MAYO CLINIC LABORATORIES

Test Definition: EOIBD

Early Onset Monogenic Inflammatory Bowel Disease (IBD) Gene Panel, Varies

Overview

Useful For

Providing a comprehensive genetic evaluation for patients with a personal or family history suggestive of an inherited inflammatory bowel disorder

Establishing a diagnosis of a monogenic early onset inflammatory bowel disease, allowing for appropriate management and surveillance for disease features based on the gene or variant involved

Identifying variants within genes known to be associated with monogenic early onset inflammatory bowel disease, allowing for predictive testing of at-risk family members

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
CULAF	Amniotic Fluid Culture/Genetic Test	Yes	No
_STR1	Comp Analysis using STR (Bill only)	No, (Bill only)	No
_STR2	Add'l comp analysis w/STR (Bill Only)	No, (Bill only)	No
CULFB	Fibroblast Culture for Genetic Test	Yes	No
MATCC	Maternal Cell Contamination, B	Yes	No

Genetics Test Information

This test utilizes next-generation sequencing to detect single nucleotide and copy number variants in 108 genes associated with monogenic early onset inflammatory bowel disease: *ADA, ADAM17, AICDA, AIRE, ALPI, ANKZF1, ARPC1B, ASAH1, BACH2, BTK, CARMIL2, CASP8, CD3G, CD40LG, CD55, COL7A1, CTLA4, CYBA, CYBB, CYBC1, DCLRE1C, DEF6, DGAT1, DKC1, DOCK8, DUOX2, EPCAM, FCHO1, FERMT1, FOXP3, G6PC (G6PC1), G6PC3, GUCY2C, HPS1, HPS3, HPS4, HPS6, ICOS, IFIH1, IKBKG, IL10, IL10RA, IL10RB, IL21, IL21R, IL2RA, IL2RB, IL2RG, IL7R, ITCH, ITGB2, JAK1, LCT, LIG4, LRBA, MALT1, MEFV, MVK, MYO5B, NCF1, NCF2, NCF4, NEUROG3, NFKBIA, NLRC4, PAX1, PCSK1, PIK3CD, PIK3R1, PLCG2, PLVAP, POLA1, RAG1, RAG2, RIPK1, RTEL1, SH2D1A, SI, SKIV2L (SKIC2), SLC10A2, SLC26A3, SLC37A4, SLC39A4, SLC51B, SLC5A1, SLC9A3, SPINT2, STAT1, STAT3, STAT5B, STIM1, STX3, STXBP2, TGFB1, TGFBR1, TGFBR2, TLR3, TNFAIP3, TRIM22, TRNT1, TTC37 (SKIC3), TTC7A, UNC45A, WAS, WIPF1, XIAP, ZAP70,* and *ZBTB24*. See Targeted Genes and Methodology Details for Early Onset Monogenic Inflammatory Bowel Disease (IBD) Gene Panel for details regarding the targeted gene regions evaluated by this test.

Identification of a disease-causing variant may assist with diagnosis, prognosis, clinical management, recurrence risk assessment, familial screening, and genetic counseling for monogenic early onset inflammatory bowel disease.

Testing Algorithm

Skin biopsy:

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

Cord blood:

For cord blood specimens that have an accompanying maternal blood specimen, maternal cell contamination studies will be performed at an additional charge.

For more information see Inflammatory Bowel Disease Diagnostic Testing Algorithm.

Special Instructions

- 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
- Early Onset Inflammatory Bowel Disease Patient Information
- Targeted Genes and Methodology Details for Early Onset Monogenic Inflammatory Bowel Disease (IBD) Gene Panel
- Inflammatory Bowel Disease Diagnostic Testing Algorithm

Method Name

Sequence Capture and Amplicon-Based Next-Generation Sequencing (NGS), Polymerase Chain Reaction (PCR), and Droplet Digital Polymerase Chain Reaction (ddPCR)/Quantitative Real-Time Polymerase Chain Reaction (qPCR) and Sanger Sequencing as needed

NY State Available

Yes

Specimen

Specimen Type

Varies

Ordering Guidance

Patients who have had a previous bone marrow transplant from an allogenic donor should not have testing performed on blood, bone marrow, or saliva because any results generated will reflect the genome of the donor rather than the recipient. Testing on patients who have an active hematologic malignancy or hematologic disorder with clonal proliferation may identify both somatic mutations and germline variants, which may result in test failure or necessitate follow-up testing to determine whether the detected variant is germline or somatic. For these patients, testing a skin biopsy or cultured fibroblasts is recommended. For instructions for testing patients who have received a bone marrow transplant or have an active hematologic disorder, call 800-533-1710. For more information see Cautions.

Customization of this panel and single gene analysis for any gene present on this panel are available. For more information see CGPH / Custom Gene Panel, Hereditary, Next-Generation Sequencing, Varies. To modify this panel via CGPH, use the Inborn Errors of Immunity/Bone Marrow Failure/Telomeropathy/Pulmonary Fibrosis/Very Early Onset IBD/Pancreatitis disease state for step 1 on the Custom Gene Ordering Tool.

Targeted testing for familial variants (also called site-specific or known variants 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.

Additional Testing Requirements

For cord blood specimens: Maternal cell contamination (MCC) studies are available. Order MATCC **/** Maternal Cell Contamination, Molecular Analysis, Varies **on both the cord blood and maternal specimens under separate order numbers**. Cord blood testing will proceed without MCC studies, but results may be compromised if MCC is present.

Specimen Required

Patient Preparation: A previous hematopoietic stem cell transplant from an allogenic donor will interfere with testing. For information about testing patients who have received a hematopoietic stem cell 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.**
3. Whole blood collected postnatal from an umbilical cord is also acceptable. See Additional Information
Specimen Stability Information: Ambient (preferred) 4 days/Refrigerated 4 days/Frozen 4 days
Additional Information:
1. Specimens are preferred to be received within 4 days of collection. Extraction will be attempted for specimens received after 4 days, and DNA yield will be evaluated to determine if testing may proceed.
2. To ensure minimum volume and concentration of DNA are met, the requested volume must be submitted. Testing may be canceled if DNA requirements are inadequate.
3. For postnatal umbilical cord whole blood specimens, maternal cell contamination studies are recommended to ensure test results reflect that of the patient tested. A maternal blood specimen is required to complete maternal cell contamination studies. Order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on both the cord blood and maternal blood specimens under separate order numbers.

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: Ambient (preferred) <24 hours/Refrigerated <24 hours
Additional Information:
1.Specimens are preferred to be received within 24 hours of collection. Culture and extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.
2.A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks are required to culture fibroblasts before genetic testing can occur.

Specimen Type: Cultured fibroblasts
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) <24 hours/Refrigerated <24 hours
Additional Information:
1. Specimens are preferred to be received within 24 hours of collection. Culture and extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.
2. A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical and Molecular Testing, Tissue. An additional 3 to 4 weeks are required to culture fibroblasts before genetic testing can occur.

Specimen Type: Extracted DNA
Container/Tube:
Preferred: Screw Cap Micro Tube, 2 mL with skirted conical base
Acceptable: Matrix tube, 1 mL
Collection Instructions:
1. The preferred volume is at least 100 mcL at a concentration of 75 ng/mcL.
2. Include concentration and volume on tube.
Specimen Stability Information: Frozen (preferred) 1 year/Ambient/Refrigerated
Additional Information: DNA must be extracted in a CLIA-certified laboratory, or equivalent, and must be extracted from a specimen type listed as acceptable for this test (including applicable anticoagulants). Our laboratory has experience with Chemagic, Puregene, Autopure, MagnaPure, and EZ1 extraction platforms and cannot guarantee that all extraction methods are compatible with this test. If testing fails, one repeat will be attempted, and if unsuccessful, the test will be reported as failed and a charge will be applied. If applicable, specific gene regions that were unable to be interrogated due to DNA quality will be noted in the report.

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 filter paper or blood spot collection card
Specimen Volume: 2 to 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 a Dried Blood Spot Sample.
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. Blood spot specimens are acceptable but not recommended. Multiple extractions will be required to obtain sufficient yield for supplemental analysis, and there is significant risk for test failure due to insufficient DNA.
2. Due to lower concentration of DNA yielded from blood spot, it is possible that additional specimen may be required to complete testing.
3. For collection instructions, see Blood Spot Collection Instructions.
4. For collection instructions in Spanish, see Blood Spot Collection Card-Spanish Instructions (T777).
5. 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:

DNA Saliva Kit High Yield (T1007)
Saliva Swab Collection Kit (T786)

Container/Tube:

Preferred: High-yield DNA saliva kit
Acceptable: Saliva swab
Specimen Volume: 1 Tube if using T1007 or 2 swabs if using T786
Collection Instructions: Collect and send specimen per kit instructions.
Specimen Stability Information: Ambient (preferred) 30 days/Refrigerated 30 days
Additional Information: Saliva specimens are acceptable but not recommended. Due to lower quantity/quality of DNA yielded from saliva, some aspects of the test may not perform as well as DNA extracted from a whole blood sample. When applicable, specific gene regions that were unable to be interrogated will be noted in the report. Alternatively, 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 in:
-Informed Consent for Genetic Testing (T576)
-Informed Consent for Genetic Testing (Spanish) (T826)
2. Early Onset Inflammatory Bowel Disease Patient Information
3. If not ordering electronically, complete, print, and send a Gastroenterology and Hepatology Test Request (T728) 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

Inflammatory bowel disease (IBD) is a term encompassing numerous disorders featuring chronic intestinal inflammation. These conditions are typically classified as either Crohn disease or ulcerative colitis based on clinical features, colonoscopy findings, histologic changes, and the anatomical distribution of disease. However, some cases are not readily classified or may have overlapping features and are classified as IBD-unspecified (IBD-U). The incidence of IBD has rapidly increased in children and adults over the past few decades. Common symptoms include diarrhea, abdominal pain, fatigue, and failure to thrive or unintentional weight loss.

Inflammatory bowel disease is caused by a combination of dysregulated immune response, microbial dysbiosis, and environmental triggers and occurs in individuals with genetic susceptibility. Most IBD is thought to be either polygenic or multifactorial. However, in rare cases, IBD or IBD-like intestinal inflammation can be attributed to disease-causing variants in a single gene (ie, monogenic inheritance) that result in a highly penetrant condition that presents early in life. Many monogenic forms of IBD are disorders of immune deficiency or dysregulation. Genes associated with IBD continue to be identified with advances in sequencing technology. However, 70% to 80% of patients have IBD without a known genetic etiology.

While the peak age of onset of IBD is between the ages of 20 and 40 years, the incidence of IBD in pediatric patients is increasing. When IBD presents in children younger than 6 years, it is described as very early onset IBD (VEO-IBD). IBD that presents in children younger than 2 years is described as infantile-onset IBD. VEO-IBD differs from IBD in older patients in that it is more likely to be IBD-U and have a monogenic cause, particularly among those with infantile-onset IBD.

Conditions associated with VEO-IBD can be grouped into the following broad, sometimes overlapping categories: disorders of general immune dysregulation (eg, IL-10 signaling defects, IPEX syndrome, STAT3 gain of function); T- and B-cell defects (eg, LRBA deficiency, CTLA4 deficiency, Wiskott Aldrich syndrome, severe combined immunodeficiency [SCID]/Omenn syndrome); phagocytic defects (eg, chronic granulomatous disease); hyper- or auto-inflammatory disorders (eg, familial Mediterranean fever, familial hemophagocytic lymphohistiocytosis); epithelial barrier dysfunction (eg, TTC7A deficiency, nuclear factor kappa B essential modulator [NEMO] deficiency); and syndromic conditions (eg, trichohepatoenteric syndrome, CHAPLE [CD55 deficiency with hyper-activation of complement, angiopathic thrombosis, and severe protein-losing enteropathy] syndrome).

Previous reports indicate patients with a monogenic form of IBD may not respond as well to conventional treatment modalities. Identification of the genetic cause of disease in these individuals is important as it may change their treatment plan. Depending on the genetic cause, targeted therapies or allogeneic hematopoietic stem cell transplantation may be beneficial. Therefore, early diagnosis and identification of the specific underlying genetic alteration is important in order to inform treatment, such as medical therapy, surgery, and stem cell transplant, and to reduce the high morbidity and mortality associated with these conditions.

Individuals with polygenic or monogenic IBD may have other family members affected with IBD. A family history of IBD is more common among those with VEO-IBD. If a monogenic cause is identified in an individual, family members may be tested for the genetic variant to assess their risk of developing IBD or to guide therapy for those who are affected.

Reference Values

An interpretive report will be provided.

Interpretation

All detected variants 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 a 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 Mayo Clinic Laboratories 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 mutations 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 nonleukoreduced 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 professionals 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 are 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 judgment.

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. Uhlig HH, Schwerd T, Koletzko S, et al. The diagnostic approach to monogenic very early onset inflammatory bowel disease. Gastroenterology. 2014;147(5):990-1007
2. Uhlig HH, Schwerd T. From genes to mechanisms. The expanding spectrum of monogenic disorders associated with inflammatory bowel disease. Inflamm Bowel Dis. 2016;22(1):202-212
3. Kelsen JR, Baldassano RN, Artis D, Sonnenberg GF. Maintaining intestinal health: the genetics and immunology of very early-onset inflammatory bowel disease. Cell Mol Gastroenterol Hepatol. 20151;1(5):462-476
4. Ouahed J, Spencer E, Kotlarz D, et al. Very early onset inflammatory bowel disease: A clinical approach with a focus on the role genetics and underlying immune deficiencies. Inflamm Bowel Dis. 2020;26(6):820-842
5. 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)405-24

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 at above 99% for single nucleotide variants, above 94% for deletions/insertions (delins) less than 40 base pairs (bp), above 95% for deletions up to 75 bp and insertions up to 47 bp. NGS and/or a polymerase chain reaction (PCR)-based quantitative method is performed to test for the presence of deletions and duplications in the genes analyzed. A supplemental PCR-based method is used to detect a large deletion in *IKBKG*, and a supplemental droplet digital PCR method is used to detect the c.75_76del;p.Tyr26Hisfs*26 (delta GT) disease-causing variant in *NCF1*.

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 Early Onset Monogenic Inflammatory Bowel Disease (IBD) Gene Panel for details regarding the targeted gene regions identified by this test.(Unpublished Mayo method)

Genes analyzed: *ADA, ADAM17, AICDA, AIRE, ALPI, ANKZF1, ARPC1B, ASAH1, BACH2, BTK, CARMIL2, CASP8, CD3G, CD40LG, CD55, COL7A1, CTLA4, CYBA, CYBB, CYBC1, DCLRE1C, DEF6, DGAT1, DKC1, DOCK8, DUOX2, EPCAM, FCHO1, FERMT1, FOXP3, G6PC (G6PC1), G6PC3, GUCY2C, HPS1, HPS3, HPS4, HPS6, ICOS, IFIH1, IKBKG, IL10, IL10RA, IL10RB, IL21, IL21R, IL2RA, IL2RB, IL2RG, IL7R, ITCH, ITGB2, JAK1, LCT, LIG4, LRBA, MALT1, MEFV, MVK, MYO5B, NCF1, NCF2, NCF4, NEUROG3, NFKBIA, NLRC4, PAX1, PCSK1, PIK3CD, PIK3R1, PLCG2, PLVAP, POLA1, RAG1, RAG2, RIPK1, RTEL1, SH2D1A, SI, SKIV2L (SKIC2), SLC10A2, SLC26A3, SLC37A4, SLC39A4, SLC51B, SLC5A1, SLC9A3, SPINT2, STAT1, STAT3, STAT5B, STIM1, STX3, STXBP2, TGFB1, TGFBR1, TGFBR2, TLR3, TNFAIP3, TRIM22, TRNT1, TTC37 (SKIC3), TTC7A, UNC45A, WAS, WIPF1, XIAP, ZAP70,* and *ZBTB24*

PDF Report

Supplemental

Day(s) Performed

Varies

Report Available

28 to 42 days

Specimen Retention Time

Whole blood: 28 days (if available); Saliva: 30 days (if available); Extracted DNA: 3 months; Blood spots: 1 year (if available)

Test Definition: EOIBD

Early Onset Monogenic Inflammatory Bowel Disease (IBD) Gene Panel, Varies

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 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

81443

88233- Tissue culture, skin, solid tissue biopsy (if appropriate)

88240- Cryopreservation (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
EOIBD	Early Onset IBD Gene Panel	105196-0

Result ID	Test Result Name	Result LOINC® Value
620121	Test Description	62364-5
620122	Specimen	31208-2
620123	Source	31208-2
620124	Result Summary	50397-9
620125	Result	82939-0
620126	Interpretation	69047-9
620127	Additional Results	82939-0
620128	Resources	99622-3
620129	Additional Information	48767-8
620130	Method	85069-3
620131	Genes Analyzed	82939-0
620132	Disclaimer	62364-5
620133	Released By	18771-6