Test Definition: C9ORF

C9orf72 Hexanucleotide Repeat, Molecular Analysis, Varies

Overview

Useful For

Molecular confirmation of clinically suspected cases of c9FTD/ALS, frontotemporal dementia (FTD), or amyotrophic lateral sclerosis (ALS)

Presymptomatic testing for individuals with a family history of c9FTD/ALS and a documented expansion in the *C9orf72* gene

Testing Algorithm

For more information see Inherited Motor Neuron Disease and Dementia Testing Algorithm

Special Instructions

- Informed Consent for Genetic Testing
- Hereditary Peripheral Neuropathy Diagnostic Algorithm
- Molecular Genetics: Neurology Patient Information
- Inherited Motor Neuron Disease Testing and Dementia Algorithm
- Informed Consent for Genetic Testing (Spanish)

Method Name

Polymerase Chain Reaction (PCR)

NY State Available

Yes

Specimen

Specimen Type

Varies

Specimen Required

Patient Preparation: A previous bone marrow transplant from an allogeneic donor will interfere with testing. For instructions for testing patients who have received a bone marrow transplant, call 800-533-1710.

Submit only 1 of the following specimens:

Specimen Type: Whole blood
Container/Tube: Lavender 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**.
3. Whole blood collected postnatal from an umbilical cord is also acceptable. See Additional Information.

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. For postnatal umbilical cord whole blood specimens, maternal cell contamination studies are recommended to ensure test results reflect that of the patient tested. A maternal blood specimen is required to complete maternal cell contamination studies. Order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on both the cord blood and maternal blood specimens under separate order numbers.

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) (T826)

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 at Mayo Clinic Laboratories for test suitability.

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies		

Clinical & Interpretive

Clinical Information

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease affecting the upper and lower motor neurons. The disease is characterized by progressive spasticity, muscle wasting and paralysis, typically leading to death from respiratory failure.

Frontotemporal dementia (FTD) is a dementia syndrome that predominantly involves the frontal and temporal lobes of the brain. Clinical presentation is variable and includes progressive changes in behavior and personality and language disturbances. Affected individuals may also exhibit extrapyramidal signs.

Amyotrophic lateral sclerosis and FTD are now thought to represent an overlapping spectrum of disease. Recent literature has found that approximately 40% of familial ALS, 25% of familial FTD, and 90% of familial ALS/FTD cases have a large hexanucleotide repeat (GGGGCC) expansion in a noncoding region of *C9orf72*. At lower frequency, *C9orf72* hexanucleotide repeat expansions have also been observed in individuals with sporadic ALS, FTD, and ALS/FTD. The vast majority of individuals affected with a *C9orf72*-related disorder (c9ALS, c9FTD, or c9ALS/FTD) have hexanucleotide repeat expansions in the hundreds to thousands, while unaffected individuals have repeat sizes less than 20. The significance of repeat sizes between 20 and 100 repeats is currently unclear as both healthy controls and individuals with ALS or FTD phenotypes have been reported with repeat sizes in this range.

Reference Values

Normal alleles (reference):<20 GGGGCC repeats
Indeterminate alleles: 20-100 GGGGCC repeats
Pathogenic alleles: >100* GGGGCC repeats

*The exact cutoff for pathogenicity is currently undefined. Although additional studies are needed to confirm if 100 repeats is the cutoff for pathogenicity, most individuals affected with a *C9orf72*-related disorder have C9orf72 hexanucleotide repeat expansions with hundreds to thousands of repeats.

An interpretive report will be provided.

Interpretation

The interpretive report includes an overview of the findings as well as the associated clinical significance.

Cautions

For predictive testing, it is important to first document the presence of the hexanucleotide repeat expansion in the *C9orf72* gene in an affected family member to confirm that the repeat expansion is the underlying mechanism of disease in the family.

It is strongly recommended that patients undergoing predictive testing receive genetic counseling both prior to testing and after results are available.

Predictive testing of an asymptomatic child is not recommended.

Test results should be interpreted in the context of clinical findings, family history, and other laboratory data. Errors in the interpretation of results may occur if information given is inaccurate or incomplete.

Due to somatic mosaicism, repeat size identified in the peripheral blood specimen may not reflect the repeat size in untested tissues (eg, central nervous system). In addition, a negative result does not rule out the presence of a mutation in the mosaic state that may be present but below the limit of detection of this assay (approximately 5%).

Rare sequence variants immediately downstream of the *C9orf72* repeat region may interfere with genotype results but are not expected to affect repeat-primed peaks.

Rare undocumented variants (ie, polymorphisms) in the polymerase chain reaction primer binding regions may lead to false-negative results.

This test does not assess the methylation status of the *C9orf72* gene.

Clinical Reference

1. DeJesus-Hernandez M, Mackenzie IR, Boeve BF, et al. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron. 2011;72(2):245-256
2. Renton AE, Majounie E, Waite A, et al. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron. 2011;72(2):257-268
3. Gijselinck I, Van Langenhove T, van der Zee J, et al. A C9orf72 promoter repeat expansion in a Flanders-Belgian cohort with disorders of the frontotemporal lobar degeneration-amyotrophic lateral sclerosis spectrum: a gene identification study. Lancet Neuron. 2012;11(1):54-65
4. Majounie E, Renton AE, Mok K, et al. Frequency of the C9orf72 hexanucleotide repeat expansion in patients with amyotrophic lateral sclerosis and frontotemporal dementia: a cross-sectional study. Lancet Neurol. 2012;11(4):323-330
5. Boeve BF, Boylan KB, Graff-Radford NR, et al. Characterization of frontotemporal dementia and/or amyotrophic lateral sclerosis associated with the GGGGCC repeat expansion in C9ORF72. Brain. 2012;135(Pt 3):765-783
6. van Blitterswijk M, DeJesus-Hernandez M, Niemantsverdriet E, et al. Association between repeat sizes and clinical and pathological characteristics in carriers of C9ORF72 repeat expansions (Xpansize-72): a cross-sectional cohort study. Lancet Neurol. 2013;12(10):978-988
7. Nordin A, Akimoto C, Wuolikainen A, et al. Extensive size variability of the GGGGCC expansion in C9orf72 in both neuronal and non-neuronal tissues in 18 patients with ALS or FTD. Hum Mol Genet. 2015;24(11):3133-3142
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9. Gami P, Murray C, Schottlaender L, et al. A 30-unit hexanucleotide repeat expansion in C9orf72 induces pathological lesions with dipeptide-repeat proteins and RNA foci, but not TDP-43 inclusions and clinical disease. Acta Neuropathol. 2015;130(4):599-601
10. Ng ASL, Tan EK. Intermediate C9orf72 alleles in neurological disorders: does size really matter? J Med Genet. 2017;54(9):591-597

11. Nordin A, Akimoto C, Wuolikainen A, et al. Sequence variations in C9orf72 downstream of the hexanucleotide repeat region and its effect on repeat-primed PCR interpretation: a large multinational screening study. Amyotroph Lateral Scler Frontotemporal Degener. 2017;18(3-4):256-264
12. Van Mossevelde S, van der Zee J, Cruts M, Van Broeckhoven. Relationship between C9orf72 repeat size and clinical phenotype. Curr Opin Genet Dev. 2017;44:117-124
13. Breevoort S, Gibson S, Figueroa K, Bromberg M, Pulst S. Expanding clinical spectrum of *C9ORF72*-related disorders and promising therapeutic strategies. A review. Neurol Genet. 2022;8(3):e670. 24;8(5):e200028

Performance

Method Description
A combined amplicon-length and repeat-primed polymerase chain reaction-based assay is utilized to size alleles up to approximately 145 repeats and detect expansions of GGGGCC hexanucleotide repeat region in the *C9orf72* gene.(Ida CM, Lundquist PA, Bram E, et al. Evaluation of single-tube combined amplicon-length and repeat-primed long-read PCR assay for clinical detection and characterization of *C9orf72* hexanucleotide repeat expansion. Abstract 731. 2017 ACMG Annual Clinical Genetics Meeting. Phoenix, AZ, March 23, 2017)

PDF Report
No

Day(s) Performed
Varies

Report Available
21 to 28 days

Specimen Retention Time
Whole blood: 30 days (if available); Extracted DNA: 3 months

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

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
C9ORF	C9orf72, Molecular Analysis	81846-8

Result ID	Test Result Name	Result LOINC® Value
52852	Result Summary	50397-9
52853	Result	77635-1
52854	Interpretation	69047-9
52855	Reason for Referral	42349-1
52856	Specimen	31208-2
52857	Source	31208-2
52858	Released By	18771-6
55158	Method	85069-3