MAYO CLINIC LABORATORIES

NEW TEST

Notification Date: December 17, 2024
Effective Date: December 17, 2024

Panel to Whole Exome Sequencing Reflex Test, Varies

Test ID: WESPR

Useful for:

- Serving as a second-tier test for patients in whom previous genetic testing was negative or inconclusive
- Identifying causative variants in genes that were not included on panel testing which can allow for:
  -Better understanding of the natural history/prognosis
  -Targeted management (anticipatory guidance, management changes, specific therapies)
  -Predictive testing of at-risk family members
  -Testing and exclusion of disease in siblings or other relatives
  -Recurrence risk assessment
- Additionally, this testing may be useful in the context of a patient's evolving clinical features.

Genetics Information:

- Whole exome sequencing utilizes next-generation sequencing (NGS) to detect variants within the protein-coding regions of approximately 20,000 genes. In patients who have had negative or inconclusive postnatal hereditary gene panel testing, analysis of previously generated sequencing data, expanded to include the whole exome, has the potential to identify new or additional variants associated with the patient's phenotype and increase the diagnostic yield of testing.
- This test is available for patients who have had hereditary panel testing performed on a postnatal sample via NGS utilizing the Integrated DNA Technologies chemistry performed by Mayo Clinic Laboratories and would like to reflex to whole exome sequencing. Most panels performed at Mayo Laboratories since 2023 are eligible for this test to be added. In addition, some panels performed between 2021 and 2023 are eligible. To confirm that it is possible to add this test for a specific patient, contact the laboratory at 800-533-1710.
- It is highly recommended that testing is performed alongside specimens submitted from the patient's biological mother and the patient's biological father as part of a trio analysis. However, testing for singletons (patient only), duos (patient and one relative to be used as a comparator), and nontraditional trios (patient and two relatives to be used as comparators) will also be accepted if the patient's biological mother and biological father are not available for testing.
- This test may be ordered by the individual who ordered the original hereditary NGS panel or by a new healthcare professional if the patient is currently under their care. Results will be sent only to the individual who placed the current order.

Highlights:

This test expands variant analysis from the targeted regions initially evaluated in panel testing previously performed by Mayo Clinic Laboratories using next-generation sequencing (NGS) to include protein-coding

regions of approximately 20,000 genes (the exome). Reflexing to whole exome sequencing offers a potentially cost-effective alternative to establishing a molecular diagnosis compared to performing multiple independent molecular assays.

Ordering Guidance:

- The American College of Medical Genetics and Genomics recommends that whole exome sequencing be considered as a first-tier or second-tier test for patients with one or more congenital anomalies, or developmental delay, or intellectual disability with onset prior to age 18 years.
- This test is only appropriate for patients who have had hereditary panel testing performed on a postnatal sample via next-generation sequencing (NGS) utilizing the Integrated DNA Technologies chemistry performed by Mayo Clinic Laboratories. To confirm that this test is possible for a specific patient, contact the laboratory at 800-533-1710.
- If the patient has not had an appropriate test previously performed by Mayo Clinic Laboratories that can be reflexed but whole exome sequencing is desired, order either WESDX / Whole Exome Sequencing for Hereditary Disorders, Varies or WESMT / Whole Exome and Mitochondrial Genome Sequencing, Varies. If whole genome sequencing is desired, order WGSDX / Whole Genome Sequencing for Hereditary Disorders, Varies. A new specimen may be required.
- This test is for affected patients (probands) only. It is possible to add family member comparators. For family member specimens being sent as comparators, order CMPRE / Family Member Comparator Specimen for Exome Sequencing, Varies. If WESPR is ordered on a family member comparator, this test will be canceled and CMPRE added as the appropriate test.
- This test cannot support detection of deep intronic variants, trinucleotide repeat variants, or variants in the mitochondrial genome.
- If separate mitochondrial genome testing is needed, order MITOP / Mitochondrial Full Genome Analysis, Next-Generation Sequencing (NGS), Varies
- This test is not appropriate for identification of somatic variants in solid tumors. If this testing is needed, order MCSTP / MayoComplete Solid Tumor Panel, Next-Generation Sequencing, Tumor. A new specimen may be required.
- This testing does not provide genotyping of patients for pharmacogenomic purposes. For an assessment for genes with strong drug-gene associations, order PGXQP / Focused Pharmacogenomics Panel, Varies. A new specimen may be required.
- Targeted testing for familial variants (also called site-specific or known variant testing) is available for variants identified by this test. See FMTT / Familial Variant, Targeted Testing, Varies. To obtain more information about this testing option, call 800-533-1710.
- Prenatal specimens (amniocentesis or chorionic villi) are not currently accepted for this test.

Reflex Tests:

Test ID	Reporting Name	Available Separately	Always Performed
CULFB	Fibroblast Culture for Genetic Test	Yes	No
MATCC	Maternal Cell Contamination, B	Yes	No
G237	Panel to Whole Exome Sequencing Reflex Test, Varies	No	No

Testing Algorithm:

- If a cord blood specimen is received, maternal cell contamination testing will be added and performed at an additional charge.
- 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.

Methods:

Reanalysis of Whole Exome Next-Generation Sequencing (NGS) followed by Sanger Sequencing or Quantitative Polymerase Chain Reaction (qPCR), as needed

Reference Values:

An interpretive report will be provided.

Specimen Requirements:

For most patients, a new specimen submission will not be required. Testing can be performed using stored DNA from the original whole exome sequencing test. To order testing on the stored specimen, see Additional Testing Requirements.

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.

Specimen Type:	**Whole blood**
Container/Tube:	Lavendar 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 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 samples 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 is met, the preferred volume of blood must be submitted. Testing may be canceled if DNA requirements are inadequate.
Minimum Volume:	1 mL

Specimen Type:	**Cord blood**
Preferred:	Lavendar top (EDTA) or yellow top (ACD)
Acceptable:	Green top (sodium heparin)
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 4 days
Additional Information:	1. Specimens are preferred to be received within 4 days of collection. Extraction will be attempted for samples received after 4 days and DNA yield will be evaluated to determine if testing may proceed. 2. If a cord blood specimen is received, MATCC / Maternal Cell Contamination, Molecular Analysis, Varies will be performed at an additional charge.

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/or 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 is required to culture fibroblasts before genetic testing can occur.

Specimen Type:	**Cultured fibroblasts**
Source:	Skin
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/or 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 is required to culture fibroblasts before genetic testing can occur.

Specimen Type:	**Blood spot**
Supplies:	Card-Blood Spot Collection (Filter Paper) (T493)
Preferred:	Collection card (Whatman Protein Saver 903 Paper)
Acceptable:	PerkinElmer 226 (formally Ahlstrom 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 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. 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:	Saliva Collection Kit (T786)
Specimen Volume:	1 Swab
Collection Instructions:	Collect and send specimen per kit instructions.
Specimen Stability Information:	Ambient (preferred) 30 days/Refrigerated 30 days
Additional Information:	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.

Additional Testing Requirements:

Patient DNA is required to allow for confirmation of any new reportable variants, based on internal laboratory criteria. For most patients, stored DNA from the original panel test should be available for this testing. If a DNA sample is depleted or discarded, testing will proceed, however, a new sample will be requested from the ordering provider to attempt any necessary confirmatory testing. If a new sample is not provided, any findings that require confirmation will be reported with a disclaimer that confirmation was not performed due to lack of a DNA specimen.

To order whole exome sequencing for the patient and family member comparator specimens after a negative or inconclusive hereditary next-generation sequencing gene panel performed at Mayo Clinic Laboratories, perform the following steps:

1. Order WESPR / Panel to Whole Exome Sequencing Reflex Test, Varies

2. Call Mayo Clinic Laboratories at 800-533-1710 to request that the remaining DNA specimen be added to the WESPR order.
3. Complete the required paperwork and informed consent: Whole Exome Sequencing: Ordering Checklist.
4. Attach clinic notes from specialists relevant to patient's clinical features, if available.
5. Attach pedigree, if available.
6. If submitting family member comparator samples, order CMPRE / Family Member Comparator Specimen for Exome Sequencing, Varies for each family member.
 a. When available, the patient's biological mother and biological father are the preferred family member comparators.
 b. If one or both of the patient's biological parents are not available for testing, specimens from other first-degree relatives (siblings or children) can be used as comparators. Contact the laboratory at 800-533-1710 for approval to send specimens from other, non-first-degree relatives.
 c. The cost of analysis for family member comparator specimens is applied to the patient's (proband's) test. Family members will not be charged separately.
7. If needed, collect specimens. Label specimens with full name and birthdate. **Do not** label family members' specimens with the proband's name.
8. Send paperwork to the laboratory along with the specimens. If not sent with the specimen or if no specimen is being sent, fax a copy of the paperwork to 507-284-1759, Attn: WES Genetic Counselors.

For more information see Whole Exome and Genome Sequencing Information and Test Ordering Guide.

Necessary Information:

Whole Exome Sequencing: Ordering Checklist is required. Fill out one form for the family and send with the specimens or fax to 507-284-1759, Attn: WES Genetic Counselors.

Specimen Stability Information:

Specimen Type	Temperature	Time	Special Container
Varies	Varies		

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.
- 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:

- Whole exome 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. 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 applicable) 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.

- If a DNA specimen is no longer available for confirmation, Mayo clinic laboratory will contact the client and ask that they submit a new sample to be used for this purpose. If no sample has been received by the time testing is ready to be reported, results will be reported with a disclaimer that confirmation studies were not performed.
- This test is not designed to detect low levels of mosaicism or 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.
- If the patient has had an allogeneic hematopoietic stem cell transplant or a recent 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:

- The classification of all previously reported variants will be reassessed at the time of reporting. Once reported, 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 are performed using published American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology recommendations as a guideline. 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 outside of the genes recommended by the ACMG may implicate another predisposition or presence of active disease. These findings will be carefully reviewed to determine whether they will be reported.

Data Sharing:

- Deidentified variant information may be shared in public genetic databases, such as GeneMatcher or ClinVar

CPT Code:

81417-Patient only
81417, 81416-Patient and one family member comparator sample (duo) (as appropriate)
81417, 81416 x 2-Patient and two family member comparator samples (trio or non-traditional trio) (as appropriate)
81417, 81416 x 3-Patient and three family member comparator samples (quad) (as appropriate)

Day(s) Performed: Varies **Report Available:** 10 weeks

Questions

Contact Michelle Raths, Laboratory Resource Coordinator at 800-533-1710.