Performance goals for the laboratory testing of antithrombin, protein C and protein S

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Summary
To achieve a reliable analytical quality for both monitoring and diagnostic testing, laboratories need to fulfill the widely accepted analytical performance goals based on the biological variation of the analytes of testing. Not only is the short-term analytical performance, which regularly is assessed by internal quality control procedures, of importance, but also the long-term analytical performance. To assess the long-term analytical performance, data obtained from an external quality assessment programme can be used. In this study we have used the evaluation model designed by the ECAT Foundation for the assessment of the long-term analytical performance, including imprecision, bias and total analytical error. The model was applied to the data from 136 different laboratories for the assay of antithrombin (activity), protein C (activity and antigen) and protein S (activity, total and free antigen). The imprecision (median; range), reflected by the long-term analytical coefficient of variation (LCV \(_A\)), was the lowest for antithrombin (7.6%; 2.6 – 43.8%) and the highest for protein S activity (17.2%; 4.3 – 88.6%). For bias and total error the same pattern was observed (antithrombin: 3.8%; 0.3 – 17.1% and 9.1%; 3.4 – 34.3%, respectively; protein S activity: 12.8%; 3.1 – 34.8% and 24.5%; 9.9 – 87.0%, respectively). For the majority of the laboratories (70 – 85%) the imprecision contributes considerably more to the total error than the bias. However the effect of the bias on the analytical quality is not negligible. Assays for antithrombin, protein C and protein S are mainly used for diagnostic testing. About 70 – 100% of the laboratories can fulfill the desirable performance goal for imprecision. The desirable performance goal for bias was reached by 50 – 95% of the laboratories. In all cases the highest numbers of laboratories fulfilling performance goals was obtained for the protein C variables. To improve the analytical quality in assays of antithrombin, protein C and protein S it is highly recommended that primarily imprecision (non-systematic failures) be suppressed. However the effect of the bias (systematic failures) on the analytical quality should not be neglected. A useful tool for determining the imprecision (LCV \(_A\)) and bias is the long-term analytical performance evaluation model as used by the ECAT Foundation.

Keywords
Thrombophilia, analytical performance, long-term, EQA

Introduction
Congenital conditions causing a tendency to venous thrombosis, are predominantly related to deficiencies of the inhibitors of the procoagulant pathway, i.e. antithrombin, protein C and protein S (1). Clinical laboratories involved in the diagnosis of thrombophilia should at least be able to diagnose functional defects of antithrombin, protein C and protein S (2–4). Guidelines for appropriate diagnostic testing of thrombophilia are nowadays available (5).

Today, a wide variety of different methods, reagents and equipments exists to serve the laboratory in testing antithrombin, proteins C and S. In general these methods can be subdivided into methods based on measuring the activity of the protein, and antigenic methods, measuring the amount of protein available. After the introduction of automated clotting analysers, not only specialised laboratories are able to perform tests for thrombophilia screening anymore. To guarantee an appropriate quality of test performance each laboratory should have available an appropriate analytical quality control system (6). Analytical quality is controlled by internal quality control procedures (IQC) and external quality assessment (EQA). IQC disclose deviations from the stable performance of the laboratories own test system reflected by the within-day and day-to-day variation. EQA disclose errors in the individual laboratories’ stable performance in relation to the performance of other laboratories (6, 7).

For the use of reference values for the diagnosis of patients, the longitudinal monitoring of patients and the comparability
Table 1: The equations for minimum, desirable and optimum performance goals as based on the biological variation.

<table>
<thead>
<tr>
<th>Performance goal</th>
<th>Analytical CV (CV&lt;sub&gt;A&lt;/sub&gt;) (monitoring)</th>
<th>Analytical CV (CV&lt;sub&gt;A&lt;/sub&gt;) (diagnostic testing)</th>
<th>Analytical bias (B&lt;sub&gt;A&lt;/sub&gt;)</th>
<th>Analytical total error (TE&lt;sub&gt;A&lt;/sub&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum quality</td>
<td>CV&lt;sub&gt;A&lt;/sub&gt; &lt; 0.75 CV&lt;sub&gt;W&lt;/sub&gt;</td>
<td>CV&lt;sub&gt;A&lt;/sub&gt; &lt; 0.87 CV&lt;sub&gt;T&lt;/sub&gt;</td>
<td>B&lt;sub&gt;A&lt;/sub&gt; &lt; 0.375 (CV&lt;sub&gt;A&lt;/sub&gt;&lt;sup&gt;2&lt;/sup&gt; + CV&lt;sub&gt;B&lt;/sub&gt;&lt;sup&gt;2&lt;/sup&gt;)&lt;sup&gt;1/2&lt;/sup&gt;</td>
<td>TE&lt;sub&gt;A&lt;/sub&gt; &lt; 0.375(CV&lt;sub&gt;A&lt;/sub&gt;&lt;sup&gt;2&lt;/sup&gt; + CV&lt;sub&gt;B&lt;/sub&gt;&lt;sup&gt;2&lt;/sup&gt;)&lt;sup&gt;1/2&lt;/sup&gt; + 1.65 (0.75 CV&lt;sub&gt;W&lt;/sub&gt;)</td>
</tr>
<tr>
<td>Desirable quality</td>
<td>CV&lt;sub&gt;A&lt;/sub&gt; &lt; 0.50 CV&lt;sub&gt;W&lt;/sub&gt;</td>
<td>CV&lt;sub&gt;A&lt;/sub&gt; &lt; 0.58 CV&lt;sub&gt;T&lt;/sub&gt;</td>
<td>B&lt;sub&gt;A&lt;/sub&gt; &lt; 0.250 (CV&lt;sub&gt;A&lt;/sub&gt;&lt;sup&gt;2&lt;/sup&gt; + CV&lt;sub&gt;B&lt;/sub&gt;&lt;sup&gt;2&lt;/sup&gt;)&lt;sup&gt;1/2&lt;/sup&gt;</td>
<td>TE&lt;sub&gt;A&lt;/sub&gt; &lt; 0.250(CV&lt;sub&gt;A&lt;/sub&gt;&lt;sup&gt;2&lt;/sup&gt; + CV&lt;sub&gt;B&lt;/sub&gt;&lt;sup&gt;2&lt;/sup&gt;)&lt;sup&gt;1/2&lt;/sup&gt; + 1.65 (0.50 CV&lt;sub&gt;W&lt;/sub&gt;)</td>
</tr>
<tr>
<td>Optimum quality</td>
<td>CV&lt;sub&gt;A&lt;/sub&gt; &lt; 0.25 CV&lt;sub&gt;W&lt;/sub&gt;</td>
<td>CV&lt;sub&gt;A&lt;/sub&gt; &lt; 0.29 CV&lt;sub&gt;T&lt;/sub&gt;</td>
<td>B&lt;sub&gt;A&lt;/sub&gt; &lt; 0.125 (CV&lt;sub&gt;A&lt;/sub&gt;&lt;sup&gt;2&lt;/sup&gt; + CV&lt;sub&gt;B&lt;/sub&gt;&lt;sup&gt;2&lt;/sup&gt;)&lt;sup&gt;1/2&lt;/sup&gt;</td>
<td>TE&lt;sub&gt;A&lt;/sub&gt; &lt; 0.125(CV&lt;sub&gt;A&lt;/sub&gt;&lt;sup&gt;2&lt;/sup&gt; + CV&lt;sub&gt;B&lt;/sub&gt;&lt;sup&gt;2&lt;/sup&gt;)&lt;sup&gt;1/2&lt;/sup&gt; + 1.65 (0.25 CV&lt;sub&gt;W&lt;/sub&gt;)</td>
</tr>
</tbody>
</table>

CV<sub>W</sub> = within-subject biological variation; CV<sub>B</sub> = between-subject biological variation; CV<sub>T</sub> = total biological variation.

and transferability of laboratory data between hospitals, a stable laboratory performance over time is of major importance (8, 9). To control stable laboratory performance over a prolonged period of time, the use of IQC results is limited. Here, the evaluation of EQA results should be used (10–12). Recently we developed an evaluation model for the assessment of the long-term analytical performance of an individual laboratory based on the data obtained by participation in the EQA programme of the ECAT Foundation (13). This model compares by linear regression the results of each individual laboratory with the corresponding consensus values of surveys over a particular period of time. By applying this model the imprecision, bias and total analytical error of each laboratory can be calculated. Imprecision is the random error included in each test result caused by non-systematic influences. Bias is the systematic deviation of the test result from the conventional true value. Bias is the net result of a constant component, caused by insufficient analytical specificity of the test procedure, and a proportional component, caused by incorrect calibration. The total error is the combined effect of random and systematic errors.

In a previous study we have already shown a considerable between-laboratory variation in the long-term analytical performance, reflected by the long-term analytical coefficient of variation (LCV<sub>A</sub>) for the assay of antithrombin and proteins C and S (14). The question is which quality is desirable for a reliable application of these tests for the screening and monitoring of thrombophilia patients. Nowadays, it is widely accepted that analytical performance goals (analytical quality specifications) should be based on the biological variation (15, 16). This concept was introduced by Cotlove et al. (17) and Harris (18) for monitoring patients and by Gowans et al. (19) for diagnostic testing. Starting with analytical goals for imprecision, this concept was further explored resulting now in analytical performance goals both for imprecision and inaccuracy (20–23). Because in practice it was not possible to reach the desirable analytical performance goals for all laboratory methods, Fraser et al. had introduced in 1997 the concept of minimum, desirable and optimum quality goals for imprecision and inaccuracy for clinical laboratory tests (24). Recently, application of these performance goals for data obtained by an EQA program was shown for endocrinology parameters, tumour markers and glycohaemoglobin (25).

Inasmuch as analytical performance goals are derived from the biological variation, knowledge about this is essential for establishing the border values for minimum, desirable and optimum performance. Although a comprehensive study on the short-term and long-term biological variation of haemostatic parameters is still lacking, several studies have investigated the biological variation on one or more of those haemostatic parameters (26–35). Published data on biological variation was compiled in a database by Ricos et al. (36) which is now available at the website of Westgard (37).

The aim of this study was to establish analytical performance goals based on the biological variation for antithrombin, protein C and S and to evaluate laboratory performance for imprecision, bias and total error by application of the long-term evaluation model on a dataset obtained from the international external quality assessment programme of the ECAT Foundation.

Materials and methods

External quality assessment programme

The ECAT Foundation, an independent non-profit organisation, provides an international external quality assessment programme (EQAP) in the field of thrombosis and haemostasis (38–40). Lyophilised plasma samples were distributed quarterly to 136 participants for laboratory testing. The participants were asked to handle the samples as regular patient samples using their routine laboratory methods for antithrombin (activity), protein C (activity and antigen) and protein S (activity, total and free antigen) [for further details see (14)].

Long-term evaluation

For the evaluation of the analytical performance goals for antithrombin, proteins C and S we have used the same dataset as described elsewhere (14), including data from 136 different laboratories. Recently we designed a model for the long-term evaluation of data obtained by an EQAP to assess the long-term analytical performance of an individual laboratory (13). This model allows us to assess the long-term within-laboratory analytical coefficient of variation (LCV<sub>A</sub>), which is represented here, imprecision, as well as bias and total analytical error. Briefly, for each participant, linear regression was applied using the consensus values of each survey as the denominator (dependent variable) and the corresponding laboratory values as the numerator (dependent variable). The slope and the variability of the regression line, the mean and the standard error of the consensus valu-
es as well as the mean value of the laboratory results were calculated. For the equations used and the legend, see appendix. Calculations were performed in Excel97.

### Biological variation

The performance goals used in this study are based on the biological variation. The biological variation of an analyte consists of two components, the within-subject variation (CV\(_W\)) and the between-subject variation (CV\(_B\)). The total biological variation (CV\(_T\)) is expressed by CV\(_T\) = (CV\(_W\)^2 + CV\(_B\)^2)^{1/2}. For the calculation of the allowable performance goals we have used the data on the biological variation obtained from the website of Westgard (37). The values for the CV\(_W\), CV\(_B\) and CV\(_T\) of antithrombin, proteins C and S are summarised in Table 2. Because no specific data is given for the different test methodologies for protein C (activity and antigen) and protein S (activity, total and free antigen), we have used the data on the biological variation for the proteins C and S as provided by Westgard for all methodologies.

### Performance goals

The laboratory performance is evaluated against established analytical performance goals for minimum, desirable and optimum quality for imprecision, bias and total error. The numerical limits for analytical performance are based on the concept of Fraser et al. (34) and the data for the biological variation as obtained from the website of Westgard (37). The equations for the calculation of the border values of each of the quality levels are given in Table 1.

### Results

#### Performance goals

Based on the equations for the minimum, desirable and optimum performance goals given in Table 1 and the data of the within-subject (CV\(_W\)), between-subject (CV\(_B\)) and total biological variation (CV\(_T\)) given in Table 2, the performance goals for antithrombin, proteins C and S were calculated (Table 3). For imprecision, different goals for monitoring and screening patients were calculated (17–19).

#### Imprecision, bias and total error

By applying the linear regression model for the evaluation of the long-term analytical performance (13), the imprecision, bias and total error were calculated for antithrombin, proteins C and S (Table 4). As reported in an earlier study (14) the lowest median imprecision was observed for antithrombin activity (7.6%) and the highest median imprecision for protein S activity (17.2%). A similar pattern is now also observed for bias and total error, 3.8% and 12.8%, respectively, for bias and 9.1% and 24.5%, respectively, for total error. It was further observed that on average the random error (imprecision) is higher than the systematic error (bias). On the level of the individual laboratory in 82% of the cases the imprecision of the assay of antithrombin was higher than the bias. For protein C activity and antigen this was 86% and 88%, respectively. For protein S activity, total antigen and free antigen this was, 74%, 70% and 85%, respectively. This resulted in a significantly higher contribution of imprecision to the total error than that of bias, except for total protein S antigen (Table 5).

#### Fulfilment of performance goals

Furthermore it was investigated how many laboratories could fulfil the criteria for a minimum, desirable and optimum test performance. First the border values for minimum, desirable and optimum performance were established (Table 3). Then the percentage of laboratories which can fulfil these performance goals for imprecision, bias and total error was calculated. The results of this evaluation are shown in Figure 1.

It is clear that almost none of the laboratories can fulfil the criteria for imprecision in monitoring patients for all the parameters investigated. This can be explained by the fact that this criterion is only based on the within-subject biological variation, which is rather low for antithrombin, proteins C and S (see Table 2).

### Table 2: Data on the biological variation for antithrombin, protein C and protein S obtained from reference 37.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Biological variation</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CV(_W)</td>
<td>CV(_B)</td>
</tr>
<tr>
<td>Antithrombin</td>
<td>5.2</td>
<td>15.3</td>
</tr>
<tr>
<td>Protein C</td>
<td>5.8</td>
<td>55.2</td>
</tr>
<tr>
<td>Protein S</td>
<td>5.8</td>
<td>63.4</td>
</tr>
</tbody>
</table>

CV\(_W\) = within-subject biological variation; CV\(_B\) = between-subject biological variation; CV\(_T\) = total biological variation.

### Table 3: The goals for minimum, desirable and optimum performance for antithrombin, protein C and protein S based on the biological variation. All data are given as %.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Performance goals based on the biological variation</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimum goal (M)</td>
<td>Desirable goal (D)</td>
</tr>
<tr>
<td></td>
<td>CV(_W) monitoring</td>
<td>CV(_M) diagnostic</td>
</tr>
<tr>
<td>Antithrombin</td>
<td>3.9</td>
<td>14.1</td>
</tr>
<tr>
<td>Protein C</td>
<td>4.4</td>
<td>48.3</td>
</tr>
<tr>
<td>Protein S</td>
<td>4.4</td>
<td>55.2</td>
</tr>
</tbody>
</table>
The vast majority of the participants (> 80%) can fulfil the criteria of desirable performance for imprecision in diagnostic testing for the proteins C and S. For antithrombin this percentage is slightly lower (72%). For the optimum goal for diagnostic testing only 10% of the participants can fulfil this criterion for antithrombin. For protein C this is about 80% and for protein S 50–70%.

For the bias also the best performance is obtained for protein C. Here 50–70% can even fulfil the criterion for optimum performance. For antithrombin this is only 20%. For protein S intermediate results were obtained. For the total error a same pattern was obtained, although the results for antithrombin and protein S were more similar.

### Discussion

In this study we have applied the principles for minimum, desirable and optimum test performance for imprecision, bias and total error (24) for the inhibitors of the procoagulant pathway, antithrombin and proteins C and S using a long-term evaluation model of EQA data (13). To our knowledge this is the first large-scale inter-laboratory evaluation for long-term analytical test performance of this type for antithrombin and proteins C and S.

This study clearly shows that random error (imprecision) is the major component of the total analytical error. This suggests that laboratories should focus on a more stable test performance over time. In a previous study we showed that the imprecision seems to be mainly caused by the performance of a particular test by the individual laboratory, independently of the method used (14). For improvement laboratories should therefore mainly focus on the variable factors influencing test performance, such as the preparation of reagents, reconstitution of lyophilised reagents, calibrators and quality control samples, pipetting, maintenance of pipettes and equipment, etc. But also the variation in test procedure between personnel may influence the test outcome. Here standardisation of operating procedures may improve analytical quality.

### Table 4: The median and range of the long-term analytical coefficient of variation (LCV<sub>A</sub>, %), bias (B, %) and total error (TE, %) for antithrombin, proteins C and S.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Number of laboratories</th>
<th>LCV&lt;sub&gt;A&lt;/sub&gt; (%)</th>
<th>Bias (%)</th>
<th>Total error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Median</td>
<td>Range</td>
<td>Median</td>
</tr>
<tr>
<td>Antithrombin (activity)</td>
<td>136</td>
<td>7.6</td>
<td>2.6 – 43.8</td>
<td>3.8</td>
</tr>
<tr>
<td>Protein C (activity)</td>
<td>132</td>
<td>8.6</td>
<td>3.3 – 33.3</td>
<td>4.4</td>
</tr>
<tr>
<td>Protein C (antigen)</td>
<td>48</td>
<td>10.8</td>
<td>4.7 – 33.4</td>
<td>6.8</td>
</tr>
<tr>
<td>Protein S (activity)</td>
<td>69</td>
<td>17.2</td>
<td>4.3 – 88.6</td>
<td>12.8</td>
</tr>
<tr>
<td>Protein S (total antigen)</td>
<td>79</td>
<td>13.4</td>
<td>5.9 – 52.1</td>
<td>10.7</td>
</tr>
<tr>
<td>Protein S (free antigen)</td>
<td>65</td>
<td>14.1</td>
<td>5.4 – 91.8</td>
<td>9.2</td>
</tr>
</tbody>
</table>

The median and range of the long-term analytical coefficient of variation (LCV<sub>A</sub>, %), bias (B, %) and total error (TE, %) for antithrombin, proteins C and S.

Table 5: The median (range) proportion of imprecision (%) and bias (%) contributing to the total analytical error for antithrombin, proteins C and S.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Imprecision (%)</th>
<th>Bias (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antithrombin</td>
<td>76 (14 – 100)</td>
<td>24 (0 – 87)</td>
</tr>
<tr>
<td>Protein C</td>
<td>78 (6 – 100)</td>
<td>22 (2 – 94)</td>
</tr>
<tr>
<td>Protein C (antigen)</td>
<td>74 (0 – 99)</td>
<td>26 (1 – 100)</td>
</tr>
<tr>
<td>Protein S (activity)</td>
<td>67 (10 – 98)</td>
<td>33 (2 – 90)</td>
</tr>
<tr>
<td>Protein S (total antigen)</td>
<td>53 (18 – 99)</td>
<td>47 (2 – 82)</td>
</tr>
<tr>
<td>Protein S (free antigen)</td>
<td>67 (20 – 98)</td>
<td>33 (2 – 80)</td>
</tr>
</tbody>
</table>

Table 5: The median (range) proportion of imprecision (%) and bias (%) contributing to the total analytical error for antithrombin, proteins C and S.
Clinical laboratory tests are generally used for diagnostic purposes or monitoring patients. Due to the lack of sufficient precision, the measurement of antithrombin, protein C and protein S is less useful for monitoring patients for changes in the concentration and/or activity of these analytes (Fig. 1). Only by significantly reducing the imprecision, can the performance goals for monitoring be reached. However, in clinical practice the laboratory tests for antithrombin, proteins C and S are mainly used for diagnostic testing. This information was obtained by a questionnaire circulated to the participants in the thrombophilia module of the ECAT EQA programme. Therefore, fulfilment of this analytical goal is of more importance. Here it is clearly observed that the vast majority of participants can fulfil the criteria for desirable test performance. One of the major reasons for this is the high total biological variation for at least protein C and protein S. Because the total biological variation for antithrombin is three to four times lower than for proteins C and S, a more precise test performance for antithrombin is required. Although antithrombin is the analyte with on average the lowest LCVₐ (Table 4), further improvement is still necessary, because 30% of the participants are unable to reach the level of desirable performance.

Since the vast majority of participants can fulfil the minimum goal for bias, and even 50–95% of participants can also fulfil the desirable goal for bias, systematic errors do not seem to be the major problem in the quality of test performance. This is confirmed by the fact that 50–80% of the total error is caused by imprecision. However, on the level of the individual laboratory bias may play an important role. In 15–30% of the laboratories the bias is higher than the imprecision. For these laboratories systematic deviations from the consensus values, caused by insufficient calibration or interference, is a major problem. This datum indicates that the role of the bias is not negligible. Because no information is available on the calibrator used by these laboratories, this problem could not be further investigated. For the analytes investigated, international standards exist (41–43). It is advisable to use only reference plasmas calibrated against these international standards.

It can be noticed that the imprecision, bias and total error observed are more dependent on the variable measured than on the assay principle used for its measurement. For example, the LCVₐ for protein C antigen is closer to those of protein C activity than to those of protein S antigen (total and free). Here the behaviour of different proteins in test systems may play a role. For example, the development of suitable assays for protein S is hampered by the fact that protein S circulates in plasma both as free and as complexed protein with C4b-binding protein (44).

It would be interesting to evaluate within a variable also the effect of the use of particular assay types, e.g. ELISA test versus latex-immuno test, and methods. However, the number of laboratories included in this study was too small to draw conclusion based on this sub-analysis.

The performance goals used in this study are based on the biological variation. This is nowadays the most widely accepted approach for analytical performance goals. The use of this concept is also recommended by the European organisation of External Quality Assessment organisers in Laboratory Medicine (EQALM) (15). Important for the establishment of the performance goals is the availability of reliable data on the biological variation of the analytes under investigation. Although several studies reported data on the biological variation for some haemostatic variables (26–35), a comprehensive study on both the short-term and long-term biological variation of a wide variety of haemostatic variables is still lacking. Here the ECAT Foundation has taken the initiative to set up such a study. In the near future we therefore expect to be able to establish for most of the haemostatic variables the analytical performance goals for both monitoring patients and diagnostic testing. At that time specific performance goals for the different analytes of protein C and protein S will be established.

Based on the results of this study the following recommendations to improve the analytical quality in assays of antithrombin, protein C and protein S can be made:

- Laboratories can improve their analytical quality predominantly by suppressing imprecision (non-systematic failures).
- The effect of bias (systematic failures) should not be neglected. The use of reference plasmas calibrated to the international standards for antithrombin, proteins C and S is highly recommended.

In conclusion, application of a long-term evaluation model based on the data of an EQA programme is a useful tool for the assessment of analytical performance of an individual laboratory including information both on imprecision and bias.

**Appendix**

Here the equations and the legend are given for the calculation of the imprecision (LCVₐ), bias (B) and total error (TE). All variables are expressed as a coefficient of variation.

\[
\text{Total error: } \text{TE} = \sqrt{\frac{\sum_{i=1}^{n} (Y_i - X_i)^2}{n}} \times 100\%
\]

\[
\text{Bias: } B = \frac{\sum_{i=1}^{n} (Y_i - X_i)^2}{n} \times 100\%
\]

\[
\text{Imprecision: } LCV_a = \frac{(s_{ys} / b)}{X} \times 100\%
\]

(X) is the consensus value and (X) is the mean value for X. (sₐ) is the standard error of (X).
(Y) is the laboratory value and (Y) is the mean value for Y.
(b) is the slope and (sₐₙ) is the variability of the regression line, which is calculated based on the least-square method.
The number of laboratory results included is expressed by (n).
References