When to collect blood specimens: midmorning vs fasting samples

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This study was carried out to define the consequences of collecting blood specimens during the forenoon instead of using fasting specimens collected early in the morning. Extensive laboratory data were obtained from specimens collected from fasting participants at 0800, after breakfast at 0930, and again at 1100. The subjects were inpatients in medical and surgical wards (n = 51; 13 women and 38 men; ages, 32–87 years) and subjectively healthy volunteers corresponding to outpatients (n = 51; 31 women and 20 men; ages, 18–63 years). The coefficient of variation (CV, \%) of the patient results was compared with the analytical CV. The observed CVs of the subjects’ results far exceeded the analytical CV (%), the average being 3.5-fold and up to 14-fold for some analytes. In individual results the observed change often exceeded the medically derived clinical critical difference. Laboratory data should always be interpreted in the context in which they were obtained. Clinical decisions should be based on objective data (observations) more than on experience and educated guesses. Different medical specialities and different clinical situations may require different kinds of procedures.

Laboratory data are routinely expressed numerically without comments on their uncertainty. Furthermore, the specimens may have been collected any time during the working day for convenience and to equalize the need for personnel during working hours. However, insufficient data exist on the optimal quality:cost ratio of such elective routine specimen collection. Our study was designed to determine the magnitude of changes induced by deviating from the recommended and standardized procedures of collecting fasting specimens early in the morning. The purpose of this study was to provide a rational basis for deciding the regimen for routine specimen collection.

\textbf{Materials and Methods}

\textbf{Analytes}

The analytes studied were the 32 most common blood tests (excluding HbA1c) according to the laboratory statistics of the Helsinki City Hospitals, which perform \textasciitilde3,000,000 laboratory studies yearly. These 32 analytes account for \textasciitilde>50\% of the total amount.

The analytes were as follows: the blood picture (leukocytes, erythrocytes, hemoglobin, hematocrit, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, and platelet counts) and leukocyte differential count (neutrophils, lymphocytes, monocytes, eosinophils, and basophils); erythrocyte sedimentation rate; thromboplastin time; the serum concentrations of total calcium, potassium, sodium, creatinine, glucose, total cholesterol, triglycerides, thyrotropin (S-TSH),\textsuperscript{3} free thyroxine (S-T\textsubscript{4}F), and C-reactive protein; and the serum activities of aspartate aminotransferase, alanine aminotransferase, \(g\)-glutamyltransferase, total creatine kinase (S-CK), and subunit B of creatine kinase (S-CK-B). Inorganic phosphate was measured only on inpatients.

\textbf{Subjects}

The study was performed on consenting subjects. The protocol of the study was approved by the ethics committee of Helsinki City Hospital, and we obtained written informed consent from every subject. The subjects formed two groups: group 1 (ambulatory subjects corresponding to outpatients) consisting of subjectively healthy individuals with no medication other than oral contraceptives for 1 week before the experiment (n = 51; 31 women, ages 18–60 years; mean, 36.7 years; 20 men, ages 23–63 years; mean, 42.4 years). Group 2 (inpatients) consisted of inpatients in different wards in Maria Hospital, Helsinki (n = 51; 13 women, ages 65–87; mean, 75.2 years; 38 men, ages 32–80; mean, 58.6 years.). The nature of the inpatients’ illnesses was not recorded because the study was per-

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\textsuperscript{3} Nonstandard abbreviations: S-TSH, serum thyrotropin; S-T\textsubscript{4}F, serum free thyroxine; and S-CK, serum creatine kinase.
formed explicitly on a general inpatient population to assess practical guidelines for specimen collection in general hospitals. However, there were equal numbers of patients from surgical and medical wards. No pediatric or gynecological patients were included. All inpatients had to be conscious and oriented to obtain informed consent.

**STUDY PROTOCOL AND SPECIMEN COLLECTION**
The study protocol consisted of three collections of specimens and a meal, all during the same day:

(a) 0800, first specimen from fasting subjects (specimen 1);
(b) breakfast;
(c) 0930, second specimen (40–80 min after breakfast; specimen 2);
(d) sitting or walking around indoors, no eating, smoking, intake of liquid, or exercise; the inpatients were mostly supine; and
(e) 1100, third specimen (specimen 3).

All specimens from ambulatory subjects were collected by an experienced laboratory technician, according to the Scandinavian recommendations (1), i.e., the subject sat for at least 15 min before venipuncture with no tourniquet, whereas inpatients were mostly supine. Blood was collected from a cubital vein with Venoject needles (20 G) in vacuum serum and EDTA tubes (Terumo Co.). No serum-separator tubes were used. The outpatients were instructed to have a breakfast similar to that of a routine working day from a selection of foodstuffs commonly used in Finland (corresponded roughly to a continental breakfast). The energy intake was <500 kJ for 11 participants, 500-1000 kJ for 20 participants, and >1000 kJ for 20 participants. The inpatients were given a routine hospital breakfast (1500 kJ).

**ANALYSIS**
The thromboplastin time and erythrocyte sedimentation rate were analyzed immediately after specimen collection (thromboplastin time with ACL1000, Instrumentation Laboratory Spa; and erythrocyte sedimentation rate with the vacuum tube method, Terumo). The complete blood count and differential count were analyzed within 3 h with a Coulter Max M analyzer (Coulter Corp.). For serum samples, the tubes were left to stand at room temperature for 30 min and then centrifuged at 1600g for 10 min. The serum was separated and frozen (−20 °C) for 2–5 days and then analyzed with a Hitachi 704 (Hitachi Ltd.) analyzer using routine procedures in the clinical chemistry laboratory of Maria Hospital. Cholesterol and triglycerides were analyzed with Kone Progress Plus (Kone Instruments Oy). All the samples of one patient were analyzed in the same series, and routine quality-control procedures were carried out. S-TSH and S-T₄F were analyzed in the laboratory of Laakso Hospital, Helsinki, S-T₄F with an AxSYM analyzer (Abbott Laboratories) and S-TSH with Delfia (Wallac Oy).

**DATA PROCESSING AND STATISTICS**
The data obtained were analyzed by ANOVA for repeated measurements and by the Scheffe test for calculating statistically significant changes. The multicomparison significance level was at 99%. The analytical CV (CV anal) was calculated with the same analyzers as the patient samples by analyzing a sample in the middle of the health-related reference interval 20 times in the same analytical run. The total CV (CV total) was calculated as the average of the individual within-person CVs from the three measurements performed per subject. The clinical significance of the observed changes was estimated by comparing them to previously published data (2–4). For the experimentally derived critical difference, the “ultrashort-term biological variation” of Costongs and co-workers (2, 3) and the 95% level for individual changes were chosen for comparison. The homogeneity of data was estimated by calculating the index of heterogeneity (i) according to Harris and Fraser (5, 6).

**Results**
The results show statistically significant changes occurring in 22 of the 32 analytes studied. The within-person CVs were systematically greater (up to 14-fold) than the analytical CVs (Tables 1 and 2).

The results of C-reactive protein for the outpatients (group 1) are not shown because the values were below the detection limit (10 mg/L) in all subjects. The leukocyte count was higher in inpatients, and both populations showed a similar, constant rise during the study. The differential count revealed a similar behavior in both groups, with increasing neutrophil and decreasing lymphocyte and monocyte counts. The hemoglobin and hematocrit values were stable in inpatients but increased in outpatients. The total calcium concentration rose in outpatients and was stable in inpatients. Sodium, creatinine, aspartate aminotransferase, alanine aminotransferase, γ-glutamyltransferase, cholesterol, and S-T₄F all revealed a change in the opposite direction in the two test groups, with the outpatient group showing more statistically significant changes. Similar changes of means in both groups were observed in S-CK, glucose, triglyceride, and S-TSH concentrations.

In >50% of the analytes, some individual changes exceeded the level of clinical significance (Table 3). It should be noted that diabetics were also included in the inpatient population, which explains the higher mean fasting glucose concentration.

**Discussion**
Quality control and quality assurance are necessary and inextricable parts of laboratory work. To ensure the quality of laboratory results, all factors having an effect on the results should be minimized. Traditionally, this is (or should be) done by quality control and perhaps by issuing standing rules concerning specimen collection. The collection should, for example, be done after 15 min of sitting
without a tourniquet, and preferably in the morning and fasting. The timing of specimen collection and the physiological state of the patient during the specimen collection session are unfortunately often neglected, and many of the recommendations are based on “educated guess”.* Furthermore, in a cost-effective laboratory, the collection of specimens should be allocated to working hours as evenly as possible. Circadian intraindividual variations are known for a number of analytes (e.g., iron and S-TSH); however, most of the analytes studied here are considered to be stable enough to be measured any time of day. Circadian rhythms are reported to be related to several aspects of daily life (7) but, according to some authors (6), are of no interest for most of the common analytes. Unfortunately, it is very unlikely that the patients are still fasting if the specimens are collected late in the morning or during the lunch break.

Our data now establish the consequences of deviating from the traditional morning fasting-state sample collection procedure. Especially worth noting is that the magnitude of the within-person CV caused by delaying specimen collection for 3 h may be up to 14-fold compared with the analytical CV. Although the absolute changes are relatively small, the focus of quality-control procedures should be reconsidered.

Clinical and statistical significance are different concepts and should not be mixed. However, the existence of statistically significant changes of the means in this study prompts one to take a stand in regard to the matters of timing and fasting status when issuing standing rules.

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concerning specimen collection. In addition, the desired level of precision must be defined separately for different clinical situations. The concept of laboratory imprecision also must include patient-derived factors to be clinically useful. Clinical usefulness relates to clinical decision limits. There are several approaches to these decision limits, and different approaches have been discussed (4). However, the main question remains: who should decide what is considered a significant clinical change? From a practical point of view, the opinions of clinicians should be more pragmatic; however, they often are stricter (4) than those based on statistics of the within-person or total biological variation. Clinicians are to judge what kind of change in the value of a laboratory test is medically important. However, they are usually unaware of the magnitude of biological variation. On the other hand, the biologically derived critical difference is calculated for 95% of the population. Therefore, when multiple univariate measurements are analyzed, there is an increased risk of getting a significantly deviating result [for 1 measurement the risk is 5%, for n measurements the risk is 100 \times (1 - 0.95^n)\%]. However, this approach has been useful for adaptive forecasting based on models that describe time-related biological processes such as tumor growth (13). It should also be noted that most of the published biological variations are based on the variability in healthy persons and therefore may not be valid in sick subjects.

In the present study, most of the changes observed...
were below the biological (experimental) and medically derived clinical decision limits; however, individual results exceeded them in >50% of the analytes investigated. The percentages of individuals exceeding the critical differences (Table 3), however, were not more than the 5% expected from the data of Costongs and co-workers (2, 3).

In the majority of cases, the experimentally derived critical differences were broader than the clinically derived critical differences of Skendzel et al. (4).

All volunteers (outpatients) considered themselves healthy and had C-reactive protein values <10 mg/L. One of the volunteers had an S-CK value of 11 500 U/L (upper reference limit, 270 U/L), which was the only result excluded from the data processing. The reason for the high enzyme activity was found to be strenuous physical exercise on the day before specimen collection, and the S-CK had returned to the health-related reference interval 2 weeks later. The age of the inpatient population was higher than in the ambulant (outpatient) population. However, according to Fraser and co-workers (8, 9), the estimates of within-subject biological variation are similar in old and young subjects, indicating that this aspect of homeostasis is not compromised in the elderly. Therefore, the smaller change in hematological indicators of inpatients most likely reflects the lack of physical exercise and upright posture in comparison with outpatients. This difference underlines the sensitivity of the changes, because the outpatients were instructed to sit or walk indoors and to avoid physical stress during the test session. However, the concentrations of the blood components showed no clear differences between the two groups. In the outpatient group, we found one undiagnosed case of hypercholesterolemia and one case of subclinical hypothyroidism. These results were included in the calculations because such patients are also encountered in the laboratory routine. These deviating results did not have an effect on the statistical significance of the differences; however, the index of heterogeneity was affected. Therefore, the indexes calculated without these “outliers” are shown in the tables.

The preanalytical phase has a greater effect on the outcome of laboratory results than the analysis itself. We feel that quality control for a laboratory should include preanalytical factors more than is the current practice. International standards such as ISO25 do not comment on the pathophysiological state or preparation of the patient before obtaining (blood) specimens in sufficient detail. The clinician should know that a change in the laboratory results does not necessarily imply a change in the health status of the patient. Different medical specialities and different clinical situations may require different standards and procedures. Thus, the appropriate level of precision for laboratory tests should be better defined for different situations in patient care. Clinical decision making should be based on facts more than experience and educated guesses.

Table 3. Comparison of the individual CVs obtained from three measurements in the morning with the experimentally* and clinically derived (4) critical differences.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>% of cases exceeding level of clinical significance</th>
<th>Exp. a crit. difference</th>
<th>Clin. crit. difference</th>
<th>Exp. crit. difference</th>
<th>Clin. crit. difference</th>
<th>Exp. crit. difference</th>
<th>Clin. crit. difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>20</td>
<td>33.4</td>
<td>14.0–16.4</td>
<td></td>
</tr>
<tr>
<td>Neutrophils</td>
<td>2</td>
<td>ND</td>
<td>4</td>
<td>ND</td>
<td>18.8</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>8</td>
<td>ND</td>
<td>0</td>
<td>ND</td>
<td>28.7</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>0</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>8.0</td>
<td>4.9</td>
<td></td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>8.2</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>Platelets</td>
<td>2</td>
<td>ND</td>
<td>0</td>
<td>ND</td>
<td>19.8</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>ND</td>
<td>6</td>
<td>ND</td>
<td>0</td>
<td>13.3</td>
<td>9.6</td>
<td></td>
</tr>
<tr>
<td>Potassium</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>2.4</td>
<td>1.7–2.7</td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>10.6</td>
<td>10.1–19.8</td>
<td></td>
</tr>
<tr>
<td>Creatinine</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>13.5</td>
<td>14.3–26.3</td>
<td></td>
</tr>
<tr>
<td>ASAT</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>15.2</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>CK</td>
<td>2</td>
<td>ND</td>
<td>2</td>
<td>ND</td>
<td>24.0</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>CK-B</td>
<td>8</td>
<td>ND</td>
<td>4</td>
<td>ND</td>
<td>40.1</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>0</td>
<td>76</td>
<td>4</td>
<td>46</td>
<td>37.9</td>
<td>11.2–17.2</td>
<td></td>
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<tr>
<td>Triglycerides</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>8</td>
<td>41.2</td>
<td>16.1</td>
<td></td>
</tr>
<tr>
<td>Free T₄</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>17.0</td>
<td>8.3–28.7</td>
<td></td>
</tr>
</tbody>
</table>

a Derived from ultra-short-term variation and the 95% level for individual changes (2, 3).

b Exp., experimentally derived; crit., critical; Clin., clinically derived; ND, no data available; TT, thromboplastin time; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase.
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References


