different antigenicity of both assays. Accordingly, the serum assay seems to be more restrictive than the urinary assay for detecting products derived from the telopeptide region of collagen type I.

The decrease in bone remodeling that occurs in older girls with respect to younger girls is a generally accepted fact. In accordance with the results found in this work in β-CTX and bAP concentrations, it would be possible to suggest that serum β-CTX assay (Serum CrossLaps) reflects better than urinary β-CTX assay (CrossLaps) the changes in bone remodeling that take place in young girls. It is possible that, independent of the differences attributable to renal clearance, the serum assay can be more specific for bone than the urinary assay because of the different antigenicity of the assays.

More work is necessary to investigate this point, but the observed differences between the two β-CTX methods constitute an interesting point to consider when selecting a serum or a urinary assay to perform a particular study.

We thank the students of Ntra. Sra. de las Maravillas School and the Nursery School of the Fundación Jiménez Díaz (Madrid) for their kind collaboration.

References

Distribution of Hemoglobin F, A, S, C, E, and D Quantities in 4 Million Newborn Screening Specimens, John W. Eastman,1* Fred Lorey,2 John Arnopp,3 Robert J. Currier,2 John Sherwin,1 and George Cunningham2 (1 California Department of Health Services, Genetic Disease Laboratory, 700 Heinz Ave., Suite 100, Berkeley, CA 94710, and 2 California Department of Health Services, Genetic Disease Branch, Berkeley, CA 94704; * author for correspondence: fax 510-540-2228, e-mail jeastman@dhs.ca.gov)

The California newborn screening program requires that all newborns be screened for selected hemoglobinopathies. Dried blood spot (DBS) specimens are analyzed using an automated 2-min cation-gradient HPLC method that is sensitive and specific (1, 2). The standardized method selected by the State allows the screening program to match the quantitative analytical performance of the assay at eight private contract laboratories and a central laboratory. Information technology is designed to derive phenotypes automatically and to use quantitative acceptability limits for quality control and proficiency testing.

The distribution of hemoglobin (Hb) quantities determined by cation-exchange HPLC in cord blood specimens has been published previously (3, 4). We have determined the frequency distributions for the analysis of DBS specimens using the rapid HPLC screening method. In this study, we examined screening data reported on 4 million nontransfused newborns tested within 2 days of birth. The frequency distributions were determined for the percentages of Hb concentrations in 14 phenotypes containing Hbs F, A, S, C, E, and D. The frequency distributions are available on request. The medians only are given in Table 1.

The percent concentrations in the current study are lower than the published cord blood data by 15% for all Hb F and by a median 36% for the Hb in other hetero- and homozygous patterns (range, 22–40%). This may be caused by differences in the HPLC methodology and by chemical degradation of Hb during formation of the dried blood spots (5, 6). Approximately 28–34% of the total area of the DBS chromatograms is eluted before the quantified species F, A, S, C, E, and D. These rapidly eluted species include unidentified degradation products and Ftr, as well as a compound that elutes, in some samples, at the void volume and is correlated with Hb Barts (leftmost peak in the dashed curve in Fig. 1).

The relationships found among the concentrations of adult Hbs in carrier patterns are consistent with published cord blood data. As a fraction of the total adult Hb present in the newborn blood spots, the results are 0.41–0.45 for Hbs S, C, and D in patterns FAS, FAC, and FAD, and 0.27 for Hb E in FAE.

The chromatographic integration parameters have been adjusted to assure that all Hbs are reported in the pattern when the concentrations exceed 1% for Hbs F, A, E, and D and 0.5% for Hbs S and C (1). In the event that concentrations are below the limits, the software will continue to include the Hb in the pattern as long as the signal-to-noise criteria are satisfied (1). We have found that the one-

<table>
<thead>
<tr>
<th>Pattern</th>
<th>F only</th>
<th>A</th>
<th>V = S, C, or D</th>
<th>E</th>
<th>Ftr + other</th>
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<tr>
<td>F only</td>
<td>67.5</td>
<td></td>
<td></td>
<td></td>
<td>32.5</td>
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<td>32.4</td>
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<td>61.6</td>
<td>10.3</td>
<td></td>
<td></td>
<td>28.1</td>
</tr>
<tr>
<td>FAS, FAC, FAD</td>
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<td>5.3</td>
<td>4.0</td>
<td>3.0</td>
<td>29.1</td>
</tr>
<tr>
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<td>61.6</td>
<td>5.3</td>
<td></td>
<td>2.0</td>
<td>31.1</td>
</tr>
<tr>
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<td>4.0</td>
<td></td>
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<td>6.8</td>
<td></td>
<td></td>
<td>31.6</td>
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<tr>
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<td>3.9</td>
<td></td>
<td>33.9</td>
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</tbody>
</table>

a V, variant.

b Only for cases in which the Hb A is detected.
percentile tails of the Hb frequency distributions are located above the specification limits in most cases. The one-percentile tail for Hb E in FAE, 0.7%, is not far below the detection limit of 1%, and we expect the Hb E to be reported accurately in the large majority of specimens. In the FSA patterns for $S/\beta^+$-thalassemia, the median Hb S is 5.1% and Hb A is 0.9%. Thus, one-half the detected Hb A in FSA patterns is below the specification of 1% for the detection limit. For this reason, we expect that many cases of $S/\beta^+$-thalassemia will be misclassified as FS. (Because all newborns with FS patterns are referred to follow-up, the differential diagnosis is made there.)

Typically, the shape of the frequency distributions and the relative standard deviations are approximated by a gaussian fit and are similar for the Hb concentrations in most patterns. However, anomalies were found in patterns FC and FAD. In FC patterns, the concentration of Hb C is unusually high at the 95th to 99th percentiles. In a span of 5 years, five of the eight specimens with the most Hb C were collected and tested at one clinical site. We have not been able to identify an analytical or demographic variable that would explain the high results, and more investigation is needed.

The frequency distributions for FAD show tails at the low end for Hb F and the high end for Hb A. These unexpected distributions are caused by nonspecificity of the HPLC method. True FAD patterns have a ratio of Hb D/Hb A similar to unity and always $>0.5$ (solid curve in Fig. 1). In the tails of the distributions for Hbs F and A, the chromatograms have a ratio $<0.5$. The low ratio is typical of an $\alpha$-chain variant such as Hb G-Philadelphia, where the expected four chromatographic peaks are unresolved by the rapid screening method (7, 8). One-half of the $\alpha$-variant Hb F is eluted in the Hb A window, and the added two chromatographic peaks elute with Hbs A and D (Fig. 1). The two types of chromatograms are readily differentiated by calculating the ratio of the concentrations Hb D/Hb A.

Algorithms based on this ratio can be used to report the $\alpha$-chain variants as such instead of as FAD patterns.

In summary, blood spot results using rapid cation-exchange HPLC give the following values. The median Hb F0 is 67.5% in F-only patterns and 61.6% in all other patterns. (The concentration of Hb F0 does not include the concentration of the acetylated Hb F1.) The median Hb A is 10.3% in FA and approximately one-half of that value, or 5.3%, in FAS, FAC, and FAE. The expression of Hbs S, C, and D in FAS, FAC, FAD, FSC, FSE, and FSD patterns is somewhat lower, at 4.0–4.2%, than the 5.3% Hb A. The Hb E is considerably lower, being only 2.0% in FAE and 1.6% in FSE. These results are consistent with published data that show that Hb variants have slightly lower concentrations than Hb A and that Hb E occurs at significantly lower concentrations than S, C, and D (3, 4). Because the co-inheritance of $\alpha$-thalassemia affects the percentages of Hb found in carrier patterns such as FAS, clinical studies are needed to determine the frequency distributions for the different conditions.

In FS and FC patterns, the concentrations of Hb S and Hb C are 6.8%, which is less than twice the 4.0–4.2% expression in the heterozygotes and well below the 10.3% Hb A in normal FA patterns. The Hb E in FE is only 4.5%, which is less than one-half of the 10.3% Hb A in FA normals. We note that, in addition to homozygous hemoglobinopathies, the patterns FS, FC, FD, and FE reported by the rapid screening method include cases of $\beta^+$-thalassemia and some cases of $\beta^+$-thalassemia with Hb A below detection, as well as S with hereditary persistence of fetal Hb. Clinical studies are needed to determine the frequency distributions for the different conditions. One study has shown that in patterns FE, the phenotype EE can be differentiated in many cases from E/\beta-thalassemia by the percentage of Hb E, which is 2.4–12.6% for EE and 1.4–4.5% for E/\beta-thalassemia (9).

References
Altered Composition of Lipoproteins in Liver Cirrhosis Compromises Three Homogeneous Methods for HDL-Cholesterol, Jordi Camps, Josep Mª Simó, Sandra Guaita, Natàlia Ferré, and Jorge Joven (Centre de Recerca Biomèdica, Hospital Universitari de Sant Joan, Catalunya, Spain; * address correspondence to this author at: C/. Sant Joan s/n, 43201-Reus, Catalunya, Spain; fax 34-77-312569, e-mail jcamps@correu.grupsgs.com)

Despite the obvious clinical advantages, the measurement of HDL-cholesterol (HDL-C) by reliable and easy-to-perform methods is not yet completely free of problems. Several reports have described homogeneous (direct) assays for HDL-C that are readily adaptable to automated analyzers as online procedures (1–3). These methods have proved to be effective and inexpensive tools for the routine screening of HDL-C in large populations. However, in a recent article (4) we observed that one of these techniques significantly undervalued the concentrations of HDL-C in patients with liver cirrhosis, a condition in which alterations in lipoprotein structure and composition are commonly found (5). Although HDL-C is not a clinically important determination in liver cirrhosis, our finding may have consequences for research groups investigating lipoprotein metabolism and its alterations.

The aims of the present study were (a) to compare three different techniques for homogeneous HDL-C measurement with a reference method [single vertical-spin ultracentrifugation (SVS)] in a group of patients with cirrhosis; and (b) to investigate whether there was a relationship between the method biases and abnormal composition of lipoproteins.

The study was performed on 58 control subjects and 37 patients with liver cirrhosis. Control subjects were chosen randomly from the routine health and safety-at-work checks conducted in several industrial companies in our area. Excluded were those subjects with clinical or laboratory evidence of diabetes, neoplasia, renal disease, hepatic damage, and cardiovascular disease. Cirrhotic patients were diagnosed by liver biopsy and proceeded from the outpatient clinics of the Hospital Universitari de Sant Joan de Reus. The etiology of cirrhosis was alcoholic in 23 patients (62%), viral in 12 (32%), and cryptogenic in 2 (6%). Twelve of the 23 alcoholic cirrhotic patients had quit alcohol consumption at least 3 months prior to the study. The other 11 patients had continued drinking. In three patients, cirrhosis was associated with diabetes mellitus. All procedures were in accordance with the ethics standards of our Institution. Blood samples were drawn in the fasted state into glass tubes containing EDTA; the plasma was separated by centrifugation at 1500g for 25 min and stored at −20 °C for batched analysis.

Three homogeneous HDL-C assays were used. All three methods contained an initial reagent to block lipoproteins other than HDL and a second reagent to measure HDL-C by slight modifications of the CHOD/PAP technique (6). In the method that used polymers, polyanions, and detergent (PPD), reagent 1 was a mixture of polyanions and synthetic polymers, forming LDL-, VLDL-, and chylomicron-polymer-polyanion and HDL-polymer complexes (Daichii; supplied in Spain by ITC Diagnostics, Izasa, Barcelona, Spain). The method that uses polyethylene glycol-modified enzymes (PEGME; Boehringer Mannheim, Mannheim, Germany) used α-cyclodextrin sulfate as a sequestering agent of apolipoprotein (apo) B-containing lipoproteins and modified enzymes that specifically react with HDL-C. The method that uses antibodies (AB; Sigma Diagnostics, St. Louis, MO) used anti-human apo B antibodies to bind lipoproteins other than HDL. SVS ultracentrifugation was performed as published previously (7). Cholesterol, triglycerides, and phospholipids in lipoprotein fractions and in patients’ plasma were determined by standard methods (ITC Diagnostics, Izasa). Plasma concentrations of apo A-I and B were analyzed by immunoturbidimetry (Biokit, Izasa). apo measurements were calibrated according to the IFCC standard (8). Liver-related tests were also measured in the plasma of all subjects by standard techniques (ITC Diagnostics, Izasa). These tests included total protein, albumin, total and esterified bilirubin, alanine aminotransferase, alkaline phosphatase, γ-glutamyltransferase, and prothrombin time. All measurements were performed on an Ilab® 900 automatic analyzer (Instrumentation Laboratories), except for prothrombin time, which was performed on an ACL 1000 automated coagulation analyzer (Instrumentation Laboratories). Results are presented as means and ranges. The presence of lipoprotein X in the plasma of cirrhotic patients was tested qualitatively by agarose gel electrophoresis (9). Bias between the homogeneous assays and the reference method was calculated as the homogeneous HDL-C result minus the ultracentrifugation method result. Differences between means were estimated by the Student t-test. The association between variables was measured by linear regression analysis. Statistical significance was set at P <0.05.

The results of the analytical determinations are summarized in Table 1. Cirrhotic patients appeared to be a very heterogeneous group, with hepatic function indices ranging from within the health-related reference intervals to frankly altered values. Although the presence of cholestasis might be suggested by an increase in esterified bilirubin in some of the patients, alkaline phosphatase activity was never higher than twice the upper reference limit, and lipoprotein X was not detected in any of the patients’ plasma. Lipoprotein composition was also very heterogeneous in cirrhotic patients, with values above and below those of the control subjects for all the indices measured both in plasma and in SVS-obtained lipoprotein fractions. This is not surprising because lipoprotein concentrations and composition in cirrhotic patients are known to be affected by a variety of derangements, including the degree of hepatocellular damage, the possible associated