the wild-type β-globin gene between the second nucleotide of codon 112 and the first nucleotide of codon 116 (Table 1). We hypothesized that β116(G18)His was deleted and the peptide sequence Arg-Val-Leu-Ala-His was inserted between β115(G17) and β117(G19) of the normal β-globin chain (Table 1). MALDI-TOF MS was performed on a tryptic digest of the β-globin chain to confirm this interpretation. The β3-chain should contain 150 amino acid residues vs 146 for the normal β chain with a theoretical mass shift of +439.29. Theoretical masses of tryptic peptides from β3- and β5-chains (8) were compared with the experimentally obtained peptide masses (Table 1).

The experimentally obtained masses of different peptides were very close to their theoretical masses. The two tryptic peptides, βT12 and βT13, generated by the five-amino acid insertion were characterized by MALDI-TOF MS (Fig. 1B). The present Hb variant was called Hb Antibes-Juan-Les-Pins and was associated with hematologic abnormalities: erythrocytes, 5.41 × 1012/L; Hb, 137 g/L; mean corpuscular volume, 78 fL; and mean corpuscular Hb, 25.3 pg. The variant was also found in two children of the same carrier. They presented with the same hematologic abnormalities without iron deficiency. Oxygen dissociation curves performed after stripping with or without the addition of 2,3-diphosphoglycerate or chloride ions were normal.

Among MS techniques for studying Hb variants, ESI-MS is the most frequently used and can be associated with peptide sequencing using tandem MS, but it often gives multiply charged fragment ions (4, 5). On the other hand, MALDI-TOF MS gives single-charge peptide ions and has been used for identification of some single-mutation Hb variants (9, 10). The present report shows that MALDI-TOF MS can be used to identify Hb variants. This MALDI-TOF peptide mass fingerprinting method is currently used in our laboratory for abnormal globin chains analysis.

The presence of the 12-nucleotide repeat strongly suggests that the origin of this insertion is probably based on a slipped mispairing by DNA polymerase during replication. Such an explanation has been proposed for different Hb variants (11). Amino acid residues at positions β115–β119 at the end of helix G in the normal β-globin chain are involved either in α1β1 subunit links or externally on the Hb molecule (12). The insertion of five amino acid residues leads to the addition of a complete helix turn that has no effect on oxygen binding but decreases molecule stability.


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Improved Reliable of Measurement of Lactate Dehydrogenase by IFCC Method in Heparin Plasma, Andries J. Bakker,* Anneke Bakker, Ageeth Bierna-Ram, Johannes T. Dijkstra, Henkie Renting-Wiering, Haye Superda, and Apple Zijlstra (Department of Clinical Chemistry, Klinisch Chemisch Laboratorium, PO Box 850, 8901 BR Leeuwarden, The Netherlands; *author for correspondence: fax 31-58-2882227, e-mail a.j.bakker@kcl.znb.nl)

Our laboratory recently reported (1) an excessively high frequency of duplicate errors in measurements of lactate dehydrogenase (LD; l-lactate:NAD+ oxidoreductase; EC 1.1.1.27) activity with the Roche IFCC-recommended method (2). This unacceptably high frequency of duplicate errors was found with various Hitachi analyzers (Roche GmbH) with primary lithium-heparin tubes with plasma separator from Becton Dickinson but not with serum or EDTA plasma. Such duplicate errors were not seen with the German- and French-recommended LD methods (3, 4), nor were such duplicate errors seen when nonevacuated heparin tubes from Sarstedt were used (5). In addition to the effects of methods, we investigated the effects of sample preparation, method modifications, mixing procedures, and the use of different lithium-heparin-containing tubes with and without plasma separator, lithium-heparin glass and plastic tubes, different lithium-heparin concentrations, and subsampling into secondary tubes. The results of all of these experiments are shown in Table 1 of the Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem.org/content/vol51/issue1/.

Previously, we showed that the high frequency of duplicate errors was not caused by carryover from reagents for other tests and was not restricted to one specific
type of Hitachi analyzer (1). In the present study, we demonstrate that the high frequency of duplicate errors is not restricted to the use of Roche reagents, confirming that the duplicate errors are caused by the combination of evacuated lithium-heparin tubes from Becton Dickinson and the IFCC method for LD. According to the various recommendations for LD assays, heparin plasma preferably should not be used because it “may contain thrombocytes, which have a high LDH concentration” (2). The presence of platelets or platelet aggregates was therefore suspected to be responsible for the duplicate errors, particularly because the preanalytical module of our Modular analyzer (Roche) prepared the plasma for analysis by a shorter than recommended centrifugation time. The authors of a recent study (6) and we suspected that the presence of platelets or platelet aggregates is the cause of duplicate errors. Consequently, reducing the platelet content of heparin plasma seems to be necessary.

Use of various heparin tubes from Becton Dickinson with (prod. nos. 367794 and 367376) or without plasma separator (prod. no. 367685), tubes constructed of glass (prod. no. 367793) or plastic (prod. no. 3677943), and tubes with increasing heparin concentrations had no significant effect. Efficient and extra mixing also did not alter the number of duplicate errors. For complete removal of platelets, the NCCLS centrifugation specifications are 15 min at 1500g (7). Our data showed that decreasing the number of platelets in the plasma did not reduce the frequency of duplicate errors. On the contrary, when we reduced the number of platelets (counted with a Cell-Dyne 1300; Abbott Laboratories), the frequency of duplicate errors increased slightly. We also could not relate the number of platelets to the magnitude of the individual duplicate errors (Fig. 1 in the online Data Supplement). Although the presence of excessive numbers of platelets was suspected to be responsible for the high frequency of duplicate errors, we could not confirm that hypothesis with these experiments. The higher frequency of duplicate errors for samples that were centrifuged for longer times at higher speeds suggested that these conditions increased the concentration some duplicate-error-causing matter in the top layer of the plasma (the Modular analyzer samples 2 mm below the plasma surface). The nature of this material has not been established.

The presence of cells was thought to be responsible for the duplicate errors, and the composition of the reagents for the IFCC method for LD seemed rather hostile for cells because of the low solute concentrations. We therefore tried, in one experiment, to stabilize cells, if present, by adding 100 mmol/L NaCl to the buffer for the IFCC method. This addition of salt did not lower the frequency of duplicate errors. In another experiment, we looked for LD-independent (blank) reactivity by substituting a reagent in which lactate was omitted from the IFCC reagent. The low blank reactivity confirmed that there was no interfering non-LD reaction that incidentally influenced the LD reaction. Furthermore, because the concentrations of potassium (measured by indirect ion-selective electrode) and asparagine aminotransferase (method according to IFCC recommendations; Roche prod. no. 1876848) in cells (platelets and erythrocytes) are much higher than in plasma, both analytes were measured in duplicate with the Modular analyzer. These general markers of cell content release did not show duplicate errors; the presence of cells in the plasma could not, therefore, be supported by these indirect experiments.

Manipulating the plasma by transferring it to a secondary sample cup lowered the frequency of duplicate errors. Therefore, in cooperation with Roche, we studied the effect of sample predilution by prediluting the sample fourfold online with saline and then increasing the sample volume in the reagent mixture fourfold to keep the sample/reagent ratio the same. This dilution was very effective in reducing the frequency of duplicate errors (Fig. 1). Reproducible measurements with no duplicate errors were obtained as a consequence of the predilution step or the transfer of plasma to a secondary cup. Apparently, both procedures eliminated the inhomogeneity that was present in the top layer of the plasma sample, thereby allowing reproducible measurements. If cells were present, the resulting LD concentration after predilution would be expected to be a little higher than the baseline concentration in the assay without the predilution step. However, when we compared the means (SD) of the lowest results for each duplicate measurement between the tests with and without the predilution step [no predilution, 388 (144) U/L; with predilution, 326 (111) U/L; n = 208], the lower values for the test with the predilution step were striking (Fig. 2 in the online Data Supplement). Because higher LD results for the same sample logically could be caused by the presence of cells or cell fragments or aggregates, the modification with the predilution step seemed to give more accurate results for LD according to the IFCC recommendations, although we did not confirm this by comparison with serum values. Recently, Roche introduced this predilution procedure for their modular application for measurement of LD.

Although the recommendations for LD measurements state that serum is the preferred sample for LD, in daily practice heparin plasma is preferred because it is more rapidly available, delayed clotting is absent, and cell lysis...
is lower than in serum (8). Therefore, a reliable method for measuring LD in heparin plasma is necessary. We have shown that in contrast to the nonevacuated heparin tubes from Sarstedt (5), the evacuated lithium-heparin tubes from Becton Dickinson cause a high frequency of duplicate errors in the IFCC method for LD. Increased attention to the preanalytical steps (additional mixing before centrifuging) and adapting the analytical procedure by introducing a sample predilution step improved the reliability of the LD results for heparin samples by the IFCC method, but the precise nature of the cause of the duplicate errors remains unknown.

References

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