I. Intended Use
Pacific Hemostasis SickleScreen Sickle Hemoglobin Screening Kit and SickleScreen Control Set are intended for use in screening for sickle cell disease and sickle cell trait. SickleScreen Controls can be used with procedures based on differential solubility of reduced hemoglobin, or with enzyme immunosassays specific for Hemoglobin S.

II. Summary and Principles
Sickle cell disease is a chronic hemolytic anemia seen in individuals homozygous for the Hemoglobin S gene (SS). In these individuals, Hemoglobin S constitutes 70-99% of the total hemoglobin. When Hemoglobin S is reduced to deoxyhemoglobin, it forms filamentous tactoids that cause red blood cells of these individuals to “sickle”. Repeated vascular occlusion in sickle cell anemia can lead to accumulated damage in a variety of organs, including kidney, heart, lung, and eyes.

Heterozygous (AS) individuals are carriers of the sickle cell trait and have up to 50% Hemoglobin S. While they are usually asymptomatic, these patients should be identified for genetic counseling purposes.

Under conditions of reduced oxygen pressure, such as anemia, flight in poorly pressurized airplanes, and severe pneumonia, sickle cell syndrome may occur.

The SickleScreen Kit is a modified Nalbandian procedure based upon differential solubility. Red blood cells are lysed by a surfactant. The released hemoglobin is reduced by sodium hydrosulfite. Reduced Hemoglobin S is insoluble and forms a turbid suspension in concentrated phosphate solutions. Normal Hemoglobin A and most other hemoglobins remain in solution under these conditions. Both sickle cell disease and sickle cell trait can be detected with this procedure.

III. Reagents

For in vitro diagnostic use.

A. Reaction Vials (30 determination kit): Prefilled with sodium hydroxysulfite powder. Store at room temperature (15-30°C). Do not expose to light for excessive periods. Best stored as supplied in kit. Use uncapped vials within 12 hours.

NOTE: Keep trays protected from light.

B. Patients with multiple myeloma, cryoglobulinemia, and other dysproteinemias.

C. Elevated levels of Hemoglobin F can cause false negative results. If Hemoglobin S or any other sickling hemoglobin is present the solution may be cloudy. The lines on the Tube Reading Rack will be easily seen through the tube contents.

D. Reactant fluctuations. Some samples analyzed with hemoglobin electrophoresis. All A/S samples were correctly reported as positive. Multiple kit lots were used.

E. Positive Control: Lyophilized hemoglobin A/S. Store at 2-8°C. Reconstitute with 0.5 mL Reconstitution Fluid. Let stand undisturbed for 30 minutes then vortex. Reconstituted control is stable for 21 days at 2-8°C.

F. Negative Control: Lyophilized hemoglobin A/A. Store at 2-8°C. Reconstitute with 0.5 mL Reconstitution Fluid. Let stand undisturbed for 30 minutes then vortex. Reconstituted control is stable for 21 days at 2-8°C.

IV. Procedure

Materials Provided:


B. Sodium Hydrosulfite Powder Vials: (120 determination kit): Store at room temperature (15-30°C). Do not expose to light for excessive periods. Best stored as supplied in kit.

C. Phosphate Buffer: A concentrated solution containing surfactant, with 0.02% sodium azide as a preservative. Store at room temperature (15-30°C).

D. Reconstitution Fluid: Deionized water with sodium azide as a preservative. Store at 2-8°C.

WARNING: Phosphate Buffer and Reconstitution Fluid contain sodium azide. Sodium azide under acid conditions yields hydrazoic acid, an extremely toxic compound. Dilute with running water before discarding, and then flush with large volumes of water. These precautions are recommended to avoid deposits in metal piping in which explosive conditions may develop.

E. Positive Control: Lyophilized hemoglobin A/S. Store at 2-8°C. Reconstitute with 0.5 mL Reconstitution Fluid. Let stand undisturbed for 30 minutes then vortex. Reconstituted control is stable for 21 days at 2-8°C.

F. Negative Control: Lyophilized hemoglobin A/A. Store at 2-8°C. Reconstitute with 0.5 mL Reconstitution Fluid. Let stand undisturbed for 30 minutes then vortex. Reconstituted control is stable for 21 days at 2-8°C.

Caution: Each unit of source material used in the preparation of Positive and Negative Controls has been tested by an FDA licensed method and found non-reactive for HBsAg and negative for antigens to HIV and HCV. However, no known test method can offer complete assurance that products derived from human blood will not transmit hepatitis, AIDS, or other infectious diseases. This product, like all materials of human origin, should be handled as potentially infectious biological material.

Lack of vacuum (lyophilized controls), unexpected results, or reagent color variations could indicate product deterioration.

V. Sample Collection
Collect whole blood in EDTA, Heparin, Sodium citrate, or ACD anti-coagulant. Samples can be stored at 2-8°C for up to 2 weeks before testing.

VI. Results

Materials Required, But Not Provided:
Tube reading rack
Clear 12 x 75 test tubes and plug stoppers (120 det. kit)
50 μL pipet

Controls are not provided with the SickleScreen Kit. They must be ordered separately.

A. Bring all reagents and samples to room temperature.

B. Run a known positive and negative control with each group of samples.

C. Run a positive control with each newly opened tray of tubes.

D. Label one test tube for each patient and control. Use prefilled Reaction Vials for 30 det. kit and 12 x 75 test tubes for 120 det. kit.

Place in Tube Reading Rack.

E. In the 10 det. kit, add 4 mL Phosphate Buffer to the prefilled reaction vial and mix well. In the 120 det. add 4 mL Sodium Hydrosulfite to a test vial.

F. Add 50 μL of whole blood or control. Cap and shake vigorously immediately after adding the whole blood or control to each tube.

G. Incubate in Tube Reading Rack at room temperature for 10-20 minutes.

H. Do not report patient results if the positive control appears negative.

VI. Results

Negative
Weakly Positive
Positive

If no sickling hemoglobin is present the solution will be clear to slightly cloudy. The lines on the Tube Reading Rack will be easily seen through the tube contents.

Weakly Positive:
The differentiation between weakly positive samples and negative samples may depend upon subjective evaluation. Some will describe a sample giving a slightly cloudy solution with the lines on the Tube Reading Rack visible as very faint lines as positive whereas others describe it as negative. All positive or questionable results should be further evaluated with hemoglobin electrophoresis.

Positive:
If Hemoglobin S or any other sickling hemoglobin is present the solution will be turbid. The lines on the Tube Reading Rack will not be clearly visible when viewed through tube contents. All positive or questionable results should be further evaluated with hemoglobin electrophoresis.

VI. Limitations

A. Severe anemia can cause false negatives. If the total hemoglobin is < 4 g/dL, double the sample volume to 100 μL.

B. Patients with multiple myeloma, cryoglobulinemia, and other dysproteinemias may give false positive results. Wash patient red blood cells in physiologic saline to minimize these problems.

C. Elevated levels of Hemoglobin F can cause false negative results. Do not use test for infants under 6 months of age.

D. Recent transfusion can cause false positive or false negative results.

E. Some rare hemoglobin variants such as Hemoglobin C Hain or C Georgeton may give a positive reaction.