MAYO CLINIC LABORATORIES

NEW TEST

Notification Date: December 15, 2022
Effective Date: December 20, 2022

Whole Genome Sequencing for Hereditary Disorders, Varies

Test ID: WGSDX

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
G227	Number of Comparators for WGSDX (Bill Only)	No (Bill Only)	No
MATCC	Maternal Cell Contamination, B	Yes	No

Genetics Information:

- This test utilizes next-generation sequencing to detect single nucleotide variants, small insertions and deletions, copy number variants, mitochondrial genome variants, and select repeat expansion variants throughout the genome. 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 the patient only (singleton), the patient and one first-degree relative (duo), or the patient and two first-degree relatives (nontraditional trios) will also be accepted if the patient's biological mother and biological father are not available for testing. Testing typically includes up to two family member comparators. Contact the laboratory for approval to send the patient and three first-degree relatives (quad).
- Additional first-tier testing may be considered/recommended. For more information see the Ordering Guidance section.

Methods:

Polymerase Chain Reaction-free Next-Generation Sequencing followed by Sanger Sequencing, Quantitative Polymerase Chain Reaction (qPCR), or other methods, as needed

Reference Values:
An interpretive report will be provided.

Specimen 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**
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.**
Additional Information:	If a cord blood specimen is received, MATCC / Maternal Cell Contamination Molecular Analysis, Varies, will be performed at an additional charge; maternal blood sample is required.
Minimum Volume:	1 mL

Note:
Specimen preferred to arrive within 96 hours of collection.

Necessary Information:
Whole Genome Sequencing: Ordering Checklist **Patient Information is required for all patients**.

Specimen Stability Information:

Specimen Type	Temperature	Time	Special Container
Varies	Ambient (preferred)		
	Frozen		
	Refrigerate		

Ordering Guidance:
- The American College of Medical Genetics and Genomics recommends that whole genome 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 CMPRG / Family Member Comparator Specimen for Genome Sequencing, Varies. If this test is ordered on a family member comparator, this test will be canceled and CMPRG performed as the appropriate test.
- This test is **not appropriate for** identification of somatic variants in solid tumors or other malignancies.

Multiple oncology (cancer) gene panels are available. For more information see Oncology Somatic NGS Testing Guide. If testing for other malignancies is needed, contact the laboratory for test selection guidance.

- 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 Mutation, Targeted Testing, Varies.
- **Prenatal specimens (amniocentesis or chorionic villi) are not currently accepted for this test.**

Additional Testing Requirements:

To order whole genome sequencing for the patient and the family member comparator specimens, see the following steps:

1. Order this test (WGSDX) on the patient (proband).
2. Order CMPRG / Family Member Comparator Specimen for Genome 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. Testing typically includes up to two family member comparators. Contact the laboratory at 800-533-1710 for approval to send specimens from other relatives or to send the patient and three first-degree relatives (quad).
    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. For each family, complete the following portions of the Whole Genome Sequencing: Ordering Checklist. **A separate form is not needed for each family member**.
    a. **Patient Information is required for all clients**
    b. Informed Consent is **required for New York State clients**
    c. **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.
5. Attach clinic notes from specialists relevant to patient's clinical features, if available.
6. Attach pedigree, if available.
7. Send paperwork to the laboratory along with the specimens. If not sent with the specimens, fax a copy of the paperwork to 507-284-1759, Attn: WGS Genetic Counselors.

For more information see Whole Genome Sequencing (WGS): Questions and Answers for Providers.

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 genome sequencing may not detect all types of genomic variants; therefore, false-negative results are possible. There may be regions of genes that cannot be effectively evaluated by whole genome sequencing as a result of technical limitations of the assay, including variable depth of coverage, regions of homology, and repetitive sequences. 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. In addition, in rare cases false-positive results may occur; however, false-positive events

should be exceedingly rare as confirmation of reportable variants will be performed by alternate methodologies based on internal laboratory criteria.

Single Nucleotide Variants:

- Genome-wide sensitivity for single nucleotide variants (SNV) is greater than 98% and in noncomplex regions sensitivity is greater than 99.9%.

Deletions and Insertions (less than 1000 base pairs):

- This test is validated to detect greater than 99% of deletions and insertions (delins) up to 50 base pairs (bp) for noncomplex regions. Performance in complex regions is slightly reduced with sensitivities of 92% to 99% depending on the size of the delins event. Although detected by this assay, performance for larger delins (51-999 bp) is not comprehensively established.

Copy Number Variants (greater than or equal to 1000 base pairs):

- Genome-wide sensitivity for copy number variants (CNV) detectable by chromosomal microarray is >99.9% as established by a comprehensive comparison with clinically validated nonmosaic CNV detected by chromosomal microarray.

Additional Variant Classes:

- A variety of additional variant classes can be detected by this test, including mitochondrial variants, repeat expansions, select spinal muscular atrophy (SMA)-associated variants, balanced structural rearrangements, and mosaic variants of all classes. A limited validation of each of these additional variant classes was conducted before inclusion in this assay; however, comprehensive assessment of sensitivity and false-negative rate has not been established. These variants will be evaluated per laboratory protocol, and findings of clinical relevance will be reported following confirmation with validated laboratory methods. Importantly, the sensitivity and performance for these variant classes is not expected to meet that of current gold-standard testing methodologies; therefore, additional testing may be indicated if there is clinical suspicion for a disorder involving these variant classes. Specific technical limitations for each class are described below.

Mitochondrial Genome Variants:

- This assay can detect mitochondrial genome SNV and small delins with heteroplasmy levels above 5%; however, comprehensive sensitivity/false-negative rate is not established. Detection of large deletion and duplication events involving the mitochondrial genome is not available with the current analysis. Any clinically relevant and reportable mitochondrial variants detected will be confirmed with standard validated methods prior to reporting.

Repeat Expansion Variants:

- Select short tandem repeats (STR; also known as repeat expansions) in pathogenic ranges can be detected with this assay; however, comprehensive sensitivity/false negative rate is not established. STR loci included in this assay are: *C9orf72, CSTB, ATN1, FXN, FMR1, HTT, AR, ATXN1, ATXN2, ATXN3, CACNA1A,* and *ATXN7*. Only loci overlapping the patient's (proband's) clinical features will be evaluated and reported. All repeat expansions meeting laboratory reporting criteria will be confirmed and further characterized by standard validated methods prior to reporting.

SMA Variants:

- Absence of the definitive C nucleotide in exon 7 of *SMN1* (NM_000344.3:c.840C) indicating the homozygous loss of *SMN1* exon 7 is detectable by this assay; however, comprehensive sensitivity/false-negative rate is not established. This assay does not identify *SMN1* or *SMN2* variants outside of this specific single nucleotide change. This assay does not detect *SMN1* carrier status or phase of *SMN1/SMN2* alleles. All variants will be confirmed by validated laboratory methods before reporting.

Balanced Structural Rearrangements:

- This assay does not involve comprehensive evaluation of balanced structural rearrangements (eg, translocations and inversions). However, select genomic regions may be evaluated and balanced events reported when there is a directed clinical focus (eg, known family history or specific locus of high clinical

suspicion communicated to the laboratory). Comprehensive sensitivity/false-negative rate is not established. All reported rearrangements will be confirmed by validated laboratory methods before reporting (additional specimen may be required for further characterization).

Mosaicism:

- This assay is not designed to detect mosaicism or to differentiate between somatic and germline variants. Mosaic variants may be detected; however, comprehensive limits of detection for mosaic events are not established. All mosaic variants meeting laboratory reporting criteria will be confirmed by validated laboratory methods before reporting.
- 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:

- At this time, 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) recommendations as a guideline.(9,10) 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.
- 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 ClinVar and Matchmaker Exchange.
- A genetic consultation is recommended for patients undergoing this test, both prior to testing and after results are available.

CPT Code:

81425-Patient only
81425, 81426-Patient and one family member comparator sample (duo) (as appropriate)
81425, 81426 x 2-Patient and two family member comparator samples (trio or non-traditional trio) (as appropriate)
81425, 81426 x 3-Patient and three family member comparator samples (quad) (as appropriate)

Day(s) Performed: Varies

Report Available: 12 weeks

Questions

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