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Comparison of Sebia Capillarys Flex capillary electrophoresis with the BioRad Variant II high pressure liquid chromatography in the evaluation of hemoglobinopathies ☆

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ABSTRACT

Background: There are multiple biochemical screening techniques for assessing hemoglobinopathies. Here we compare a new instrument, the Sebia Capillarys Flex (capillary zone electrophoresis (CE)), with the BioRad Variant II (high pressure liquid chromatography (HPLC) in the evaluation of hemoglobinopathies.
Methods: This was a retrospective study using 174 whole blood samples encompassing the 5 most common (Hb A, A2′, S, C, and E) and 10 rare (Hb $G_{Philidelphia}$, D, H, Bart's, O_{Arab}, $S/G_{Philidelphia}$, Hasharoon, Q_{India}, $N_{Baltimore}$, and Malmo) hemoglobin variants. An additional 126 samples were used to establish a CE reference interval for Hb A2.
Results: Hb A measurements agreed well between the 2 methods (bias = −0.06;r = 0.999). The agreement of Hb F was also very good (bias = −0.17; r = 0.994). When samples with the highest Hb F concentration were excluded, agreement was less precise (bias = −0.44; r = 0.811). When no variant was present, the Hb A2 concentrations showed excellent agreement (bias = 0.00; r = 0.994). Positive bias for Hb A2 is seen when Hb C is present using CE. The Hb A2 reference interval using CE was <3.2%.
Conclusion: The Capillarys Flex is capable of identifying and quantifying hemoglobin species, consistent with existing HPLC methods.

1. Introduction

Hemoglobinopathies and thalassemias are globally recognized as two of the most commonly inherited genetic disorders [1,2]. Definitive diagnosis of hemoglobin abnormalities relies heavily on the clinical laboratory and there are multiple indications for screening or testing [3]. Testing is generally pursued when a provisional diagnosis is made based on clinical symptoms or hematologic results, such as microcytic anemia. Screening is mandated for newborns in all 50 states and is advantageous for pre-conception and prenatal identification of an at-risk paternal pair, or for preoperative assessments [3–5]. Identifying hemoglobin abnormalities early can improve outcomes through education, treatment, and care. Additionally, patients' hematological status may be followed throughout life to assess response to treatment such as hydroxyurea or transfusion.

The laboratory diagnosis of hemoglobin abnormalities is based on identifying structural variants, such as Hb S, or abnormal elevations in normal hemoglobins, such as Hb A2 [6]. Cation-exchange high pressure liquid chromatography (HPLC) systems have been automated for fairly rapid and accurate identification of hemoglobin species. In general, HPLC can resolve the most common hemoglobin variants while accurately quantifying Hb A2 and F, making it useful for the detection of both hemoglobinopathies and thalassemias [7–10]. However, the presence of glycated Hb S and Hb E both interfere with Hb A2 quantification, making it difficult for results obtained using HPLC to support the diagnosis of β thalassemia in patients with either S or E alleles [11–13]. Additionally, Hb A2′, a Δ-chain variant seen in 1–2% of the African American population, runs in the S-window when separated using HPLC, further confounding diagnosis of β-thalassemia [14,15]. More recently, capillary zone electrophoresis (CE) instruments have been adapted for high throughput analysis of hemoglobin variants [16–21]. Clinical studies comparing HPLC and CE have found that they are complementary methods, and can be used in tandem for accurate and precise hemoglobin variant quantification [20,22].

In the current study, the performance characteristics of the Capillarys Flex CE method (Sebia, Norcross, GA) were evaluated. The Capillarys Flex CE method was compared to the BioRad Variant HPLC system (BioRad, Hercules, CA) for detection of the 5 most common (Hb A, A2′, S, C, and E) and 9 rare (Hb $G_{Philidelphia}$, D, H, Bart's, O_{Arab}, Hasharoon, Q_{India}, $N_{Baltimore}$, and Malmo) hemoglobin variants. A compound heterozygous Hb $S/G_{Philidelphia}$ was also evaluated. We defined rare variants as those occurring in <0.1% of our submitted samples. In our population, Hb S, C, and E are observed in ~1–2% of submitted samples, while Hb A2′ is detected in ~0.5%. The ability of

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the methods to accurately quantify Hb A2 and Hb F was assessed in specimens heterozygous and homozygous for Hb A, S, C, and E. All variants were confirmed using isoelectric focusing electrophoresis (IEF). Additionally, a reference interval for Hb A2 using the Capillarys Flex was established.

2. Materials and methods

2.1. Study design

This was a retrospective study using data from 174 whole blood samples sent to the Kaiser Permanente Northern California clinical laboratory for physician-ordered hemoglobinopathy screening. The samples included patients being evaluated due to anemia, prenatal/antenatal screening, or for sickle cell disease treatment/monitoring and were analyzed using HPLC, IEF, and CE. 164 of these samples were collected over a period of 3 weeks, and analyzed using HPLC and CE within 24 h of each other. The 10 samples corresponding to the rare hemoglobin variants were collected over a period of 10 years and were stored in small aliquots at −80 °C. The latter samples were only used for a qualitative assessment of migration patterns for rare variants and not a quantitative inter-assay comparison. An additional 126 whole blood samples representing 61 males and 65 female with an age span 10–70 y were used to establish a reference interval for Hb A2 on the Capillarys Flex. These samples were received for routine complete blood count analysis, and had hemoglobin, hematocrit, and MCV concentrations within the normal range when analyzed on the Sysmex XE 2100 (Sysmex America, Mundelein, IL). The mean and standard deviation for the hemoglobin, hematocrit, and MCV were 15.0 ± 0.9 g/dl, 44.0 ± 2.0%, and 90.0 ± 4.4 fl, respectively for males and 11.9 ± 0.3 g/dl, 35.3 ± 0.5%, and 89.7 ± 4.1 fl for females. This study was considered a quality assessment project and was therefore deemed exempt by the Kaiser Permanente Northern California Institutional Review Board.

2.2. HPLC

HPLC analysis was performed using the manufacturer's instructions for the BioRad Variant II β-thalassemia Short Program (BioRad, Hercules, CA), which separates hemoglobin variants by cation-exchange chromatography using a salt gradient [13,23]. The instrument is equipped to resuspend, lyse, separate, and analyze EDTA whole blood for hemoglobin variants. The change in absorbance at 415 nm is monitored for hemoglobin detection. An additional filter at 690 nm corrects the background absorbance. Hb A2 and Hb F single point calibrators are performed daily to adjust and ensure proper retention times and to establish calibration parameters for accurate quantification. Low and high controls are evaluated at the front and end of each run. The Variant II Clinical Data Management software performs reduction of raw data collected from each analysis and generates a sample report with a chromatogram for each sample. Our laboratory determined the normal ranges for adults as follows: Hb A 95–98%, Hb A2 < 3.3%, and Hb F < 2%.

2.3. Capillary zone electrophoresis (CE)

CE was performed following the manufacturer's guidelines for the Sebia Capillarys Flex system using reagents provided in the Capillarys Hemoglobin (E) kit (Sebia, Norcross, GA). The instrument is equipped to resuspend, lyse, separate, and analyze EDTA whole blood for hemoglobin variants. Samples were tracked using a built in bar code reader, and electropherograms were produced automatically. The lysed red cells are electrophoresed in alkaline buffer (pH 9.4) allowing separation to be directed by pH and endosmosis. Detection of eluting hemoglobin species is accomplished using the change in absorbance at 415 nm. CE does not require daily calibration, but normal Hb A and Hb A2 migration controls are analyzed through each capillary daily before additional QC or patient samples are run to ensure proper charge and function of the capillaries. BioRad level 1 (Hb A, F, and A2) and level 2 (Hb A, F, A2, and S) QC materials (Hercules, CA) were also run daily before patient samples were analyzed.

2.4. IEF

IEF was performed using a pH gradient of 6.0–8.0, a 1 mm precast agarose gel, and reagents found in the RESOLVE Hb Neonatal Hemoglobin Screen Kit (Perkin Elmer, Waltham, MA). A 1:10 dilution was performed on each specimen using the elution solution provided in the RESOLVE kit. Three to 5 μl of the resulting mixture was electrophoresed using a Multiphor II (Model 17) Horizontal Electrophoresis Unit (Pharmacia LKB Bio Technologies) under 30 W for 80 min, followed by 5 min at 35 W. Hemoglobin bands were visualized by an o-dianisidine stain using the JB-2 Staining System (Perkin Elmer, Waltham, MA). Staining is a multi-step process that includes fixing the gel in 10% TCA for 10 min, washing the gel under continuously running deionized water for 15 min, allowing the gel to soak in freshly prepared staining solution for 15 min, washing the gel under continuously running deionized water for 30 min, and finally drying the gel for 30–45 min. Hb HAFSC hemoglobin electrophoresis controls are included on all gels; Hb N, Hb E, Hb D, and Hb G controls are used as needed (Analytical Control Systems Inc, Fishers, IN).

2.5. Analytical performance of Capillarys Flex

Total imprecision for migration times and concentrations of Hb fractions were determined by analyzing the Sebia A2 normal and BioRad level 1 and level 2 QC materials at least 8 times/run for 12 consecutive runs and over 2 different buffer lots. The linearities of Hb A2 and Hb F were evaluated by mixing high and low samples to obtain 5 or 8 samples (Hb A2 and Hb F, respectively) with concentrations spanning clinically-relevant ranges, which were then analyzed in triplicate. Hb A2 samples ranged from 1.4% to 6.3%; Hb F samples spanned a range of 0% to 20.2%. To measure lot to lot variation, 40 samples were evaluated for their A2 concentration using two different buffer lots on the same day. Carryover was evaluated for both the cap piercer and the capillaries. For the cap piercer, five high (~20%) Hb F samples were flanked by 2 sets of 5 low (<1.0%) Hb F samples. Each set of 5 samples was run consecutively. For capillary carryover, 8 patients (1 for each capillary) with at least one abnormal hemoglobin variant (Hb C, S, or E) were evaluated followed by 8 normal patients.

2.6. Reference values and statistical analysis

Reference values for Hb A2 were calculated using the nonparametric CLSI C-28A method. Nonparametric statistics were used because the data skewed slightly to the right (Fig. 5). However, when the same data was analyzed parametrically, the normal range was identical. All statistical calculations were performed by EP evaluator (Data Innovations, Burlington, VT).

3. Results

3.1. CE reproducibility

The between run variability was evaluated over 12 consecutive runs and 2 buffer lots using 3 different control materials all of which contained Hb A and Hb A2. Two of the control materials also contained Hb F; one of the controls contained Hb S. The performance characteristics are shown in Table 1.