MAYO CLINIC LABORATORIES

Test Definition: FXS

Fragile X Syndrome, Molecular Analysis, Varies

Overview

Useful For

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

Determining 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

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
_STR1	Comp Analysis using STR (Bill only)	No, (Bill only)	No
_STR2	Add'l comp analysis w/STR (Bill Only)	No, (Bill only)	No

Testing Algorithm

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

When sending in prenatal specimens:

-If amniotic fluid (nonconfluent cultured cells) is received, amniotic fluid culture will be added at an additional charge.

-If chorionic villus specimen (nonconfluent cultured cells) is received, fibroblast culture for genetic test will be added at an additional charge.

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)

Method Name

Polymerase Chain Reaction (PCR)

NY State Available

Yes

Specimen

Specimen Type

Varies

Ordering Guidance

Due to the complexity of prenatal testing, consultation with the laboratory is required. To speak with a genetic counselor about this testing option, call 800-533-1710.

***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. **This must be a different order number than the prenatal 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. 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:
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) 4 days/Refrigerated

Prenatal Specimens
Due to its complexity, consultation with the laboratory is required for all prenatal testing; call 800-533-1710 to speak to a genetic counselor.

Specimen Type: Amniotic fluid
Container/Tube: Amniotic fluid container
Specimen Volume: 20 mL
Specimen Stability Information: Refrigerated (preferred)/Ambient
Additional information:
1. A separate culture charge will be assessed under CULAF / Culture for Genetic Testing, Amniotic Fluid. An additional 2 to 3 weeks is required to culture amniotic fluid before genetic testing can occur.
2. All prenatal specimens must be accompanied by a maternal blood specimen; order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on the maternal specimen.

Specimen Type: Chorionic villi
Container/Tube: 15-mL tube containing 15 mL of transport media
Specimen Volume: 20 mg
Specimen Stability Information: Refrigerated
Additional Information:
1. A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 2 to 3 weeks is required to culture chorionic villi before genetic testing can occur.
2. All prenatal specimens must be accompanied by a maternal blood specimen; order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on the maternal specimen.

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
Additional Information: **All prenatal specimens must be accompanied by a maternal blood specimen;** order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on the maternal specimen.

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: Congenital Inherited Diseases Patient Information (T521)
3. If not ordering electronically, complete, print, and send a Neurology Specialty Testing Client Test Request (T732) with the specimen.

Specimen Minimum Volume

Blood: 0.5 mL
Amniotic fluid: 10 mL
Chorionic villi: 5 mg

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

Fragile X syndrome is an X-linked disorder with variable expression in male and female patients. 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 intellectual disability, 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 a 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 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 years.

Premature Ovarian Insufficiency:
Female patients with a premutation are at risk for increased follicular stimulating hormone levels, early menopause, and POI. Penetrance and early onset of female reproductive symptoms correlate 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 the interpretation of results may occur if information given is inaccurate or incomplete.

Methylation status will not be assessed on chorionic villus specimens or 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

Day(s) Performed

Monday, Wednesday

Report Available

8 to 10 days

Specimen Retention Time

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

Performing Laboratory Location

Rochester

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

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	Test Result Name	Result LOINC® Value
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