Assay Summary

DMD Gene Mutation Analysis
Duchenne and Becker Muscular Dystrophy

Synopsis
Alterations of the DMD gene, encoding dystrophin, are causative for Duchenne (DMD) or Becker (BMD) muscular dystrophies. Large deletions or duplications in the dystrophin gene are found in approximately 60-70% of patients diagnosed with DMD or BMD; while sequence variants are detected in ~25-35% of patients. Identification of causative mutations can provide an accurate diagnosis without the need for muscle biopsy. For cases in which a proband is not available for testing, analysis may be performed for an obligate carrier or otherwise at-risk relative. Mutation detection in an affected individual permits accurate determination of carrier status of at-risk females in these families and provides options for prenatal diagnosis.

Indications for testing
- Individuals with a diagnosis/suspected diagnosis of DMD/BMD
- Obligate carriers or appropriate at-risk relatives of DMD/BMD patients
- Known carriers of familial DMD/BMD mutations desiring prenatal diagnosis (Please contact the laboratory in advance of sending prenatal orders).

Methodology
MLPA Analysis for large deletion/duplication: DNA is amplified in 80 segments using the DMD MLPA probe mixes, which targets all 79 coding exons of the DMD gene and the alternative exon 1 (DP427c). The MLPA products are analyzed by DNA fragment analysis on an automated fluorescent sequencer. The absence or presence of deletions of one or more exons is confirmed by MLPA analysis using an independently amplified segment.

Sequence analysis: Coding exons and associated intron junctions are captured and enriched using custom Agilent SureSelect technology. Next-generation sequencing is performed on Illumina MiSeq. Additional Sanger sequencing is performed for any regions with insufficient depth of coverage or for verification of suspect variant calls. Targeted testing for known familial mutation is performed by Sanger sequencing.

Performance
The analytical sensitivity and specificity for detection of deletion or duplication of one or more exons of the DMD gene or for the detection of coding sequence alterations is greater than 99%. Point mutations have been detected in ~75% of Duchenne muscular dystrophy patients who have previously tested negative for a large deletion. Multiple factors, including genetic heterogeneity and mutations outside of the coding regions, may account for the lack of detection in the remaining patients. Overall, MLPA analysis and full mutation analysis together is expected to detect a mutation in at least 90% of patients with Duchenne or Becker muscular dystrophy. Once a causative mutation is identified, the sensitivity and specificity for carrier detection and for prenatal diagnosis are estimated to be greater than 99%.
**Limitations**

MLPA is designed to detect deletions/duplications of one or more exons of the gene, but does not determine precise breakpoints of alterations detected. MLPA analysis will not detect certain genetic alterations, such as point mutations or small deletions/insertions and inversions. Additionally, MLPA analysis may be sensitive to DNA sample purity and other experimental conditions. Probe signals may also be adversely affected by sequence variants situated in the vicinity of, or at the probe ligation site; therefore, apparent deletions/duplications detected by a single probe should be confirmed by other methods. Partial exonic deletions/duplications outside of the probe target sequence may not be detected.

Full gene sequencing analysis will not detect large deletion in females nor any large duplications, or mutations outside of the regions analyzed. Some sequence alterations that may be detected (such as those causing missense or synonymous changes) will be of unknown clinical significance. Interpretation of test results should be in the context of the patient’s ethnicity, clinical and family histories, and other laboratory test results.

**Specimen Requirements**

(a) **Blood samples**: 2 tubes with a total of 6 ccs in ACD (yellow top) or EDTA (lavender top) tubes.  
Keep at ambient temperature and ship by overnight courier. Samples must be received in our laboratory within 72 hours of draw.  
**Note:**  
1) for infants, a minimum of 3 ccs is sufficient.  
2) we accept DNA; at least 10 micrograms is required.

(b) **Prenatal samples**: 2 T25 flasks of confluent amniocytes or CVS sent padded to arrive on M/Tu/W.  
**Maternal cell contamination studies** (required and performed concurrently for prenatal): If not previously tested in our lab, a blood sample from the mother is required, as described in (a).

**Test Request Form (TRF)**

A completed CMDL TRF is required for each specimen. Please submit the completed TRF with the specimen. Complete testing and billing information must be provided before the specimen is processed.

<table>
<thead>
<tr>
<th>Order Codes</th>
<th>CPT Codes</th>
<th>TAT</th>
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</thead>
<tbody>
<tr>
<td>DMD-SEQ (Dystrophin gene, full gene sequencing by NGS)</td>
<td>81408, G0452</td>
<td>3 wks</td>
</tr>
<tr>
<td>DMD-CAS (Dystrophin gene, targeted mutation analysis, known mutation)</td>
<td>81403, G0452</td>
<td>2 wks</td>
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<tr>
<td>DMD-PD (Dystrophin gene, prenatal sequence analysis for known mutation, with maternal cell contamination studies)</td>
<td>81403, G0452</td>
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<tr>
<td>DMD-DEL (Dystrophin gene, MLPA analysis (79 Exons))</td>
<td>81161, G0452</td>
<td>3 wks</td>
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<tr>
<td>DMD-DEL-CAS (Dystrophin gene, MLPA analysis, known deletions/duplications (79 exons))</td>
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<td>3 wks</td>
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<tr>
<td>DMD-DEL-PD (Dystrophin gene, prenatal MLPA analysis for known deletion/duplication, with maternal cell contamination studies)</td>
<td>811161, G0452</td>
<td>2 wks</td>
</tr>
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**References**