41.7 (13.5) years. The study was approved by the local ethics committee. Compared to the healthy controls, median concentrations of cholesten (interquartile range) in patients with ileal resection were significantly higher [87.8 μg/L (range 42.1–150.5 μg/L) vs 11.9 μg/L (range 9.2–16.9 μg/L), P < 0.001, Mann–Whitney Rank–Sum test].

This method retains the advantages of other HPLC-based methods, while eliminating the need for temperature-controlled SPE. Silica cartridges offer higher binding capacity and the possibility of analyte extraction at ambient temperature, which, compared to previously used C8 cartridges, results in a wider linear range (up to 1000 μg/L vs 200 μg/L) (4). Except for the chloroform:methanol extraction, the analysis can theoretically be automated. Additionally, the initial chloroform:methanol extraction ensures quantitative extraction of both cholesten and the internal standard and thus potential imprecision caused by either internal standard precipitation or incomplete extraction of protein-bound cholesten can be avoided.

The increased availability of a laboratory diagnosis of bile acid malabsorption is of considerable importance, especially for patients with chronic diarrhea and irritable bowel syndrome. These disorders belong to the most common gastrointestinal conditions, and it is estimated that bile acid malabsorption might be present in about half of these patients (5). Because the majority of patients with bile acid malabsorption respond to bile acid sequestrants (5), targeted therapy, based on serum cholesten concentrations, should both improve outcomes and lower treatment costs.

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Unexpected Hemoglobin A Results after an Erythrocyte Exchange: Importance of Specimen Mixing

To the Editor:

Erythrocyte exchange, a procedure in which blood is removed and replaced with donor cells, is often used to prevent or treat severe vasocclusion in patients with sickling hemoglobinopathies. This procedure increases the percentage of hemoglobin A without dramatically increasing the hematocrit or viscosity. The efficacy of erythrocyte exchange is often measured by hemoglobin electrophoresis and densitometry to determine the posttransfusion percentages of hemoglobin A and S.

We performed a manual erythrocyte exchange on a 28-year-old pregnant woman with hemoglobin SD-Punjab. Five units of whole blood were withdrawn and replaced with packed erythrocytes and saline. The expected percentage of hemoglobin A in each unit withdrawn was calculated in an iterative fashion. We calculated the total volume of hemoglobin SD-Punjab erythrocytes using the patient’s total blood volume estimated by nomogram, measured hematocrit, and assuming 100% hemoglobin SD-Punjab. The volume of erythrocytes removed was subtracted from the hemoglobin SD-Punjab erythrocyte volume tally, and the volume of erythrocytes administered (assumed to be an average of 160 mL) was added to the hemoglobin A erythrocyte volume tally. Volumes and percentages of hemoglobin A and SD-
Punjab erythrocytes in the patient and in the next volume of blood withdrawn could be estimated and the calculations repeated for each successive cycle. After the exchange, percentage hemoglobin A was measured by alkaline electrophoresis with densitometry, and a significant discrepancy between the calculated and measured value was noted. We report an investigation to elucidate whether the discrepancy was due to a methodological problem in the Sebia Hydrasys assay used at this institution.

We noted the manufacturer did not recommend a mixing step for sample preparation before withdrawal of erythrocytes from the washed cell pellet to prepare the hemolysate. Sickle erythrocytes have a heterogeneous density profile depending on the degree of dehydration, erythrocyte age, and other factors (1). In addition, transfused patients will have a dimorphic erythrocyte population of sickle cells and normal transfused cells. Given this heterogeneity, samples containing sickle cells may form a density gradient if allowed to settle. Depending on where the tip of the pipette lands in this gradient, different values for the percent of hemoglobin A could be obtained from the same patient specimen.

To test the theory that different values for the percentage of hemoglobin A could be obtained from the same patient specimen owing to a density gradient, we prepared 1 bag (bag 1) with the patient’s pretransfusion blood and 4 bags (bags 2–5) containing increasing amounts of transfused blood and therefore increasing percentages of hemoglobin A. Two samples were taken from bags 2, 3, and 5, and hemolysate was prepared from each using 2 methods: the manufacturer’s procedure for sample preparation and a variant in which a vortex-mixing step was added before withdrawal of the aliquot for hemolysis. Also, 12 hemolysates were prepared from bag 4 (6 mixed and 6 unmixed) and analyzed in replicate.

Results for the hemoglobin electrophoreses of unmixed samples taken from the same bag yielded widely variable results (see Fig. 1). Not only was there a wider variation (range of SDs 2.9–13.5 for unmixed vs 0.6–2.2 for mixed), but the results were drastically different than those from the mixed sample from the same unit. The calculated hemoglobin A values were also closer to the results from the mixed samples. These findings suggest that thorough mixing before withdrawing erythrocyte aliquots during sample preparation distributes the cells more evenly and yields a more representative estimation of the percentage of hemoglobin A.

With a heterogenous erythrocyte population, the formation of a density gradient seems logical. However, current sample preparation methods do not address this potential source of error. According to the College of American Pathologists 2006 Survey (2), 58% of respondents use electrophoresis to measure the percentage of hemoglobin A. Three kits dominate: Sebia Hydrasys, Helena, and Beckman Coulter. In reviewing the manufacturers’ sample preparation methods (3–5), we found that none included a vortex-mixing step during hemolysate preparation. However, we noted a recent change in the package insert for Sebia Hydrasys: the phrase “vortex-mix them before taking 10 μL to hemolyze” was added after instructions to discard the excess saline over the erythrocytes.

The problem brought to light by our investigation of the results for this single patient could represent a more pervasive issue, especially pertinent to those with sickling hemoglobinopathies. The omission of a vortex-mixing step seems not to be unique to a single kit or manufacturer but a common problem in hemoglobin electrophoresis sample preparation. Because the percentage of hemoglobin A after an erythrocyte exchange is often used to guide treatment, recognition and correction of this omission are important.

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Insufficient Standardization of a Direct Carbohydrate-Deficient Transferrin Immunoassay

To the Editor:

Measurement of carbohydrate-deficient transferrin (CDT) can reveal alcohol-related changes in the serum transferrin glycosylation pattern. CDT is a more alcohol-specific indicator than liver-function tests and is used for identification and follow-up of chronic high alcohol consumption (1). Therefore stable calibration of the assays is very important. Comparison of CDT results between methods has often been hindered by method-dependent discrepancies in the definition of the measurand (the transferrin glycoforms covered) and the way results are expressed. With some methods there has been an increased risk for false-positive results (2). The lack of CDT standardization prompted initiation of a working group under the International Federation of Clinical Chemistry and Laboratory Medicine, whose aim was to define the measurand, select and validate a reference method, and work out procedures for the production of reference materials. The first recommendation was that the fraction of disialotransferrin to total transferrin (%disialotransferrin) should be the primary target for CDT testing, with HPLC as the candidate reference method (3).

The performance of individual laboratories and agreement of different methods can be determined through external quality assessment (EQA). A Swedish EQA scheme for CDT has been run by EQUALIS (External Quality Assurance in Laboratory Medicine in Sweden) since 1996. Each year, 10 samples (human serum pools without preservative) whose target %disialotransferrin values are set by selected expert laboratories that use HPLC (3) are distributed to the participants.

In-house or commercial HPLC assays are the most common CDT assays (n = 19) in the EQUALIS EQA scheme. HPLC methods involve separation of iron-saturated transferrin glycoforms by anion-exchange chromatography, and quantification by selective absorbance of the iron-transferrin complex at approximately 460 nm (4). The disialotransferrin fraction is calculated as relative peak area using baseline integration, in agreement with the recommendations of the CDT standardization work (3). The second most common method (n = 15) is the N Latex CDT direct immunonephelometric assay (Siemens, formerly Dade Behring). The N Latex CDT assay uses a monoclonal antibody that recognizes transferrin glycoforms lacking 1 or 2 complete N-glycans (i.e., disialo-, monosialo-, and asialotransferrin) and on a simultaneous transferrin immunoassay (5). The %CDT value (fraction of disialo-, monosialo-, and asialotransferrin to total transferrin) is calculated automatically.

In a multicenter evaluation of N Latex CDT (5), the %CDT results correlated well with the %disialotransferrin results by HPLC, but owing to the different measurands the numerical values were not interchangeable. For healthy controls, the upper 97.5th percentile for %disialotransferrin by HPLC was approximately 1.7% (4) compared with approximately 2.35% for %CDT by N Latex CDT (5). A discrimination limit of 2.5% (99th percentile) is proposed in the N Latex CDT package insert, and is commonly applied in clinical practice. However, starting in 2006, the relation between the HPLC and N Latex CDT values in the EQUALIS EQA surveys has gradually changed. At %disialotransferrin values around 2% by HPLC, the corresponding %CDT results by N Latex CDT were on average 0.4% higher in April 2006, but roughly identical in September 2007 (Fig. 1A). This change was observed over the entire measuring range (Fig. 1B).

Four serum pools with %disialotransferrin target values of 1.02%, 1.75%, 2.61%, and 3.52% that were stored frozen since their use in a Swedish CDT harmonization program in 2002–2003 were reanalyzed by HPLC in November 2007. The resulting values were almost identical to the original ones (1.00%, 1.83%, 2.58%, 3.53%; r² = 0.997), confirming that the