Proficiency Testing of Therapeutic Drug Monitoring Techniques

To the Editor:
In this issue of the Journal, Jenny and Jackson-Tarentino (1) have carefully analyzed data from the New York State Department of Health therapeutic drug monitoring proficiency testing program to determine some causes of unsatisfactory performance. In regard to the Beckman Coulter SYNCHRON® Systems, it was noted that 9 results (out of 270) that were flagged “Out of Instrument Range” (OIR) were incorrectly reported as less than the reportable range when in fact they exceeded the range. A software change introduced in 1997 made the OIR flags more explicit by indicating OIR LO or OIR HI.

These authors note that the product precision claim is usually greater than that which a user would typically encounter. Most laboratories do not routinely perform precision runs, but use ongoing laboratory quality-control data to determine whether the instrument is performing satisfactorily. I'm not sure what benefit the customer would derive from a tighter advertised precision claim.

Reference

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Early Detection of Leptomeningeal Metastasis by PCR Examination of Tumor-derived K-ras DNA in Cerebrospinal Fluid

To the Editor:
Leptomeningeal metastasis (LMM) occurs in 3–8% of all cancer patients. Of the solid tumors, breast cancer, lung cancer, and malignant melanoma are the most common to metastasize to the leptomeninges (1). The prognosis for patients with LMM is poor: most individuals survive a median of only ~4 months. Early diagnosis may improve the clinical response to radiotherapy and (intrathecal) chemotherapy, and may lead to more effective palliation and prolonged survival (1).

Traditionally, a definitive diagnosis of LMM requires cytological detection of malignant cells in the cerebrospinal fluid (CSF). Interpretation is often aided by immunocytochemical techniques. Unfortunately, these CSF samples often contain very few morphologically identifiable malignant cells. In these cases, no definitive diagnosis can be established, leading to “suspicious” or “atypical” diagnoses (1).

Molecular detection of tumor-derived DNA in CSF can potentially improve early and sensitive detection of LMM because no intact cells are required for diagnosis (2, 3). Here we report the detection of K-ras gene mutations in the CSF of two patients with clinical features of LMM and negative cytology.

Both patients (A and B) suffered from lung adenocarcinoma, which had metastasized to the cerebellum. Treatment consisted of resection of

Reference
metastatic tumor followed by radiotherapy. Clinically suspected LMM emerged 24 (patient A) and 2 weeks (patient B) after the resection. Patient A experienced radicular pain in both arms. Although MRI scanning revealed LMM, cytology of the first CSF specimen was negative. It was only 4 weeks after this initial investigation, at the second CSF puncture, that malignant cells in the CSF could be detected. Patient B suffered from radicular pain in both legs and diplopia. The clinically suspected LMM initially could not be confirmed by cytological examination of the CSF. After 11 weeks, patient B had developed an organic psychiatric syndrome and multifocal radiculopathy. At that time, a second CSF examination was diagnostic for LMM.

CSF of both patients was collected at the initial clinical presentation of LMM and centrifuged. The pellet was used for cytology, the results of which were negative. Free DNA was isolated from 0.5 mL of the supernatant. A K-ras mutation (codon 12; GGT→GTT) common for lung adenocarcinoma was found for both patients with mutant-allele-specific amplification (MASA; Fig. 1B) (5, 6). Identical mutations had been found previously in the lung primary (Fig. 1A) and the cerebellum metastasis. Other K-ras mutations were absent.

In controls, including two patients with LMM without a K-ras mutation in the tumor and three patients with nonneoplastic disease (multiple sclerosis, meningitis, and Alzheimer disease), no mutant K-ras could be detected in the CSF supernatant.

We conclude that detection of K-ras mutations in the CSF of clinically suspected LMM patients is a promising tool for early diagnosis, follow-up, and treatment monitoring of LMM derived from primary adenocarcinoma of the lung. Moreover, with sensitive methods such as MASA, it is relatively easy to screen for K-ras point mutations in the CSF supernatant, which is not used for cytology, even without previous knowledge of the tumor mutations.

Because K-ras mutations are found in only 30% of the adenocarcinoma of the lung (4) and are rare in other solid tumors that frequently metastasize to the leptomeninges (7), additional molecular markers for the management of LMM are of potential interest (8, 9).

References

To the Editor:

Professionally set quality specifications are needed as major considerations in development of new reagents (1). Two years ago, the Dutch Lipid Reference Laboratory, a permanent international member of the CDC Cholesterol Reference Method Laboratory Network (2, 3), in collaboration with Roche Diagnostics Nederland B.V. (formerly Boehringer Almere, The Netherlands), began evaluating lot-to-lot differences of the new HDL-cholesterol (HDL-C) reagent from Kyowa Medex (cat. no. 1731157) and of the HDL-C/LDL-cholesterol cfds calibrator (cat. no. 1778501) before distribution on the Dutch market. To this end, a scaled down split-sample comparison protocol was used, essentially according to the description given in the HDL Cholesterol Method Evaluation Protocol for Manufacturers (4, 5). The accuracy platform was the CDC Designated Comparison Method (DCM) for HDL-C (2, 4); the test method was run on an Hitachi 911 analyzer (Boehringer Mannheim/Roche). A split-sample comparison was done with six specimens covering the HDL-C concentration range. All specimens were from individual donors. The matrix types investigated with the test method were serum and heparin plasma, whereas serum was used in combination with the DCM. Fresh specimens, intermittently stored at 4 °C, were analyzed with the test method, whereas frozen split sera were analyzed with the HDL-C DCM (5, 6). With the test method, all specimens were run in duplicate during 3 consecutive days for a total of 36 measurements (6 × 3 × 2 measurements); with the DCM, duplicate analyses were performed in one analytical run for a total of 12 measurements (6 × 2 measurements). Both test and reference data were produced in the

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Lipid Reference Laboratory in Rotterdam. Analytical performance was checked against the National Cholesterol Education Program guidelines (4). New lot combinations were acceptable when the total error criterion of 13% was met. Total error can be considered as an error budget that can be divided between imprecision and bias (4, 7).

During a 2-year period, 31 reagent/calibrator lot combinations for direct HDL-C were evaluated. For serum, the mean (± SD) results were as follows: mean bias (%), −1.6% ± 3.0%; mean absolute bias (%), 3.0% ± 1.9%; overall imprecision, 1.5% ± 0.7%; and total error, 5.5% ± 2.5%. For heparin plasma, the mean (± SD) results were as follows: mean bias (%), −2.7% ± 3.1%; mean absolute bias (%), 3.8% ± 2.1%; overall imprecision, 1.6% ± 0.7%; and total error, 6.2% ± 2.7%. In four reagent/calibrator lot combinations, the mean bias exceeded ± 5%, i.e., the excessive bias was related to one specific lot of calibrator in three combinations and to a specific reagent lot only once. In none of the combinations did the total error exceed 13%, the maximum total error being 11.1% for one serum matrix and 12.1% for the corresponding heparin plasma. Hence, all tested combinations were sold on the Dutch market. In the HDL-C reagent kit, a “Document of Comparison” issued by the Lipid Reference Laboratory Rotterdam is inserted, stating the overall analytical CV (%), the percentage of bias, and the percentage of total error for both matrices and for the tested reagent/calibrator lot numbers on a Hitachi 911 system.

It is concluded that the lot-to-lot differences of the direct HDL-C reagent and calibrator during the past 2 years were acceptable and in agreement with the 1998 National Cholesterol Education Program recommendations for HDL-C method performance (4). We have been assured that Roche will make further efforts to decrease the method bias to less than ± 5% in all combinations. The inserted Document of Comparison with the stated method bias and total error is objective evidence that should aid clinical chemists in the field to judge whether the quality of different lots of direct HDL-C reagent and calibrator meets recommended standards (4). In general, the results obtained for analytical performance characteristics should be compared to well-documented, objective quality specifications. We believe that assessment of the analytical performance by independent bodies across different sales lots should become part of (inter)national reagent release.

References


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High Prevalence of Factor V Mutation (Leiden) in the Eastern Mediterranean

To the Editor:
Factor V (FV), a 330-kDa procofactor, is a plasma protein that acts in concert with other plasma factors in regulating the blood coagulation cascade (1). Upon its proteolysis by factor Xa and/or thrombin, factor Va is inactivated by proteolysis by activated protein C (1, 2). A defect in FV hydrolysis brought about in part by resistance to activated protein C hydrolysis translates into poor anticoagulant activity, leading to thrombosis and other disorders of poor anticoagulation (3–5). Factor V mutation-Leiden (FV-Leiden) is a specific point mutation, identified as a G-A substitution in nucleotide 1691 in the factor V gene that leads to Arg506-Gln conversion (2) and is associated with hypercoagulability and increased risk of venous thromboembolism. The prevalence of FV-Leiden is high among venous thromboembolism patients (20–60%) compared with otherwise healthy individuals (2–13%) (6, 7). FV-Leiden has been described as a predisposing as well as an additional risk factor for venous thrombosis (3), pulmonary embolism (5), and to a lesser extent coronary artery disease (8). However, the latter remains to be established in light of reports disputing any link between FV-Leiden and coronary artery disease (3, 9).

High prevalence of FV-Leiden has been reported in Caucasians (Europeans and Arabs) but not in non-Caucasians (Africans, Asians, and Eskimos) (10–12), thus suggesting a single origin of the mutation (13). In view of its role as the most common
ordered probes and detection by DNA enzyme immunoassay according to the manufacturer’s instruction (SORIN). The results were interpreted as follows: FV-Leiden non-carriers, positive G-negative A; FV-Leiden heterozygote, positive G-positive A; and FV-Leiden homozygote, negative G-positive A.

The prevalence of FV-Leiden was determined for 155 healthy, unrelated Lebanese subjects (mean age, 11.3 years). The subjects, recruited from different provinces, were of both sexes (81 males and 74 females) and represented the two major religious groups (60 Moslems and 95 Christians). All participants were free of blood coagulation disorders, and none was on anticoagulant therapy. FV-Leiden was present in 22 of 155 participants (14.2%; Table 1). The mean age of FV-Leiden carriers was similar to that of FV-Leiden-negative individuals (32.2 ± 11.4 years and 32.3 ± 11.3 years, respectively; \( P = 0.96 \)). The frequencies of FV-Leiden were similar in females and males (13 of 74 vs 9 of 81, respectively; \( P = 0.25 \)) and in Christians and Moslems (16 of 95 vs 6 of 60, respectively; \( P = 0.39 \)). Heterogeneity in FV-Leiden distribution was noted among the different provinces of Lebanon, exemplified by the high prevalence in Mount Lebanon and the low prevalence in South Lebanon (7 of 43 and 1 of 32, respectively; \( P = 0.21 \); Table 1), thus suggesting differential demographic distribution of FV-Leiden. We found 21 heterozygotes and 1 homozygote. The homozygote, a 61-year-old male, had no signs of thrombosis at the time of specimen collection. The observed homozygote-to-heterozygote ratio was consistent with Hardy-Weinberg equilibrium (\( p = 0.858 \); 2pq = 0.135, and \( q = 0.00645 \)).

The high prevalence of FV-Leiden in Lebanon (14.2%) was similar to prevalences in Syria (13.6%; A. Nehme, personal communication), Greece-Cyprus (13.4%) (6), Jordan (12.3%) (16), and Turkey (7.4%) (17), further supporting the concept that the FV-Leiden mutation arose in the Eastern Mediterranean basin. FV-Leiden is common in countries with predominantly Caucasian populations [e.g., Sweden (11.1%) (18) and Germany (7.13%) (19)] and also in countries associated with significant migrations of Lebanese/Syrians and Greeks [e.g., UK (8.9%) (6), Argentina (5.12%) (11), Australia (20), and Spain (3.33%) (4)]. In contrast, FV-Leiden is nearly absent in Senegalese (6), African-Americans (15), Koreans (21), Japanese (22), and among Greenland Inuits (23), further con-

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\( a \) SE in parentheses.

\( b \) OR, odds ratio; CI, confidence interval.

\( c \) Two-tailed Student’s t-test.

\( d \) Pearson \( \chi^2 \) test.

\( e \) Compared with Greater Beirut.
firming the preferential prevalence of FV-Leiden among Caucasians, as suggested (10, 12, 15).

FV-Leiden is the largest inherited risk factor of venous thrombosis (14). However, the pathogenesis of venous thrombosis is multifactorial, including inherited and non-inherited risk factors (24). Whereas our results do not support random and indiscriminate screening for FV-Leiden, they do recommend screening for FV-Leiden in relatives of FV-Leiden carriers.

References


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Use of Turbidity-Correction Algorithm Eliminates the Effect of Perflubron Emulsion on CO-Oximeter Results

To the Editor:

Shepherd and Steinke (1) reported that perflubron emulsion, a potential blood substitute soon to be in clinical use, interferes with measurements by several CO-oximeters. The analyzer affected most was the AVL Omni: at 29 g/L perflubron, oxyhemoglobin was decreased by 12.3%, carboxyhemoglobin was decreased by 3.5%, and methemoglobin was increased by 1.0%. At higher concentrations of perflubron, the AVL Omni gave error messages without results. Although the Radiometer OSM3 Hemoximeter reported results at all concentrations of perflubron studied, it too was affected: at 29 g/L perflubron, oxyhemoglobin was decreased by 5.3%, carboxyhemoglobin was increased by 4.1%, and methemoglobin was increased by 2.3%.

At about the time of this report, we observed that an occasional heparinized blood sample analyzed by the AVL Omni would give a valid total hemoglobin result but report “interference” for the oxy-, carboxy-, and methemoglobin measurements. We assessed these results to patients who were receiving Propofol, a sedative used in intensive care settings and administered as a turbid emulsion. In discussions with AVL Scientific Corporation, they believed the interference was attributable to turbidity and had developed an algorithm to correct for turbidity. Because perflubron emulsion is also turbid, we investigated whether the algorithm for the AVL Omni would also correct the interferences caused by perflubron.

We obtained perflubron-based emulsion (600 g/L; product no. AF0144) from Alliance Pharmaceutical Corporation, San Diego, CA. The blood gas and CO-oximeter analyzers were an AVL Omni 9 blood gas analyzer and a CO-oximeter from AVL Scientific, and a Radiometer OSM3 hemoximeter attached to an ABL 505 blood gas analyzer from Radiometer America.
To heparinized whole blood samples from patients in operating rooms or intensive care, we added perflubron within 5 min after the sample was analyzed for blood gases. We anaerobically transferred 0.2 mL of either saline or perflubron mixed with saline to 0.8 mL of each sample of whole blood to give final perflubron concentrations of 10, 30, 40, and 60 g/L. Each specimen was kept thoroughly mixed. We made no other adjustments to the samples, which had Po2 values ranging from 5.1 to 30.7 kPa (38 to 231 mmHg) and oxyhemoglobin values of 87–98%. The difference in the CO-oximeter or blood gas results between the sample mixed with saline and the same sample mixed with perflubron was used to determine the analytical effects of perflubron.

To minimize changes in the sample during the analyses, we analyzed individual blood samples to which we added saline and perflubron rather than a series of samples prepared from a larger pool of blood. With only ~2 mL of blood available in each leftover sample, we used 60 different samples for the study: 15 different blood samples for each of the four concentrations of perflubron studied.

We used the paired t-test to determine the significance of differences in means between results for samples with saline and those with perflubron (2).

The use of perflubron has the therapeutic effect of both replacing blood volume and supplementing the oxygen and carbon dioxide transport capabilities of blood. The concentrations of perflubron we studied covered the range expected in clinical practice. Based on an expected dosage of 0.9–2.7 g of perflubron per kilogram of body weight, the concentration of perflubron in blood is estimated to be 10–40 g/L (1).

Perflubron up to 40 g/L produced no significant changes in the blood gas or CO-oximeter measurements on the AVL (Table 1). At 60 g/L, all changes were statistically significant (all P values <0.005), and changes for pH, Pco2, Po2, hematocrit, and total hemoglobin were greater than the SDs of the respective methods. These changes at 60 g/L represent changes of marginal clinical significance.

Our results on the Radiometer OSM3 (not shown) confirmed the reported effects (1) on oxyhemoglobin, carboxyhemoglobin, and methemoglobin.

The algorithm for turbidity correction has been incorporated in our analyzers for nearly 1 year. With this algorithm in place, we have had only two or three samples that have given error messages. Therefore, we conclude that this algorithm has significantly improved the ability of the AVL Omni to report valid CO-oximeter data in turbid samples and has virtually eliminated the effect of perflubron emulsion in blood at concentrations up to 40 g/L.

We appreciate the help of Shari Morgan and Dr. Peter Keipert of Alliance Pharmaceutical Corporation for providing the perflubron emulsion used in this study. We also appreciate the information provided by Dr. A. P. Shepherd regarding his protocol. Dr. Toffaletti receives funding for research from AVL Scientific Corporation.

### References


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### Abbreviated Direct and Indirect ELISAs: A New and Simple Format

To the Editor:

Many laboratories, including our own, perform ELISAs using immobilized antibody or antigen. A common format is to coat the ELISA plate with
antibody for direct assays, or antigen for indirect assays. ELISA plates are subsequently “blocked” to prevent any further nonspecific absorption.

Direct assays often are performed by incubation of antibody-coated microtiter plate wells with calibrators or patient samples and then washing the plate and detecting bound antigen with a mouse monoclonal antibody, followed by washing and the addition of anti-mouse immunoglobulin-peroxidase, further washing, and the addition of substrate.

We compared our direct sandwich ELISA for sex hormone-binding globulin (SHBG), using this format (1), to a procedure that differs in that the supernatant contains both monoclonal antibody to SHBG and the anti-mouse immunoglobulin-peroxidase (1.20 and 1:500, respectively; cat. no. NA 931; Amersham). The combined antibodies are then added to washed polyclonal anti-SHBG antibody-coated ELISA plate wells containing bound calibrator or sample for a 30-min incubation before washing and addition of the substrate. This is in contrast to the two separate 30-min steps—one using a 1:20 dilution for the SHBG monoclonal antibody supernatant, and the second using a 1:1000 dilution of anti-mouse immunoglobulin-peroxidase—before the wells are washed and substrate added. The calibration curves were identical, and the correlation between the one- and two-step protocols is shown in Fig. 1A over a wide physiological range.

We have extended this concept to our indirect ELISAs for steroid hormones, using immobilized steroid conjugates. Cortisol and dehydroepiandrosterone sulfate (DHEAS) had been assayed previously by adding plasma, diluted plasma samples, or calibrators (50 µL) directly to either cortisol-thyroglobulin-coated or DHEAS-thyroglobulin-coated plates (2, 3). This was followed by the addition of 50 µL/well of either cortisol monoclonal antibody supernatant diluted 1:50 or DHEA monoclonal antibody supernatant diluted 1:100 and incubation at room temperature, followed by washing and the addition of anti-mouse immunoglobulin-peroxidase (100 µL/well) at a 1:1000 dilution. After an additional incubation, plates were washed and substrate was added. We now combine the monoclonal antibody supernatant, at a dilution similar to that for the two-step method, with anti-mouse immunoglobulin-peroxidase (NA 931) at a 1:500 dilution and then add 50 µL to each well for a single 1-h incubation at room temperature, followed by washing and the addition of substrate. The calibration curves are similar to the two-step protocol, with excellent correlation between the one- and two-step formats for cortisol and DHEAS (Fig. 1, B and C, respectively) over a wide physiological range.

The final dilution of either monoclonal antibody or anti-mouse immunoglobulin-peroxidase is identical for both the one- and two-step protocols for the indirect formats described. The direct format also uses the same dilution of monoclonal antibody with double the concentration of anti-mouse immunoglobulin-peroxidase. Optimization experiments also showed that doubling the concentrations of monoclonal antibody used in the two-step protocol and/or the anti-mouse immunoglobulin-peroxidase produced similar results for both the direct and indirect ELISAs. This suggests that these systems are particularly robust and implies that the anti-mouse immunoglobulin-peroxidase used neither binds to the antigen-binding sites of the monoclonal antibodies nor is in close enough proximity to produce steric hindrance. The finding that the dilutions of antibodies for the one-step methods are similar to those for the two-step method suggests that this could be a relatively common finding, and hence, it could act as a guide when optimizing other assays to the one-step protocol. The obvious caveat is avoiding sodium azide in all reagents.

There are many advantages of the procedures described. The need for purifying monoclonal antibody from culture supernatants and covalent conjugation to enzyme is abolished together with the tendency for procedural losses and the need for regular syntheses because of the limited shelf-life of enzyme conjugates. The use of a common and relatively inexpensive anti-mouse immunoglobulin-peroxidase combined with monoclonal antibody supernatant is an
attractive, simple alternative that overcomes these difficulties and allows a one-step protocol. This concept has direct application to the laboratory as well as to ELISA kit manufacturers, whose dependence on antibody isolation and enzyme conjugation could be minimized.

References
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tumor of blood sampling or a prominent review in the September issue of Clinical Laboratory News (2). Hicks presents a horror story from the “Doctor, BE your Patient” standpoint. Her recital of her prolonged hospital stay in which 10 mL of blood was drawn twice daily for 2 weeks for only a complete blood count and white cell differential (defended as the “computer-dictated” volume) shocked me as much as it did her. I can vouch for the commercial availability of 4-mL blood collection tubes for hemato logic measurements as early as the 1960s, and tubes of varying sizes for other specific uses have become available since then. (Has “Hal” taken over the decisions of the clinical laboratory?)

Undoubtedly, the great majority of current clinical laboratorians were not trained in the fading era of universal manual laboratory tests, when patient sample collection was performed not by “phlebotomists”, but by trained laboratory staff. The importance of the improvement between 10 mL and the miniscule samples now drawn for red blood cells and white blood cells in dilution pipettes from ear lobe- or finger-sticks is beyond estimation. Capillary sampling for the hematocrit has supplanted the several milliliters required by the old graduated tubes, and the Drummond system, with its guaranteed-bore small capillaries and high gravity centrifugation (as a percentage of an unmeasured negligible sample), and microscopic readers now permit precise measurements of (arterial) red cells and plasma hematocrit by sharp delineation of the white blood cell pack.

Of course, finger-stick sampling has the disadvantage of requiring an adequate puncture to avoid milking the site, and the samples represent capillary blood, giving somewhat higher cell/plasma ratios than venous samples. In addition, precise automated sample dilutors have limited drawn sample requirements for multiple assays.

Microchemical methods were being developed rapidly, notably by Manny Sanz in Berne, when they were overtaken by automated, and particularly “multiphasic”, testing for ever larger panels of analytes—most of which were ignored by the physicians who needed only a few critical results. These requirements appear to be perpetuated in excessive draws for modern technologies. Not that we can return to the “good old days”, but there are ways of drastically reducing this awful waste. All of this illustrates the fallacy of the assumption that real progress necessarily correlates positively with time over the years.

Elution of Hemoglobin αMontgomery2βS2 Hybrid Tetramers by the Variant Apparatus

To the Editor:
Hemoglobin (Hb) Montgomery (α48 Leu→Arg) is an uncommon variant first reported in 1975 (1). We report here a case of the association of homozygous HbS (β6 Glu→Val) with this variant identified by the late Dr. T.H.J. Huisman in an 8-year-old black girl with a life-long sickling disorder.

We quantified Hb fractions by cation-exchange HPLC with the Variant™ Hemoglobin Testing System Beta-Thalassemia Short Program (Bio-Rad Diagnostics) (2, 3). The complete blood count was determined by a Beckman-Coulter STK-S™. Alkaline Hb electrophoresis was performed on cellulose acetate (Helena Laboratories). Solubility testing was done with phosphate-buffered saline using SickleScreen™ (Pacific Hemostasis). The Hb was 88 g/L [reference interval (RI), 110–160 g/L], mean cell volume was 83.1 fl (RI, 80–98 fl), and the red cell distribution width was 19.5% (RI, 11.5–18.0%). HbF, HbA2, HbS, and “HbC” were 8.9%, 3.8%, 67.7%, and 15.8%, respectively, by HPLC (Fig. 1). The blood sample was solubility positive. Alkaline Hb electrophoresis had an “HbSCF” pattern with an abnormal HbA2 band.

Although Hb Montgomery is not rare in African Americans (4), it is less common than HbG-Philadelphia (α68 Asn→Lys); hence, the combination of homozygous HbS and Hb Montgomery in this patient (A. Kutlar, personal communication) is very
uncommon, although an accurate prevalence estimate of this condition is unknown to us. The lack of HbA by both HPLC and alkaline electrophoresis confirms homozygosity for HbS; therefore, the HbC window by HPLC and the band on cellulose acetate are a Montgomery2 bS hybrid tetramers. The elution of these tetramers in the HbC window on the Variant has not been reported. This window cannot contain Hb Montgomery (aMontgomery2bS2) because only bS chains were synthesized. Hb Montgomery is a stable variant of the a2-globin gene (a\(^2\)a/aa) (5); the relatively high concentration of hybrid tetramers (15.8%; aMontgomery2bS2) here reflects the high degree of a-chain variant stability and its affinity for the bS chains (5,6). In alkaline Hb electrophoresis, the aMontgomery2bS2 hybrid tetramers behave similarly to aG-Philadelphia2bS2 hybrid tetramers, which also migrate to the HbC position (6).

References

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