ASD-PCR assay described here can be easily adapted for the identification of other drug-resistant mutations in *M. tuberculosis*. It can also serve as a simple and efficient tool for general single-nucleotide polymorphism analysis.

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References


Estimate of Biological Variation of Laboratory Analytes Based on the Third National Health and Nutrition Examination Survey, David A. Lacher,’ Jeffry P. Hughes, and Margaret D. Carroll (Division of Health and Nutritional Examination Surveys, National Center for Health Statistics, Centers for Disease Control and Prevention, Hyattsville, MD; * address correspondence to this author at: National Center for Health Statistics, 3311 Toledo Rd, Room 4215, Hyattsville, MD 20792; fax 301-458-4028, e-mail dol2@cdc.gov)

Laboratory analytes for individuals are subject to several sources of variation, including biological variation, pre-analytical variation (specimen collection), analytical variation (bias and imprecision), and postanalytical variation (reporting of results). Biological variation consists of within-person (WP) and between-person (BP) variation. These components of biological variation are used to set analytical quality specifications for bias and imprecision, evaluate serial changes in individual analytes, and assess the clinical utility of population-based reference intervals.

Desirable quality specifications for imprecision (I), bias (B), and total error have been related to the WP CV (CVw) and the BP CV (CVg) of laboratory analytes (1–3). Imprecision should be ideally less than one half of the CVw, and bias should be <0.25[(CVw)2 + (CVg)2]1/2. The quality specification for total error is to be less than kI + B, where k = 1.65 at α = 0.05. The total CV (CVt) can be estimated assuming that the CVs of all sources are measured at the same analyte mean and that pre- and postanalytical sources of variation are negligible. The CVt = [(CVw)2 + (CVg)2]1/2, where the analytical CV (CVa) equals the laboratory method imprecision (CVI) if there is no bias present.

Estimates of CVw and CVg for laboratory analytes were derived from the Third National Health and Nutrition Examination Survey (NHANES III) conducted from 1988 to 1994 (4, 5). NHANES III was a cross-sectional survey that collected data on the civilian noninstitutionalized US population through questionnaires and medical examinations, including laboratory analytes. NHANES III used a stratified, multistage probability design to collect a nationally representative sample. The laboratory methods including imprecision (CVI) for NHANES III have been described (6).

The BP and WP means, SDs, and CVs for 42 general biochemical, nutritional, immunologic, environmental, and hematoletic analytes are listed in Table 1. The BP and WP variations were estimated on 24 978 and 2426 sample persons, respectively. The WP sample, ~10% of the sample persons, was recruited for a second analyte measurement. The WP sample was not selected randomly, but with the goal for obtaining approximately equal proportions of males and females with one half between 20 and 39 years of age and one half over 40 years of age. When possible, the second examinations were scheduled at the same time of day as the first examinations. Compared with the BP sample, the WP sample was older (mean age, 42.9 vs 30.8 years), had more non-Hispanic whites (42.2%
The BP variations were estimated from a non-normal distribution with more stable estimates of variation. Outliers were excluded to obtain an approximately gaussian distribution. Several analytes were non-gaussian, and extreme outliers were deleted by the Tukey method.

The \( CV_g \) values were estimated using a weighted, complex sample design by Taylor series linearization (7). The BP standard deviation was calculated as \( \text{SE}^2 + \text{SD}_{\text{ars}}^2 \), where SE is the standard error of the mean obtained with the complex design and \( \text{SD}_{\text{ars}}^2 \) is the square of the standard deviation assuming a simple random sample. The WP variations were estimated from a non-random, unweighted sample with a mean (SD) of 17 (8) days between two analyte measurements. The \( CV_w \) was calculated as \( \left[ (CV_g)^2 - (CV_w)^2 \right]^{1/2} \). The distributions of several analytes were non-gaussian, and extreme outliers were excluded to obtain an approximately gaussian distribution with more stable estimates of variation. Outliers

<table>
<thead>
<tr>
<th>Analyte (units)</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>CV_g,%</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>CV_w,%</th>
<th>Index of individuality</th>
<th>Method CV_w,%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine aminotransferase (U/L)</td>
<td>15 535</td>
<td>15.3</td>
<td>7.7</td>
<td>50.2</td>
<td>1976</td>
<td>15.1</td>
<td>3.6</td>
<td>23.7</td>
<td>0.47</td>
<td>3.2</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>16 118</td>
<td>42.2</td>
<td>3.7</td>
<td>8.9</td>
<td>2076</td>
<td>41.2</td>
<td>1.8</td>
<td>2.8</td>
<td>0.31</td>
<td>3.4</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/L)</td>
<td>15 281</td>
<td>84.1</td>
<td>28.0</td>
<td>33.4</td>
<td>1996</td>
<td>87.8</td>
<td>6.9</td>
<td>4.4</td>
<td>0.13</td>
<td>6.5</td>
</tr>
<tr>
<td>Apolipoprotein A (g/L)</td>
<td>10 128</td>
<td>1.41</td>
<td>0.25</td>
<td>17.8</td>
<td>1060</td>
<td>1.44</td>
<td>0.12</td>
<td>7.0</td>
<td>0.39</td>
<td>4.8</td>
</tr>
<tr>
<td>Aspartate aminotransferase (U/L)</td>
<td>15 614</td>
<td>20.0</td>
<td>5.8</td>
<td>29.1</td>
<td>1993</td>
<td>20.4</td>
<td>3.2</td>
<td>15.1</td>
<td>0.52</td>
<td>3.4</td>
</tr>
<tr>
<td>β-Carotene (μmol/L)</td>
<td>20 002</td>
<td>0.32</td>
<td>0.22</td>
<td>67.4</td>
<td>2202</td>
<td>0.32</td>
<td>0.08</td>
<td>24.2</td>
<td>0.36</td>
<td>7.4</td>
</tr>
<tr>
<td>Fibrinogen, plasma (g/L)</td>
<td>16 135</td>
<td>2.32</td>
<td>0.11</td>
<td>4.7</td>
<td>1416</td>
<td>2.31</td>
<td>0.08</td>
<td>3.3</td>
<td>0.70</td>
<td>2.2</td>
</tr>
<tr>
<td>Gamma-glutamyl transferase (U/L)</td>
<td>11 784</td>
<td>22.0</td>
<td>13.2</td>
<td>59.8</td>
<td>1554</td>
<td>24.6</td>
<td>4.0</td>
<td>16.2</td>
<td>0.27</td>
<td>1.7</td>
</tr>
<tr>
<td>Glucose, plasma (mg/dL)</td>
<td>15 341</td>
<td>5.07</td>
<td>0.63</td>
<td>12.5</td>
<td>1950</td>
<td>5.14</td>
<td>0.43</td>
<td>8.3</td>
<td>0.66</td>
<td>1.7</td>
</tr>
</tbody>
</table>

\(^a\) Specimen type is serum unless noted.

\(^b\) Index of individuality is the ratio of \( CV_w \) to \( CV_g \).

\(^c\) Outliers were deleted by the Tukey method.
were eliminated by use of Tukey’s method, which defines outliers as three interquartile ranges below the 25th percentile or above the 75th percentile (8). Statistical analyses were carried out with SAS for Windows software (SAS Institute) and SUDAAN software (Research Triangle Institute).

The \( CV_w \) and \( CV_g \) exceeded the \( CV_r \) for laboratory analytes (Table 1). For most laboratory analytes, the mean BP and WP analyte values were similar despite some demographic differences between the two groups. The analytical quality specifications for imprecision and bias can be judged by use of the \( CV_w \) and \( CV_g \). For example, the total cholesterol imprecision should be less than one half of \( CV_w \) (8.2%), or 4.1%. The method imprecision was 2.3%. The bias for total cholesterol should be less than 0.25\((CV_w)^2 + (CV_g)^2\)\(^{1/2}\), or 0.25\((0.079)^2 + (0.226)^2\)\(^{1/2}\), or \(-6.0\). The quality specification for total error is estimated as \( B + 1.65(I) \), or 6.0% + 1.65(4.1%), or 12.8%. This compares with the National Cholesterol Education Program (NCEP) performance criteria of <3% for imprecision and bias and 9% for total error (9).

Serum sodium had the lowest \( CV_g \) (1.6%) and lowest \( CV_w \) (1.3%). This reflects the narrow homeostatic range for sodium that the body maintains. High \( CV_w \) and \( CV_g \) values were seen for several analytes. High \( CV \) values could result from natural population or individual variations, diurnal variations, disease, outlying analyte values, and relatively lower analyte values.

The ratio of \( CV_w \) to \( CV_g \), also known as the index of individuality, is important in determining the use of population-based reference (normal) intervals in detecting changes of disease status in individuals (10, 11). When the index of individuality is low (<0.6), the individual results stay within a narrow range compared with the population reference interval. Hence, a low index suggests the utility of evaluating serial changes in analyte values in an individual, and population-based reference intervals would be of limited use. A high index (>1.4) suggests that the reference interval is appropriate. The index of individuality ranged from 0.13 for serum alkaline phosphatase to 0.83 for serum bicarbonate (Table 1).

In this study, BP and WP estimates of \( CV \) were obtained for some selective environmental and nutritional analytes. NHANES III provides a better estimate of \( CV_g \) than do other short-term local studies because the NHANES III sample was nationally representative (1, 12, 13). The \( CV_w \) estimate was limited by the nonrandom, self-selected design and reflected a mean of 17 days between two measurements. The estimate of \( CV_w \) could be improved by use of a stratified, multistage probability design over different time periods. Differences for \( CV_w \) and \( CV_g \) among subpopulations (gender, age, race, and ethnicity) can be further investigated by use of NHANES III data.

References


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Breast Cancer Progression and Host Polymorphisms in the Chemokine System: Role of the Macrophage Chemoattractant Protein-1 (MCP-1)−2518G Allele, Giorgio Ghilardi,1† Maria Luisa Biondi,2 Anna La Torre,2 Lodovica Battagliolo,2 and Roberto Scorza1 (1 Dipartimento MCO, Clinica Chirurgica Generale, Università degli Studi di Milano–Polo S. Paolo, Milan, Italy; 2 Laboratorio di Chimica Clinica e Microbiologia, Ospedale S. Paolo–Polo Universitario, Milan, Italy; * address correspondence to this author at: Dipartimento MCO, Clinica Chirurgica Generale, Università degli Studi di Milano–Polo S. Paolo, Via A. Di Rudini, 8, I-20142 Milan, Italy; e-mail giorgio.ghilardi@unimi.it)

Interaction between tumor cells and stroma is essential for tumor growth. Tumor cells stimulate the formation of stromal tissue, which excretes a variety of growth factors, cytokines, and proteases. Tumor-associated macrophages (TAMs) are on the major components of tumor stromal tissue and are capable of eliciting diverse aspects of tumor growth as either a positive or negative regulator (1). In breast carcinoma, large numbers of infiltrating T cells and TAMs are often observed. The leukocyte infiltrate is found within the tumor stromal areas as well as in the epithelial areas that constitute the tumor mass (2–13).

Recent reports suggest that the inflammatory reaction at the breast tumor site affects tumor growth and progression. Whereas lymphocytes have been shown to have divergent effects on development of breast cancer (2, 10–