

**Dystrophin Protein Quantification as a Duchenne Muscular Dystrophy Diagnostic Biomarker in Dried Blood Spots Using Multiple Reaction Monitoring Tandem Mass**

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**Abstract:** Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder characterized by progressive muscle loss, leading to difficulties in movement. Mutations in the DMD gene that code for the protein dystrophin are responsible for the development of DMD disorder, where the synthesis of this protein is completely halted. Therefore, circulating dystrophin protein could be a promising biomarker of DMD disease. Current methods for diagnosing DMD have sensitivity, specificity, and reproducibility limitations. Herein, a quantitative liquid chromatography-tandem spectrometry (LC-MS/MS) technique in multiple reaction monitoring (MRM) mode was designed and validated for accurate dystrophin protein measurement in a dried blood spot (DBS). The method was successfully validated on the basis of international guidelines regarding calibration curves, precision, and accuracy. In addition, patients and healthy controls were used to test the amount of dystrophin protein circulating in DBS samples as a potential biomarker for DMD disorders. DMD patients were found to have considerably lower levels than controls. To the best of our knowledge, this is the first study to report dystrophin levels in DBS through LC-MS/MS as a diagnostic marker for DMD to the proposed MRM method, providing a highly specific and sensitive approach to dystrophin quantification in a DBS that can be applied in DMD screening.