MAYO CLINIC LABORATORIES

# Test Definition: HPLC

High-Performance Liquid Chromatography (HPLC) Hemoglobin Variant, Blood (Non-Orderable)

## Overview

### Useful For

**Only orderable as part of a profile**. For more information see:
HAEV1 / Hemolytic Anemia Evaluation, Blood
HBEL1 / Hemoglobin Electrophoresis Evaluation, Blood
MEV1 / Methemoglobinemia Evaluation, Blood
REVE2 / Erythrocytosis Evaluation, Blood
THEV1 / Thalassemia and Hemoglobinopathy Evaluation, Blood and Serum

Providing additional information, which aids in the identification of hemoglobin variants

### Method Name

Only orderable as part of a profile. For more information see:
-HAEV1 / Hemolytic Anemia Evaluation, Blood
-HBEL1 / Hemoglobin Electrophoresis Evaluation, Blood
-MEV1 / Methemoglobinemia Evaluation, Blood
-REVE2 / Erythrocytosis Evaluation, Blood
-THEV1 / Thalassemia and Hemoglobinopathy Evaluation, Blood and Serum

Cation Exchange High-Performance Liquid Chromatography (HPLC)

### NY State Available

Yes

## Specimen

### Specimen Type

Whole Blood EDTA

### Specimen Required

Only orderable as part of a profile. For more information see:
-HAEV1 / Hemolytic Anemia Evaluation, Blood
-HBEL1 / Hemoglobin Electrophoresis Evaluation, Blood
-MEV1 / Methemoglobinemia Evaluation, Blood
-REVE2 / Erythrocytosis Evaluation, Blood
-THEV1 / Thalassemia and Hemoglobinopathy Evaluation, Blood and Serum

### Specimen Minimum Volume

1 mL

### Reject Due To

All specimens will be evaluated at Mayo Clinic Laboratories for test suitability.

### Specimen Stability Information

| Specimen Type | Temperature | Time | Special Container |
|---|---|---|---|
| Whole Blood EDTA | Refrigerated | 10 days | |

## Clinical & Interpretive

### Clinical Information

A large number of variants of hemoglobin have been recognized. Although many do not result in clinical or hematologic effects, clinical symptoms that can be associated with hemoglobin disorders include microcytosis, sickling disorders, hemolysis, erythrocytosis/polycythemia, cyanosis/hypoxia, anemia (chronic, compensated, or episodic), and increased methemoglobin or sulfhemoglobin results (M-hemoglobins).

For common, and many uncommon, hemoglobin variants, protein studies will be sufficient for definitive identification. High-performance liquid chromatography is a method that provides useful and supplementary information on most hemoglobin variants.

### Reference Values

Only orderable as part of a profile. For more information see:
-HAEV1 / Hemolytic Anemia Evaluation, Blood
-HBEL1 / Hemoglobin Electrophoresis Evaluation, Blood
-MEV1 / Methemoglobinemia Evaluation, Blood
-REVE2 / Erythrocytosis Evaluation, Blood
-THEV1 / Thalassemia and Hemoglobinopathy Evaluation, Blood and Serum

An interpretive report will be provided.

### Interpretation

This test is not interpreted in isolation but as a part of a profile.

### Cautions

Some hemoglobin disorders and variants, including common alpha-thalassemia conditions, are not detected by screening methods and require further reflex testing to identify. If there is 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 to the laboratory so appropriate reflex testing can be added.

Recent transfusion may mask protein results, including hemoglobin electrophoresis, hereditary persistence of fetal

hemoglobin 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 from the tested recipient. These are typically found in low percentages.

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

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

### Clinical Reference

1. Hoyer JD, Hoffman DR. The thalassemia and hemoglobinopathy syndromes. In: McClatchey KD, eds. Clinical Laboratory Medicine. 2nd ed. Lippincott, Williams and Wilkins; 2002:866-895
2. Szuberski J, Oliveira JL, Hoyer JD. A comprehensive analysis of hemoglobin variants by high-performance liquid chromatography (HPLC). Int J Lab Haematol. 2012;34(6):594-604
3. Van Delft P, Lenters E, Bakker-Verweij M, et al. Evaluating five dedicated automatic devices for haemoglobinopathy diagnostics in multi-ethnic populations. Int J Lab Haematol. 2009;31(5):484-495
4. Oliveira JL. Diagnostic strategies in hemoglobinopathy testing, the role of a reference laboratory in the USA. Thalassemia Reports. 2018;8(1). doi:10.4081/thal.2018.7476

## Performance

### Method Description

Hemolysate of whole blood is injected into an analysis stream passing through a cation exchange column using high-performance liquid chromatography. A pre-programmed gradient controls the elution buffer mixture that also passes through the analytical cartridge. The ionic strength of the elution buffer is raised by increasing the percentage of a second buffer. As the ionic strength of the buffer increases, the more strongly retained hemoglobins elute from the cartridge. Absorbance changes are detected by a dual-wavelength filter photometer. Changes in absorbance are displayed as a chromatogram of absorbance versus time.(Huismann TH, Schroeder WA, Brodie AN, et al. Microchromotography of hemoglobins. III. A simplified procedure for the determination of hemoglobin A2. J Lab Clin Med. 1975;86[4]:700-702; Ou CN, Buffone GJ, Reimer GL, Alpert AJ. High-performance liquid chromatography of human hemoglobins on a new cation exchanger. J Chromatogr. 1983;266:197-205; instruction manual: Variant II Beta-thalassemia Short Program Instructions for Use, L70203705. Bio-Rad Laboratories, Inc; 11/2011)

### PDF Report

No

### Day(s) Performed

Monday through Friday

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High-Performance Liquid Chromatography (HPLC) Hemoglobin Variant, Blood (Non-Orderable)

### Report Available
Same day/1 day

### Specimen Retention Time
Normal: 7 days; Abnormal: 14 days

### Performing Laboratory Location
Mayo Clinic Laboratories - Rochester Main Campus

## Fees & Codes

### Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

### Test Classification
This test has been modified from the manufacturer's instructions. Its performance characteristics were determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the US Food and Drug Administration.

### CPT Code Information
83021

### LOINC® Information

| Test ID | Test Order Name | Order LOINC® Value |
|---|---|---|
| HPLC | HPLC Hb Variant, B | No LOINC Needed |

| Result ID | Test Result Name | Result LOINC® Value |
|---|---|---|
| 65615 | HPLC Hb Variant, B | No LOINC Needed |