UCSF Benioff Children's Hospital Oakland

Requisition form for Hemoglobinopathy Testing
Hemoglobinopathy Reference Laboratory
UCSF Benioff Children's Hospital & Research Center Oakland

747 52nd Street, Oakland, CA 94609
Phone: (510) 450-7688
Fax: (510) 601-3928
Email: shabnam.tavassoli@ucsf.edu

* **Patient name:** _____ ***DOB:** _____ **Ethnicity:** _____

* **Date of Sample Collection:**_____**Type of Sample:** ◯ **Whole Blood** ◯ **DNA**** (page 3)

Recent Transfusion: ◯ **Yes** ◯ **No** **If yes, date of transfusion:** _____

* **Ordering Physician/ Sender's information:**_____

* **Billing information, email address:** _____

Address: _____

City/State/Zip: _____ Phone: _____ Fax: _____

Please provide the following laboratory information (if available):

Hb: _____g/dL	HbA:_____%	Serum Iron: _____ ug/dL
RBC:_____x10^9 /ul	HbA2:_____%	Ferritin: _____ ug/dL
MCV:_____fL	HbF: _____%	
MCH:_____Pg	Others:_____%	

Check tests requested:

☐ Hb variant identification by Isoelectric Focusing (IEF) and/or Capillary Electrophoresis (CE)

☐ Alpha thalassemia, common deletions (Alpha Multiplex GAP-PCR)

☐ Alpha globin gene sequencing (Alpha Multiplex GAP-PCR will be performed, unless results provided)

☐ Beta Globin gene sequencing

☐ Gamma Globin gene sequencing

☐ Alpha Globin gene Triplication

☐ HPFH (Hereditary Persistence of Fetal Hemoglobin), most common deletions

☐ MLPA (Multiplex Ligation-dependent Probe Amplification) for alpha-globin gene cluster

☐ MLPA (Multiplex Ligation-dependent Probe Amplification) for beta-globin gene cluster

☐ Xmn1 (screening for the polymorphism)

☐ Other specialized tests (please specify, page 2): _____

* required

Thalassemia and Hemoglobinopathy Evaluation:

- **Alpha thalassemia, common deletions (Alpha Multiplex GAP-PCR):** If genetic testing for alpha thalassemia is indicated based on RBC indices, Alpha thalassemia, common deletions, would be the PREFERRED initial genetic test. This assay detects 7 of the most common alpha thalassemia deletions ($--^{SEA}/$, $--^{FIL}/$, $--^{THAI}/$, $--^{MED}/$, $--^{20.5}/$, $-\alpha^{3.7}/$, $-\alpha^{4.2}/$), and Hb Constant Spring (α^{CS}) point mutation.

- **Alpha globin gene sequencing**: This assay detects alpha thalassemia mutations and alpha chain variants by Sanger sequencing. If alpha thalassemia is suspected and "Alpha Thalassemia, Common Deletions" is negative, alpha-globin gene sequencing should be the next test. Alpha Multiplex GAP-PCR for genotyping will be performed unless results provided.

- **Alpha MLPA (Multiplex Ligation-dependent Probe Amplification):** Detecting deletions and duplications (copy number variation) within the α-globin gene cluster.

- **Beta globin gene sequencing:** This assay detects beta thalassemia mutations and beta chain variants (e.g., Hb S, Hb C, Hb D, Hb E, Hb O-Arab, etc.) by Sanger sequencing.

- **Beta MLPA (Multiplex Ligation-dependent Probe Amplification)**: Detecting deletions (delta-beta deletions) and duplications within the β-globin gene cluster.

- **Alpha Globin gene Triplication**: This assay detects additional α-gene copy, (ααα-anti 3.7) and (ααα-anti 4.2) by Multiplex PCR assay.

- **HPFH (Hereditary Persistence of Fetal Hemoglobin), most common deletions:** This assay detects common HPFH deletions (HPFH-1, HPFH-2, HPFH-3, HPFH-7, SEA-HPFH, and Hb Lepore's by deletion-specific PCR assay.

- **Xmn1 (screening for the polymorphism):** This assay detects a polymorphism associated with increase in fetal Hb, a beneficial finding in many patients with thalassemia or sickle-cell disease.

- **Other specialized tests**:
  - **Hepcidin**, for sample collection visit: https://www.testmenu.com/UBCHOLab/Tests/1061714
  - **Pitted RBC**, for sample collection visit: https://www.testmenu.com/UBCHOLab/Tests/924899

Test Classification:

- These molecular assays were developed and validated by the Hemoglobinopathy Reference Laboratory according to CLIA requirements. These assays have not been cleared or approved by the US Food and Drug Administration.
- Test results should be interpreted in the context of clinical findings, family history, and other laboratory data.
- Rare polymorphisms exist that could lead to false-negative or false-positive results. If results obtained do not match the clinical findings, additional testing should be considered.
- Bone Marrow transplants from allogenic donors will interfere with testing.

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Sample Collection and Shipping

- Please complete the requisition form. Visit https://hemoglobinlab.ucsf.edu or contact the laboratory (Monday - Friday 7:00 AM to 4 PM) for detailed information on the tests provided.
- Collect a minimum of 0.6-2 ml whole blood into a purple (or lavender) EDTA tube. Immediately invert the tube several times to ensure complete mixing of blood with anticoagulant in the tube. Label the tube with the patient's name, date of birth and collection date.
- When the temperature in the sample collection tube decreases below freezing point, the blood cells will hemolyze!! Use sufficient airspace between ice packs and tube (bubble wrap, inserted in secondary tube) to avoid direct contact. **Avoid freezing the samples!!!**

  ** DNA must be extracted by a qualified laboratory with one or more of the following certifications / accreditations: CLIA certification, CAP accreditation, accreditation equivalency as determined by CMS, accreditations, and certifications from established international organizations and/or government agencies.

Shipping

- We are not open on the weekend. Ship early in the week (Monday-Wednesday) using a next day deliver option to make sure your sample gets to us before the weekend. For Thursday shipments use overnight delivery.
- Samples collected on Friday or Saturday should be held in refrigeration (2-8°C) and shipped on Sunday or Monday. Address:

Attn: Shabnam Tavassoli Hemoglobinopathy
Reference Laboratory UCSF Benioff
Children's Hospital Oakland 747 52nd Street,
Oakland, CA 94609 Phone: (510) 450-7688

CPT codes:

Alpha Thalassemia, Common deletion	81257
Alpha Gene Sequencing	81259
Alpha MLPA	81404
Beta Gene Sequencing	81364
Beta MLPA	81403
Alpha Gene Triplication	81404
Hereditary Persistent Fetal Hemoglobin	81403
Gamma Gene Sequencing	81404
Xmn1 polymorphism	81403
Hepcidin (immunoassay)	86849
Pitted RBC count	85999
Isoelectric focusing (IEF)	82664
Solubility testing	85660