and disappearance of circulating DNA seems to be a characteristic phenomenon for the nature of this DNA.

Using DNA separated on a polyacrylamide gel, we have demonstrated that circulating DNA in healthy volunteers shows ladders typical for apoptosis. Circulating DNA seems to be mainly a result of apoptosis. In support of our hypothesis, Fournie et al. (16) concluded in a previous study that degradation of leukocytes in the artificial kidney was responsible for the increase in circulation of extracellular DNA.

Our data for 7AAD and annexin V in CD45+ leukocytes, measured immediately after blood draw, demonstrate apoptosis of leukocytes in HD. Previous in vitro studies (9) have demonstrated increased apoptosis of leukocytes after cultivation mimicking uremic conditions. In our study, increases in the MF of 7AAD in cell populations and in circulating DNA during HD mainly reflect apoptosis induced by contact with the dialysis membrane.

The source of increased DNA concentrations seems to be apoptotic leukocytes. Possibly, DNA fragments are leaking from the nuclei of leukocytes. In our study, the increase in uptake of 7AAD in leukocytes indicates increased permeability. The rapid increase in circulating DNA during HD and the increase in 7AAD in leukocytes suggest that both are caused by apoptosis.

We thank Grandits Ernestine for excellent technical assistance.

References

Calculating Uncertainty of Measurement for Serology Assays by Use of Precision and Bias, Wayne Dimech, Barbara Francis, Jennifer Kox, and Graham Roberts, for the Serology Uncertainty of Measurement Working Party (1 Na
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Background: In many countries, regulatory authorities that use International Organization for Standardization Standards to assess laboratory competence require an estimate of the uncertainty of measurement (MU) of assay test results. This estimate can be determined by identifying all sources of variation, calculating the extent of variation, and using established methods to combine the uncertainty. Alternatively, laboratory staff may use existing data generated from evaluations, proficiency testing, or external run controls to determine MU.

Methods: A quality-control (QC) sample with low reactivity was tested by laboratories participating in a national QC program. The results of testing the QC sample were entered into a shared database by use of an Internet-based program, EDCNet. Using a statistical approach that accounts for imprecision and bias of test results, we estimated the MU of the laboratories.

Results: A total of 2167 test results of a single QC sample reported by 18 laboratories were analyzed, and the MU of 1 laboratory was estimated by the statistical model described.

Conclusion: Using peer-group run control data, MU of serologic testing can be estimated by taking into account both imprecision and bias.

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Most regulatory authorities that use International Organization for Standardization (ISO) Standards to assess
laboratory competence require an estimate of the uncertainty of measurement (MU) of assay test results. MU may be estimated by considering the bias and precision of a test result. Laboratory staff may use existing data, including external quality-control (QC) results, to determine MU. When the run controls are calibrated to an international standard, the result can be compared with an expected result and adjusted accordingly to correct for bias. When the run controls are not traceable, it is more difficult to correct for bias. In this report, we describe an approach using peer-group run control data to estimate both precision and bias and use these measurements to estimate MU. The terminology used in this document conforms to the International Vocabulary of Basic and General Terms in Metrology (1). Unless stated otherwise, the mathematical calculations are derived from the EURACHEM/CITAC guide (2).

Currently, only quantitative assays or qualitative assays that use a quantitative basis to express results require an estimation of MU under the requirements of ISO/IEC 17025 (2005) clause 5.4.6 (3, 4). In serology assays, patient sample results can be divided by the manufacturer’s defined cutoff to give a sample-to-cutoff ratio. Random or systematic variation is introduced into serology assays from many sources (Table 1).

In clinical chemistry, the calibrators of many assays are traceable to an International Standard. Where a traceable calibrator is used, analytical bias should not introduce uncertainty into the testing system (5). However, assays without traceable calibrators may experience significant bias; therefore, total error (imprecision and bias) should be considered when estimating MU. Data obtained from the National Serology Reference Laboratory, Australia’s peer comparison QC program conducted through an Internet-based program, EDCNet (https://www.nrlqa.net) was used to estimate bias and precision. Additional details are provided in the “Example” file included in the Data Supplement that accompanies the online version of this Technical Brief (http://www.clinchem.org/content/vol52/issue3/).

QC samples were tested in the same manner as patient samples. Variations in the QC and patient samples were assumed to be equivalent. A low-concentration positive QC sample was selected to mimic the variation experienced at the cutoff concentration. Validation ranges for the QC sample results were implemented, and outlying results were excluded. It is suggested that results reported by each laboratory are checked for normality by use of a bar graph (See Fig. 1 in the online Data Supplement) or a statistical method such as Grubbs test. If the results are not normally distributed, another method of estimating MU should be considered. Laboratories reporting <10 results should be excluded from the estimation. The precision of a laboratory is determined as the standard deviation of the observed results ($S_{\text{obs}}$), where $S$ is the standard deviation:

$$\text{Precision} = S_{\text{obs}}$$

The relative standard uncertainty (relative standard deviation) of precision ($RSD_{\text{prec}}$) is calculated by use of the formula:

$$RSD_{\text{prec}} = \frac{\text{precision}}{x_{\text{obs}}} = \frac{S_{\text{obs}}}{x_{\text{obs}}}$$

where $x_{\text{obs}}$ is the mean of the observed results from a specific laboratory.

Major contributors to variation are the differences in reactivity of reagent batches and changes in the process attributable to different operators (6). Therefore, the results of QC samples must include multiple batches of reagents and operators to provide an accurate estimate of precision.

A laboratory can be precise but inaccurate. Inaccuracy in such cases is attributable to lack of trueness, a measure of systematic error quantified as bias. EURACHEM/CITAC guide clause 7.15.2 (IV) states “Where the bias is significant compared to the combined uncertainty, additional action is required. Appropriate action may be . . . report the observed bias and its uncertainty in addition to the result.”

To calculate bias, the result produced by the laboratory must be compared mathematically with a “true result”. ISO 15189:2003 details the methods of verification of trueness (7). Section 5.6.3 indicates that when a traceable standard is unavailable, trueness can be derived from “participation in a suitable program of interlaboratory comparison.”

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**Table 1. Differentiation of random and systemic sources of variation in serology assays.**

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Random variation</th>
<th>Systemic variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>Differences associated with use of different pipettes to dispense the same volume</td>
<td>Deterioration of function of a single pipette after calibration</td>
</tr>
<tr>
<td>Time</td>
<td>Run-to-run variation in incubation time</td>
<td>Timing of an assay incubation set at the extremes of the manufacturer’s limits</td>
</tr>
<tr>
<td>Temperature</td>
<td>Day-to-day temperature fluctuation of an incubator</td>
<td>Difference in ambient temperature of laboratories in different climates</td>
</tr>
<tr>
<td>Reading</td>
<td>Run-to-run variation of reader</td>
<td>Failing light source, producing consistent low readings</td>
</tr>
<tr>
<td>Operator</td>
<td>Operator-to-operator variation</td>
<td>Operator consistently performing assay in a manner different from other operators but within manufacturer’s instructions</td>
</tr>
<tr>
<td>Reagent batch</td>
<td>Batch-to-batch variation</td>
<td>One lot of reagent consistently performing differently because of poor storage or transport</td>
</tr>
</tbody>
</table>
The consensus values of results from multiple laboratories testing the same QC sample and assay can be used to define a true result, as detailed in ISO/TS 21748:2004, section 7.2.2.4 (8, 9).

Bias is defined as the difference between the observed result and the expected (or true) result (1, 10). To eliminate the units of measurement and use the quotient rule for combining uncertainty, a measure of bias can be expressed as a ratio, as in recovery experiments (2, 11, 12), and is expressed as bias $(B)$ to a multilaboratory consensus:

$$B = \frac{\text{observed result}}{\text{expected result}} = \frac{x_{\text{obs}}}{x_{\text{exp}}} \quad (3)$$

where $x_{\text{exp}}$ is the expected value, which in this report is taken to be the consensus value from a multilaboratory QC program. The relative standard uncertainty (relative standard deviation) of $B$ ($RSD_B$) can be calculated from the formula:

$$RSD_B = \frac{u_B}{B} = \sqrt{\left(\frac{u_{\text{obs}}}{x_{\text{obs}}}\right)^2 + \left(\frac{u_{\text{exp}}}{x_{\text{exp}}}\right)^2} = \sqrt{\text{RSD}_{\text{obs}}^2 + \text{RSD}_{\text{exp}}^2} \quad (4)$$

It is not practical to estimate bias within each analytical run; however, an estimate of the average day-to-day bias influencing a laboratory’s results may be obtained from QC data.

For a particular laboratory, the uncertainty of the observed value ($u_{\text{obs}}$) is the standard deviation ($S_{\text{obs}}$) of the QC results produced by that laboratory divided by the square root of the number of results ($n$) on which the standard deviation is based:

$$u_{\text{obs}} = \frac{S_{\text{obs}}}{\sqrt{n}} \quad (5)$$

The relative standard uncertainty of the observed value ($RSD_{\text{obs}}$) is the $u_{\text{obs}}$ divided by the mean of the laboratory’s observed results ($x_{\text{obs}}$):

$$\text{RSD}_{\text{obs}} = \frac{u_{\text{obs}}}{x_{\text{obs}}} \quad (6)$$

Because the individual laboratory means are based on different numbers of measurements and each laboratory has different precision, the expected (true) result ($x_{\text{exp}}$) is calculated by applying weighting factors that take into account the number of results and the precision applicable to each laboratory mean (the weighted average of all results). The Mean$_{\text{exp}}$, or $x_{\text{exp}}$, is the weighted mean of the results of the QC sample produced from all laboratories testing the QC sample with the same assay (13):

$$W_{\text{lab } 1} = \frac{n_{\text{lab } 1}}{S_{\text{lab } 1}^2}, \quad W_{\text{lab } 2} = \frac{n_{\text{lab } 2}}{S_{\text{lab } 2}^2}, \quad W_{\text{lab } n} = \frac{n_{\text{lab } n}}{S_{\text{lab } n}^2}$$

$$W_{\text{lab } 1} \cdot x_{\text{lab } 1} + W_{\text{lab } 2} \cdot x_{\text{lab } 2} + \ldots + W_{\text{lab } n} \cdot x_{\text{lab } n}$$

where $W_{\text{lab } n}$ is a weighting factor inversely proportional to the respective variances calculated for each laboratory, $n_{\text{lab } n}$ is the number of QC results reported by laboratory $n$, and $S_{\text{lab } n}^2$ is the variance of the population for laboratory $n$.

The weighted mean ($x_{\text{exp}}$) of all results can be used as an estimate of the true or expected result, taking into account the different numbers of results and precision of each laboratory contributing to the population, and can be calculated by use of the formula (13):

$$x_{\text{exp}} = \frac{W_{\text{lab } 1} \cdot x_{\text{lab } 1} + W_{\text{lab } 2} \cdot x_{\text{lab } 2} + \ldots + W_{\text{lab } n} \cdot x_{\text{lab } n}}{W_{\text{lab } 1} + W_{\text{lab } 2} + \ldots + W_{\text{lab } n}} \quad (8)$$

where $x_{\text{lab } n}$ is the mean of the results contributed to the population by laboratory $n$.

Using the same weighting principle, the standard deviation of all results in the total population, $S_{\text{exp}}$, or $u_{\text{exp}}$, is calculated using Eq. 9 (13):

$$u_{\text{exp}} = S_{\text{exp}} = \sqrt{\frac{1}{W_{\text{lab } 1} + W_{\text{lab } 2} + \ldots + W_{\text{lab } n}}} \quad (9)$$

The relative standard uncertainty of the expected results ($RSD_{\text{exp}}$) is calculated by:

$$RSD_{\text{exp}} = \frac{u_{\text{exp}}}{x_{\text{exp}}} \quad (10)$$

from Eqs. 8 and 9 above.

Rearranging the formula in Eq. 4, the uncertainty of $B(u_B)$ can now be calculated:

$$u_B = B \sqrt{\text{RSD}_{\text{obs}}^2 + \text{RSD}_{\text{exp}}^2} \quad (11)$$

where $B$ is obtained from Eq. 3, $\text{RSD}_{\text{obs}}$ is obtained from Eq. 6, and $\text{RSD}_{\text{exp}}$ is obtained from Eq. 10.

Once $u_B$ is calculated, a test should be carried out to establish whether $B$, taking into account its associated uncertainty, is significantly different from 1.0. Because $B$ is a ratio that should be symmetric around 1.0, a test for significance is performed.

To test for significance, the value of:

$$\frac{|B - 1|}{u_B} \quad (12)$$

is compared with the statistical value $t^*_{n-1}$%, which is nominally taken to be 2.0 (2).

If the value is $>2.0$, then the $B$ is significant. Ideally, results should be corrected for significant bias (14). However, if results are not corrected for significant bias, then the estimate of uncertainty should be increased to ensure that the true result is encompassed by the confidence interval (95% confidence). There are several ways to account for bias in estimates of uncertainty (15, 16). In this example, the approach suggested by Barwick and Ellison (17) is used because of its simplicity. Extra allowance for the uncertainty associated with results uncorrected for significant bias ($u_B^{\text{extra}}$) is made by use of the following formula:

$$u_B^{\text{extra}} = \sqrt{\left(\frac{|B - 1|}{2}\right)^2 + (u_B)^2} \quad (13)$$
where $B$ is obtained from Eq. 3, and $u_B$ is obtained from Eq. 11.

The relative standard uncertainty of $B$ may then be expressed as:

$$RSD_{B\, \text{extra}} = \frac{u_B\, \text{extra}}{B} \quad (14)$$

The combined standard uncertainty of the observed value is calculated by combining the relative standard uncertainty of precision ($RSD_{\text{prec}}$, Eq. 2) and the relative standard uncertainty of $B$ ($RSD_{B\, \text{extra}}$, Eq. 14):

$$\left( \frac{u_{\text{obs}}}{x_{\text{obs}}} \right)^2 = \left( \frac{u_{\text{prec}}}{x_{\text{prec}}} \right)^2 + \left( \frac{u_{B\, \text{extra}}}{B} \right)^2 = RSD_{\text{prec}}^2 + RSD_{B\, \text{extra}}^2$$

where $RSD_{\text{prec}}$ is obtained from Eq. 2, and $RSD_{B\, \text{extra}}$ is obtained from Eq. 14. Therefore:

$$u_{\text{obs}} = x_{\text{obs}} \sqrt{RSD_{\text{prec}}^2 + RSD_{B\, \text{extra}}^2} \quad (15)$$

To determine the expanded uncertainty ($U_{\text{RESULT [95%CI]}}$), the $u_{\text{obs}}$ is multiplied by a factor of 2 to provide a level of confidence of ~95% that the true result will be within the reported range. The result is therefore expressed as $x \pm U_{\text{RESULT [95%CI]}}$.

Imprecision (random error) and bias (systematic error) can be combined to obtain the total error (15, 18). The estimation of MU in serology assays must account for both imprecision and bias. The precision of serology assays can be estimated easily from the results of external QC sample testing. Imprecision derived from the performance of a laboratory in an internal quality assurance program is not recommended for estimating MU, because fewer data points are available on which to base the uncertainty estimate relative to the number available from external QC (5). However, an estimation of bias is less easy to calculate and not frequently attempted because there are few certified reference materials available for serology (15). By comparing the results of peer-group QC programs, in which all participants use the same QC sample and assay, an approximation of a "true" value can be determined and an estimate of bias made.

With the use of collaborative peer-group QC testing results, a true consensus value can be assigned to the QC sample and an estimate of bias can be included in the MU estimation. A worked example using the method outlined here can be found in the "Example" file included in the online Data Supplement.

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References


