MAYO CLINIC LABORATORIES

# Test Definition: PHEGP

Phenylalanine Disorders Gene Panel, Varies

## Overview

### Useful For

Follow up for abnormal biochemical results suggestive of a phenylalanine disorder

Establishing a molecular diagnosis for patients with phenylalanine disorders

Identifying variants within genes known to be associated with phenylalanine disorders, allowing for predictive testing of at-risk family members

### Reflex Tests

| Test Id | Reporting Name | Available Separately | Always Performed |
|---|---|---|---|
| CULFB | Fibroblast Culture for Genetic Test | Yes | No |

### Genetics Test Information

This test utilizes next generation sequencing to detect single nucleotide and copy number variants in 10 genes associated with phenylalanine disorders: *DDC*, *DNAJC12*, *GCH1*, *PAH*, *PCBD1*, *PTS*, *QDPR*, *SLC18A2*, *SPR*, and *TH*. See Targeted Genes and Methodology Details for Phenylalanine Disorders Gene Panel and Method Description for additional details.

Identification of a disease-causing variant may assist with diagnosis, prognosis, clinical management, familial screening, and genetic counseling for phenylalanine disorders.

### Testing Algorithm

For skin biopsy or cultured fibroblast specimens, fibroblast culture testing will be performed at an additional charge. If viable cells are not obtained, the client will be notified.

### Special Instructions

- Molecular Genetics: Biochemical Disorders Patient Information
- Informed Consent for Genetic Testing
- Blood Spot Collection Card-Spanish Instructions
- Blood Spot Collection Card-Chinese Instructions
- Informed Consent for Genetic Testing (Spanish)
- Blood Spot Collection Instructions
- Targeted Genes and Methodology Details for Phenylalanine Disorders Gene Panel

### Method Name

Sequence Capture and Targeted Next-Generation Sequencing followed by Polymerase Chain Reaction (PCR) and Sanger Sequencing

### NY State Available

Yes

## Specimen

### Specimen Type
Varies

### Ordering Guidance
The recommended first-tier test for disorders of phenylalanine metabolism is quantitative plasma amino acids (AAQP / Amino Acids, Quantitative, Plasma), as well as neurotransmitters in cerebrospinal fluid and pterin metabolite analysis in blood and urine.

Customization of this panel and single gene analysis for any gene present on this panel is available. For more information see CGPH / Custom Gene Panel, Hereditary, Next-Generation Sequencing, Varies.

Targeted testing for familial variants (also called site-specific or known mutations testing) is available for the genes on this panel. See FMTT / Familial Variant, Targeted Testing, Varies. To obtain more information about this testing option, call 800-533-1710.

### Shipping Instructions
Specimen preferred to arrive within 96 hours of collection.

### Specimen Required
**Patient Preparation:** A previous bone marrow transplant from an allogenic donor will interfere with testing. For instructions for testing patients who have received a bone marrow transplant, call 800-533-1710.

**Submit only 1 of the following specimens:**

**Specimen Type:** Whole blood
**Container/Tube:** Lavender top (EDTA) or yellow top (ACD)
**Specimen Volume:** 3 mL
**Collection Instructions:**
1. Invert several times to mix blood.
2. Send whole blood specimen in original tube. **Do not aliquot**.
**Specimen Stability Information:** Ambient (preferred) 4 days/Refrigerated 14 days

**Specimen Type**: Skin biopsy
**Supplies**: Fibroblast Biopsy Transport Media (T115)
**Container/Tube**: Sterile container with any standard cell culture media (eg, minimal essential media, RPMI 1640). The solution should be supplemented with 1% penicillin and streptomycin.
**Specimen Volume**: 4-mm punch
**Specimen Stability Information**: Refrigerated (preferred)/Ambient
**Additional Information:** A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks is required to culture fibroblasts before genetic testing can occur.

**Specimen Type**: Cultured fibroblast
**Container/Tube**: T-25 flask
**Specimen Volume**: 2 Flasks
**Collection Instructions**: Submit confluent cultured fibroblast cells from a skin biopsy from another laboratory. Cultured cells from a prenatal specimen will not be accepted.
**Specimen Stability Information**: Ambient (preferred)/Refrigerated (<24 hours)
**Additional Information:** A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks is required to culture fibroblasts before genetic testing can occur.

**Specimen Type**: Blood spot
**Supplies:** Card-Blood Spot Collection (Filter Paper) (T493)
**Container/Tube**:
**Preferred**: Collection card (Whatman Protein Saver 903 Paper)
**Acceptable**: PerkinElmer 226 (formerly Ahlstrom 226) filter paper or blood spot collection card
**Specimen Volume**: 5 Blood spots
**Collection Instructions**:
1. An alternative blood collection option for a patient older than 1 year is a fingerstick. For detailed instructions, see How to Collect Dried Blood Spot Samples.
2. Let blood dry on the filter paper at ambient temperature in a horizontal position for a minimum of 3 hours.
3. Do not expose specimen to heat or direct sunlight.
4. Do not stack wet specimens.
5. Keep specimen dry.
**Specimen Stability Information**: Ambient (preferred)/Refrigerated
**Additional Information:**
1. Due to lower concentration of DNA yielded from blood spot, it is possible that additional specimen may be required to complete testing.
2. For collection instructions, see Blood Spot Collection Instructions
3. For collection instructions in Spanish, see Blood Spot Collection Card-Spanish Instructions (T777)
4. For collection instructions in Chinese, see Blood Spot Collection Card-Chinese Instructions (T800)

**Specimen Type:** Saliva
**Patient Preparation:** Patient should not eat, drink, smoke, or chew gum 30 minutes prior to collection.
**Supplies:** Saliva Swab Collection Kit (T786)
**Specimen Volume:** 1 Swab
**Collection Instructions:** Collect and send specimen per kit instructions.
**Specimen Stability Information:** Ambient 30 days
**Additional Information:** Due to lower concentration of DNA yielded from saliva, it is possible that additional specimen may be required to complete testing.

## Forms

1. **New York Clients-Informed consent is required.** Document on the request form or electronic order that a copy is on file. The following documents are available:
-Informed Consent for Genetic Testing (T576)
-Informed Consent for Genetic Testing (Spanish) (T826)

2. Molecular Genetics: Biochemical Disorders Patient Information (T527)
3. If not ordering electronically, complete, print, and send a Biochemical Genetics Test Request (T798) with the specimen.

### Specimen Minimum Volume
See Specimen Required

### Reject Due To
All specimens will be evaluated at Mayo Clinic Laboratories for test suitability.

### Specimen Stability Information

| Specimen Type | Temperature | Time | Special Container |
|---|---|---|---|
| Varies | Varies | | |

## Clinical & Interpretive

### Clinical Information
Hyperphenylalaninemia is a heterogeneous disorder of phenylalanine catabolism caused by a deficiency of any one of 6 enzymes involved in the conversion of phenylalanine to tyrosine.

Phenylketonuria (PKU) is the most frequent inherited disorder of amino acid metabolism (about 1:10,000-1:15,000) and was the first successfully treated inborn error of metabolism and included in newborn screening programs worldwide. It is inherited in an autosomal recessive manner and is caused by a defect in the enzyme phenylalanine hydroxylase (PAH), which converts the essential amino acid phenylalanine to tyrosine. Deficiency of PAH results in decreased levels of tyrosine and an accumulation of phenylalanine in blood and tissues. Untreated, PKU leads to severe brain damage with intellectual impairment, behavior abnormalities, seizures, and spasticity. The level of enzyme activity differentiates classic PKU (PAH activity <1%) from other milder forms; however, all are characterized by increased levels of phenylalanine (hyperphenylalaninemia). Treatment includes the early introduction of a diet low in phenylalanine.

Approximately 2% of patients with hyperphenylalaninemia have a deficiency of tetrahydrobiopterin (BH4), which causes a secondary deficit of the neurotransmitters, dopamine and serotonin. There are 4 autosomal recessive disorders associated with BH4 deficiency plus hyperphenylalaninemia; guanosine triphosphate cyclohydrolase deficiency (GCH1), 6-pyruvoyl tetrahydropterin synthase deficiency (PTS), dihydropteridine reductase deficiency (QDPR), and pterin-4 alpha carbinolamine dehydratase (PCD) deficiency (PCBD1). This group of disorders, with the exception of PCD, is characterized by progressive dystonia, truncal hypotonia, extremity hypertonia, seizures, and intellectual disability though milder presentations exist. PCD has no symptoms other than transient alterations in tone. Treatment may include administration of BH4, L-dopa (and carbidopa) 5-hydroxytryptophan supplements, and a low phenylalanine diet.

Recently, variants in *DNAJC12*, which encodes a heat-shock protein that interacts with the phenylalanine, tyrosine, and tryptophan hydroxylases to help catalyze the conversion of the substrates to their respective products, has been shown to cause hyperphenylalaninemia, progressive neurodegeneration, and dystonia. Treatment may include early administration of BH4 and/or neurotransmitter precursors.

Related additional disorders of neurotransmitter metabolism include:

-Aromatic l-amino acid decarboxylase (AADC) deficiency, caused by variants in *DDC*, is an autosomal recessive inborn error in neurotransmitter metabolism that leads to combined serotonin and catecholamine deficiency.
-Patients with dopa-responsive dystonia due to variants in *SPR* causing sepiapterin reductase deficiency have progressive psychomotor retardation and dystonia.
-Variants in tyrosine hydroxylase (TH) prevent the conversion of L-tyrosine to L-dopa resulting in Segawa syndrome.
-Variants in *SLC18A2*, a vesicular transporter of dopamine, cause infantile parkinsonism-dystonia-2 (PKDYS2)

### Reference Values

An interpretive report will be provided.

### Interpretation

All detected alterations are evaluated according to American College of Medical Genetics and Genomics recommendations.(1) Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance.

### Cautions

Clinical Correlations:
Test results should be interpreted in the context of clinical findings, family history, and other laboratory data. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

If testing was performed because of a clinically significant family history, it is often useful to first test an affected family member. Detection of at least one reportable variant in an affected family member would allow for more informative testing of at-risk individuals.

To discuss the availability of additional testing options or for assistance in the interpretation of these results, contact the Mayo Clinic Laboratory genetic counselors at 800-533-1710.

Technical Limitations:
Next-generation sequencing may not detect all types of genomic variants. In rare cases, false-negative or false-positive results may occur. The depth of coverage may be variable for some target regions; assay performance below the minimum acceptable criteria or for failed regions will be noted. Given these limitations, negative results do not rule out the diagnosis of a genetic disorder. If a specific clinical disorder is suspected, evaluation by alternative methods can be considered.

There may be regions of genes that cannot be effectively evaluated by sequencing or deletion and duplication analysis as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences. Confirmation of select reportable variants will be performed by alternate methodologies based on internal laboratory criteria.

This test is validated to detect 95% of deletions up to 75 base pairs (bp) and insertions up to 47 bp. Deletions-insertions (delins)) of 40 or more bp, including mobile element insertions, may be less reliably detected than smaller delins.

Deletion/Duplication Analysis:
This analysis targets single and multi-exon deletions/duplications; however, in some instances single exon resolution cannot be achieved due to isolated reduction in sequence coverage or inherent genomic complexity. Balanced structural rearrangements (such as translocations and inversions) may not be detected.

This test is not designed to detect low levels of mosaicism or to differentiate between somatic and germline variants. If there is a possibility that any detected variant is somatic, additional testing may be necessary to clarify the significance of results.

Genes may be added or removed based on updated clinical relevance. For detailed information regarding gene-specific performance and technical limitations, see Method Description or contact a laboratory genetic counselor.

If the patient has had an allogeneic hematopoietic stem cell transplant or a recent heterologous blood transfusion, results may be inaccurate due to the presence of donor DNA. Call Mayo Clinic Laboratories for instructions for testing patients who have received a bone marrow transplant.

Reclassification of Variants:
Currently, it is not standard practice for the laboratory to systematically review previously classified variants on a regular basis. The laboratory encourages healthcare providers to contact the laboratory at any time to learn how the classification of a particular variant may have changed over time. Due to broadening genetic knowledge, it is possible that the laboratory may discover new information of relevance to the patient. Should that occur, the laboratory may issue an amended report.

Variant Evaluation:
Evaluation and categorization of variants is performed using published American College of Medical Genetics and Genomics and the Association for Molecular Pathology recommendations as a guideline.(1) Other gene-specific guidelines may also be considered. Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance. Variants classified as benign or likely benign are not reported.

Multiple in silico evaluation tools may be used to assist in the interpretation of these results. The accuracy of predictions made by in silico evaluation tools is highly dependent upon the data available for a given gene, and periodic updates to these tools may cause predictions to change over time. Results from in silico evaluation tools should be interpreted with caution and professional clinical judgement.

Rarely, incidental or secondary findings may implicate another predisposition or presence of active disease. These findings will be carefully reviewed to determine whether they will be reported.

## Clinical Reference

1. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-424
2. Burgard P, Luo X, Levy HL, Hoffmann GF: Phenylketonuria. In: Sarafoglou K, Hoffmann GF, Roth KS, eds. Pediatric Endocrinology and Inborn Errors of Metabolism. 2nd ed. McGraw-Hill Education; 2017:251-258
3. Blau N, Thony B: Hyperphenylalanemias: Disorders of tetrahydrobiopterin metabolism. In: Sarafoglou K, Hoffmann GF, Roth KS, eds. Pediatric Endocrinology and Inborn Errors of Metabolism. 2nd ed. McGraw-Hill Education; 2017:259-266
4. Anikster Y, Haack TB, Vilboux T, et al. Biallelic mutations in DNAJC12 cause hyperphenylalaninemia, dystonia, and intellectual disability. Am J Hum Genet. 2017;100(2):257-266. doi:10.1016/j.ajhg.2017.01.002
5. OMIM. Johns Hopkins University; Updated March 7, 2024. Accessed March 8, 2024. Available at https://omim.org/

## Performance

### Method Description

Next-generation sequencing (NGS) and/or Sanger sequencing is performed to test for the presence of variants in coding regions and intron/exon boundaries of the genes analyzed, as well as some other regions that have known disease-causing variants. The human genome reference GRCh37/hg19 build was used for sequence read alignment. At least 99% of the bases are covered at a read depth over 30X. Sensitivity is estimated to be over 99% for single nucleotide variants, over 94% for insertions/deletions (indels) less than 40 base pairs (bp), and over 95% for deletions up to 75 bp and insertions up to 47 bp. NGS and/or a polymerase chain reaction based quantitative method is performed to test for the presence of deletions and duplications in the genes analyzed.

There may be regions of genes that cannot be effectively evaluated by sequencing or deletion and duplication analysis as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences. See Targeted Genes and Methodology Details for Phenylalanine Disorders Gene Panel for details regarding the targeted genes analyzed and specific gene regions not routinely covered.(Unpublished Mayo method)

Genes analyzed: *DDC*, *DNAJC12*, *GCH1*, *PAH*, *PCBD1*, *PTS*, *QDPR*, *SLC18A2*, *SPR*, and *TH*

### PDF Report

Supplemental

### Day(s) Performed

Varies

### Report Available

14 to 21 days

### Specimen Retention Time

Whole blood: 2 weeks (if available); Extracted DNA: 3 months; Blood spots/Saliva; 1 month

### Performing Laboratory Location

Rochester

## 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 was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA

requirements. It has not been cleared or approved by the US Food and Drug Administration.

## CPT Code Information

81405
81406 x 2
81479
88233-Tissue culture, skin, solid tissue biopsy (if appropriate)
88240-Cryopreservation (if appropriate)
81479 (if appropriate for government payers)

## LOINC® Information

| Test ID | Test Order Name | Order LOINC® Value |
|---|---|---|
| PHEGP | Phenylalanine Disorders Gene Panel | 105265-3 |

| Result ID | Test Result Name | Result LOINC® Value |
|---|---|---|
| 608788 | Test Description | 62364-5 |
| 608789 | Specimen | 31208-2 |
| 608790 | Source | 31208-2 |
| 608791 | Result Summary | 50397-9 |
| 608792 | Result | 82939-0 |
| 608793 | Interpretation | 69047-9 |
| 608794 | Resources | 99622-3 |
| 608795 | Additional Information | 48767-8 |
| 608796 | Method | 85069-3 |
| 608797 | Genes Analyzed | 48018-6 |
| 608798 | Disclaimer | 62364-5 |
| 608799 | Released By | 18771-6 |