III. Reagents

I. Intended Use

Pacific Hemostasis SickleScreen Sickle Cell 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 immunoassays 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 1, it forms filamentous tetramers that cause red blood cells to become “sickled.” Repeated cessation occlusion in sickle cell anemia can lead to accumulated damage in a variety of organs including kidney, heart, lung, and spleen.

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, febrile illness, and severe intravascular or sickle cell syndromes, they may develop acute exacerbations.

The SickleScreen Kit is a modified Haldex® 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 soluble with this procedure. Both sickle cell disease and sickle cell trait can be detected with this procedure.

III. Reagents

A. Reaction Vials [3 determination kit]: supplied in kit. Use uncapped vials within 12 hours.

B. Sodium Hydrosulfite Powder Vial (130 determination kit): Store at room temperature (15-30°C). Do not expose to light for excessive periods. Best stored as supplied in kit. Expiration date is 12 months from date of manufacture.

C. Phosphate Buffer: 4 x 125 mL vials with dispensing caps. Phosphate Buffer: 1 x 125 mL vial with dispensing cap

D. Deionized water with sodium azide as a preservative. Store at 2°C to 8°C.

E. Phosphate Buffer and Reconstitution Fluid: Let stand undisturbed for 30 minutes then vortex to mix.

F. Reconstitution Fluid: Let stand undisturbed for 30 minutes then vortex to mix.

IV. Procedure

A. Reaction Vials [3 determination kit]: Place the entire contents of one Sodium Hydrosulfite vial into one bottle of Phosphate Buffer. Powder must be dry and free flowing. Mix well and let stand 15 minutes to dissolve.

B. Reaction Fluid: Place 1.0 mL concentrate into each reaction vial. Add 0.5 mL Reconstitution Fluid and mix well.

C. Deionized water with sodium azide as a preservative. Store at 2°C to 8°C.

D. Phosphate Buffer: 1 x 125 mL vial with dispensing cap

VII. Performances Characteristics

A. Sickle Screening

1. FISH GR Diagnostics (FD) was asked to independently screen the sample of 120 identical samples done with the SickleScreen Kit for sickle cell disease.

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

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

D. Read and interpret results as follows:

   Negative
   No error: if 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
   May cause slight eye irritation
   Interactions with other chemicals
   No information available.

   Positive
   Sickle Screen Controls can be used with procedures based on differential solubility of reduced hemoglobin, or with enzyme immunoassays specific for hemoglobin S.

VI. Results

A. Binging any samples and samples to room temperature.

B. Reaction Fluid: Place the entire contents of one Sodium Hydrosulfite vial into one bottle of Phosphate Buffer and mix well.

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

D. Read and interpret results as follows:

   Negative
   May cause slight eye irritation
   Interactions with other chemicals
   No information available.

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

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

G. Deionized water with sodium azide as a preservative. Store at 2°C to 8°C.

H. Reaction Fluid: Place 1.0 mL concentrate into each reaction vial. Add 0.5 mL Reconstitution Fluid and mix well.

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

J. Read and interpret results as follows:

   Negative
   No error: if 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
   May cause slight eye irritation
   Interactions with other chemicals
   No information available.

   Positive
   Sickle Screen Controls can be used with procedures based on differential solubility of reduced hemoglobin, or with enzyme immunoassays specific for hemoglobin S.

VI. Results

A. Binging any samples and samples to room temperature.

B. Reaction Fluid: Place the entire contents of one Sodium Hydrosulfite vial into one bottle of Phosphate Buffer and mix well.

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

D. Read and interpret results as follows:

   Negative
   May cause slight eye irritation
   Interactions with other chemicals
   No information available.

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

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

G. Deionized water with sodium azide as a preservative. Store at 2°C to 8°C.

H. Reaction Fluid: Place 1.0 mL concentrate into each reaction vial. Add 0.5 mL Reconstitution Fluid and mix well.

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

J. Read and interpret results as follows:

   Negative
   No error: if 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
   May cause slight eye irritation
   Interactions with other chemicals
   No information available.

   Positive
   Sickle Screen Controls can be used with procedures based on differential solubility of reduced hemoglobin, or with enzyme immunoassays specific for hemoglobin S.

VI. Results

A. Binging any samples and samples to room temperature.

B. Reaction Fluid: Place the entire contents of one Sodium Hydrosulfite vial into one bottle of Phosphate Buffer and mix well.

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

D. Read and interpret results as follows:

   Negative
   May cause slight eye irritation
   Interactions with other chemicals
   No information available.

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

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

G. Deionized water with sodium azide as a preservative. Store at 2°C to 8°C.

H. Reaction Fluid: Place 1.0 mL concentrate into each reaction vial. Add 0.5 mL Reconstitution Fluid and mix well.

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

J. Read and interpret results as follows:

   Negative
   No error: if 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
   May cause slight eye irritation
   Interactions with other chemicals
   No information available.

   Positive
   Sickle Screen Controls can be used with procedures based on differential solubility of reduced hemoglobin, or with enzyme immunoassays specific for hemoglobin S.

VI. Results

A. Binging any samples and samples to room temperature.

B. Reaction Fluid: Place the entire contents of one Sodium Hydrosulfite vial into one bottle of Phosphate Buffer and mix well.

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

D. Read and interpret results as follows:

   Negative
   May cause slight eye irritation
   Interactions with other chemicals
   No information available.

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

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

G. Deionized water with sodium azide as a preservative. Store at 2°C to 8°C.

H. Reaction Fluid: Place 1.0 mL concentrate into each reaction vial. Add 0.5 mL Reconstitution Fluid and mix well.

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

J. Read and interpret results as follows:

   Negative
   No error: if 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
   May cause slight eye irritation
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   No information available.