Lab Dept:	**Hematology**
Test Name:	**HEMOGLOBIN ELECTROPHORESIS CASCADE REFLEX**

General Information

Lab Order Codes:	MELP
Synonyms:	Hemoglobin Electrophoresis Blood; Isoelectric Focusing
CPT Codes:	83020 – Hemoglobin fractionation and quantitation; electrophoresis 83021 – Hemoglobin fractionation and quantitation; chromatography 82664 – Electrophoresis, agar (if appropriate) 83068 - Unstable hemoglogin (if appropriate) 83789 – Hemoglobin variant by mass spectrophotometry (if appropriate) 88184 - Hemoglobin F, RBC distribution (if appropriate) 83020-26 – Interpretation (if appropriate)
Testing includes:	This evaluation will always include hemoglobin (Hb) A2 and HbF and hemoglobin electrophoresis utilizing capillary electrophoresis and cation exchange high-performance liquid chromatography methods. Reflex testing, performed at additional charge, may include any or all of the following to identify rare hemoglobin variants present: sickle solubility (hemoglobin S screen); hemoglobin heat and isopropanol stability studies (unstable hemoglobin); isoelectric focusing, intact globin chain mass spectrometry (hemoglobin variant by mass spectrometry); HbF distribution by flow cytometry; DNA Sanger sequencing assays for: 1) beta-chain variants and the most common beta thalassemias (beta-globin gene sequencing), 2) alpha-chain variants and less common nondeletional alpha thalassemias (alpha-globin gene sequencing), or 3) gamma-chain variants and nondeletional hereditary persistence of fetal hemoglobin (HPFH) (gamma-globin full gene sequencing); multiplex ligation-dependent probe amplification assays for: 1) large deletional alpha thalassemias and alpha-gene duplications (alpha-globin gene analysis), or 2) beta-globin gene cluster locus large deletions and duplications, including large deletional HPFH, delta-beta thalassemia, gamma-delta-beta thalassemia, epsilon-gamma-delta-beta thalassemia and large deletional beta or delta thalassemia (beta-globin cluster locus deletion/duplication). If test results in the profile are abnormal, results may be reviewed by a hematopathology consultant, and a summary interpretation provided.

Logistics

Test Indications:	Useful for the diagnosis and classification of hemoglobin disorders, including thalassemias and hemoglobin variants.

Lab Testing Sections:	Hematology - Sendouts
Referred to:	Mayo Clinic Laboratories (MML Test: HBEL1)
Phone Numbers:	MIN Lab: 612-813-6280 STP Lab: 651-220-6550
Test Availability:	Daily, 24 hours
Turnaround Time:	2 – 25 days
Special Instructions:	Metabolic Hematology Patient Information **(T810) is strongly recommended**. Testing may proceed without this information, however if the information requested is received, any pertinent reported clinical features and data will drive the focus of the evaluation and be considered in the interpretation.

Specimen

Specimen Type:	Whole blood
Container:	Lavender (EDTA) top tube Alternate: ACD (Yellow solution B), Green (NaHep/Sodium Heparin
Draw Volume:	10 mL (Minimum: 1 mL) blood The minimum volume will limit reflex testing possibilities; 3 mL is minimum if multiplex ligation-dependent probe amplification is needed
Processed Volume:	Same as Draw Volume.
Collection:	Routine blood collection
Special Processing:	Lab Staff: **Do Not** centrifuge. Specimen should remain in original collection container. **Do not** freeze. Send refrigerated. Specimen stable refrigerated for seven days.
Patient Preparation:	None
Sample Rejection:	Testing cannot be performed on clotted samples; frozen specimens; mislabeled or unlabeled specimens

Interpretive

Reference Range:

LEVEL 1	**Hgb A**	0 - 30 days:	5.9 – 77.2%

		1 – 2 months:	7.9 – 92.4%
		3 – 5 months:	54.7 – 97.1%
		6 – 8 months:	80.0 – 98.0%
		9 – 12 months:	86.2 – 98.0%
		13 – 17 months:	88.8 – 98.0%
		18 – 23 months:	90.4 – 98.0%
		> or = 24 months:	95.8 – 98.0%
	Hgb A_2	0 - 30 days:	0.0 – 2.1%
		1 – 2 months:	0.0 – 2.6%
		3 – 5 months:	1.3 – 3.1%
		> or = 6 months:	2.0 – 3.3%
	Hgb F	0 – 30 days:	22.8 – 92.0%
		1 – 2 months:	7.6 – 89.8%
		3 – 5 months:	1.6 – 42.2%
		6 – 8 months:	0.0 – 16.7%
		9 – 12 months:	0.0 – 10.5%
		13 – 17 months:	0.0 – 7.9%
		18 – 23 months:	0.0 – 6.3%
		>or = 24 months:	0.0 – 0.9%
	Variants	No abnormal variants	
Reflexes:	**Hemoglobin F, Red Cell Distribution**	Reported as heterogeneous or homogenous	
	IEF Confirmation	Noted when performed	

	Hemoglobin, Unstable	Normal (reported as normal [stable] or abnormal [unstable])
	Hemoglobin Variant by Mass Spec	Noted when performed
	Hemoglobin S, Scrn	Negative
	Alpha Globin Gene Analysis	Interpretive report
	Beta Globin Gene Sequencing	Interpretive report
	Beta Globin Cluster Locus Del/Dup	Interpretive report
	Gamma Globulin Full Gene Sequencing	Interpretive report

Critical Values: N/A

Limitations: Some hemoglobin disorders and variants are not detected by the screening methods including common alpha thalassemia conditions and require further reflex testing to identify. If a family history of a known hemoglobin disorder, prior therapy for a hemoglobin disorder, or otherwise unexplained lifelong/familial symptoms such as hemolysis, microcytosis, erythrocytosis/polycythemia, cyanosis, or hypoxia are present, this should be clearly communicated via the requested form T810.

Recent transfusion may mask protein results including hemoglobin electrophoresis, hereditary persistence of hemoglobin F (HPFH) by flow cytometry, stability studies, and sickle solubility studies depending on percentage of transfused cells present.

Some hemoglobin variants can originate from the donor blood product and not the tested recipient. These are typically found in low percentage.

If the patient has undergone a bone marrow transplant, the results may show atypical results and should be interpreted in the context of clinical information.

Some therapies cause artefactual effects in protein studies, including hydroxyurea and decitabine (increased Hgb F levels), Voxelotor (artefactual peaks) and gene therapy (alternate protein detection, Beta T87Q, by mass spectrometry). Clear communication of prior therapy is strongly recommended.

Methodology:

Hemoglobin A2 and F: Cation Exchange/HPLC
Hemoglobin Electrophoresis: Capillary Electrophoresis
Hemoglogin S: Hemoglobin S Solubility
Unstable Hemoglobin: Isopropanol Stability
Hgb F, Red Cell Distribution: Flow Cytometry
Hgb Variant by Mass Spec: Mass Spectrophotometry (MS)
Polymerase Chain Reaction (PCR) Analysis/Multiplex Ligation-Dependent Probe Amplification (MLPA), Polymerase Chain Reaction (PCR)/DNA Sequencing
IEF Confirms: Isoelectric Focusing

References:

Mayo Clinic Laboratories March 2025

Updates:

1/11/2007: CPT 2007 updates
4/21/2009: Addition of Level 3 testing, additional charging/CPT's
1/25/2011: Level 2 testing now direct reflex per test. Updated reference ranges for pediatrics.
2/12/2013: CPT update
3/5/2018: Updated reflex testing
3/22/2021: Updated test per Mayo.
3/18/2025: Updated CPT codes, reference ranges to include day of birth. Added specimen stability.