Comparison of Radial Immunodiffusion and Alkaline Cellulose Acetate Electrophoresis for Quantitating Elevated Levels of Fetal Hemoglobin (HbF): Application to Evaluating Patients With Sick Cell Disease Treated With Hydroxyurea

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Radial immunodiffusion (RID), alkaline cellulose acetate electrophoresis, and high-performance liquid chromatography (HPLC) were compared for quantitating the elevated (> 10%) level of fetal hemoglobin (HbF) found in the red blood cells of sickle cell disease patients undergoing treatment with hydroxyurea. HPLC- and electrophoresis-determined values were comparable. The RID-determined values were higher, in many cases twofold higher. False high HbF values would be misleading in assessing the effectiveness of hydroxyurea therapy in sickle cell disease patients. We subsequently initiated an examination of the variation in HbF values due to the use of different HbF radial immunodiffusion QUIPlates and different positions within a single plate in an attempt to determine the cause of these discrepancies. Within-run precision studies indicated that significantly different size precipitin rings

were obtained depending upon which area of the plate the hemolysate containing antigen (HbF) was applied. A common feature associated with poor precision plates was a marked difference in degree of coloration of gel throughout the plate. Spuriously high HF concentrations were obtained with antigen (HbF) placed in wells located in the lighter colored gel area while antigen placed in wells in the darker colored area of the agarose gel bed were more in agreement with the electrophoretically determined HbF concentrations. The variation in HbF values was significantly greater in the diluted (HbF QUIPlate Diluent) samples than in the neat samples even on plates of uniform gel coloration. As a result of this study, we will continue to monitor high HbF levels by densitometry following alkaline cellulose acetate electrophoresis. *J. Clin. Lab. Anal.* 13:82–89, 1999. © 1999 Wiley-Liss, Inc.

Key words: high-performance liquid chromatography; hemoglobin F analysis; antiserum; point-of-care testing; hemoglobin electrophoresis

INTRODUCTION

Fetal hemoglobin, HbF, (composed of $\alpha_2\gamma_2$) is the predominant hemoglobin found in the fetus after the first 10 weeks of pregnancy, and is present in a concentration of 50 to 85% in umbilical cord blood. However, after the birth of the infant, the proportion of HbF diminishes, and by 4 to 6 months in the normal infant, HbF is usually less than 2% of the total hemoglobin; and then to less than 1% in adults (1,2). But increased levels of HbF occur in several conditions: for example, some malignancies and anemias, and chronic renal disease as well as some benign conditions such as hereditary persistence of fetal hemoglobin (HPFH) or during acute erythropoietic stress (3). An increase in HbF is frequently observed in inherited blood disorders such as β -hemoglobinopathies (the sickle cell disease) and β -thalassemia syndromes (3). In β -hemoglobinopathies, increasing the patient's HbF level could be a therapeutic approach to ameliorating the clinical

symptoms of the underlying disease (4). In sickle cell disease, HbF inhibits polymerization of sickle hemoglobin (HbS) directly, accounting for the lower propensity of HbF-containing cells to form intracellular polymer and undergo sickling (5). Because HbF is an effective inhibitor of polymerization of deoxyhemoglobin S, therapeutic agents that increase HbF production would be expected to benefit patients with sickle cell disease. Several pharmacological agents, including hydroxyurea (HU) and derivatives of butyric acid have been used to stimulate HbF synthesis in vitro (6) and in vivo (7). In sickle cell disease patients treated with HU, the magnitude and rapidity of the response among patients differed; some

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had drug-induced HbF levels near 40%, while others had little change in HbF concentrations (5,7–9). HU increases the number of red cells containing HbF (F cells), the percentage of HbF, and the amount of HbF per F cell (9–12).

Assessment of HbF is therefore important for the diagnosis of hemoglobinopathies and thalassemias and, in addition, because of the narrow margin between toxicity and the therapeutic effect of some HbF-stimulating agents including HU *in vivo*, it is important to monitor the response of patients during the course of treatment (10).

Hemoglobin F can be evaluated with respect to its percentage of the total hemoglobin present in whole blood. Five widely used methods currently used in the clinical setting to measure HbF quantitatively are: alkali denaturation (1,13); high-performance liquid chromatography (HPLC) (14,15); densitometry following electrophoresis (16,17); isoelectric focusing (18); and radial immunodiffusion (19,20).

Monospecific polyclonal antibodies and monoclonal antibodies specific for HbF are available and have been incorporated into enzyme-linked immunosorbent assays (ELISA) (21,22), radioimmunoassays (RIA) (23,24), and magnetic affinity immunoassay (25), but their usage has been limited to a small number of investigators. Introduction by Helena Laboratories of a ready-to-use monospecific antibody- (antiserum) based immunodiffusion assay (HbF QUIPlate) into the clinical laboratory has made immunologic testing easier and available to a greater number of laboratories (26). The Helena HbF QUIPlate is the only commercially available ready-to-use immunologic assay for quantitating HbF.

The primary reason for conducting the studies described in this report was to determine whether the HbF QUIPlate system would produce analytical results comparable with those obtained by densitometry following alkaline cellulose acetate electrophoresis used to measure elevated levels of HbF in our clinical laboratory.

MATERIALS AND METHODS

Blood Specimen

Blood samples used in this study had been previously submitted to our laboratory for hemoglobin analysis.

Procedures

Radial immunodiffusion

HbF QUIPlates, HbF QUIPlate Diluent, HbF QUIPlate Control, and HbF standards were obtained commercially (Helena Laboratories, Beaumont, TX). The enclosed instructions from the vendor were strictly followed. The antigen (HbF) in the sample reacts with the HbF antiserum incorporated in the agarose gel of the HbF QUIPlate, producing an opaque precipitin ring around the wells. If the precipitin ring was greater in diameter than that obtained with 10% HbF standard, the 1gHb/dL hemolysate required dilution with

Helena HbF QUIPlate Diluent prior to RID. (HbF Diluent contains purified HbA which has been lyophilized and reconstituted in purified water; the HbA concentration in the Diluent is 1g/dL.) The diameter of the opaque precipitin ring formed was measured with a calibrating viewer (Bausch & Lomb, Rochester, NY) which permits accurate measurement to 0.1 mm. The concentrations of the standard were plotted vs. the square of the diameter of the precipitin ring (27). The antibody to fetal human hemoglobin in the HbF QUIPlate gel is obtained from sheep antisera and purified by affinity chromatography. The affinity-purified antibody shows no cross-reactivity with human hemoglobins A, A₂, or S when used in an ELISA quantitation procedure for fetal human hemoglobin (Bethyl Laboratories, Inc., Montgomery, TX).

Electrophoresis

Hemoglobin electrophoresis was performed on Gelman Sephaphore III cellulose acetate electrophoresis strips (5.7 cm × 12.7 cm) in Tris-EDTA-glycine buffer at pH 9.2 (28) following the approved standard (29). Following electrophoresis and visualization by staining with Ponceau S, quantitation of hemoglobin was achieved by scanning densitometry (Quick Scan Jr. TLC, Helena Laboratories). AFSC Hemo Control (Helena Laboratories) was used as a position marker for the identification of HbF in the test specimens and, when quantitated, provided verification for all aspects of the procedure.

Alkali denaturation

Quantitative measurement of fetal hemoglobin was performed using the alkali denaturation method (Betke-2 minutes) (1) following the approved guideline (30).

High-performance liquid chromatography

Separation and quantitation of HbF was achieved using a cation exchange column (Poly CATA) and a gradient elution system as described by Ou et al. (31). Effluent was monitored at 436 nm. Hemoglobin fractions in patient samples were compared to known hemoglobin fractions in hemoglobin AFSC control mixture (IsoLab, Akron, OH).

Measurement of HbF QUIPlate gel layer thickness and base plate flatness

Measurement of gel thickness, unless mentioned otherwise, was made close to the actual wells and away from the plastic side and interior plastic ridges on the plates. Gel thickness was measured at six points on each plate (between wells 1 and 2, 4 and 5, 7 and 8, 10 and 11, 13 and 14, 15 and 16) by inserting a thin stainless steel ruler calibrated at a 32nd of an inch directly through the gel bed on the plate. In addition, gel thickness was also measured on some of the plates by excising a 0.5 inch square section of the gel from the base plate and measuring its thickness with the aid of a Bausch and Lomb

Stereo Zoom 7 microscope using the same ruler. All HbF QUIPlates including plates described in this study were stored in ziplock plastic bags and kept at 4°C to prevent any dehydration of gel which would result in shrinkage of the gel.

The degree of base plate warpage was determined by measuring the distance between the base plate bottom and the true flat surface the plate rests on with measurements made at different locations on the plate using a feeler gauge.

Statistical analysis

Linear-regression analysis was utilized to compare HbF concentrations determined with the different methods.

RESULTS

Performance Characteristics

High hemoglobin F level measurement: comparison studies

Peripheral blood samples were obtained from neonatal and pediatric patients less than one year of age, patients with hereditary persistence of fetal hemoglobin (HPFH), and sickle cell disease patients including responders to hydroxyurea (HU) treatment. HbF values determined by densitometry following electrophoresis were in good agreement with HPLC values (not shown). In contrast, as seen in Figure 1, results obtained with the HbF QUIPlate method failed to correlate with the % HbF values determined by alkaline cellulose acetate electrophoresis (i.e., slope was over 30% higher than the comparative method). It

Fig. 1. Comparisons of HbF quantitations by the HbF QUIPlate testing system with conventional electrophoresis: $y = 3.2088 + 1.3140 \times$ ($r^2 = 0.796$). Hemoglobin electrophoresis and quantitation of hemoglobin by densitometry were performed as described under Materials and Methods. Different lots of HbF QUIPlate were used and included plates of uniform and non-uniform agarose gel coloration. Hemolysates of patient samples were adjusted to 1g/dL of total hemoglobin and mixed with HbF Diluent in a 1:10 dilution.

should be pointed out that some HbF level measurements were in good agreement with the comparative method(s) while the remaining values differed significantly with the HbF QUIPlate values, in some instances being twofold higher than HPLC- and/or electrophoretically determined values. Further studies on the reproducibility of the HbF QUIPlate system, which are described in the following sections, may help explain why certain measurements of HbF by HbF QUIPlate were in good agreement with the comparison method(s) while other RID determined values were significantly higher than that obtained by electrophoresis and/or HPLC. As a result of the findings shown in Figure 1, an examination of the variation in HbF values due to the use of different plates and different positions within a single plate was performed as described below.

Reproducibility of the HbF QUIPlate method: variation due to the use of different plates and different positions within a single plate

Although uncertain of its importance at the time, we had observed that the agarose-antisera gel bed mixture of some of the immunodiffusion plates was not of uniform coloration throughout the plate while other plates within the same lot were. This was the case for plates used to measure HbF levels described in Figure 1. This difference between plates of the same lot was also observed with other lots of HbF QUIPlate plates. Figure 2 shows a HbF QUIPlate which does not have a uniformly colored agarose gel bed. Wells 4–9, 14, and 15 are located in the lightly colored gel region; wells 1–3, and 13 are in a darker colored gel region and wells 10–12, and 16 in the darkest colored area. Other plates showing differences

Fig. 2. Hemoglobin F QUIPlate (radial immunodiffusion plate).

in gel coloration on the plate had the same pattern and degree of varying coloration but the difference in coloration was shifted so that, for example, the wells on the left-hand side of the plate containing wells 7–12, 15 and 16 were occupying the lightly colored gel region. After finding inconsistencies in HbF measurement by HbF QUIPlate compared to electrophoresis (Fig. 1) and/or HPLC, studies were initiated to determine whether there was any relationship between HbF levels measured and gel coloration surrounding the antigen sample wells. Aliquots of a hemolysate containing antigen (HbF) were applied to wells throughout the plate (Plate 3a) on day 1 to provide a test on the influence of the position of the wells within the plate. On day 2, two plates (Plates 3b and 3c) were set up, each containing 5 μ L aliquots of the same hemolysate applied to Plate 3a. The plates would serve not only as a measure of within-run and run-to-run variation but also would allow comparison of results obtained on plates of uniform gel bed coloration versus plates having wells located in light and darker colored regions of the gel bed. Figure 3 shows the results of HbF measurements on day 1 (Plate 3a). Depending upon the well in which the sample was placed, the HbF concentration value varied from 45% (wells 6–8) to 77% (well 13), with wells located in the lighter region of the gel yielding larger precipitin ring diameters than antigen placed in wells located in the darker colored gel areas. HbF values obtained with samples spotted in wells located in the darker colored areas of the agarose gel bed were in good agreement with electrophoretically determined HbF concentrations, while spuriously high values were obtained from wells in the lighter gel region. The amount of variation due to the position occupied by the antigen wells in the plate was significant. Replicate diameters should be within ± 0.25 mm (27). Hemoglobin F concentrations measured on Plate 3b were similar to those obtained with Plate 3a, (i.e., the percentage of HbF varied depending upon the location of the well on the plate). Again, antigen placed in wells in lighter colored gel bed regions (wells 9–16) consistently gave higher values of HbF levels (54–68%) compared to wells in the darker gel colored region (wells 4–8), which yielded values ranging from 35 to 44% HbF. The HbF standards gave a linear response on each plate and the control was in the acceptable range.

The plate with gel of uniform coloration throughout the plate (Plate 3c) showed a higher degree of precision (mean % HbF $\pm$ SD = 50.5 ± 3.6 ; CV % = 7.08) than that obtained with Plates 3a (mean % HbF $\pm$ SD = 59.2 ± 11.0 ; CV% = 18.25 and 3b (mean % HbF $\pm$ SD = 53.0 ± 11.1 ; CV% = 21.07), and yielded HbF values which were within approximately 10% of the values obtained by electrophoresis and HPLC.

HbF measurement in conditions associated with slightly increased proportions of HbF

Although the data (not shown) on reproducibility and accuracy of measurements of HbF within the normal adult

Fig. 3. Within-run and run-to-run precision of HbF QUIPlate. Peripheral blood was obtained from a five-month-old patient with elevated level of fetal hemoglobin. HbF represented 43.5% of the total hemoglobin as determined by densitometry following alkaline cellulose acetate electrophoresis. A 1gHb/dL of total hemoglobin hemolysate was prepared with purified water. The hemolysate was diluted 1:10 with HbF Diluent. Five μ L aliquots of the resulting 1:10 dilution hemolysate, HbF standards, and control were applied to the HbF QUIPlate on day 1 for **Plate 3a** and day 2 for **Plates 3b** and **3c**. Plates 3a and 3b were of the same lot and had a non-uniform gel bed coloration. Plate 3c was of a different lot and had a uniform gel bed coloration.

range (0.0–2.1%) on HbF QUIPlate did not show a problem and the determined values were comparable to those obtained by alkali denaturation method, it would be informative to know if samples with concentrations of HbF above 2.1% but below 10%, (i.e., elevated but not requiring dilution with Helena HbF Diluent) when analyzed using non-uniform plates to assess intraplate variability cause errors in this range of HbF as well. Table 1 shows the results of HbF measurements using plate of uniform gel coloration

TABLE 1. Comparison of Within-Run Precision of HbF QUIPlates of Uniform and Non-Uniform Gel Bed Coloration in Measuring Slightly Increased Levels of HbF^a

	Plate 1a (uniform gel bed coloration)	Plate 1b (non-uniform gel bed coloration)
Mean % HbF $\pm$ SD for the 15 replicate samples	3.30 $\pm$ 0.27	4.33 $\pm$ 0.49
Range	2.9 – 3.9%	3.7 – 5.4%
CV%	8.26	11.27

^aPeripheral blood was obtained from the pediatric patient. Patient hemolysate was applied in replicate to wells 2–16 of each plate and plates were incubated simultaneously and under identical conditions. The Helena HbF QUIPlate control (mean = 6.8% HbF; acceptable range = 5.7–7.9%) was applied to well 1 of each plate and yielded a HbF concentration of 6.1 and 7.8 percent on plates 1 and 2, respectively. Plate 1a was of a different lot than plate 1b.

(Plate 1a) and plate of non-uniform gel bed coloration (Plate 1b) to measure HbF in a sickle cell disease patient. The amount of variation, due to the position occupied by the antigen wells, was higher using the non-uniform gel plate; the variation seen on both plates is, however, not of the magnitude obtained when measuring samples containing elevated (> 10%) levels of HbF (which required dilution with HbF Diluent prior to RID) and would not present any clinical problems.

Measurement of HbF QUIPlate base plate flatness and gel layer thickness

We attempted the measurement of gel bed thickness and flatness of the plastic base plate upon which the agarose gel was poured to determine whether the difference in gel coloration was attributed to factors such as slight variation in leveling of plates during pouring of gel and/or base plate not being truly flat. Of the 132 gel bed thickness measurements made on the 29 plates which represented five different lots and approximately equal numbers of uniform and non-uniform plates, values ranging from 1.5/32 to 2/32 inches were obtained in 130 measurements; the remaining measurements indicated a gel bed thickness of 1/32 inches. The subtle dif-

ferences in gel bed thickness and base plate flatness (not shown) did not allow a relationship between these parameters and gel bed coloration to be established. Helena Quality Assurance for plate thickness is 0.127 to 0.155 inches (approx. 4/32 to 5/32 inches) which is the combined thickness of gel bed and plastic base plate.

Reproducibility of the HbF standards and control

The results of experiments studying the effect of gel coloration on the size of the precipitin ring diameter of the 0.5%, 5%, and 10% HbF standards and Helena HbF QUIPlate control are summarized in Table 2. The standards and control provided good precision and accuracy on uniform as well as non-uniform gel bed quality plates. Within-plate and between-plate precision showed little variance. The standards and control therefore did not allow differentiation between plates yielding spuriously high HbF values and plates giving results comparable to electrophoresis on samples containing elevated HbF levels (> 10%) which required dilution with HbF Diluent.

Effect of HbS concentration on HbF measurement

To determine if HbS had any effect on the HbF QUIPlate method, the HbF level of a pediatric patient with normal phenotype was measured in the presence of increasing concentrations of HbS by addition of increasing amounts of hemolysate prepared from an adult with homozygous S (HbSS) disease. The amount of HbF introduced into the pediatric patient's hemolysate by the addition of increasing amounts of HbS-containing hemolysate was determined on separate but identical size aliquots of the sickle hemolysate by HbF QUIPlate method. The total amount of HbF measured in each of the various mixtures was in good agreement with the expected values, i.e., sum of HbF contributed by each hemolysate (Fig. 4). The results indicate that HbS at levels present in individuals with sickle cell trait (AS) and disease (SS, SC, S/ β thal., etc.) would not interfere with HbF measurement using the HbF QUIPlate method.

TABLE 2. Summary Statistics for Precipitin Ring Diameter (d) Obtained by Applying HbF QUIPlates Standards and Control in Different Regions of Plates of Uniform and Non-Uniform Gel Bed Coloration

Sample	Mean precipitin ring diameter ($\pm$ SD) ^a for the four replicate samples of each control or standard applied to			
	Plate 2a	Plate 2b	Plate 2c	Plate 2d
0.5% HbF Std	4.05 $\pm$ .06 mm	3.95 $\pm$ .06 mm	4.00 $\pm$ .00 mm	3.97 $\pm$ .05 mm
5% HbF Std	6.48 $\pm$ .13	6.35 $\pm$ .19	6.50 $\pm$ .22	6.32 $\pm$ .10
10% HbF Std	8.27 $\pm$ .17	8.25 $\pm$ .17	8.17 $\pm$ .36	8.12 $\pm$ .13
Control ^b	7.12 $\pm$ .21	7.27 $\pm$ .17	7.25 $\pm$ .35	7.17 $\pm$ .5

^aThe mean ($\pm$ SD) precipitin ring diameter values (measured in millimeters) shown above were calculated using the d values obtained in the four replicates of each sample (0.5, 5, and 10% HbF standards and HbF Control) spotted in different regions on each plate. The two plates of uniform quality (Plates 2b and 2d) and of the same lot and two plates of non-uniform quality (Plates 2a and 2c) of another lot were set up, each containing aliquots of the same standards and control.

^bHbF QUIPlate Control (6.8% HbF; acceptable range = 5.7–7.9%).

Fig. 4. Effect of sickle hemoglobin (HbS) on determination of HbF using HbF QUIPlate. Peripheral blood was obtained from a pediatric patient with normal hemoglobin phenotype (HbAA). Hemoglobin F represented 7.4% of the total hemoglobin (measured by HbF QUIPlate method). Aliquots of the 1g Hb/dL total hemoglobin hemolysate were spiked with increasing amounts of a 1g/dL total hemoglobin hemolysate prepared from a patient

with homozygous hemoglobin S (HbSS). The sickle cell disease blood contained 8.6% HbF as determined by HbF QUIPlate. The HbF QUIPlate had a uniform agarose gel bed coloration. The standard HbF samples (0.5, 5, and 10%) yielded a linear response plot and the control gave a value of 6.4% HbF which is in the acceptable range (5.7–7.9%).

DISCUSSION

The need for accurate HbF measurement is always critical, but especially so when monitoring HbF levels in sickle cell disease patients treated with HU, because of the narrow margin between toxicity and the therapeutic effect of the HbF-stimulating agent in vivo. Conventional methods used to measure the HbF level in blood such as electrophoresis, HPLC, and alkali denaturation each have their limits of usefulness, e.g., alkali denaturation methods are not accurate at low (Singer method) and high (Betke method) values for HbF (32), and densitometry following electrophoresis has been previously reported to be unreliable for normal or slightly elevated values of HbF (17,33). We routinely use cellulose acetate electrophoresis (pH 9.2) to measure elevated levels (> 10%) of HbF and alkali denaturation method for lower levels.

Our intention was to replace the time-consuming and laborious electrophoretic and alkali denaturation methods with HbF QUIPlate assay. Radial immunodiffusion and alkali denaturation were comparable (not shown) in measuring normal and mild increases (< 10%) seen in, for example, β -thalassemia trait and anemias. However, on measuring elevated (> 10%) levels of HbF which required dilution with HbF Diluent, we frequently observed significant differences in HbF values obtained by electrophoresis and HPLC compared to the HbF QUIPlate method, with the latter method consistently yielding higher HbF values. The greatest variation in accuracy and precision with RID was obtained with HbF Diluent diluted samples applied to plates of non-uniform gel bed coloration. Improper mixing of gel components

during gel bed preparation and/or slight variation in leveling when pouring gel onto plates could be sources of error to account for the pattern of gel coloration observed. No attempt was made to determine if differences in antiserum concentration existed in lighter colored gel bed area compared to darker area gel of the plate.

Uneven thickness of gel bed would affect the distance the antigen (HbF) travels before being neutralized. Thus we found a difference in precipitin ring diameter of a sample depending upon which area of the plate (thick or thin gel thickness) the antigen well was located. Samples placed in wells located in the lighter colored gel area yielded spuriously high HbF concentration values. As more antigen diffuses out of the antigen well, the precipitin ring dissolves in antigen excess and reappears at a greater distance from the well. The antigen can diffuse out radially further in thinner gel thickness areas before being completely neutralized, compared to gel areas of greater thickness where neutralization occurs earlier because the neutralizing concentration is encountered earlier. The variation in HbF values was considerably greater with HbF Diluent samples than in the neat samples. Samples containing greater than 10% HbF are diluted 10-fold with Helena HbF QUIPlate Diluent to bring them into the 0.5% to 10% range of the assay. A sample diluted 10-fold would have 10 times the variability in result compared to neat samples because the result is multiplied by ten. On plates of non-uniform gel bed coloration which showed poor precision, the variability in results obtained with HbF Diluent diluted samples would be even higher than that obtained on higher quality plates.

College of American Pathologists (CAP) Hemoglobinopathy Survey Participant Summaries from as early as 1986 (the year when the immunodiffusion Mancini method, i.e., HbF QUIPlate method, was first listed among the test methods for measuring HbF) up to the present showed good agreement between HbF measurement results obtained with immunodiffusion method (HbF QUIPlate) and comparison methods when quantitating low (< 10%) HbF levels. However, in agreement with our findings, differences in HbF values between the RID method and the other methods of detection were evident when measuring samples containing > 10% HbF. The CAP survey results showed the immunodiffusion method consistently yielded the highest mean% HbF, standard deviation, coefficient of variation, and HbF level reported by participants (34). With regard to CAP Survey samples, the discrepancy between HbF values obtained using HbF QUIPlate versus other methodologies was most evident when HbF levels exceeded 20%, such as in the case from patients with hereditary persistence of fetal hemoglobin (HPFH) including homozygous HPFH, HPFH in combination with HbS, HbC, or β^+ -thalassemia (β^+ -thal /HPFH), and several thalassemia conditions (homozygous β^+ -thalassemia and δ β -thalassemia). For a recent example of a CAP Survey Participant Summary illustrating this point see reference 35.

In conclusion, we will continue to monitor elevated (> 10%) levels of HbF by densitometry following alkaline cellulose acetate electrophoresis. In its HbF QUIPlate product insert, Helena Laboratories states that electrophoresis may also be used to quantitate high HbF levels; therefore, the Helena Sick-Cell Hemoglobinopathies Procedure is recommended.

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