

Hemoglobin Assays: Advantages and Disadvantages

High-performance liquid chromatography (HPLC)

- Readily separates some proteins that cannot be resolved by other means
- Allows for accurate quantification of normal and variant hemoglobins at low concentrations, enabling differentiation of Hb S/ β^+ -thalassemia from sickle cell trait (Hb A/S), as well as a quantitative description of compound heterozygous disorders such as Hb S/HPFH (hereditary persistence of fetal hemoglobin; see Beta-Thalassemia, [Genotype-Phenotype Correlations](#)) and Hb S/C
- Does not definitively distinguish Hb S/ β^0 -thalassemia from Hb S/S (identification requires DNA testing or integration with other laboratory studies)

Isoelectric focusing

- Capable of higher resolution than other hemoglobin electrophoresis
- Routine isoelectric focusing provides an efficient platform for high-throughput screening and thus is often used for newborn screening but is less quantitative than HPLC.
- Capillary isoelectric focusing technology allows for separation of very small samples, quantification, and automation of sampling.

Cellulose acetate electrophoresis and citrate agar electrophoresis

- Useful for quick screening of a small number of samples
- Protein bands are relatively wide and many abnormal hemoglobins overlap.
- Quantitative densitometry of abnormal hemoglobins is inaccurate at low concentrations (e.g., HbA₂ and HbF in adults).

Kleihauer-Betke test

- Acid-elution test that detects the presence of cells with high HbF content
- Can be used to characterize coexistent HPFH with SCD

Solubility test (e.g., Sickledex, Sickleprep, Sicklequik)

- Utilizes the relative insolubility of deoxygenated HbS in solutions of high molarity. HbS in hemolysates precipitate in the test solution while other hemoglobins remain in solution.
- Solubility tests alone should NEVER be used for genetic counseling assessments if one partner is known to have HbS, as this test will not detect β^0 -thalassemia, HbC, or other hemoglobin variants that can lead to compound heterozygous forms of SCD.

The solubility test has no place in the definitive diagnosis of SCD because:

- It does not differentiate SCD from sickle cell trait (Hb A/S);
- False positives have been reported [Hara 1973];
- High levels of HbF may cause false negative results in infants with SCD;

- It may miss some clinically significant forms of sickle hemoglobinopathies (e.g., Hb S/C) [Fabry et al 2003].
- The main uses for the solubility tests are:
 - A low-cost and rapid screen for the presence of HbS prior to investing in definitive testing;
 - Emergent estimation of whether a clinically significant hemoglobinopathy exists (if combined with a CBC, blood smear, and reticulocyte count).

References

Fabry ME, Archyra SA, Suzuka SM, Nagel RL. Solubility measurement of the sickle polymer. In: Nagel RL, ed. *Hemoglobin Disorders: Molecular Methods and Protocols*. Totowa, NJ: Humana Press; 2003:271-87.

Hara S. Reliability and modification of Sickledex test. *J Natl Med Assoc.* 1973;1973;65:431.