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) 85660 – Sickling of red blood cells, reduction (if appropriate) 81259 – Alpha globin gene sequencing (if appropriate) 81364 – Beta globin gene sequencing (if appropriate) 81363 – Beta globin cluster locus deletion/duplication (if appropriate) 81479 – Gamma globin full gene sequencing (if appropriate)
Testing includes:	**Level 1 Testing** - Includes: Hemoglobin A2 and F and Hemoglobin electrophoresis. **Reflex Testing** – Hemoglobin electrophoresis reflex testing performed at an additional charge, may include any or all of the following as indicated to identify rare hemoglobin variant(s) present: Sickle Solubility, Unstable Hgb, IEF confirms, Hgb variant by mass spec, Hgb F red cell distribution, alpha globin analysis, beta-globin gene sequencing, beta globin cluster locus deletion/duplication, alpha-globin gene sequencing, and gamma globulin full gene sequencing.

Logistics

Test Indications:	Diagnose thalassemias and hemoglobin variants. Evaluation of unexplained microcytosis.
Lab Testing Sections:	Hematology - Sendouts
Referred to:	Mayo Medical Laboratories (MML Test: HBEL1)
Phone Numbers:	MIN Lab: 612-813-6280 STP Lab: 651-220-6550
Test Availability:	Daily, 24 hours

Turnaround Time:	1 – 25 days, performed Monday – Saturday
Special Instructions:	A Metabolic Hematology Information Sheet (available from lab-Mayo Supply T810) may be completed and forwarded with the specimen. Include recent transfusion history and most recent CBC. This will drive the focus of the evaluation and will be considered in the interpretation.

Specimen

Specimen Type:	Whole blood
Container:	Lavender (EDTA) top tube Alternate: ACD (Yellow solution B), Green (NaHep)
Draw Volume:	10 mL (Minimum: 1 mL) blood
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.
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**	1 - 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	1 - 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	1 – 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 2021
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.