MAYO CLINIC LABORATORIES

# Test Definition: AFXN

Friedreich Ataxia, Repeat Expansion Analysis, Varies

## Overview

### Useful For

Molecular confirmation of clinically suspected Friedreich ataxia

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

### Genetics Test Information

This test assesses for GAA trinucleotide repeat expansions within the *FXN* gene to confirm a molecular diagnosis of Friedreich ataxia.

### Testing Algorithm

**For prenatal specimens only:**

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

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

For any prenatal specimen that is received, maternal cell contamination studies will be added. **A maternal whole blood specimen is required to perform this test**.

### Special Instructions

- Informed Consent for Genetic Testing
- Hereditary Peripheral Neuropathy Diagnostic Algorithm
- 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
Polymerase Chain Reaction (PCR)

### NY State Available
Yes

## Specimen

### Specimen Type
Varies

### 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 as **this must be a different order number than the prenatal specimen.**

### 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**: None
**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 is met, the preferred volume of blood must be submitted. Testing may be canceled if DNA requirements are inadequate.

**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.
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)

**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**:
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**: 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**:
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.

**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.

## 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 1 of the following forms with the specimen:
-Neurology Specialty Testing Client Test Request (T732)
-Biochemical Genetics Test Request (T798)

## 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

Friedreich ataxia (FA) is one of the most common hereditary ataxias and is characterized by progressive gait and limb ataxia, dysarthria, dysphagia, and sensory loss. The phenotypic spectrum includes non-neurologic manifestations, particularly cardiomyopathy and diabetes mellitus. Onset typically occurs between the ages of 10 to 16 years; however, late-onset and early-onset variants have been reported.

Friedreich ataxia is inherited in an autosomal recessive manner. The majority of affected individuals (96%) have homozygous GAA trinucleotide repeat expansions in intron 1 of the *FXN* gene. The remaining affected individuals have a heterozygous GAA trinucleotide repeat expansion and another disease-causing *FXN* variant detectable by sequencing or deletion and duplication analysis (order CGPH / Custom Gene Panel, Hereditary, Next-Generation Sequencing, Varies and note the Gene List ID NEUROLOGY-P9CYQH).

Correlation exists between the size of the GAA repeat and disease onset and severity, with larger alleles associated with earlier onset and more severe disease presentation. GAA expansions may demonstrate instability during meiosis and mitosis. The GAA repeat size may expand or contract during transmission to offspring, and GAA repeat size may vary in different tissues. The GAA trinucleotide repeat is polymorphic in the general population, with the number of nondisease-associated repeats ranging from 5 to 33. Repeats of 66 or greater are fully penetrant disease-associated alleles; however, the majority of affected individuals have repeat sizes in the 600 to 1200 repeat range. Repeat sizes of 34 to 65 fall within a borderline range. Borderline alleles are of unclear significance and may be associated with clinical symptoms of FA and/or a risk for expansion to a full penetrance allele when transmitted to offspring.

### Reference Values

*FXN*
Normal alleles: <34 GAA repeats
Borderline alleles: 34-65 GAA repeats
Expanded alleles: >65 GAA repeats

An interpretive report will be provided.

### Interpretation

The interpretive report will include assay information, background information, and conclusions based on the test results.

### Cautions

For familial testing, it is important to first document the molecular etiology of disease in an affected family member to confirm that a repeat expansion is the underlying mechanism of disease in the family. Specifically, this assay will not

detect nonrepeat expansion variants (eg, sequence variants, deletions, and duplications).

It is strongly recommended that patients undergoing genetic testing receive genetic counseling.

Test results should be interpreted in the context of clinical findings, family history, and other laboratory data, such as frataxin concentrations; see FFRWB / Friedreich Ataxia, Frataxin, Quantitative, Blood. Errors in test interpretation may occur if the provided information is inaccurate or incomplete.

Rare variants (ie, polymorphisms) may exist, such as intron 1 deletions, which could lead to false-negative results. If GAA-repeat expansion results do not match clinical findings, additional testing should be considered.

Due to somatic mosaicism, GAA repeat-sizes in peripheral blood specimens may not reflect GAA repeat-sizes in other tissues (eg, central nervous system).

Bone marrow transplants from allogenic donors will interfere with testing. Call Mayo Clinic Laboratories at 800-533-1710 for instructions for testing patients who have received a bone marrow transplant.

### Clinical Reference

1. Campuzano V, Montermini L, Molto MD, et al. Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. Science. 1996;271(5254):1423-1427
2. Corben LA, Collins V, Milne S, et al. Clinical Management Guidelines Writing Group. Clinical management guidelines for Friedreich ataxia: best practice in rare diseases. Orphanet J Rare Dis. 2022;17(1):415. doi:10.1186/s13023-022-02568-3
3. Lynch DR, Schadt K, Kichula E, McCormack S, Lin KY. Friedreich ataxia: Multidisciplinary clinical care. J Multidiscip Healthc. 2021;14:1645-1658. doi:10.2147/JMDH.S292945
4. Montermini L, Richter A, Morgan K, et al. Phenotypic variability in Friedreich ataxia: role of the associated GAA triplet repeat expansion. Ann Neurol. 1997;41(5):675-682
5. Pilotto F, Chellapandi DM, Puccio H. Omaveloxolone: a groundbreaking milestone as the first FDA-approved drug for Friedreich ataxia. Trends Mol Med. 2024;30(2):117-125. doi:10.1016/j.molmed.2023.12.002
6. Rummey C, Corben LA, Delatycki M, et al. Natural history of Friedreich ataxia: heterogeneity of neurologic progression and consequences for clinical trial design. Neurology. 2022;99(14):e1499-e1510. doi:10.1212/WNL.0000000000200913
7. Sharma R, De Biase I, Gomez M, Delatycki MB, Ashizawa T, Bidichandani SI. Friedreich ataxia in carriers of unstable borderline GAA triplet-repeat alleles. Ann Neurol. 2004;56(6):898-901

## Performance

### Method Description

A polymerase chain reaction-based assay is used to amplify across the region of *FXN* containing GAA repeats.(Unpublished Mayo method)

### PDF Report

No

### Day(s) Performed
Monday

### Report Available
21 to 28 days

### Specimen Retention Time
Whole blood: 2 weeks (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
81284
81265-Maternal Cell Contamination (if appropriate)
88233-Fibroblast Culture (if appropriate)
88235-Amniotic Fluid Culture (if appropriate)
88240-Cryopreservation (if appropriate)

### LOINC® Information

| Test ID | Test Order Name | Order LOINC® Value |
|---|---|---|
| AFXN | FXN, Repeat Expansion Analysis | 21762-0 |

| Result ID | Test Result Name | Result LOINC® Value |
|---|---|---|
| 609752 | Result Summary | 50397-9 |
| 609753 | Result | 21762-0 |
| 609754 | Interpretation | 69047-9 |
| 609755 | Reason for Referral | 42349-1 |
| 609756 | Specimen | 31208-2 |
| 609757 | Source | 31208-2 |

| | | |
|---|---|---|
| 609758 | Method | 85069-3 |
| 609759 | Disclaimer | 62364-5 |
| 609760 | Released By | 18771-6 |