Intended Use
For the qualitative, visual, colorimetric determination of G6PD deficiency in red blood cells. For in vitro diagnostic use only.

Clinical Significance
G6PD assays are most commonly performed to determine deficiency of G6PD, which is widely prevalent throughout the world. It has been determined that G6PD deficiency in red cells is the basis for certain drug-induced hemolytic anemias. This type of susceptibility to drug-induced hemolysis is often called “primquine sensitivity” because studies which led to its characterization were made during investigations of the hemolytic properties of this antimalarial compound.

Method History and Principle
A visual colorimetric procedure for measuring G6PD was developed in 1962 using brilliant cresyl blue as the indicator. A similar procedure was developed in which dichlorophenol indophenol was used as the indicator. The method presented here is based on the use of dichlorophenol indophenol...

Glucose-6-phosphate dehydrogenase (G6PD, D-glucose-6-phosphate: oxidoreductase, EC 1.1.1.49) catalyzes the first step in the pentose phosphate shunt, oxidizing glucose-6-phosphate (G-6-P) to 6-phosphogluconate (6-PG) and reducing NADP to NADPH. In the presence of phenazine methosulfate (PMS), NADPH reduces dichlorophenol indophenol from its blue form to its colorless form. The rate at which the blue color disappears is dependant on the G6PD present in the red cells.

\[
\text{G-6-P + NADP}^{+} \xrightarrow{\text{G6PD}} 6-\text{PG} + \text{NADPH} + H^{+} \\
\text{PMS} \xrightarrow{} \text{Colorless Dye + NADP}^{+}
\]

Reagents
G6PD Screen Reagent: Reconstituted screen reagent contains NADP, 0.5mM, Glucose-6-Phosphate, 4.55mM, Dichlorophenol indophenol, 0.55mM, Phenazine Methosulfate 0.2g/L.

G6PD Screen Buffer: Buffer to give pH of 8.5±0.1 when reconstituted with G6PD Screen Reagent, Sodium Azide (0.095%) added as preservative.

Precautions
1. These reagents are for in vitro diagnostic use only.
2. Normal precautions exercised in handling laboratory reagents should be followed. Wear suitable protective clothing. Dispose of waste observing all local, state and federal laws.
3. G6PD Screen Reagent is TOXIC. May cause genetic damage and/or irritation to eyes, respiratory system and skin.
4. G6PD Screen Buffer contains sodium azide that may react with lead and copper plumbing to form highly explosive metal azides. Avoid azide accumulation by flushing with copious amounts of water upon disposal.

Reagent Preparation
1. G6PD Screen Reagent is prepared by reconstituting with the volume of G6PD Screen Buffer indicated on vial label. Swirl gently and invert several times to dissolve contents. Wait 2-3 minutes and mix again.

Storage and Stability
1. When unopened screen reagent vials and the buffer reagent are stored at 2-8°C, they are stable until the expiration date on the labels.
2. Reconstituted screen reagent is stable for 8 hours refrigerated (2-8°C). It is suggested that a single batch run be performed within 8 hours of reconstitution.

Optional Reagents
G6PD controls: Lyophilized controls with G6PD in a stabilized human red cell hemolysate base. Pointe Scientific catalog number G7583-CTL.

Mineral Oil: Pointe Scientific catalog number G7584-MO.

Specimen Collection and Storage
1. It is recommended that specimen collection be carried out in accordance with NCCLS document M29-T2.
2. Whole blood collected in EDTA, heparin or acid-citrate-dextrose (ACD) is satisfactory.
3. Red cell G6PD is stable in whole blood for one week refrigerated (2-8°C), but is unstable in red cell hemolysates.
4. Freezing of blood is not recommended.

Interfering Substances
1. Copper completely inhibits G6PD at a concentration of 100 umol/L, and sulfate ions (0.005 mol/L) decrease observed levels of G6PD activity.
2. Certain drugs and other substances are known to influence circulating levels of G6PD.
3. Reticulocytes have higher G6PD levels than mature red cells. It is recommended that assays not be performed after a severe hemolytic crisis, since G6PD levels may appear falsely elevated. Under those conditions, detection of deficiency may require family studies. Testing may be performed after the level of mature red cells has returned to normal.
4. Under normal circumstances, activity contributed by leukocytes, platelets and serum is relatively small. However, in cases of extreme anemia, grossly elevated white counts or, very low levels of red cell G6PD activity, the contribution to the total made under these conditions may be significant.

Materials Provided
See “reagents” section

Materials Required But Not Provided
1. Deionized Water
2. Pipeting devices for delivery of volumes required for the assay
3. Timer
4. 37°C. Heating Block or Water Bath
5. Mineral Oil

Manual Procedure
1. Prepare red blood cell hemolysate by adding 0.05ml (50ul) whole blood to 2.5ml deionized water. Mix gently and allow to stand for 5 minutes.
2. Label the appropriate number of 13 x 100mm test tubes for the controls and samples that will be performed. (Pointe Scientific recommends 13 x 100mm test tubes, other sizes may also be acceptable.)
3. Pipet 0.5ml (500ul) of reconstituted G6PD Screen Reagent into each tube.
4. Add 1.0ml of the hemolysate to each tube and gently shake tube to mix.
5. Gently layer approximately 1-2ml of mineral oil on top of the reaction mixture. Do not mix the mineral oil with the reaction mixture!
6. Place the tubes into a 37°C heating block or water bath.
7. Observe the tubes at 15 minute intervals for up to 1 hour looking for a color change from the original deep blue/purple to a red/orange color. Normal blood (normal levels of G6PD) will typically reach the red/orange color within 15-60 minutes. The color change may be more easily detected if the tubes are viewed in front of a bright light or white paper.

Limitations
This assay is designed to detect samples with a significantly deficient level of G6PD from those with an essentially normal level of G6PD. It is strongly recommended that any sample requiring longer than 60 minutes before a color change occurs, be assayed using a quantitative G6PD method (e.g. Pointe Scientific product G7583) to verify the finding of deficiency.

Quality Control
Reliability of test results should be monitored by use of control materials with known levels of G6PD within each run. Pointe Scientific glucose-6-phosphatase dehydrogenase controls are suitable for this purpose. (Catalog number G7583-CTL) It is recommended that each laboratory establish its own frequency of control determination.

Expected Values
Samples were collected from 152 apparently healthy adults and assayed according to this method. Every sample reached the red/orange color change within 60 minutes.

Performance Characteristics
Precision: A known normal sample and a known deficient sample were run in duplicate on ten successive days. The known normal sample was found to be normal in 100% of the assays. The known deficient sample was found to be deficient in 100% of the assays.

Correlation: A comparison study on 164 samples between the Pointe Scientific method and that of Sigma Diagnostics (Procedure No. 400) yielded 100% agreement in the results from the two methods. The samples included 157 normal specimens and 7 deficient specimens.

This visual colorimetric procedure has also been verified in comparisons with the methemoglobin reduction method and the ascorbate-cyanide method.

References