrams per deciliter.

eight of the nine samples for IgM, the geometric standard deviation was larger for the kinetic method than for the end-point method, indicating that the latter method provides results which are more comparable between laboratories.

To determine the within-laboratory precision, the ratios of results on duplicate samples were calculated. Table 6 shows the within-laboratory precision for each method. The geometric mean ($\bar{x}_G$) of the ratios was near 1.0, which is the expected value for duplicates. When results were compared by method, there was no significant difference by the *t* test.

The percentage of the total variance which was due to within-laboratory variation for the kinetic and end-point methods was determined from component of variance analysis (Table 6). From 50 to 90% of the variation in these test results was due to reasons other than within-laboratory variation. Some of these problems, such as differences in reagents and bias between laboratories, can be reduced by standardization, which would increase the comparability of results between laboratories.

## DISCUSSION

One source of inconsistency in publications dealing with quantitative immunoglobulins is the statistical treatment of the data. Unless the data are approximately normally distributed, the commonly used statistical techniques are invalid (3). Several investigators have observed that immunoglobulin levels conform to a log gaussian distribution (1, 3–5, 8). Others have

TABLE 6. *Within-laboratory precision by method for immunoglobulin quantitation*[a]

| Method | IgG | | | | IgA | | | | IgM | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *N* | $\bar{x}_G$ | $SD_G$ | % | *N* | $\bar{x}_G$ | $SD_G$ | % | *N* | $\bar{x}_G$ | $SD_G$ | % |
| Kinetic | 152 | 0.992 | 1.097 | 61.8 | 152 | 1.001 | 1.089 | 18.8 | 151 | 0.993 | 1.115 | 19.0 |
| End-point | 140 | 0.997 | 1.101 | 45.3 | 138 | 1.011 | 1.092 | 32.2 | 137 | 0.989 | 1.065 | 8.4 |

[a] *N*, Number of paired results; $\bar{x}_G$, geometric mean of ratios of paired results; $SD_G$, geometric standard deviation; %, percentage of total variance.

reported distributions which were positively skewed but have not attempted to characterize the distribution (9).

Allansmith et al. (1) reported that the distribution of immunoglobulin levels in adults was log normal for IgG, IgA, and IgM on the basis of plots on probability paper, but no statistical tests were applied to the data to confirm this conclusion. Our data indicate that the log normal distribution gives the best fit of any of the well-known frequency distributions. Log transformation of the results allows valid use of the usual statistical tests.

The precision indicated in publications and commercial literature is usually given as a coefficient of variation of about 6 to 10% for the end-point methods and 10 to 15% for the kinetic methods (1, 3, 8, 10). Data presented here indicate that the interlaboratory precision in terms of geometric standard deviations for both the kinetic and end-point methods is equivalent to a coefficient of variation of about 10% (if the results are normally distributed).

Intralaboratory precision based on results of duplicate samples appears to be better than this, but each laboratory should determine its own precision by using log-transformed data for its quality control. Better standardization of reagents seems to be needed for interlaboratory comparability of results. Because there does not appear to be a significant difference between the precision obtained in the kinetic and end-point methods, it might be more efficient to use the quicker kinetic method. Before a method is selected, however, each should be evaluated in terms of time, ease of performance, expense, and other factors which may be important to the individual laboratory.

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