



Peiguo G. Chu, MD, PhD  
Interim Chairman, CLIA #05D0665695  
Juan-Sebastian Saldivar, MD, FACMG  
Director of Molecular Diagnostics

**Molecular Diagnostic Laboratory**  
1500 East Duarte Road  
Northwest Building, Second Floor, Room 2236  
Duarte, CA 91010-3000  
Phone 888-826-4362 Fax 626-301-8142  
mdl@coh.org <http://mdl.cityofhope.org>

## Assay Summary

### Dystrophin Gene Mutation Analysis Duchenne and Becker Muscular Dystrophy

#### *Synopsis*

Identification of causative mutations in the dystrophin gene for families with suspected Duchenne or Becker muscular dystrophy can provide an accurate diagnosis without the need for muscle biopsy. Mutation detection in an affected individual permits very accurate determination of carrier status of at-risk females in these families and provides options for prenatal diagnosis. To identify the mutation in a Duchenne or Becker muscular dystrophy family, a blood sample is required from an affected individual (proband). Because large deletions or duplications in the dystrophin gene are found in approximately 60% of patients diagnosed with Duchenne or Becker muscular dystrophy, the gene deletion analysis is initially performed on samples from patients with these diseases. For patients without a large deletion, full gene sequencing analysis of the dystrophin gene is performed. After a mutation is detected in a proband, carrier testing and prenatal diagnosis may be offered to the family. In cases in which a proband is not available for testing, analysis may be performed on a sample from an obligate carrier.

#### *Indications for testing*

Individuals with a possible diagnosis of Duchenne or Becker muscular dystrophy, appropriate at-risk female relatives of probands with identified mutations, and carriers of Duchenne or Becker muscular dystrophy with previously identified dystrophin gene mutations desiring prenatal diagnosis, with genetic counseling, are candidates for testing.

#### *Methodology*

MLPA Analysis: DMD large deletions/duplications are found in 60-70% of Duchenne muscular dystrophy/Becker muscular dystrophy (DMD/BMD). We have incorporated the SALSA Multiplex Ligation-Dependent Probe Amplification (MLPA) kit which is a rapid, high-throughput technique for copy number quantification, specifically testing for large deletions/duplications for the DMD gene in DMD/BMD. This assay should be considered for patients with DMD/BMD as first tier molecular tests for proband and carrier female. This P034/P035 DMD probemix contains probes for all 79 exons of the DMD gene on Xp21.2 chromosome. In addition, one probe is present for the alternative exon 1 DP427c. These 80 probes have been divided in two probe mixes: P034 and P035. Performing two MLPA reactions is thus sufficient to investigate the copy number of all exons. P034 kit contains two probes on Y chromosome and P035 kit has one probe on Y chromosome. As a control, several probes for other human genes on different chromosomes are included for references in each probemix.

Full gene sequencing Analysis: If a large deletion is not detected in a proband, all the coding regions and splice junctions of the dystrophin gene are analyzed by direct DNA sequence analysis. Once a specific mutation is detected in the proband, carrier testing for appropriate family members is performed by direct DNA sequence analysis.

## ***Performance***

MLPA Analysis: For the 79 exons tested, the sensitivity for detection of dystrophin gene deletions/duplications in Duchenne or Becker muscular dystrophy patients is greater than 99%; the specificity is greater than 99%. The sensitivity and specificity for prenatal diagnosis for families with identified dystrophin gene deletions/duplications are both estimated to be greater than 99%.

Full Gene Sequencing Analysis: 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 regions of likely functional significance, may account for the lack of mutations in the remaining patients.

Overall, however, MLPA analysis and full mutation analysis together will detect a mutation in at least 90% of patients with Duchenne or Becker muscular dystrophy. Once a point mutation is found, the sensitivity and specificity for carrier detection and for prenatal diagnosis for families with identified dystrophin gene mutations are both estimated to be greater than 99%.

## ***Limitations***

This SALSA MLPA kit is designed to detect deletions/duplications of one or more exons of the DMD gene. Deletions of probe recognition sequences in males will be apparent by the absence of the probe amplification product. Duplications of DMD gene in males will give a 30-50% increased relative peak area, and deletions in female carriers will cause a 35-50% reduced relative peak area of the amplification product of that probe. However, mutations and/or polymorphisms very close to the probe ligation site may also result in a reduced relative peak area. Therefore, apparent deletions/duplications detected by a single probe always require confirmation by other methods.

Full gene sequencing analysis will not detect large deletion in females and duplications (estimated to be up to 5% of cases) in males 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:**

- i) for infants, a minimum of 3 ccs is sufficient.
- ii) we accept DNA; at least 10 micrograms is required.

(b) Prenatal samples: 2 T25 flasks of confluent cells sent padded to arrive on M/Tu/W.

A blood sample from the mother maybe required (2 tubes with a total of 6 ccs in ACD (yellow top) or EDTA (lavender top) tubes) for use as positive control. Maternal cell contamination studies are not done here but are required for autosomal disorders and dosage analysis on X-linked disorders. We would be happy to assist in coordinating sending out a specimen for this purpose.

### ***Test Request Form (TRF)***

A completed MDL [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.

<b><i>Order Codes</i></b>	<b><i>CPT Codes</i></b>	<b><i>TAT</i></b>
DMD-SEQ (Dystrophin gene, full gene sequencings)	83890, 83898(x79), 83904(x79), 83894, 83912	10 wks
DMD-CAS (Dystrophin gene, targeted mutation analysis, known mutation)	83890, 83898, 83894, 83904, 83912	3 wks
DMD-PD (Dystrophin gene, known mutation detection, prenatal)	83890, 83898, 83894, 83904, 83912	2 wks
DMD-DEL (Dystrophin gene, MLPA analysis (79 Exons))	83890, 83896(x79), 83909, 83912	3 wks
DMD-DEL-CAS (Dystrophin gene, MLPA analysis, known deletions/duplications (79 exons))	83890, 83896(x79), 83909, 83912	3 wks
DMD-DEL-PD (Dystrophin gene, MLPA analysis (79 Exons), prenatal)	83890, 83896(x79), 83909, 83912	3 wks

### ***References***

1. MLPA DNA Detection/Quantification Protocol; MRC-Holland, Amsterdam, Holland
2. Mendell, J.R. et al. (2001). *Neurology* 57: 645-650.

NOTE: This test is performed pursuant to a license agreement with Roche Molecular Systems, Inc