Test Definition: WESDX

Whole Exome Sequencing for Hereditary Disorders, Varies

Overview

Useful For

Serving as a first-tier test to identify a molecular diagnosis in patients with suspected genetic disorders, 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

Serving as a second-tier test for patients in whom previous genetic testing was negative

Providing a potentially cost-effective alternative to establishing a molecular diagnosis compared to performing multiple independent molecular assays

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
CULAF	Amniotic Fluid Culture/Genetic Test	Yes	No
G226	Number of Comparators for WESDX	No, (Bill Only)	No
MATCC	Maternal Cell Contamination, B	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

Genetics Test Information

This test utilizes next-generation sequencing to detect single nucleotide and copy number variants within the protein-coding regions of approximately 20,000 genes. See 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.

It is highly recommended that specimens are submitted from the patient (proband), the patient's biological mother, and the patient's biological father (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.

Additional first-tier testing may be considered/recommended. For more information see the Ordering Guidance section.

Prior Authorization is available for this assay.

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 testing will be performed at an additional charge. If viable cells are not obtained, the client will be notified.

For more information see Epilepsy: Unexplained Refractory and/or Familial Testing Algorithm

Special Instructions

- Whole Exome Sequencing: Ordering Checklist
- Hereditary Peripheral Neuropathy Diagnostic Algorithm
- Blood Spot Collection Card-Spanish Instructions
- Blood Spot Collection Card-Chinese Instructions
- Epilepsy: Unexplained Refractory and/or Familial Testing Algorithm
- Blood Spot Collection Instructions
- Whole Exome Sequencing for Hereditary Disorders (WESDX) Prior Authorization Ordering Instructions
- Whole Exome and Genome Sequencing Information and Test Ordering Guide

Highlights

Additional information is available; see Whole Exome and Genome Sequencing Information and Test Ordering Guide.

Method Name

Sequence Capture and Targeted Next-Generation Sequencing followed by Sanger Sequencing or Quantitative Polymerase Chain Reaction (qPCR), as needed.

NY State Available

Yes

Specimen

Specimen Type

Varies

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.(1)

If a specific diagnosis is suspected, single gene or panel testing may be a more appropriate first-tier testing option.

This test is for affected patients (probands) only. For family member specimens being sent as comparators, order CMPRE / Family Member Comparator Specimen for Exome Sequencing, Varies. If this test is ordered on a family member comparator, this test will be canceled and CMPRE performed as the appropriate test.

This test cannot support detection of deep intronic variants, trinucleotide repeat variants, or variants in the mitochondrial genome.
-For whole exome sequencing plus analysis of the mitochondrial genome, order WESMT / Whole Exome and Mitochondrial Genome Sequencing, Varies.
-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.

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.

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.

Additional Testing Requirements

To order whole exome testing for the patient and the family member comparator specimens, see the following steps:
1. Order this test on the patient (proband).
2. Order CMPRE / Family Member Comparator Specimen for Exome Sequencing, Varies on all family members' specimens being submitted as comparators.
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 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.
3. Collect patient (proband) and family member specimens. Label specimens with full name and birthdate. Do not label family members’ specimens with the proband’s name.
4. Complete the signature sections of the Informed Consent (required for New York State clients) portion of Whole Exome Sequencing: Ordering Checklist.
5. If the patient wishes to opt-out of receiving secondary findings or change the DNA storage selection, select the

appropriate boxes in the Informed Consent section.
6. Attach clinic notes from specialists relevant to patient's clinical features, if available.
7. Attach pedigree, if available.
8. Send paperwork to the laboratory along with the specimens. If not sent with the specimen, 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.

Shipping Instructions

Specimen preferred to arrive within 96 hours of collection.

Necessary Information

1. Whole Exome Sequencing: Ordering Checklist is required. Fill out one form for the family and send with the specimens.
2. Prior Authorization is available, **but not required**, for this test. If proceeding with the prior authorization process, submit the required form with the specimen.

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)/Refrigerated
Additional Information: 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: 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 (Filtration 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. **Whole Exome Sequencing: Ordering Checklist is required.**
2. **New York Clients-Informed consent is required, included in the above form.** Document on the request form or electronic order that a copy is on file.
3. Whole Exome Sequencing (WESDX) Prior Authorization Ordering Instructions
4. If not ordering electronically, complete, print, and send 1 of the following forms with the specimen:

-Neurology Specialty Testing Client Test Request (T732)
-Biochemical Genetics Test Request (T798)

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	Ambient (preferred)		
	Refrigerated		
	Frozen		

Clinical & Interpretive

Clinical Information

This test uses next-generation sequencing technology to assess patients with suspected underlying genetic disorders for single nucleotide and copy number variants within the protein-coding regions (exons and splice junctions) of approximately 20,000 genes simultaneously. Indications for whole exome sequencing include but are not limited to:(1,2)
-Patients with one or more congenital anomalies
-Patients with developmental delay or intellectual disability with onset prior to age 18 years
-Patients with a phenotype and/or family history that strongly suggests an underlying genetic cause, yet genetic tests for that phenotype have failed to arrive at a diagnosis (diagnostic odyssey)
-Patients with a phenotype and/or family history that strongly suggests an underlying genetic cause, but the phenotype does not fit with one specific disorder (numerous individual genetic tests would be required for evaluation)
-Patients with a suspected genetic disorder that has numerous underlying genetic causes, making analysis of numerous genes simultaneously a more practical approach than single-gene testing (condition is genetically heterogeneous)
-Patients with a suspected genetic disorder for which specific molecular genetic testing is not yet available
-Patients with an atypical presentation of a genetic disorder

It is highly recommended that specimens are also submitted from the patient's biological mother and biological father, which are used for comparison purposes (trio analysis). Based upon published reports, a diagnosis is identified in trio-based whole exome sequencing (WES) in approximately 25% to 37% of cases, with slightly lower diagnostic yield in non-trio WES.(3-5) However, testing for singletons (patient only), duos (patient and one family member to be used as a comparator), and non-traditional trios (patient and two family members to be used as comparators) will also be accepted if both biological parents are unavailable.

Reference Values

An interpretive report will be provided.

Interpretation

Variants of interest are evaluated according to American College of Medical Genetics and Genomics (ACMG) recommendations.(6) Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance.

Variants are reported in one of the following categories:
-Likely Causative: variants with a high degree of suspicion for causing the patient's reported clinical features
-Possibly Relevant: variants that may be related to the patient's clinical features or variants in genes of uncertain significance (GUS)
-Secondary Findings: medically actionable variants unrelated to the indication for testing (see below for additional information)

It is possible that a variant may not be recognized as the underlying cause of disease due to incomplete scientific knowledge about the function of all genes in the human genome and/or the impact of variants in those genes.

Secondary Findings:
Patients are evaluated for medically actionable secondary findings and these findings are reported in accordance with the ACMG recommendations.(7) Variants in these genes will not be evaluated or reported if the patient opts out of this evaluation unless they overlap with the patient's reported clinical features.

The presence of a variant in family member comparator samples is stated on the patient's (proband's) report unless family members opt-out of secondary findings. If the patient (proband) opts out, secondary findings will not be reported for any family member. Variants that are present in family member comparators but absent from the patient (proband) are not evaluated.

The absence of a reportable secondary finding does not guarantee that there are no disease-causing or likely disease-causing variants in these genes, as portions of the genes may not be adequately covered by this testing methodology.

Reanalysis and Raw Data Requests:
Patient data is not guaranteed to be stored indefinitely. Requests for reanalysis or release of raw data may not be possible, and a new whole exome sequencing order may be required if the original patient data is no longer available or no longer compatible with current bioinformatics processes or analysis tools.

Healthcare providers may contact the laboratory at 800-533-1710 to request reanalysis of the patient's exome due to new patient clinical features, advances in genetic knowledge, or changes in testing methodology. A charge may apply for reanalysis.

Requests for the raw data obtained from whole exome sequencing should be directed to the laboratory. A separate fee may apply. Raw data will be released for individuals who complete a Mayo Clinic release of information form. If raw data for family member comparators is requested, it will only be released with an accompanying request for the proband's raw data. Contact the laboratory for instructions on completing the release of information form. The laboratory is not responsible for providing software or other tools needed to visualize, filter, or interpret this data.

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 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 detects multi-exon deletions/duplications; however, in some instances, single exon resolution can be achieved. The reliability of detection can be variable 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.

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:
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 are performed using published American College of Medical Genetics and Genomics and the Association for Molecular Pathology recommendations as a guideline (ACMG).(6) 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 findings 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 ClinVar and Matchmaker Exchange.

Clinical Reference

1. Manickam K, McClain MR, Demmer LA, et al: Exome and genome sequencing for pediatric patients with congenital anomalies or intellectual disability: an evidence-based clinical guideline of the American College of Medical Genetics and Genomes (ACMG). Genet Med. 2021 Nov;23(11):2029-2037. doi: 10.1038/s41436-021-01242-6
2. ACMG Board of Directors: Points to consider for informed consent for genome/exome sequencing. Genet Med. 2013;15(9):748-749. doi: 10.1038/gim.2013.94
3. Yang Y, Muzny DM, Xia F, et al: Molecular findings among patients referred for clinical whole-exome sequencing. JAMA. 2014 Nov;312(18):1870-1879. doi: 10.1001/jama.2014.14601
4. Lee H, Deignan JL, Dorrani N, et al: Clinical exome sequencing for genetic identification of rare Mendelian disorders. JAMA. 2014 Nov;312(18):1880-1887. doi: 10.1001/jama.2014.14604
5. Farwell KD, Shahmirzadi L, El-Khechen D, et al: Enhanced utility of family-centered diagnostic exome sequencing with inheritance model-based analysis: results from 500 unselected families with undiagnosed genetic conditions. Genet Med. 2015 Jul;17(7):578-586. doi: 10.1038/gim.2014.154
6. 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 May;17(5):405-24. doi: 10.1038/gim.2015.30
7. Miller DT, Lee K, Gordon AS, et al: Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2021 update: a policy statement of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2021Aug;23(8):1391-1398. doi: 10.1038/s41436-021-01171-4

Performance

Method Description

Next-generation sequencing (NGS) is performed on DNA extracted from the patient and all submitted comparator samples to test for the presence of variants in coding regions and intron/exon boundaries. The human genome reference GRCh37/hg19 build is used for sequence read alignment. Variants are called using an optimized bioinformatics package. 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 deletion-insertions (delins) less than 40 base pairs (bp), above 95% for deletions up to 75 bp, and insertions up to 47 bp. This assay also detects most copy number variants (deletions/duplications)

involving three or more exons. In some instances, copy number variants less than three exons may be detected, however, the reliability of this detection is variable due to isolated reduction in sequence coverage or inherent genomic complexity. Resulting variants are filtered and annotated using public and proprietary resources and presented for analysis and interpretation using a vended interpretation tool. Confirmation of select reportable variants in the proband and submitted comparator samples may be performed by alternate methodologies based on internal laboratory criteria.

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)

PDF Report
Supplemental

Day(s) Performed
Varies

Report Available
84 days

Specimen Retention Time
Whole blood: 2 weeks (if available); Extracted DNA: 3 months; Blood spots, saliva, cultured fibroblasts, skin biopsy, cord blood: 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.

CPT Code Information
81415-Patient only
81415, 81416-Patient and one family member comparator sample (duo) (as appropriate)
81415, 81416 x 2-Patient and two family member comparator samples (trio or non-traditional trio) (as appropriate)
81415, 81416 x 3-Patient and three family member comparator samples (quad) (as appropriate)

Prior Auhtorization

Insurance preauthorization is available for this testing; forms are available.

Patient financial assistance may be available to those who qualify. Patients who receive a bill from Mayo Clinic Laboratories will receive information on eligibility and how to apply.

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
WESDX	Whole Exome Sequencing	86205-2

Result ID	Test Result Name	Result LOINC® Value
616410	Interpretation	69047-9
616411	Specimen	31208-2
616412	Source	31208-2
616413	Released By	18771-6