Standardization in Abnormal Hemoglobin Detection.  
An Evaluation of Hemoglobin Electrophoresis Kits

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Three widely used commercial hemoglobin electrophoresis kits were studied to ascertain specificity, accuracy, precision, and characteristics of product information. Two hundred and ninety-six hemoglobin samples were used to evaluate each kit. Except for poor separation of hemoglobin F by one kit, all three kits gave generally satisfactory results. We give specific recommendations for improving these kits and for laboratory quality-control practices.

Additional Keyphrases: screening • sickle-cell disease • hemoglobin phenotyping

Interest in population-screening programs for abnormal hemoglobins in the United States has increased because of the National Sickle Cell Disease Program developed by the Department of Health, Education, and Welfare. Consequent activities by state, county, and city health departments, universities, hospitals, and community groups necessitate accurate, easy, economical screening tests for hemoglobin S and related abnormal hemoglobins. Many papers discuss the merits of various types of laboratory procedures that are useful for primary hemoglobinopathy-screening programs. Electrophoresis on cellulose acetate remains the initial procedure of choice in the federal screening program and, when combined with a solubility test, allows the more common abnormal hemoglobins to be identified (1).

As with any test, however, results obtained depend on a number of variables, including the reliability of commercial products marketed for their use. The Hemoglobinopathy Proficiency Testing Program of the Center for Disease Control (CDC) has shown the most important single error in abnormal hemoglobin identification to be misinterpretation of hemoglobin electrophoresis patterns.1 An evaluation of the three hemoglobin electrophoresis kits that are most commonly used, according to preliminary abnormal-hemoglobin survey reports from 227 laboratories in the United States, was undertaken at the National Hemoglobinopathy Standardization Laboratory at the CDC, to ascertain specificity, accuracy, precision, and adequacy of product information.

Materials and Methods

The kits studied were: Microzone (Beckman Instruments, Inc., Fullerton, Calif. 92634), Sepratek (Gelman Instrument Co., Ann Arbor, Mich. 48106), and Zip Zone (Helena Laboratories Corp., Beaumont, Tex. 77704). With each of these, a control containing hemoglobins A, F, S, and C, and seven unknown samples can be electrophoresed on a cellulose acetate membrane. Directions given in the manuals that accompany the kits were followed except for hemolysate preparation (2–4). Helena Supre Heme buffer, a Tris–EDTA–borate2 buffer, comes prepackaged and is diluted to 1200 ml with distilled water before use (pH 8.4). Gelman Tris–EDTA–glycine buffer, pH 9.2, is supplied in a vial for the Sepratek system and diluted to 450 ml with distilled water. Beckman uses a Tris–EDTA–borate buffer, pH 8.9–9.3.

Venous blood with EDTA anticoagulant or capillary blood collected in heparinized tubes was centrifuged, the plasma was removed, and the erythrocytes were washed three times in isotonic saline. After the last wash, all supernatant fluid was aspirated from the tubes. A volume of distilled water equivalent to the volume of packed cells and a volume of carbon tetrachloride equivalent to half the volume of packed cells were added to the tubes. All tubes were stoppered and shaken vigorously for 5 min and then centrifuged at 3000 rpm for 15 min. The hemolysate was removed with a pipette, placed in another vial, a few drops of potassium cyanide solution (30 g/liter) was added as a preservative, and these samples were


2 Nonstandard abbreviations used; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetate.

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Cellulose acetate membranes were soaked in the buffers as suggested by the manufacturer. Helena Titan III membranes were soaked for 20 min in Supre Heme buffer; Gelman Sepaphore III strips were soaked for 10 min in Tris–EDTA–glycine buffer, and Beckman cellulose acetate membranes were immersed in Tris buffer and used immediately.

Voltage and time of run varied according to the recommendations of the manufacturer: for Zip Zone strips, 450 V for 20 min; for Sepaphore strips, 350 V for 40 min; and for Microzone strips, 400 V for 1 h. A power supply, chamber, applicator, sample plate, and aligning base were used with each kit.

The Helena, Beckman, and Gelman power supplies have a maximum output of 500 V. Four Zip Zone chambers can be connected to one power supply, and 12 cellulose acetate strips can be run for a total of 96 applications (12 hemoglobins AFSC controls and 84 unknowns). A Beckman power supply can be connected to two Microzone cells containing one strip each, for a total of eight applications (two controls and 14 unknowns). A Gelman power supply can be used with four Sepratek cells containing four strips for a total of 32 applications (four controls and 28 unknowns). For routine screening it is also possible to make double or triple applications on any of the above membranes, thereby greatly increasing the number of samples tested in one run. Temperature increase within the chambers can be minimized by using refrigerated buffer and ice packs.

The Beckman applicator is the only one requiring that one sample be applied at a time to the cellulose acetate strip. The applications were spaced so that they did not run together. With the Gelman and Helena applicators, eight samples can be positioned on the sample plates at one time. Ponceau S was the stain used for all kits.

Results

Table 1 shows the estimated reagent cost per 1000 tests for the three commercial hemoglobin electrophoresis kits tested. For routine use as recommended by the manufacturer, Gelman provided the most economical reagents.

Table 2 summarizes the specificity of hemoglobin electrophoresis separation for the three commercial kits. With both the Gelman and the Helena kits, all hemoglobin samples tested were correctly identified. The Beckman electrophoresis kit does not provide routine separation of hemoglobins A and F. Only 22 of 26 hemoglobin FA samples and 6 of 10 hemoglobin AF samples could be correctly identified with the Beckman Microzone kit.

Table 3 shows the accuracy of hemoglobin electrophoresis separation for the three kits tested as shown in Table 2. Only the Microzone electrophoresis kit gave results that were less than 100% accurate.

To estimate the precision of hemoglobin electrophoresis separation by the three kits, the technologist randomly tested 100 duplicates. As a measure of precision, the percentage of duplicates giving the same result was determined. For the Sepratek and Zip Zone kits this was 100%; for the Microzone kit it was 95% (Table 4).
Although the Gelman electrophoresis method (Method B) resulted in no mis-identifications, there was difficulty in distinguishing S beta-thalassemia (SA) from SS. This difficulty was caused when various samples became streaked or when they ran together because the application points were too close together. However, the Gelman electrophoresis kit did differentiate hemoglobins A and F, while with the Beckman kit hemoglobin F was poorly separated from hemoglobin A. After the technologist became more familiar with the Beckman system, he frequently could identify samples in which hemoglobin F was greatly elevated (above 15%), because of a wide band in the hemoglobin A area.

The Zip Zone electrophoresis kit gave the best overall performance for clear, reproducible separations of hemoglobins A, F, S, and C. Hemoglobins AD and AG were differentiated from hemoglobin AS with all three kits, as indicated by a slightly more anodic migration of hemoglobins D and G than of hemoglobin S. However, this slight difference in mobility would not allow routine differentiation of hemoglobins D and G from hemoglobin S by electrophoresis on cellulose acetate.

Table 5 summarizes labeling, package-insert information, and performance characteristics for these commercial electrophoresis kits. For routine separations of hemoglobins A, F, S, and C, the Gelman and Helena electrophoresis kits are satisfactory. The Beckman electrophoresis kit could be improved by modifying it to allow the reproducible separations of hemoglobins A and F. For none of the three products is an expiration date listed.

Discussion

In this study three commercial hemoglobin electrophoresis kits evaluated by use of 296 hemoglobin samples were interpreted by two experienced investigators. Results of the study show that the current commercial products most commonly used for abnormal hemoglobin separations are satisfactory. However, this study was not designed to test the lot-to-lot variation in cellulose acetate that has frequently been observed in many laboratories. Because cellulose is a medium of natural origin, it has properties that are difficult to control. Manufacturers now produce membranes that are suitable for the separation of hemoglobins. Previously, most membranes were only tested for the reliability with which serum proteins could be separated. The lots used in this study were all satisfactory.

The Microzone system was the least satisfactory of these three kits, primarily because hemoglobin F was poorly separated from hemoglobin A. This manufacturer also claims that this system will separate hemoglobins C and A2. Although hemoglobin C occasionally moved slightly more anodically than hemoglobin A2, mobilities were insufficiently different to justify conclusions about the percentage of A2. The Beckman system is designed only for Beckman-supplied strips, which cannot be used in other systems.

With the Sepratek system, hemoglobins A and F could be separated. However, the applicator would be improved by increasing the distance between application points, which would decrease the likelihood of samples running together. Recent changes in the Gelman electrophoresis buffer system have resulted in improved separation of hemoglobins.

Zip Zone electrophoresis provides the best separation of hemoglobins A, F, S, and C. In the past we have had problems with lot-to-lot quality control of the Zip Zone cellulose acetate membranes. Recently, however, this has not been a problem.

Apparently, the high error rate in hemoglobin electrophoresis pattern interpretation observed in
the CDC Hemoglobinopathy Proficiency Testing Program cannot be attributed to deficiencies in commercial products. More likely the principal reasons are lack of quality-control systems in the laboratory and (or) inadequate education of laboratory technologists regarding abnormal hemoglobins and their prevalence in the United States.

A summary of recommendations for hemoglobin electrophoresis quality control follows:

1. Always use a hemoglobin control that contains hemoglobins A, F, S, and C, or other combinations of these fractions on each cellulose acetate strip. These can be prepared by the laboratory or obtained from commercial sources (Gelman, Helena, Hyland, Schering).

2. Check the pH of the buffer when it is first prepared and when separations are poor.

3. Have the power supplies checked periodically.

4. Refrigerate the buffer, to retard mold contamination.

5. Clean applicators thoroughly after use, and rinse with distilled water. Dry before re-using.

6. When lots of cellulose acetate are discovered that give poor results, have them replaced by the manufacturer.

7. Make certain that hemolysates are clear and free of fibrin clots and particles, which may interfere with migration patterns.

8. Adjust hemoglobin concentrations of hemolysates to provide sharp patterns (3 to 6 g/dl appears to be satisfactory).

Laboratories providing hemoglobinopathy testing services are urged to participate in the CDC Hemoglobinopathy Proficiency Testing Program and to report any problems with commercial reagents used in hemoglobinopathy testing to the National Hemoglobinopathy Standardization Laboratory at the Center for Disease Control or to the Food and Drug Administration in Vitro Diagnostics Program.

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Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U. S. Department of HEW.

References


