

Overview

Useful For

Confirmation of a diagnosis of fragile X syndrome, fragile X tremor/ataxia syndrome, or premature ovarian insufficiency caused by expansions in the *FMR1* gene

Determination of carrier status for individuals with a family history of fragile X syndrome or X-linked intellectual disability

Prenatal diagnosis of fragile X syndrome when there is a documented *FMR1* expansion in the family

Testing Algorithm

When this test is ordered, fragile X follow-up analysis testing will be performed and charged dependent upon the reported gender of the individual and on the size of the CGG repeat found by polymerase chain reaction (PCR) analysis.

When sending in prenatal specimens: If amniotic fluid (nonconfluent cultured cells) is received, amniotic fluid culture will be added and charged separately. If chorionic villus specimen (nonconfluent cultured cells) is received, fibroblast culture for genetic test will be added and charged separately. For any prenatal specimen that is received, maternal cell contamination studies will be added.

Special Instructions

- [Molecular Genetics: Congenital Inherited Diseases Patient Information](#)
- [Informed Consent for Genetic Testing](#)
- [Informed Consent for Genetic Testing \(Spanish\)](#)

Reflex Tests

| Test Id | Reporting Name | Available Separately | Always Performed |
|---------|-------------------------------------|----------------------|------------------|
| CULFB | Fibroblast Culture for Genetic Test | Yes | No |
| CULAF | Amniotic Fluid Culture/Genetic Test | Yes | No |
| MATCC | Maternal Cell Contamination, B | Yes | No |
| FUFXS | Fragile X, Follow up Analysis | No | No |

Method Name

Polymerase Chain Reaction (PCR)-Based Assay

NY State Available

Yes

Specimen**Specimen Type**

Varies

Ordering Guidance

Due to the complexity of prenatal testing, consultation with the laboratory is required for all prenatal testing.

FMR1-methylation status cannot be assessed on chorionic villus specimens. Contact a molecular genetic counselor/consultant at 800-533-1710 to discuss the limitations of testing prior to sending a chorionic villus specimen for fragile X analysis.

Additional Testing Requirements

All prenatal specimens must be accompanied by a maternal blood specimen. Order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on the maternal specimen.

Shipping Instructions

Specimen preferred to arrive within 96 hours of collection.

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 specimen in original tube. **Do not** aliquot.

Specimen Stability Information: Ambient (preferred) 4 days/Refrigerated

Prenatal Specimens

Specimen Type: Amniotic fluid

Container/Tube: Amniotic fluid container

Specimen Volume: 20 mL

Specimen Stability Information: Refrigerated (preferred)/Ambient

Specimen Type: Chorionic villi

Container/Tube: 15-mL tube containing 15 mL of transport media

Specimen Volume: 20 mg

Specimen Stability Information: Refrigerated

Acceptable:

Specimen Type: Confluent cultured cells

Container/Tube: T-25 flask

Specimen Volume: 2 flasks

Collection Instructions: Submit confluent cultured cells from another laboratory.

Specimen Stability Information: Ambient (preferred)/Refrigerated

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 in Special Instructions:

[-Informed Consent for Genetic Testing \(T576\)](#)

[-Informed Consent for Genetic Testing-Spanish \(T826\)](#)

2. [Molecular Genetics: Congenital Inherited Diseases Patient Information \(T521\)](#) in Special Instructions

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

Reject Due To

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

Specimen Minimum Volume

Blood: 0.5 mL

Amniotic Fluid: 10 mL

Chorionic Villi: 5 mg

Specimen Stability Information

| Specimen Type | Temperature | Time | Special Container |
|---------------|--------------------|------|-------------------|
| Varies | Varies (preferred) | | |

Clinical & Interpretive

Clinical Information

Fragile X syndrome is an X-linked disorder with variable expression in males and females. In greater than 99% of affected individuals, it is caused by an expansion of the CGG trinucleotide repeat in the 5'UTR (untranslated region) of the *FMR1* gene, located on the X chromosome. This trinucleotide repeat is polymorphic in the general population, with the number of repeats ranging from 5 to 44. These normal alleles are passed from generation to generation with the number of repeats remaining constant. Small expansions, called premutations, range from 55 to 200 CGG repeats. Individuals with a premutation do not exhibit features of fragile X syndrome but are at risk for other *FMR1*-related disorders such as fragile X tremor/ataxia syndrome (FXTAS) and premature ovarian insufficiency (POI). Transmission of a premutation by a man to his daughter usually results in little or no change in the CGG repeat number. Transmission of a premutation by a woman to her son or daughter usually results in further expansion, either to a larger premutation or a full mutation. The risk for a woman with a premutation to have a child affected with fragile X syndrome by expansion to a full mutation increases with the number of CGG repeats in the premutation. Full mutations are typically greater than 200 repeats long and are associated with abnormal methylation of a region adjacent to the *FMR1* gene. This is thought

to interfere with normal *FMR1* gene expression, resulting in fragile X syndrome. There are multiple clinical phenotypes associated with expansion (premutations and full mutations) in the *FMR1* gene.

Fragile X Syndrome:

Approximately 1 in 4000 individuals are affected with fragile X syndrome. Most affected male patients exhibit moderate mental retardation with affected female patients having milder, if any, cognitive deficiency. Neuropsychiatric diagnoses, such as autism spectrum and anxiety disorders, are common. Characteristic physical features include a long face with prominent jaw, protruding ears, connective tissue abnormalities, and large testicles in postpubertal male patients.

Fragile X Tremor/Ataxia Syndrome:

FXTAS is a neurodegenerative disorder that is clinically distinct from fragile X syndrome. Both male patients and female patients with a premutation are at risk for FXTAS. However, the disorder is much less common and milder in clinical presentation than fragile X syndrome and shows a later age of onset in female patients. Clinical hallmarks of the disorder include intention tremor, gait ataxia, dementia, and neuropsychiatric symptoms. The risk for FXTAS increases as the number of CGG repeats increases, and the majority of individuals with FXTAS have CGG repeat expansions of 70 or more. Penetrance of clinical symptoms is associated with increasing age, with the majority of affected men showing symptoms between age 70 and 90.

Premature Ovarian Insufficiency:

Female patients with a premutation are at risk for increased follicular stimulating hormone (FSH) levels, early menopause, and POI. Penetrance and early onset of female reproductive symptoms correlates with increasing size of the CGG repeat and reaches its highest penetrance at approximately 80 to 90 repeats. Of note, penetrance remains stable or may even decrease at approximately 100 repeats. There is no risk for increased penetrance of the POI phenotype due to maternal or paternal inheritance of the expanded CGG repeat.

Reference Values

Normal alleles: 5-44 CGG repeats

Intermediate (grey zone) alleles: 45-54 CGG repeats

Premutation alleles: 55-200 CGG repeats

Full mutation alleles: >200 CGG repeats

An interpretive report will be provided.

Methylation status:

Unmethylated: < or =20%

Partially methylated: 21-69%

Fully methylated: > or =70%

Interpretation

An interpretive report will be provided.

Cautions

For predictive testing, it is important to first document the presence of CGG-repeat amplification in the *FMR1* gene in an affected family member to confirm that molecular expansion is the underlying mechanism of disease in the family.

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

Methylation status will not be assessed on chorionic villus specimens nor will it be assessed if the reported sex is female.

Less than 1% of individuals clinically diagnosed with fragile X syndrome do not have the CGG expansion-type mutation. These individuals may have a different type of variant within the *FMR1* gene (eg, deletion or point alteration).

Due to incomplete penetrance and variable expression of the *FMR1* expansion, this test is not reliable for prenatal assessment of disease severity.

The absence of an expansion in the *FMR1* gene does not eliminate the diagnosis of other inherited disorders that have overlapping clinical features with fragile X syndrome, fragile X tremor/ataxia syndrome, or premature ovarian insufficiency.

Clinical Reference

1. Jacquemont S, Hagerman RJ, Hagerman PJ, Leehey MA: Fragile-X syndrome and fragile X-associated tremor/ataxia syndrome: two faces of *FMR1*. *Lancet Neurol*. 2007 Jan;6(1):45-55
2. Finucane B, Abrams L, Cronister A, Archibald AD, Bennett RL, McConkie-Rosell A: Genetic counseling and testing for

FMR1 gene mutations: practice guidelines of the National Society of Genetic Counselors. J Genet Couns. 2012 Dec;21(6):752-60

3. Monaghan KG, Lyon E, Spector EB: ACMG Standards and Guidelines for fragile X testing: a revision to the disease-specific supplements to the Standards and Guidelines for Clinical Genetics Laboratories of the American College of Medical Genetics and Genomics. Genet Med. 2013 Jul;15(7):575-586

4. Biancalana V, Glaeser D, McQuaid S, Steinback P: EMQN best practice guidelines for the molecular genetic testing and report of fragile X syndrome and other fragile X-associated disorders. Eur J Hum Genet. 2015 Apr;23(4):417-425. doi: 10.1038/ejhg.2014.185

Performance

Method Description

A polymerase chain reaction (PCR)-based assay is used to detect expansions of a CGG trinucleotide tract in the 5'UTR of the *FMR1* gene. Methylation status for large premutations (170+) and full mutation alleles is determined by capillary electrophoresis analysis of a PCR-amplified product from DNA that is treated with a methylation-sensitive restriction enzyme. (Grasso M, Boon EMJ, Filipovic-Sadic S, et al: A novel methylation PCR that offers standardized determination of FMR1 methylation and CGG repeat length without southern blot analysis. J Mol Diagn. 2014 Jan;16(1):23-31; Snow K, Doud LK, Hagerman R, Pergolizzi RG, Erster SH, Thibodeau SN: Analysis of a CGG sequence at the FMR-1 locus in fragile X families and in the general population. Am J Hum Genet. 1993 Dec;53(6):1217-1228)

PDF Report

No

Specimen Retention Time

Whole Blood: 2 weeks (if available); Extracted DNA: 3 months

Performing Laboratory Location

Rochester

Fees & Codes

Test Classification

This test was developed, and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information

- 81243

- 88233 (if appropriate)

- 88240 (if appropriate)

- 88235 (if appropriate)

- 81265 (if appropriate)

- 81244 (if appropriate)

LOINC® Information

| Test ID | Test Order Name | Order LOINC Value |
|---------|-----------------------------------|-------------------|
| FXS | Fragile X Syndrome, Mol. Analysis | 81856-7 |

| Result ID | Reporting Name | LOINC® |
|-----------|---------------------|---------|
| 52870 | Result Summary | 50397-9 |
| 52871 | Result | 81856-7 |
| 52872 | Interpretation | 69047-9 |
| 52873 | Reason for Referral | 42349-1 |
| 52874 | Specimen | 31208-2 |
| 52875 | Source | 31208-2 |
| 52876 | Method | 85069-3 |
| 52877 | Released By | 18771-6 |