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
***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 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:
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**.
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, 2mL with skirted conical base
Acceptable: Matrix tube, 1mL
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.

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: Ambient (preferred) <24 hours/Refrigerated <24 hours
Additional Information: Direct testing of an uncultured specimen may be attempted for this test. Contact the laboratory at 800-533-1710 if direct testing is desired.).
1. Specimens are preferred to be received within 24 hours of collection. Culture and/or 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 CULAF / Culture for Genetic Testing, Amniotic Fluid. An additional 2 to 3 weeks is required to culture amniotic fluid before genetic testing can occur.
3. **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: Prenatal cultured fibroblasts (eg, products of conception), amniocytes, or other confluent cultured cells. This does not include cultured chorionic villi.
Container/Tube: T-25 flask
Specimen Volume: 2 Flasks
Collection Instructions: Submit confluent cultured cells from another laboratory.
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/or 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.
3. **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: Ambient (preferred) <24 hours/Refrigerated <24 hours
Additional Information: Specimen can be tested from a direct extraction or culture: Contact the laboratory at 800-533-1710 if a direct testing is desired.
1. Specimens are preferred to be received within 24 hours of collection. Culture and/or 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 is required to culture fibroblasts before genetic testing can occur.
3. **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: Cultured chorionic villi
Container/Tube: T-25 flasks
Specimen Volume: 2 Full flasks
Collection Instructions: Submit confluent cultured cells from another laboratory.
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/or 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.
3. **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

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

Note: Where applicable, verbiage refers to sex assigned at birth.

Fragile X syndrome (FXS) 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 FXS but are at risk for other *FMR1*-related disorders, such as fragile X tremor/ataxia syndrome (FXTAS) and primary ovarian insufficiency (POI). Transmission of a premutation by a man to his female child usually results in little or no change in the CGG repeat number. Transmission of a premutation by a woman to her child 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 FXS 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 generally associated with abnormal methylation of a region adjacent to the *FMR1* gene. This is thought to interfere with normal *FMR1* gene expression, resulting in FXS. 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 FXS. 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:
Fragile X tremor/ataxia syndrome is a neurodegenerative disorder that is clinically distinct from FXS. 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 FXS 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 males 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 primary ovarian insufficiency (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

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 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;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;21(6):752-760
3. Spector E, Behlmann A, Kronquist K, Rose NC, Lyon E, Reddi HV. Laboratory testing for fragile X, 2021 revision: a technical standard of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2021;23(5):799-812
4. Hunter JE, Berry-Kravis E, Hipp H, et al. *FMR1* Disorders. In: Adam MP, Feldman J, Mirzaa GM, et al, eds. GeneReviews [Internet]. University of Washington, Seattle; 1998. Updated May 16, 2024. Accessed January 28, 2025. Available at www.ncbi.nlm.nih.gov/books/NBK1384/

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;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;53[6]:1217-1228)

PDF Report

No

Day(s) Performed

Varies

Report Available

8 to 10 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

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