MAYO CLINIC LABORATORIES

Test Definition: IMMAU

Inborn Errors of Immunity with Immune Dysregulation and Autoimmunity Gene Panel, Varies

Overview

Useful For

Providing a comprehensive genetic evaluation for patients with a personal or family history suggestive of an inborn error of immunity (IEI) associated with immune dysregulation or autoimmunity

Establishing a diagnosis of an IEI, allowing for appropriate management and surveillance for disease features based on the gene and/or variant involved

Identifying variants within genes known to be associated with immune dysregulation or autoimmunity, 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 30 genes associated with immune dysregulation and autoimmunity: *AIRE, BACH2, CARD11, CASP10, CASP8, CD3G, CTLA4, DEF6, FADD, FASLG, FERMT1, FOXP3, IL10, IL10RA, IL10RB, IL2RA, IL2RB, ITCH, JAK1, LRBA, ORAI1, PEPD, PRKCD, RIPK1, STAT3, STAT5B, STIM1, TET2, TGFB1,* and *TPP2*. See Targeted Genes and Methodology Details for Inborn Errors of Immunity with Immune Dysregulation and Autoimmunity Gene Panel and Method Description for additional details.

Identification of a disease-causing variant may assist with diagnosis, prognosis, clinical management, recurrence risk assessment, familial screening, and genetic counseling for inborn errors of immunity with immune dysregulation and autoimmunity.

Testing Algorithm

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.

Special Instructions

- Informed Consent for Genetic Testing
- Informed Consent for Genetic Testing (Spanish)
- Targeted Genes and Methodology Details for Inborn Errors of Immunity with Immune Dysregulation and Autoimmunity Gene Panel
- Inborn Errors of Immunity, Autoimmunity, and Autoinflammatory Disease Patient Information

Method Name

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

NY State Available

Yes

Specimen

Specimen Type

Varies

Ordering Guidance

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.

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. Call 800-533-1710 for instructions for testing patients who have received a bone marrow transplant.

Submit only 1 of the following specimens:

Specimen Type: Whole blood
Container/Tube:
Preferred: Lavender top (EDTA) or yellow top (ACD)
Acceptable: Any anticoagulant
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

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 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)/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.

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: Congenital Inherited Diseases Patient Information (T521)
3. Inborn Errors of Immunity, Autoimmunity, and Autoinflammatory Disease Patient Information

Specimen Minimum Volume

Blood: 1 mL; Skin biopsy or cultured fibroblasts: 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

Primary immunodeficiencies or inborn errors of immunity (IEI) were originally defined by an increased risk of infections. Now it is clear that these diseases can also present with autoimmunity, autoinflammation, atopy, lymphoproliferation or malignancy, and infections are not always the leading cause of morbidity and mortality. This gene panel includes IEI with presentations characterized by autoimmunity. Examples of conditions where this gene panel is useful include immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome and other regulatory T-cell (Treg) defects; autoimmune polyendocrinopathy with candidiasis and ectodermal dystrophy (APECED or APS-1); and lymphoproliferation, solid organ autoimmunity, recurrent infections associated with gain-of-function *STAT3* defects.

The development of autoimmune diseases can be caused by the dysregulation of the immune system, leading to defects in regulatory mechanisms that normally control the immune response. Thymic selection is critical for T-cell development and includes positive and negative selection of the maturing T cells. The positive selection ensures that mature T cells can recognize antigen-presenting molecules and thus carry out their function, whereas the negative selection eliminates developing T cells that are strongly autoreactive, including T cells directed against tissue-restricted antigens. The autoimmune regulator (AIRE) is responsible for intrathymic presentation of tissue-restricted antigens that would otherwise not be expressed in the thymus, and their absence in the thymus would allow the development of autoreactive T cells against these tissue-restricted antigens. Variants in the *AIRE* gene cause APECED because the self-antigens are not properly expressed in the thymus.

IPEX syndrome is characterized by systemic autoimmunity presenting in infancy. It typically presents with the triad of enteropathy (watery diarrhea), endocrinopathy (eg, insulin-dependent diabetes mellitus), and eczematous dermatitis. IPEX is caused by defects in the transcription factor *FOXP3*, which is required for the development of regulatory T cells. The regulatory (suppressive) actions of Tregs control autoimmunity. Tregs have different suppressive mechanisms, including cell contact-mediated cytotoxicity, sequestration of interleukin (IL)-2, and cytokine-mediated inhibition. Defects in the genes encoding these suppressive cytokines and cytokine receptors (eg, IL-10, IL-10 receptor alpha and beta, or transforming growth factor-beta [TGF-beta]) also lead to autoimmune manifestations. Cell-to-cell contact of membrane-bound molecules, such as CTLA-4, can transmit an inhibitory signal. In the absence of the inhibitory signal, CTLA-4 deficiency can manifest with recurrent infections, inflammatory bowel disease, in addition to autoimmunity. Increased (gain) of function in STAT3 signaling also decreases Treg numbers and function, leading to recurrent infections, lymphoproliferation, and, mainly, solid organ autoimmunity, such as thyroiditis, insulin-dependent diabetes mellitus, as well as autoimmune cytopenias.

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 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. Refer to the Targeted Genes and Methodology Details for Inborn Errors of Immunity with Immune Dysregulation and Autoimmunity Gene Panel for the most up to date list of genes included in this test. 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 non-leukoreduced 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 health care 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 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. Richards S, Aziz N, Bale S, et al; ACMG Laboratory Quality Assurance Committee: 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 May;17(5):405-424
2. Tangye SG, Al-Herz W, Bousfiha A, et al: Human inborn errors of immunity: 2022 update on the classification from the International Union of Immunological Societies Expert Committee. J Clin Immunol. 2022 Oct;42(7):1473-1507. doi: 10.1007/s10875-022-01289-3
3. Azizi G, Yazdani R, Rae W, et al: Monogenic polyautoimmunity in primary immunodeficiency diseases. Autoimmun Rev. 2018 Oct;17(10):1028-1039. doi: 10.1016/j.autrev.2018.05.001
4. Baxter SK, Walsh T, Casadei S, et al: Molecular diagnosis of childhood immune dysregulation, polyendocrinopathy, and enteropathy, and implications for clinical management. J Allergy Clin Immunol. 2022 Jan;149(1):327-339. doi: 10.1016/j.jaci.2021.04.005
5. Cepika AM, Sato Y, Liu JM, Uyeda MJ, Bacchetta R, Roncarolo MG: Tregopathies: Monogenic diseases resulting in regulatory T-cell deficiency. J Allergy Clin Immunol. 2018 Dec;142(6):1679-1695. doi: 10.1016/j.jaci.2018.10.026
6. Consonni F, Favre C, Gambineri E: IL-2 signaling axis defects: How many faces? Front Pediatr. 2021 Jul 2;9:669298. doi: 10.3389/fped.2021.669298
7. Bjorklund G, Pivin M, Hangan T, Yurkovskaya O, Pivina L: Autoimmune polyendocrine syndrome type 1: Clinical manifestations, pathogenetic features, and management approach. Autoimmun Rev. 2022 Aug;21(8):103135. doi: 10.1016/j.autrev.2022.103135

Performance

Method Description

Next-generation sequencing (NGS) and/or Sanger sequencing are 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), and above 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.(Unpublished Mayo method)

See Targeted Genes and Methodology Details for Inborn Errors of Immunity with Immune Dysregulation and Autoimmunity Gene Panel for details regarding the targeted genes analyzed for each test and specific gene regions not routinely covered.

Confirmation of select reportable variants may be performed by alternate methodologies based on internal laboratory criteria.

Genes analyzed: *AIRE, BACH2, CARD11, CASP10, CASP8, CD3G, CTLA4, DEF6, FADD, FASLG, FERMT1, FOXP3, IL10, IL10RA, IL10RB, IL2RA, IL2RB, ITCH, JAK1, LRBA, ORAI1, PEPD, PRKCD, RIPK1, STAT3, STAT5B, STIM1, TET2, TGFB1,* and *TPP2*

PDF Report
Supplemental

Day(s) Performed
Varies

Report Available
28 to 42 days

Specimen Retention Time
Whole blood: 2 weeks (if available); Extracted DNA: 3 months; Cultured fibroblasts, skin biopsy: 1 month

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.

Test Definition: IMMAU

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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
IMMAU	Dysregulation/Autoimmune GenePanel	103741-5

Result ID	Test Result Name	Result LOINC® Value
619859	Test Description	62364-5
619860	Specimen	31208-2
619861	Source	31208-2
619862	Result Summary	50397-9
619863	Result	82939-0
619864	Interpretation	69047-9
619865	Additional Results	82939-0
619866	Resources	99622-3
619867	Additional Information	48767-8
619868	Method	85069-3
619869	Genes Analyzed	82939-0
619870	Disclaimer	62364-5
619871	Released By	18771-6