Spinal muscular atrophy (SMA) is the second most common lethal, autosomal recessive disorder in Caucasians, with an incidence of approximately 1/4000 to 1/6000 in the general population, and is a leading genetic cause of intellectual disability. Fragile X syndrome is associated with a triplet (CGG) repeat expansion in the promoter of the FMR1 gene on the X chromosome. CGG expansion leads to methylation and subsequent inactivation of the FMR1 gene. Normal alleles have ~5-44 repeats. Intermediate alleles have ~45-54 repeats and may expand in subsequent generations to premutation alleles. Alleles with ~55-200 repeats are called premutation alleles and may expand in subsequent generations to full mutations, especially when passed from mother to child. Alleles over ~200 repeats are full mutations and will cause fragile X syndrome in males.

Please note this panel will be performed and reported on both male and female specimens. Because of the nature of X-linked inheritance, this test, if positive, may be diagnostic for male patients in rare cases. If you do not wish to have X-linked conditions assessed in male patients, please contact the laboratory.

Genes

ABC8, ASPA, BCKDHB, BLM, CFTR, CLRN1, DLD, ELP1, FANCC, FKTN, FMR1, G6PC, GBA, HEXA, MCOLN1, NEB, PCDH15, SMN1, SMPD1, TMEM216

Indications

Appropriate for:

- Carrier testing in individuals of Ashkenazi Jewish descent.

Methodology

**Next Generation Sequencing:** In-solution hybridization of all coding exons is performed on the patient’s genomic DNA. Although some deep intronic regions may also be analyzed, this assay is not meant to interrogate most promoter regions, deep intronic regions, or other regulatory elements, and does not detect single or multi-exon deletions or duplications. Direct sequencing of the captured regions is performed using next generation sequencing. The patient's gene sequences are then compared to a standard reference sequence. Only known pathogenic variants will be reported.

**Fragile X Syndrome Repeat Analysis:** Both normal CGG repeat tracts and expanded CGG repeat tracts are detected by PCR amplification, using a CGG repeat-specific probe, and capillary electrophoresis. Expanded CGG repeat tracts will be reflexed to a gene specific PCR and sized by agarose electrophoresis.
gel electrophoresis.

**Spinal Muscular Atrophy (SMA) Testing:** SMN1 gene deletions were quantified by multiplex ligation polymerase chain reaction amplification (MLPA) of exons 7 and 8. Gene dosage ratios of SMN1 are calculated relative to the average of 16 reference loci and are expressed as gene dosage, and/or copy number. Diploid gene dose or 2 copies of SMN1 indicates normal (not affected) status, 1x gene dosage or 1 copy of the SMN1 gene most likely indicates carrier status and deletions (less than 0.1x) of SMN1 or 0 copies of the SMN1 gene designates affected status. This carrier assay tests for the common SMN1 deletion only; other pathogenic variants will not be detected. SMN2 copy number is not assessed.

**Detection**

**Next Generation Sequencing:** Next Generation Sequencing: Clinical Sensitivity: See results report. Pathogenic variants in regions other than the targeted area, including the promoter region, some mutations in the introns and other regulatory element mutations, cannot be detected by this analysis. Large deletions/duplications will not be detected by this analysis. Results of molecular analysis should be interpreted in the context of the patient's clinical/biochemical phenotype.

Analytical Sensitivity: ~99%.

**For Fragile X Syndrome Repeat Analysis:** All cases of fragile X syndrome caused by CGG expansion will be detected by this assay. Rare cases of fragile X syndrome caused by pathogenic variants in the FMR1 gene will not be detected by this assay.

**For Spinal Muscular Atrophy (SMA) Testing:** Deletions of the SMN1 gene are found in approximately 95% of individuals with SMA. This carrier assay tests for the common SMN1 deletion only; other pathogenic variants will not be detected. Approximately 5-8% of carrier individuals will have a normal SMN1 copy number of two, but both copies will be on the same chromosome (in cis) with a deletion on the second chromosome. This assay will not detect these carrier individuals. SMN2 copy number is not assessed.

**Reference Range**

**For Fragile X Testing:**
Normal: Approximately 5-44 CGG repeats.
Intermediate: Approximately 45-54 unmethylated CGG repeats.
Premutation: Approximately 55-200 CGG repeats and methylation of expanded allele.
Affected: Over 200 CGG repeats and methylation of expanded allele.

**Specimen Requirements**

Submit only 1 of the following specimen types

**Type: Saliva**

Specimen Requirements:

Oragene™ Saliva Collection Kit.

Specimen Collection and Shipping: Store sample at room temperature. Ship sample within 5 days of collection at room temperature with overnight delivery.

**Type: Whole Blood**

Specimen Requirements:

In EDTA (purple top) tube:
Infants (2 years): 3-5 ml
Older Children & Adults: 5-10 ml.

Specimen Collection and Shipping: Ship sample at room temperature with overnight delivery.

**Type: Isolated DNA**

Specimen Requirements:

In microtainer: 60 ug

Isolation using the Qiagen™ Puregene kit for DNA extraction is recommended.

Specimen Collection and Shipping: Refrigerate until time of shipment in 100 ng/ul of TE buffer. Ship sample at room temperature with overnight delivery.

**Related Tests**

- Ashkenazi Jewish Carrier Screen: Targeted Mutation Panel
- Pan-Ethnic Carrier Screen: Gene Sequencing Panel
- ACOG/ACMG Carrier Screen: Gene Sequencing Panel