**Clinical Genomics Laboratory**
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# Fragile X with Reflex to Methylation:

## Disease Overview:

Fragile X syndrome (FXS) is the most common inherited form of intellectual disability, with an estimated prevalence of 1 in 4000 males and 1 in 8000 females. In more than 99% of cases, FXS is caused by an expansion of CGG repeats in the 5' untranslated region of exon 1 of FMR1 gene located on Xq27.3. Most individuals in the general population have 5-44 CGG repeats, while the individuals with FXS have more than 200 CGG repeats called full mutation. This CGG repeat expansion results in the hypermethylation of FMR1 promoter, which in turn leads to gene silencing and deficiency or loss of FMRP protein. Some individuals carry 55-200 CGG repeats called premutation alleles, which are unstable at meiosis and have an increased risk of expanding to full mutation in the next generation. This expansion generally occurs during maternal transmission depending on the number of maternal CGG repeats and the presence of interspersed AGG repeats. The premutation alleles are not hypermethylated and are not associated with FXS. However, individuals with premutation alleles are at risk for adult onset disorders such as Fragile X-associated tremor and ataxia syndrome (FXTAS) and premature ovarian insufficiency (FXPOI).

## Indications:

- Developmental delays
- Intellectual disability of unknown etiology
- Autism spectrum disorders or features
- Family history of Fragile X Syndrome
- Women with premature ovarian failure

## Testing Methodology:

The Fragile X test is performed using triplet repeat-primed polymerase chain reaction on DNA extracted from the submitted specimen. The PCR products are then size-separated by capillary electrophoresis to determine the number of CGG repeats within the FMR1 gene. If alleles are observed with greater than 54 CGG repeats, the reflex test (methylation-specific PCR) is performed to assess the methylation status and expression of the repeats found.

## Specimen Type:

- Minimum: 800ul of Whole Blood in an EDTA (lavender-top) Tube
- Oragene Buccal Swab (OCD-100)

## Genetic Testing Order Requirements:

- An **Informed Consent for Genetic Testing** must be signed by the ordering provider and the patient's guardian, and must be received by the lab before testing can begin, per Arizona law.
- This form is available on PCH4U – Forms (Form PCH11437 and PCH11437S for the Spanish version)
- This form is also available within the patient dashboards:
  - Sunrise Dashboard for Inpatient Care Areas
  - Outpatient Dashboard for Clinics

## Turnaround Time:

- For Fragile X tests completed at PCH Clinical Genomics Lab, the results will appear in the patient's chart and in SCM 7-14 days after receipt of consent form, patient specimen, and insurance authorization (if required).

**Order Name in SCM/AllScripts:**

- In SCM, select Fragile X w/ Reflex to Methylation.

**Test Limitations:**

- Reported CGG repeat size may vary by 1-2 repeats in case of normal and intermediate alleles (up to 54 repeats) and up to 4 in case premutation alleles (55-200 repeats).
- This test does not detect rare forms of Fragile X syndrome caused pathogenic variants in the FMR1 gene.
- This test does not assess the AGG trinucleotide interruptions within the CGG repeat track in the FMR1 gene.
- Diagnostic errors are rare, but can occur due to low level mosaicism, rare sequence variants at the primer site.

**Results:**

Results will be reported to the referring physician and/or genetic counselor as specified on the electronic requisition.