

Overview

Useful For

Establishing a molecular diagnosis in individuals with features of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) and *NOTCH3*-related disorders

Identifying disease-causing variants within the *NOTCH3* gene known to be associated with CADASIL and *NOTCH3*-related disorders, 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 the *NOTCH3* gene associated with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) and other *NOTCH3*-related disorders. See Method Description for additional details.

Identification of a disease-causing variant may assist with diagnosis, prognosis, clinical management, familial screening, recurrence risk assessment, and genetic counseling for *NOTCH3*-related disorders.

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.

Special Instructions

- [Informed Consent for Genetic Testing](#)
- [Molecular Genetics: Neurology Patient Information](#)

- [Blood Spot Collection Card-Spanish Instructions](#)
- [Blood Spot Collection Card-Chinese Instructions](#)
- [Informed Consent for Genetic Testing \(Spanish\)](#)
- [Blood Spot Collection Instructions](#)

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 *NOTCH3* gene. 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:**

**Preferred:** Lavender 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/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.

**Specimen Type:** Cord blood

**Container/Tube:**

**Preferred:** Lavender 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 cord blood specimen in original tube. Do not aliquot.

**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. While a properly collected cord blood sample may not be at risk for maternal cell contamination, unanticipated complications may occur during collection. Therefore, maternal cell contamination studies are recommended to ensure the test results reflect that of the patient tested and are available at an additional charge. Order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on the maternal specimen.

**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:** 2 Swabs

**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.

**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.

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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, 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.

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:** Cultured fibroblasts

**Source:** Skin

**Container/Tube:** T-25 flask

**Specimen Volume:** 2 Flasks

**Collection Instructions:** Submit confluent cultured fibroblast cells from a skin biopsy. 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 or Molecular Testing. An additional 3 to 4 weeks are required to culture fibroblasts before genetic testing can occur.

**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:** 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.

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 \(Spanish\) \(T576\)](#)

2. [Molecular Genetics: Neurology Patient Information](#)

3. If not ordering electronically, complete, print, and send a [Neurology Specialty Testing Client Test Request \(T732\)](#) with the specimen.

Specimen Minimum Volume

See Specimen Required

Reject Due To

All specimens will be evaluated by Mayo Clinic Laboratories for test suitability.

Specimen Stability Information

| Specimen Type | Temperature | Time | Special Container |
|---------------|-------------|------|-------------------|
| Varies        | Varies      |      |                   |

Clinical & Interpretive

Clinical Information

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a hereditary small vessel disorder and common genetic cause of stroke and dementia in adults. The onset of clinical symptoms typically occurs in mid-adulthood and may include recurrent ischemic stroke and transient ischemic attacks, cognitive decline that progresses to dementia, migraines with aura, and psychiatric disturbances. Symmetric and progressive white matter hyperintensities, lacunes of presumed vascular origin, and subcortical infarcts are characteristic neuroimaging findings. Granular osmophilic material (GOM) detected by electron microscopy on skin fibroblasts is considered an indicative finding for CADASIL.

Disease-causing variants in the *NOTCH3* gene cause CADASIL. Most individuals with CADASIL inherit the condition from a parent, but rare *de novo* variants have been reported. The family history may appear negative due to variable expressivity of the condition and failure to recognize symptoms in other affected family members. Further, *NOTCH3* is comprised of repetitive epidermal growth factor-like repeat (EGFr) domains. Reported disease-causing variants typically

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result in either loss or gain of cysteine residues within EGFr domains; those impacting EGFr domains 1-6 are fully penetrant, while those impacting EGFr domains 7-34 may be associated with mild disease or incomplete penetrance.

Heterozygous disease-causing variants in *NOTCH3* also cause autosomal dominant lateral meningocele syndrome (LMS). LMS is a rare condition associated with multiple lateral meningoceles, hearing loss, developmental delay, hypotonia, joint hyperlaxity, and variable additional congenital malformations. LMS typically occurs due to a *de novo* disease-causing variant, but rare instances of inheritance from an affected parent have been reported.

**Reference Values**

An interpretive report will be provided.

**Interpretation**

All detected variants are evaluated according to American College of Medical Genetics and Genomics recommendations.<sup>(1)</sup> 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.

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.

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-leukocyte reduced blood transfusion, results may be inaccurate due to the presence of donor DNA. Call Mayo Clinic Laboratories for instructions regarding 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.<sup>(1)</sup> 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.

#### Clinical Reference

1. 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):405-424
2. Ferrante EA, Cudrici CD, Boehm M. CADASIL: new advances in basic science and clinical perspectives. Curr Opin Hematol. 2019;26(3):193-198
3. Rutten JW, Van Eijdsden BJ, Duering M, et al. The effect of NOTCH3 pathogenic variant position on CADASIL disease severity: NOTCH3 EGFr 1-6 pathogenic variant are associated with a more severe phenotype and lower survival compared with EGFr 7-34 pathogenic variant. Genet Med. 2019;21(3):676-682
4. Canalis E. The skeleton of lateral meningocele syndrome. Front Genet. 2021;11620334

#### 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 *NOTCH3* gene 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 deletion/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-based quantitative method is performed to test for the presence of deletions and duplications in the *NOTCH3* gene.

There may be regions of the *NOTCH3* gene 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.

The reference transcript for the *NOTCH3* gene is NM\_000435.3. Reference transcript numbers may be updated due to transcript re-versioning. Always refer to the final patient report for gene transcript information referenced at the time of testing. Confirmation of select reportable variants may be performed by alternate methodologies based on internal laboratory criteria.

### PDF Report

Supplemental

### Day(s) Performed

Varies

### Report Available

14 to 21 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)

### 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

81479  
88233-Tissue culture, skin, solid tissue biopsy (if appropriate)  
88240-Cryopreservation (if appropriate)

LOINC® Information

| Test ID | Test Order Name                 | Order LOINC® Value |
|---------|---------------------------------|--------------------|
| NTC3Z   | NOTCH3 Gene, Full Gene Analysis | 103950-2           |

| Result ID | Test Result Name       | Result LOINC® Value |
|-----------|------------------------|---------------------|
| 616564    | Test Description       | 62364-5             |
| 616565    | Specimen               | 31208-2             |
| 616566    | Source                 | 31208-2             |
| 616567    | Result Summary         | 50397-9             |
| 616568    | Result                 | 82939-0             |
| 616569    | Interpretation         | 69047-9             |
| 616570    | Resources              | In Process          |
| 616571    | Additional Information | 48767-8             |
| 616572    | Method                 | 85069-3             |
| 616573    | Genes Analyzed         | 82939-0             |
| 616574    | Disclaimer             | 62364-5             |
| 616575    | Released By            | 18771-6             |