


23. Uhlig HH, Lichtenfeld J, Osman AA, Richer T, Mothes T. Evidence for the integrity of platelets and erythrocytes. In this study all plasma contamination with blood cells, nor LD measurements without concurrent measurements of other analytes to eliminate possible interferences lowered the reported high rate of duplicate errors. The problem was also not attributable to the clinical chemistry analyzer because measurements on both Hitachi 911 and 717 analyzers (Roche Diagnostics GmbH) showed results similar to those obtained with the previously used Modular analyzer (Roche Diagnostics GmbH). Only when serum samples or secondary tubes for centrifuged plasma samples were used was there a significantly lower frequency of duplicate errors: 2.6% (3 of 114) for serum and 1.1% (1 of 94) for secondary plasma tubes. The authors pointed out that the differences in buffer composition of the IFCC (pH 9.40 ± 0.05) (2) and SFBC (pH 7.4–7.8) methods influenced the integrity of platelets and erythrocytes. In this case, the presence of cells might play a role in the high frequency of duplicate errors.

We have been measuring LD activity on three Roche Hitachi 917 analyzers according to the recommendations of the IFCC (2) in our laboratory since February 2003. The within-run imprecisions (CV) for a heparin-plasma sample (n = 20) were 0.8% (197.4 U/L) and 0.7% (321.3 U/L), and the between-run CV were 1.4% for the Roche Precipath U (178.3 U/L; n = 28) and 1.1% for the Roche Precinorm U calibrators (241.0 U/L; n = 28). During this period of time we observed no obviously erratic LD measurements for controls or patients when several samples from the same patient where analyzed within a few hours for follow-up. All measurements were routinely performed with plasma samples from plastic lithium-heparin-gel tubes (Monovette prod. no. 03.1631.001; Sarstedt). Blood sample collection was done without vacuum drawing. After centrifugation for 10 min at 3000g, the primary tubes were used for analysis. To reevaluate the reliability of the IFCC-recommended LD method in our laboratory with special regard to the reported high frequency of duplicate errors, we performed 140 LD duplicate measurements and studied the possible interferences from platelet contamination, cuvette and reagent probe/stirrer carryover effects, and different primary plasma tubes. To avoid long time delays between duplicate measurements, the analyses of the 140 samples were performed in the same run in groups of 5 with reruns.

The statistical analysis of the results from the duplicate measurements was calculated according to the Bland–Altman procedure (3–5). Fig. 1 shows the Bland–Altman plot with the means of the duplicate LD measurements (U/L) plotted against the absolute differences between duplicate measurements (U/L). For the differences the values of the second measurements were subtracted from the values of the first. The limits of agreement [mean (2 SD), 0.3 (26.6) U/L] (3–5) are shown as solid lines in Fig. 1, and the mean of the differences as a dashed line. The frequency of duplicate errors [differences exceeding mean (2 SD)] under these conditions was 3.6% (5 of 140). This is more than the reported 1.4% for the SFBC method but less than the 17.8% reported for the IFCC method (1). The 95% confidence interval (−1.95 to 2.54 U/L; mean ± 2 SE) includes 0, so there is no evidence of systematic bias (3–5).

[When comparing our data with the results published by]
Bakker et al. (1), please be aware that Bakker et al. introduced a conversion factor for the IFCC method.

The interferences from carryover effects have been reported before (2, 6). To test cuvette carryover effects, we measured LD activity in patient samples in plastic lithium-heparin-gel tubes. The Hitachi analyzer we used was the Hitachi 917 Rack analyzer, which uses a cuvette ring of 160 cuvettes. Therefore, after cleaning and rinsing the cuvettes, we measured 160 LD samples to use every cuvette of the analyzer in one series of measurements. After that, 160 subsequent measurements of alanine aminotransferase (ALT), LD, aspartate aminotransferase (AST), and again LD were done. Thus, each cuvette was used for subsequent measurements of LD, ALT, LD, AST, and LD. Use of 160 samples in each run meant that each of the cuvettes had been used once for each analyte and that cuvette rinsing was performed only once in between. This was controlled by reporting both the cuvette numbers and the corresponding results. Interferences from previous AST or ALT measurements were expressed as absolute and relative differences (LD\textsubscript{AST or ALT} – LD\textsubscript{clean})/LD\textsubscript{clean} for each measurement (Table 1). The LD values measured after ALT or AST measurements tended to be higher than the first LD values with the “clean analyzer”. However, the absolute differences were small, with means of 3.3 U/L for AST and 1.4 U/L for ALT (Table 1). This small systematic difference (Table 1) (3–5) may be the result of minimal carryover from LD-containing AST (LD\textsubscript{AST IFCC; Roche Diagnostics GmbH}) and ALT (LD\textsubscript{ALT IFCC; Roche Diagnostics GmbH}) reagents (2).

Interferences from reagent probe/stirrer carryover effects were tested by measuring alternately LD and the suspected “offender” test (n = 100) without interruption from other tests. Possible offenders were defined as tests that could cause pH changes (lactate or calcium) or contamination with LD-containing reagents (ALT). All three tests caused small positive differences (Table 1), with mean relative differences of 3.9% (lactate), 2.6% (ALT), and 1.7% (calcium). Rinsing the pipetter with an acidic solution (SMS/Acid wash; Roche GmbH) after the calcium test did not change the performance of the following LD measurements. These results point to minimal reagent probe/stirrer carryover effects, but would not explain the high frequency of duplicate errors reported by Bakker et al. (1), especially because the frequency was even higher when no other tests were run on the analyzer.

It has been shown that both platelet contamination of

![Fig. 1. Bland–Altman plot with the means of the duplicate LD measurements (U/L) plotted against the absolute differences between duplicate measurements (U/L).](image)

For the differences, the values of the second measurements were subtracted from the values of the first. The limits of agreement (mean [2 SD], 0.3 (26.6) U/L) are shown as solid lines and the mean of the differences as a dashed line.

### Table 1. Interferences in LD measurements (IFCC method) on Hitachi 917.

<table>
<thead>
<tr>
<th>Cuvette carryover effects(^a)</th>
<th>LD</th>
<th>After ALT</th>
<th>After AST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (95% CI) relative difference, %</td>
<td>0.7 (0.3–1.2)</td>
<td>1.6 (1.1–2.1)</td>
<td></td>
</tr>
<tr>
<td>Mean (95% CI) absolute difference, U/L</td>
<td>1.4 (0.1–2.7)</td>
<td>3.3 (2.0–4.6)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reagent probe/stirrer carryover effects (n = 100)</th>
<th>LD</th>
<th>After ALT</th>
<th>After lactate</th>
<th>After calcium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean, U/L</td>
<td>194.2</td>
<td>199.3</td>
<td>201.7</td>
<td>197.1</td>
</tr>
<tr>
<td>Mean (95% CI) relative difference, %</td>
<td>2.6 (2.4–2.8)</td>
<td>3.9 (3.7–4.1)</td>
<td>1.7 (1.5–1.9)</td>
<td></td>
</tr>
<tr>
<td>Mean (95% CI) absolute difference, U/L</td>
<td>5.0 (4.7–5.4)</td>
<td>7.6 (7.2–7.9)</td>
<td>3.3 (3.0–3.7)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Platelet contamination(^c)</th>
<th>Serum-Gel</th>
<th>Lithium-Heparin-Gel</th>
<th>Lithium-Heparin</th>
<th>Serum-Gel</th>
<th>Lithium-Heparin-Gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centrifugation conditions</td>
<td>5 min; 500g</td>
<td>5 min; 500g</td>
<td>5 min; 500g</td>
<td>10 min; 3000g</td>
<td>10 min; 3000g</td>
</tr>
<tr>
<td>Platelets, 10(^9)/L</td>
<td>2</td>
<td>321</td>
<td>148</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Mean LD, U/L</td>
<td>133.8</td>
<td>142.8</td>
<td>141.8</td>
<td>136.0</td>
<td>165.1</td>
</tr>
<tr>
<td>CV, %</td>
<td>1.6</td>
<td>2.8</td>
<td>3.2</td>
<td>1.1</td>
<td>1.2</td>
</tr>
</tbody>
</table>

\(^a\) 160 patient samples; LD range, 104–1258 U/L.

\(^b\) CI, confidence interval.

\(^c\) Blood samples from one person (77 measurements).
the samples and hemolysis can be responsible for LD measurements with poor precision in addition to higher LD activity values (7, 8). To investigate these effects, we performed LD measurements with serum-gel (Sarstedt Monovette prod. no. 02.1388), lithium-heparin-gel (same as given above), and lithium-heparin tubes (Sarstedt Monovette prod. no. 01.1604.400). Blood samples were collected from the same person in five different tubes and centrifuged differently to obtain various degrees of platelet contamination. Serum-gel, lithium-heparin-gel, and lithium-heparin samples were centrifuged for 10 min at 3000g or for 5 min at 500g. Platelet counts were done for all samples (Max M; Beckman-Coulter). IFCC-recommended serum samples had very low platelet contamination, even after reduced centrifugation (see Table 1). Standard-centrifugation lithium-heparin samples contained platelet-poorn plasma, whereas reduced centrifugation produced platelet-rich plasma (Table 1). LD activity was measured (n = 77) for each of the five types of sample.

The higher platelet contamination of the samples in case of reduced centrifugation caused only a small increase of the within-run CV: 2.8% (142.9 U/L) and 3.2% (141.8 U/L) compared with 1.1–1.6% (133.9–165.2 U/L) for samples with low platelet contamination (Table 1). The mean LD activity for standard-centrifugation lithium-heparin-gel samples was higher than the activity for the corresponding serum-gel samples (mean difference, 28.8 U/L) and the corresponding reduced-centrifugation lithium-heparin-gel samples (Table 1). This may be the result of platelet destruction with subsequent release of intracellular LD. Lithium-heparin samples showed the same LD activity as lithium-heparin-gel samples despite higher platelet contamination. These results demonstrate that very high platelet contamination may have a small influence on the performance of the LD assay, but again, it would not account for the reported high frequency of duplicate errors.

Bakker et al. (1) found different frequencies of duplicate errors for heparin-plasma samples with (19%) and without separator (35%), and they could show that after heparin-plasma samples were transferred to secondary tubes (efficient mixing), the frequencies of duplicate errors dropped to 1.1%. We speculate that in the case of the Becton Dickinson heparin-plasma tubes used, both partial instability of the gel as well as inhomogeneities attributable to platelets and platelet aggregation might have caused the described problems. In combination with the specific sampling tubes, different variables, including blood sample collection, time between blood draw and centrifugation, reduced centrifugation, temperature, or specific analyzer features (e.g., rinsing program, sample and reagent volumes, or timing) could also contribute to the high frequency of duplicate errors.

We conclude that the IFCC method from Roche for LD measurement in heparin plasma is reliable, at least when performed under the conditions described here with Sarstedt Monovette tubes.

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References

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Comparison of Gene Expression Profiles in Laser-Microdissected, Nonembedded, and OCT-Embedded Tumor Samples by Oligonucleotide Microarray Analysis, Marta Sanchez-Carbayo,†‡† Fabien Saint,† Juan Jose Lozano,‡ Agnes Viale,§ and Carlos Cordon-Cardo† (1 Division of Molecular Pathology and 2 Genomics Core Laboratory, Memorial Sloan Kettering Cancer Center, 1275 York Ave., New York, NY 10021; 3 Grup de Recerca en Informática Biomèdica, Universitat Pompeu Fabra, Barcelona, Spain; † these authors contributed equally to this manuscript; § authors for correspondence: fax 212-794-3186, e-mail sanchezm@mskcc.org)

In the study and interpretation of expression profiles in tumor samples, the quantity and quality of the RNA and the heterogeneity of the tissue specimen from which it is extracted are both important. Optimization of amplification protocols has allowed researchers to use fewer tumor cells to obtain expression profiles for some clinical samples (1, 2). Assessment of RNA quality control has been improved by the use of highly sensitive gel analysis (3), and flow cytometry and laser microdissection techniques allow isolation of tumor cells from nontumorous connective tissue as well as inflammatory infiltrates (4, 5). Several groups have reported the use of gene expression profiles from laser-microdissected tumors (6–8). However, the extent of the effect of the laser beam on the quality of the RNA and on the expression profile results remains unclear. Because the amount of RNA obtained after laser microdissection is generally low, opportunities for additional quality control and validation are limited (9, 10). These issues led us to investigate the potential differences in gene expression profiles among samples obtained by different sample manipulation procedures.