

## Overview

### Useful For

Providing a comprehensive genetic evaluation for patients with a personal or family history suggestive of primary immunodeficiency due to phagocytic defects, chronic granulomatous disease, or related disorders

Establishing a diagnosis and, in some cases, allowing for appropriate management and surveillance for disease features based on the gene involved

Identifying variants within genes known to be associated primary immunodeficiency due to phagocytic defects, chronic granulomatous disease, or related disorders allowing for predictive testing of at-risk family members

### Genetics Test Information

This test includes next-generation sequencing and supplemental Sanger sequencing to evaluate for the genes listed on the panel.

### Highlights

This test uses next-generation sequencing to test for variants in the *CEBPE*, *CSF2RA*, *CTSC*, *CYBA*, *CYBB*, *FERMT3*, *FPR1*, *G6PD*, *ITGB2*, *MPO*, *NCF2*, *NCF4*, *PMM2 (CDG1)*, *RASGRP2*, *SPINK5* genes.

Identification of a pathogenic variant may assist with prognosis, clinical management, familial screening, and genetic counseling.

### Reflex Tests

Test ID	Reporting Name	Available Separately	Always Performed
FIBR	Fibroblast Culture	Yes	No
CRYOB	Cryopreserve for Biochem Studies	No	No

### Testing Algorithm

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

### Special Instructions

- [Informed Consent for Genetic Testing](#)
- [Primary Immunodeficiencies Patient Information](#)
- [Informed Consent for Genetic Testing \(Spanish\)](#)

### Method Name

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

### NY State Available

Yes

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## Specimen

### Specimen Type

Varies

### Ordering Guidance

[Targeted testing for familial variants \(also called site-specific or known mutations testing\) is available for the genes on this panel. See FMTT / Familial Mutation, Targeted Testing, Varies.](#)

### Necessary Information

1. [Primary Immunodeficiencies Patient Information \(T791\)](#) is required. See Special Instructions.

**Note:** Testing may proceed without the Patient Information however it aids in providing a more thorough interpretation. Ordering physicians are strongly encouraged to fill out the form.

2. Include physician name and phone number with specimen.

### Specimen Required

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

#### Preferred:

**Specimen Type:** Whole blood

**Container/Tube:** Lavender top (EDTA)

**Specimen Volume:** 3 mL

#### Collection Instructions:

1. Invert several times to mix blood.
2. Send specimen in original tube.

**Specimen Stability Information:** Ambient (preferred)/Refrigerated

**Specimen Type:** Blood spot

**Supplies:** Card-Blood Spot Collection Filter Paper (T493)

#### Container/Tube:

**Preferred:** Collection card (Whatman Protein Saver 903 Paper)

**Acceptable:** Whatman FTA Classic paper, Ahlstrom 226 filter paper, or Blood Spot Collection Card (T493)

**Specimen Volume:** 2 to 5 blood spots on collection card

**Collection Instructions:**

1. An alternative blood collection option for a patient <1 year of age is finger stick.
2. Let blood dry on the filter paper at ambient temperature in a horizontal position for 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**Specimen Type:** Peripheral blood mononuclear cells (PBMCs)**Container/Tube:** Cell pellet**Collection Instructions:** Send as a suspension in freezing medium or cell pellet frozen on dry ice.**Specimen Stability Information:** Frozen**Specimen Type:** Cultured fibroblasts**Container/Tube:** T-75 or T-25 flask**Specimen Volume:** 1 Full T-75 or 2 full T-25 flasks**Additional Information:** Indicate the tests to be performed on the fibroblast culture cells. A separate culture charge will be assessed under FIBR / Fibroblast Culture. An additional 4 weeks is required to culture fibroblasts before genetic testing can occur.**Specimen Stability Information:** Ambient (preferred)/Refrigerated <24 hours**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. Tubes of culture media can be supplied upon request (Eagle's minimum essential medium with 1% penicillin and streptomycin [T115]).**Specimen Volume:** 4-mm punch**Additional Information:** A separate culture charge will be assessed under FIBR / Fibroblast Culture. An additional 4 weeks is required to culture fibroblasts before genetic testing can occur.**Specimen Stability Information:** Refrigerated (preferred)/Ambient

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**Specimen Type:** DNA

**Container/Tube:** 2 mL screw top tube

**Specimen Volume:** 100mcL (microliters)

**Collection Instructions:**

1. The preferred volume is 100 mcL at a concentration of 250 ng/mcL
2. Include concentration and volume on tube.

Specimen Stability Information: Frozen (preferred)/Ambient/Refrigerated

**Forms**

**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 in Special Instructions:

[-Informed Consent for Genetic Testing](#) (T576)

[-Informed Consent for Genetic Testing-Spanish](#) (T826)

**Specimen Minimum Volume**

Whole blood: 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
Varies	Varies		

**Clinical and Interpretive**

**Clinical Information**

Primary immunodeficiencies (PID) that affect the function of phagocytes (neutrophils, monocytes, macrophages, and eosinophils) predispose patients to a narrow spectrum of specific infections as a result of impaired killing of bacteria and fungi.

Chronic granulomatous disease (CGD), due to impaired production of reactive oxygen intermediates, is characterized by infections (ie, *Staphylococcus aureus*, *Burkholderia cepacia complex*, *Serratia marcescens*, *Nocardia*, and *Aspergillus* sp.) that involve the skin, lungs, lymph nodes, liver, and bones, although any organ or tissue can be affected. Patients may also experience immune dysregulation, resulting in granuloma formation, colitis, and other inflammatory disorders. While most affected individuals are diagnosed prior to 5 years of age, patients may

present into late adulthood. Tests that measure neutrophil superoxide production by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, including the dihydrorhodamine (DHR) or nitroblue tetrazolium (NBT) tests, may be used in establishing a diagnosis. X-linked CGD, the most common form, is caused by pathogenic variants in *CYBB*. In some cases, a contiguous gene deletion may result in CGD along with McLeod neuroacanthocytosis syndrome. In cases of a large contiguous gene deletion, patients may also inherit *RPGR*-related retinitis pigmentosa, Duchenne muscular dystrophy, and ornithine transcarbamylase deficiency. A chromosomal microarray may be indicated if a contiguous gene deletion is suspected. In addition to the X-linked form, CGD may also be inherited in an autosomal recessive pattern, due to biallelic pathogenic variants in the other genes that encode the remainder of the subunits of phagocyte NADPH, including *CYBA*, *NCF1*, *NCF2*, and *NCF4* (**Note:** *NCF1* is not currently included on this panel). Similarly to CGD, complete glucose 6-phosphate dehydrogenase (G6PD) deficiency can result in an increased susceptibility to infection due to impaired neutrophil respiratory burst. G6PD deficiency is also inherited in an X-linked pattern due to pathogenic variants in *G6PD*. Chronic nonspherocytic hemolytic anemia occurs in severe deficiency, while acute hemolytic episodes (typically triggered by some medications, ingestion of fava beans, viral or bacterial infections, etc) are observed in less severe G6PD deficiency. Patients with myeloperoxidase deficiency also show a reduced ability of the neutrophil to generate a respiratory burst, as evidenced by abnormal DHR results, but show normal superoxide production levels and NBT staining.

Neutrophils contain azurophilic (or primary) granules, specific (or secondary) granules, and tertiary granules that contain antimicrobial substances. Azurophilic granules contain myeloperoxidase, bactericidal/permeability-increasing protein, defensins, neutrophil elastase, and cathepsin G. Specific granules contain lactoferrin, lysozyme, NADPH oxidase, alkaline phosphatase, collagenase, histaminase, and cathelicidin. Tertiary granules contain cathepsin, gelatinase, and collagenase. Deficiency of myeloperoxidase can occur as an autosomal recessive condition due to variants in myeloperoxidase (*MPO*) and results in a susceptibility to *Candida* infections. Papillon-Lefevre Syndrome (PALS) is an autosomal recessive disorder due to pathogenic variants in *CTSC* (lysosomal cysteine protease cathepsin C, also known as dipeptidyl peptidase I [DPPI]). DPPI is necessary for posttranslational modification of the serine proteases in the neutrophil azurophilic granules, activation of granzymes A and B of cytotoxic lymphocytes, and activation of mast cell chymases. PALS typically presents with severe periodontal disease and keratosis palmoplantaris, along with mild immunodeficiency. In specific granule deficiency (SGD), neutrophils lack expression of secondary and tertiary granule proteins, have an atypical bilobed nuclear morphology, and demonstrate defects in chemotaxis and bactericidal activity. SGD is due to pathogenic variants in *CEBPE*, which is a myeloid-specific transcription factor.

Leukocyte adhesion deficiencies (LAD) are characterized by recurrent bacterial infections due to reduced ability of neutrophils to adhere to various substances and migrate to sites of infection, as well as defective phagocytic and respiratory burst response to bacteria and yeast. Patients often are first noticed due to omphalitis, but later gingivitis/periodontitis, pneumonia, peritonitis, and deep abscesses may develop. LAD can be caused by pathogenic variants in *ITGB2*, which encodes for the CD18 antigen (LAD1); and *SLC35C1*, which encodes for a GDP-fucose transporter (LAD2) (**Note:** *SLC35C1* is not currently included on this panel) or *FERMT3* (LAD3). Although the neutrophil functional studies are similar between LAD1 and LAD2, the clinical course in LAD2 is milder, though patients may also present with other features (ie, mental and growth retardation) due to abnormal fucose metabolism. LAD3 presents similarly to LAD1, but platelets are also affected resulting in clotting defects. Pathogenic variants in *RASGRP2* (also inherited in a recessive pattern) mimic the phenotype of LAD3. Recessively inherited defects in *PMM2* (congenital disorder of glycosylation type IA) show diminished neutrophil chemotaxis resulting in severe infections.

Granulocyte-macrophage colony-stimulating factor (GM-CSF) stimulates the formation of colonies of neutrophils and macrophages from bone marrow precursors, but is also required for proper neutrophil function. Recessive inheritance of pathogenic variants in *CSF2RA*, which encodes for the alpha chain of the GM-CSF receptor, disrupts GM-CSF signaling and results in defects in neutrophil adhesion, phagocytosis, superoxide formation, and microbial killing. Clinically, this manifests as pulmonary alveolar proteinosis and increased susceptibility to infections (pulmonary and extrapulmonary).

The fMet-Leu-Phe receptor, encoded by *FPR1*, is located on the cell surface and is involved in chemotaxis phagocytic cells. Variants in *FPR1* may present with aggressive periodontitis and *FPR1* governs neutrophil function during acute inflammation.

Netherton syndrome (NS), due to pathogenic variants in *SPINK5* encoding LEKTI (lymphoepithelial Kazal-type-related inhibitor), is characterized by extensive skin inflammation, hair abnormalities, atopic manifestations, and recurrent bacterial infections. Although various immunologic defects have been suggested to contribute to the immune deficiency, in some cases NK cells demonstrate an immature phenotype with impaired degranulation and cytotoxic effects. Patients may also have decreased circulating B-cells and elevated IgE and IgA.

**Table1. Genes included in the Phagocytic / Chronic Granulomatous Disease PID Gene Panel**

GENE SYMBOL (ALIAS)	PROTEIN	OMIM	INHERITANCE	PHENOTYPE DISORDER
<i>CEBPE</i>	CCAAT/enhancer-binding protein epsilon	600749	AR	Specific granule deficiency
<i>CSF2RA</i>	Granulocyte-macrophage colony-stimulating factor receptor subunit alpha isoform a precursor	306250	XL	Pulmonary surfactant metabolism dysfunction 4 (SMDP4), pulmonary alveolar proteinosis
<i>CTSC</i>	Dipeptidyl peptidase 1 isoform a preproprotein	602365	AR	Haim-Munk syndrome, Papillon-Lefevre syndrome, Periodontitis 1, juvenile
<i>CYBA</i>	Cytochrome b-245 light chain	608508	AR	Chronic granulomatous disease
<i>CYBB</i>	Cytochrome b-245 heavy chain	300481	XL	Chronic granulomatous disease, immunodeficiency 34, mycobacteriosis
<i>FERMT3</i>	Fermitin family homolog 3 short form	607901	AR	Leukocyte adhesion deficiency, type III
<i>FPR1</i>	fMet-Leu-Phe receptor	136537	AR	Juvenile periodontitis
<i>G6PD</i>	Glucose-6-phosphate 1-dehydrogenase isoform b	305900	XL	Hemolytic anemia, chronic granulomatous disease
<i>ITGB2</i>	Integrin beta-2 precursor	600065	AR	Leukocyte adhesion deficiency type 1



<i>MPO</i>	Myeloperoxidase precursor	606989	AR	Myeloperoxidase deficiency
<i>NCF2</i>	Neutrophil cytosol factor 2 isoform 1	608515	AR	Chronic granulomatous disease
<i>NCF4</i>	Neutrophil cytosol factor 4 isoform 2	601488	AR	Chronic granulomatous disease
<i>PMM2 (CDG1)</i>	Phosphomannomutase 2	601785	AR	Congenital disorder of glycosylation, type Ia
<i>RASGRP2</i>	RAS guanyl-releasing protein 2 isoform a	605577	AR	Bleeding disorder, platelet-type, 18, LAD-III
<i>SPINK5</i>	Serine protease inhibitor Kazal-type 5 isoform b preproprotein	605010	AD/AR	Atopy(AD), Netherton syndrome (AR)

AD=autosomal dominant AR=autosomal recessive XL=X-linked

## Reference Values

An interpretive report will be provided.

## Interpretation

Evaluation and categorization of variants is performed using the most recent published American College of Medical Genetics and Genomics (ACMG) recommendations as a guideline. Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance.

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 predictions made by these tools may change over time. Results from in silico evaluation tools should be interpreted with caution and professional clinical judgment.

## Cautions

Clinical Correlations:

This panel contains genes that are primarily associated with a phagocytic defect (including chronic granulomatous disease). Although leukocyte adhesion deficiency types 1 and 3 are included, type 2 is not. In addition, due to phenotypic overlap, *PMM2* is included in this panel.

Some individuals who have involvement of one or more of the genes on the panel may have a variant that is not identified by the methods performed (eg, promoter variants, deep intronic variants). The absence of a variant, therefore, does not eliminate the possibility of disease. Test results should be interpreted in context of clinical findings, family history, and other laboratory data. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

For predictive testing of asymptomatic individuals, it is often useful to first test an affected family member. Identification of a pathogenic variant in an affected individual allows for more informative testing of at-risk individuals.

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**Technical Limitations:**

Next-generation sequencing may not detect all types of genetic variants. The variant detection software has lower detection efficiency for insertion/deletion variants as compared to single nucleotide variants. Therefore, small deletions and insertions greater than 8 nucleotides in length may not be detected by this test. Copy number variations (CNV) are not currently reported for any of the genes on this panel. Additionally, rare polymorphisms may be present that could lead to false-negative or false-positive results. In some cases, DNA variants of undetermined significance may be identified. If results do not match clinical findings, consider alternative methods for analyzing these genes, such as Sanger sequencing or large deletion/duplication analysis. If the patient has had an allogeneic blood or bone marrow transplant or a recent (ie, <6 weeks from time of sample collection) heterologous blood transfusion, results may be inaccurate due to the presence of donor DNA. Call 800-533-1710 for instructions for testing patients who have received a bone marrow transplant.

**Reclassification of Variants-Policy:**

At this time, it is not standard practice for the laboratory to systematically review likely deleterious alterations or variants of uncertain significance that are detected and reported. The laboratory encourages health care providers to contact the laboratory at any time to learn how the status of a particular variant may have changed over time. Consultation with a healthcare provider, or team of healthcare providers, with expertise in genetics and primary immunodeficiencies, is recommended for interpretation of this result.

A list of benign and likely benign variants detected for this patient is available from the lab upon request.

Contact the laboratory if additional information is required regarding the transcript or human genome assembly used for the analysis of this patient's results.

**Clinical Reference**

1. Picard C, Gaspar HB, Al-Herz W, Bousfina A, et al: International Union of Immunological Societies: 2017 Primary Immunodeficiency Disease Committee Report on Inborn Errors of Immunity, *J Clin Immunol*. 2018;38:96-128
2. Roos D, de Boer M: Molecular diagnosis of chronic granulomatous disease. *Clin Exp Immunol*. 2014;175:139-149
3. Hanna S, Etzioni A: Leukocyte adhesion deficiencies. *Ann N Y Acad Sci*. 2012;1250:50-55
4. Taqhzade-Mortezaee F, Esmaeli B, Badalzadeh M, et al: Investigation of ITGB2 Gene in 12 New Cases of Leukocyte Adhesion Deficiency-Type I Revealed Four Novel Mutations from Iran, *Archives of Iranian Med*. 2015;18:11:760-764
5. Trapnell BC, Carey BC, Uchida K, Suzuki T: Pulmonary alveolar proteinosis, a primary immunodeficiency of impaired GM-CSF stimulation of macrophages. *Curr Opin Immunol*. 2009;21(5):514-521
6. Gombart AF, Koeffler HP: Neutrophil specific granule deficiency and mutations in the gene encoding transcription factor C/EBP(epsilon). *Curr Opin Hematol*. 2002;9(1):36-42
7. Nagy N, Valyi P, Csoma Z, et al: CTSC and Papillon-Lefevre syndrome: detection of recurrent mutations in Hungarian patients, a review of published variants and database update, *Mol Gen and Genomic Med*. 2014;2(3):217-228
8. Lozano ML, Cook A, Bastida JM, et al: Novel mutations in *RASGRP2*, which encodes CALDAG-GEF1, abrogate Rap1 activation, causing platelet dysfunction. *Blood*. 2016;128:1282-1289
9. Pasvolsky R, Feigelson SW, Kilic SS, et al: A LAD-III syndrome is associated with defective expression of the

Rap1 activator, CALDAG-GEF1, in lymphocytes, neutrophils and platelets. J Exp Med. 2007;204:1571-1582

10. Notarangelo LD, Pessach I: Out of breath: GM-CSFRa mutations disrupt surfactant homeostasis. J Exp Med. 2008;205:2693-2697

11. Kuhns DB, Alvord WG, Heller T: Residual NADPH oxidase and survival in Chronic Granulomatous Disease. New Engl J Med. 2010;363:2600-2610

12. Dorward DA, Lucas CD, Chapman GB, et al: The role of formylated peptides and formyl peptide receptor 1 in governing neutrophil function during acute inflammation. Am J Pathol. 2015;185:1173-1184

## Performance

### Method Description

Next-generation sequencing (NGS) is performed using an Illumina instrument with paired-end reads. The DNA is prepared for NGS using a custom Agilent SureSelect Target Enrichment System. Data is analyzed with a bioinformatics software pipeline. Supplemental Sanger sequencing may be performed occasionally in regions where NGS is insufficient for data capture or not specific enough to correctly identify a variant. (Unpublished Mayo method)

The following genes are evaluated in this multigene panel:

*CEBPE, CSF2RA, CTSC, CYBA, CYBB, FERMT3, FPR1, G6PD, ITGB2, MPO, NCF2, NCF4, PMM2 (CDG1), RASGRP2, SPINK5*

### PDF Report

No

### Day(s) Performed

Monday

### Report Available

4 to 8 weeks

### Specimen Retention Time

Extracted DNA: 2 months

### Performing Laboratory Location

Rochester

## Fees and 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 Regional Manager. 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. This test has not been cleared or approved by the U.S. Food and Drug Administration.

**CPT Code Information**

81479

**LOINC® Information**

Test ID	Test Order Name	Order LOINC Value
PHAGP	Phagocytic PID Gene Panel	In Process

Result ID	Test Result Name	Result LOINC Value
BA3902	Gene(s) Evaluated	48018-6
BA3903	Result Summary	50397-9
BA3904	Result Details	82939-0
BA3905	Interpretation	69047-9
BA3906	Additional Information	48767-8
BA3907	Method	85069-3
BA3908	Disclaimer	62364-5
BA3909	Reviewed by	18771-6