

**Requisition form**  
**Hemoglobinopathy Reference Laboratory**  
**UCSF Benioff Children's Hospital Oakland**  
**Department of Pathology and Laboratory Medicine**  
**744 52nd Street, Oakland, CA 94609**  
**Phone: (510) 450-7688**  
**Fax: (510) 601-3928**  
**Email: [shabnam.tavassoli@ucsf.edu](mailto: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 <sup>9</sup> /ul | HbA2: _____ %   | Ferritin: _____ ug/dL   |
| MCV: _____ fL                   | HbF: _____ %    |                         |
| MCH: _____ Pg                   | Others: _____ % |                         |

***Check tests requested:***

- ☐ Hemoglobin 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 genes sequencing
- ☐ Gamma Globin genes 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): \_\_\_\_\_

## 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 ( $\alpha^{CS}$ ) 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  $\alpha$ -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  $\beta$ -globin gene cluster.
- **Alpha Globin gene Triplication:** This assay detects additional  $\alpha$ -gene copy, ( $\alpha\alpha\alpha$ -anti 3.7) and ( $\alpha\alpha\alpha$ -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:** Hepcidin is a serum test and the sample must be transported on dry ice. For sample collection visit:  
<https://www.testmenu.com/UBCHOLab/Tests/1061714>
  - **Pitted RBC:** Pitted RBC is a morphology test performed on red blood cells fixed in 1% glutaraldehyde. 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 allogeneic donors will interfere with testing.

## 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.
- For the two specialized tests—**Hepcidin and Pitted RBC**—please refer to the link on page 2 for detailed sample collection and transportation instructions.

## 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  
Department of Pathology and Laboratory Medicine  
744 52nd Street, Oakland, CA 94609

### CPT codes:

|                                                    |       |
|----------------------------------------------------|-------|
| Alpha Thalassemia, <a href="#">Common deletion</a> | 81257 |
| Alpha Gene Sequencing                              | 81259 |
| Alpha MLPA                                         | 81269 |
| Beta Gene Sequencing                               | 81364 |
| Beta MLPA                                          | 81403 |
| Alpha Gene Triplication                            | 81404 |
| Hereditary Persistent Fetal Hemoglobin             | 81403 |
| Gamma Gene Sequencing                              | 81404 |
| Xmn1 polymorphism                                  | 81400 |
| Hepcidin (immunoassay)                             | 86849 |
| Pitted RBC count                                   | 85999 |
| Isoelectric focusing (IEF)                         | 82664 |
| Solubility testing                                 | 85660 |