

Rapid lateral-flow “dipstick” immunoassays to detect and measure levels of assembled OXPHOS enzyme complexes in inherited and acquired mitochondrial disease

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The majority of inherited mitochondrial diseases affect OXPHOS enzyme Complexes I and/or IV, and most of these are assembly defects. Therefore, simple, reliable tests to measure the levels of assembled Complex I and Complex IV would be very useful for diagnosis and study of these disorders. We have developed a set of simple lateral-flow immunoassays (dipsticks) for this purpose. These dipsticks are 2-site “sandwich” assays in which a pair of monoclonal antibodies bind simultaneously to two non-overlapping epitopes on a single target antigen molecule. A signal is generated only if the intact target antigen is present, e.g., assembled Complex I or IV. The assays are extremely simple, rapid, quantitative and reproducible with CVs <10%. Complex I and/or Complex IV can be measured in very small samples of clinically relevant tissues such as skeletal muscle, whole blood, fat cultured fibroblasts, and most intriguingly, in cheek swab-derived cells. Importantly, crude extracts of whole cells or tissue are suitable samples (only 0.1–2 µg protein is needed for Complex IV assays and only 2–20 µg protein is needed for Complex I assays). Analysis of patient-derived cultured fibroblasts confirm that the assays specifically and accurately detect and quantitate isolated inherited defects in both complexes. Moreover, both assays accurately measure the rate and extent of reduction of Complex I and IV associated with mtDNA depletion caused by in vitro exposure of normal cells to mitotoxic drugs such as NRTIs or ethidium bromide. In summary, these novel dipstick assays have great potential as simple tests to facilitate rapid diagnosis and study of many OXPHOS disorders, including inherited and degenerative diseases and mitotoxicities of therapeutic drugs such as NRTIs and other anti-retroviral drugs used to treat AIDS.

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