Gene Sequencing testing can provide confirmation of a clinical diagnosis, characterization of the gene mutation, and carrier testing for female family members and prenatal testing. The combined EmArray DMD and DMD full gene sequence analysis detects ~98-99% of mutations in both males and females, thereby providing the most comprehensive and robust analysis of the DMD gene.

Deletion/Duplication by EmArray DMD

Testing begins with EmArray DMD (test code EG), a targeted CGH array which consists of overlapping probes covering the entire 2.2MB of the DMD gene. Deletion and duplication mutations account for 65% of mutations in the DMD gene, and EmArray DMD testing will detect deletions and duplications in both males and females. This testing is indicated for individuals suspected to carry a DMD gene mutation who have not yet had testing or for individuals with previous deletion/duplication test results that do not clearly identify the breakpoints and size of the deletion or duplication. EmArray DMD testing can also be performed for female carrier testing even when an affected male is not available for testing.

Methods other than CGH array that have been used to test for DMD deletions and duplications have inherent drawbacks. These methods include multiplex PCR, Southern blotting, and MLPA. Drawbacks of these methods include difficulties in detecting small deletions, difficulties in detecting most duplications, difficulties in detecting female carriers, and the inability to determine precise boundaries of deletions and duplications. Use of these other methodologies will fail to identify a mutation in ~5-10% of individuals tested (Prior and Bridgeman, 2005). EmArray DMD testing can be performed when an individual has had previous negative deletion/duplication results by one of these other methods.

Full Gene Sequence Analysis

If no deletion or duplication is identified by EmArray DMD, testing can continue with full gene sequence analysis (test code EE). The remaining 35% of mutations in the DMD gene are point mutations and small deletions and duplications that can be detected by direct sequencing. Sequence analysis interrogates the 14kb coding region, 1.4kb of intronic sequence flanking the exon/intron boundaries, and 5 cryptic deep intronic mutations. This test is indicated for individuals suspected to carry a DMD mutation in whom previous testing did not identify a deletion or duplication. Rarely, novel missense changes or changes in introns other than at standard splice consensus sites are discovered. Testing of additional family members may be necessary for further interpretation in these cases. EGL follows the ACMG recommendations for interpretation and reporting of sequence variations (Richards et al., 2008).

Advantages of Using EGL’s Comprehensive DMD Testing

- Equal sensitivity and detection for males and females
- Deletions and duplications mapped to the exact nucleotide breakpoint using CGH array
- Enhanced detection of duplications that may be missed by other methods
- Rapid turn-around time
- Improved access to carrier and prenatal testing
- Carrier risk adjustment using Bayesian analysis can be performed if appropriate family history information is provided

EGL Genetics, Parent Project Muscular Dystrophy (PPMD), leading researchers, and Duchenne muscular dystrophy (DMD) clinicians are working together to offer improved testing for DMD and to develop a mutation and clinical data collection system based on the CETT Program model of collaboration.


References:

Genes

DMD

Indications

This test is indicated for:
- Males with a clinical diagnosis or symptoms of Duchenne or Becker muscular dystrophy in whom deletion/duplication testing was negative.
- Females who are at risk to be a carrier or have a family history of Duchenne or Becker muscular dystrophy in whom deletion/duplication testing
was negative.
  • Prenatal testing is available to females who carry an identified \textit{DMD} mutation.

\textbf{Methodology}

\textbf{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. Potentially causative variants and areas of low coverage are Sanger-sequenced. Sequence variations are classified as pathogenic, likely pathogenic, benign, likely benign, or variants of unknown significance. Variants of unknown significance may require further studies of the patient and/or family members.

\textbf{Detection}

Clinical Sensitivity: In approximately 35\% of Duchenne muscular dystrophy and approximately 15\% of Becker muscular dystrophy, point mutations and mutations in the promoter or intronic regions are identified in the \textit{DMD} gene, which are detectable by full gene sequence analysis. Sequence analysis will not detect larger deletions and duplications.

\textbf{Specimen Requirements}

Submit only 1 of the following specimen types

* Preferred specimen type: Whole Blood

\textbf{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: Refrigerate until time of shipment. Ship sample within 5 days of collection at room temperature with overnight delivery.

\textbf{Type: Saliva}

Specimen Requirements:

Oragene\textsuperscript{TM} Saliva Collection kit (available through EGL) used according to manufacturer instructions.

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

\textbf{Special Instructions}

\textbf{Click here} for the Dystrophin Clinical Information Form to send with the sample.

\textbf{Related Tests}

• \textbf{EmArray DMD CGH} is available to test for deletions and duplications of the \textit{DMD} gene.