Assessment of drug-induced liver mitochondrial dysfunction in primary human hepatocytes using XF Mito Tox Assay

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SOT 2023

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Introduction

Mitochondrial toxicity is a common issue with therapeutic development, as most eukaryotic cells employ mitochondria to produce the majority of ATP required for metabolic function and regulate key cellular processes.

Using the Seahorse XF Mito Tox Assay kit in conjunction with the XF Pro Analyzer and dedicated software features enables streamlined, sensitive detection and characterization of mitochondrial toxicants. It delivers a standardized quantitative parameter, the Mito Tox Index (MTI), derived from the oxygen consumption rate (OCR) through a customized software tool, Seahorse Analytics.

The use of cryopreserved primary human hepatocytes is a standard model of *in-vitro* assessment toxicity in predicting *in-vivo* drug-induced liver injury especially when the drug metabolism is considered.

In this study, the use of human primary hepatocytes in Seahorse XF Mito Tox Assay was validated. The workflow requires optimization of the cell seeding density and appropriate FCCP concentration. The results demonstrate that the XF Mito Tox Assay solution is a robust way to screen and evaluate mitochondrial toxicity using primary human hepatocytes.

Assay Design

Parametric assessment of mitochondrial toxicity using the Mito Tox Index and detection of OxPhos inhibitors

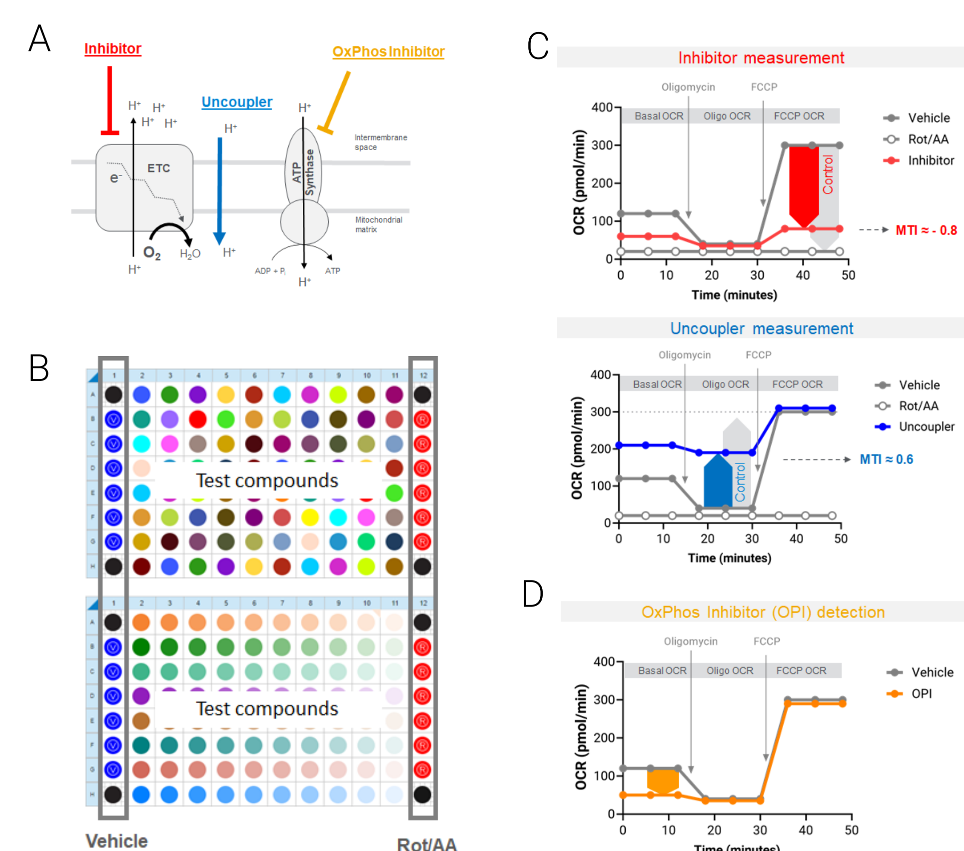

Figure 1. XF Seahorse Mito Tox Assay design and concept of calculating Mito Tox Index (MTI) and detecting potential OxPhos inhibitors (OPIs). A. Three types of mitochondrial toxicity are assessable by measuring OCR with the XF Seahorse technology. B. XF Mito Tox Assay template designs for screening and dose-response analysis. The assay requires two control groups: Vehicle and Rot/AA. C. The MTI is quantitative measurement of mitochondrial toxicity. Toxicity due to inhibition is defined as a decrease in FCCP OCR compared to the vehicle group, resulting in a negative MTI value (between 0 and -1). Toxicity due to uncoupling is defined as an increase in Oligo OCR compared to the vehicle group, resulting in a positive MTI value (between 0 and 1). D. If a test compound treatment results in a decrease in Basal OCR, but does not result in significant decrease in FCCP OCR, then the compound is categorized as a potential OPI. It is suggested to perform downstream assays (dose-response or other orthogonal assays) to further investigate and characterize this type of toxicity.

Results and Discussion

Cell seeding density optimization

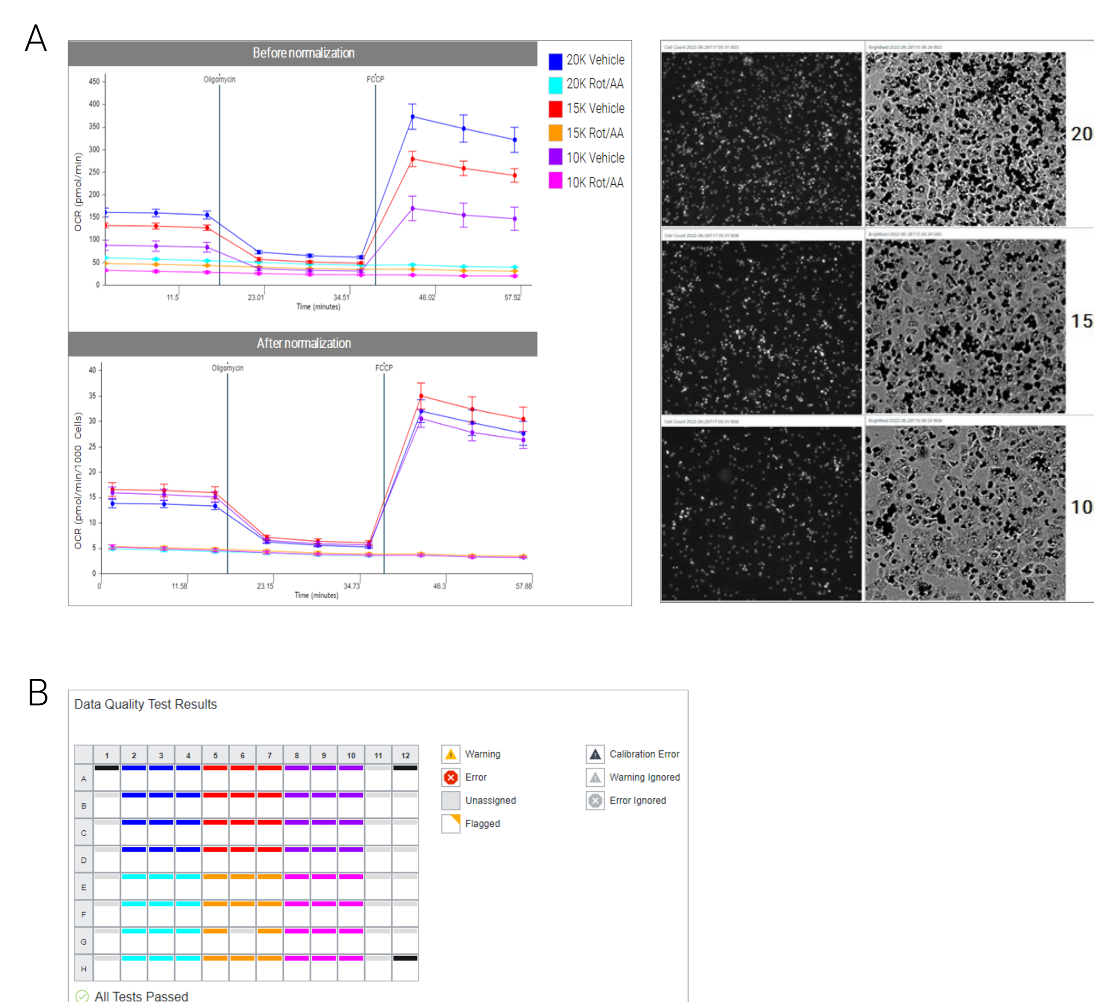

Figure 2. XF Mito Tox assay using primary human hepatocytes in optimal seeding conditions. Primary hepatocytes were seeded at 3 different densities (10 K, 15 K, and 20 K cells/well) and the XF Mito Tox assay was performed. A. OCR kinetic data showed a linear correlation to the seeding density (upper graph). Data normalization indicates equivalent OCR across seeding density on a per cell basis. Representative fluorescence and bright field images indicate cells are nearly confluent at 20K density. B. No data quality errors or warnings were reported in the data QC software.

Non-optimal seeding conditions

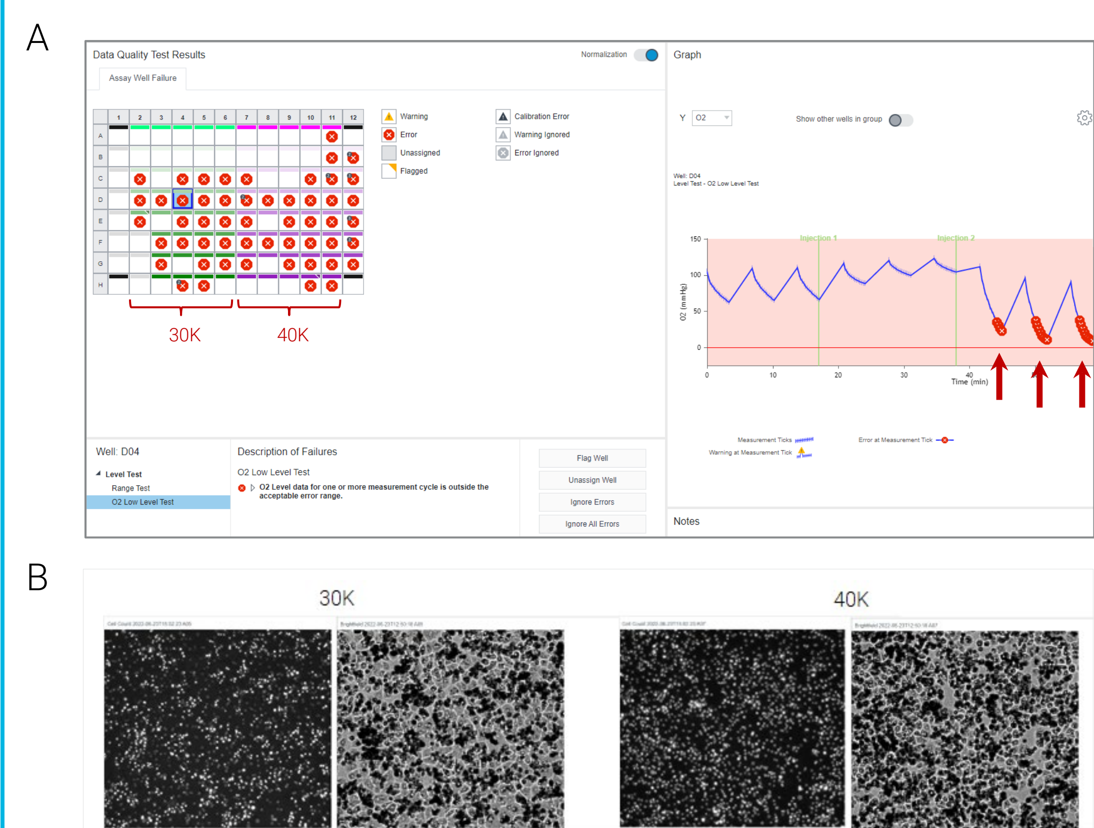

Figure 3. XF Mito Tox assay using primary human hepatocytes with non-optimal seeding conditions. Primary hepatocytes were seeded at non-optimal seeding densities, 30 K and 40 K cells/well. A. Data QC reported errors of high OCR and low O_2 level data (arrows), indicating the OCR is beyond the dynamic range of the instrument. B. Bright field and fluorescence images show inconsistent cell monolayers, over-confluence and a significant number of cell clusters.

FCCP titration and assay performance evaluation

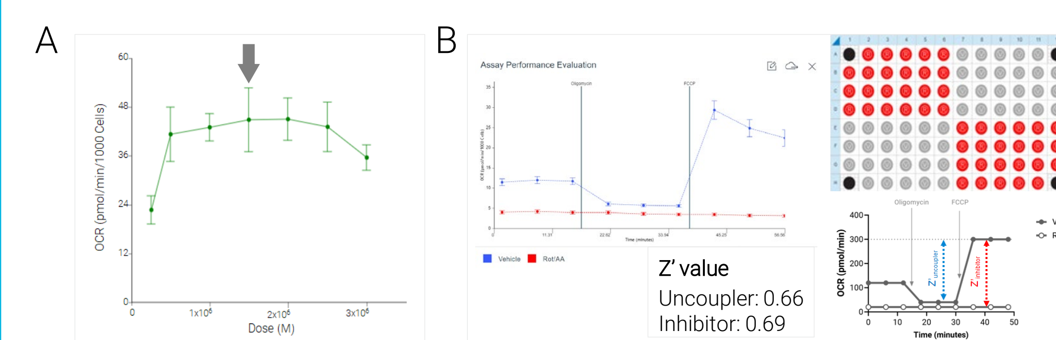

Figure 4. FCCP titration and the assay performance evaluation. A. FCCP was titrated at the optimal cell density of 20 K cells/well and the optimal FCCP concentration was determined to be 1.5 µM (arrow). B. Primary hepatocytes plated on an XF96 well plate were divided into two groups, Vehicle control (gray) and Rot/AA (red) and the XF Mito Tox assay was performed. The Z' values for inhibitor and uncoupler are automatically calculated using Seahorse Analytics.

Mitochondrial toxicity test using primary human hepatocytes

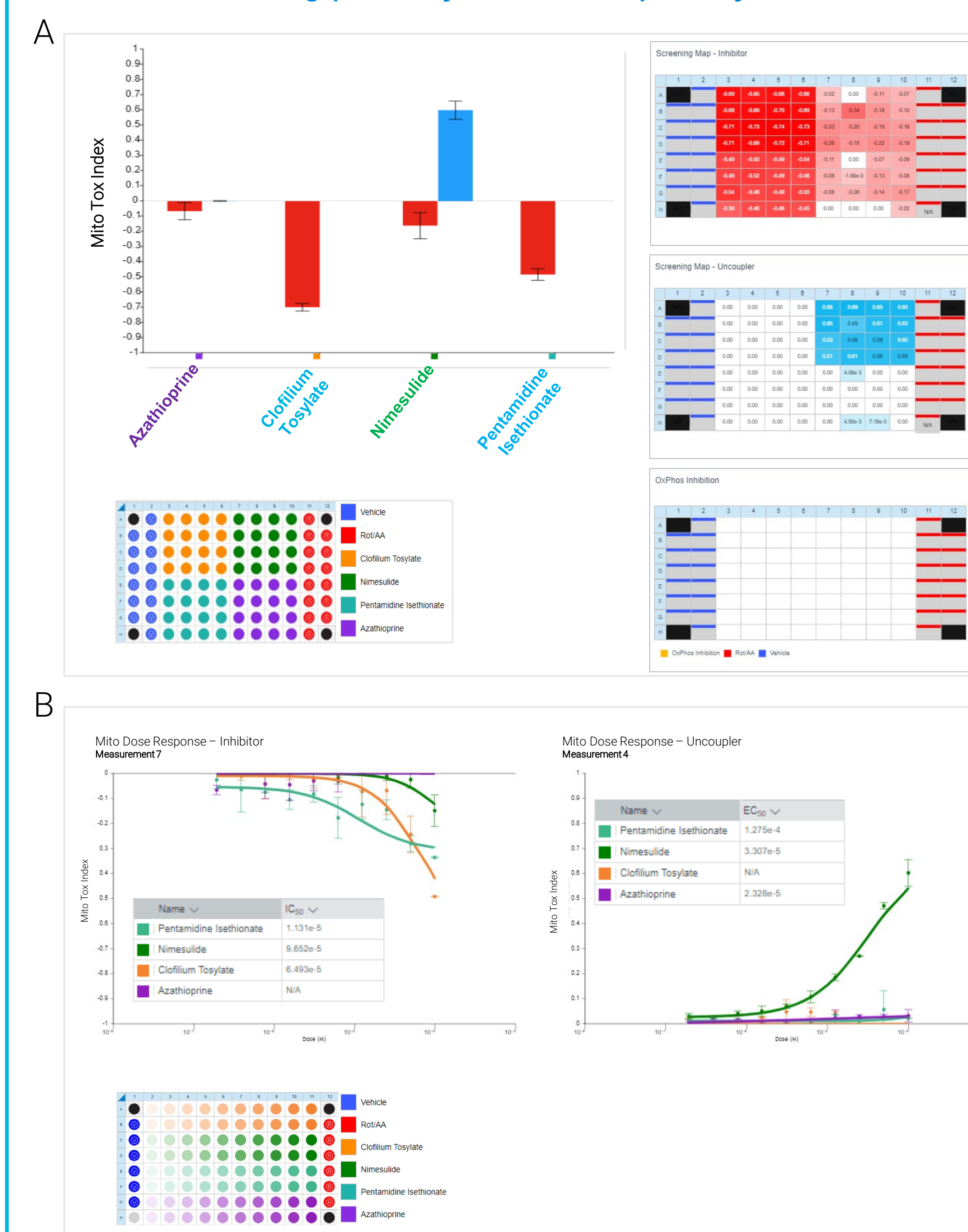

Figure 5. Mitochondrial toxicity evaluation using primary human hepatocytes. Select model compounds known to elicit mitochondrial toxicity were tested by screening and dose-response assays. A. The screening assay identified 2 inhibitors and 1 uncoupler, as expected. B. The IC_{50} of the inhibition and EC_{50} of uncoupling were obtained by using the dose-response template design tool in Wave Pro software and the dose-response companion view in Seahorse Analytics.

Conclusions

Primary hepatocytes are the preferred cell type for drug safety tests predicting drug-induced liver injury. XF Mito Tox assay solution can successfully screen and evaluate the mitochondrial toxicity of test compounds on primary human hepatocytes when using optimal assay conditions.

Results indicate optimal conditions for primary human hepatocytes are 20 K/well and a concentration of 1.5 µM FCCP. The use of primary human hepatocytes in the XF Mito Tox assay is robust for the detection of mitochondrial toxicity, with Z' values > 0.5 for both inhibition (0.69) and uncoupling (0.66).

Among 4 model drugs tested, 2 inhibitors and 1 uncoupler were identified in agreement with the previous reports as reported by bar charts and heat maps. The amplitude of toxicity was also successfully evaluated by the dose-response assay.

The optimization steps are highly recommended for any cell type that has not been optimized previously as summarized below.

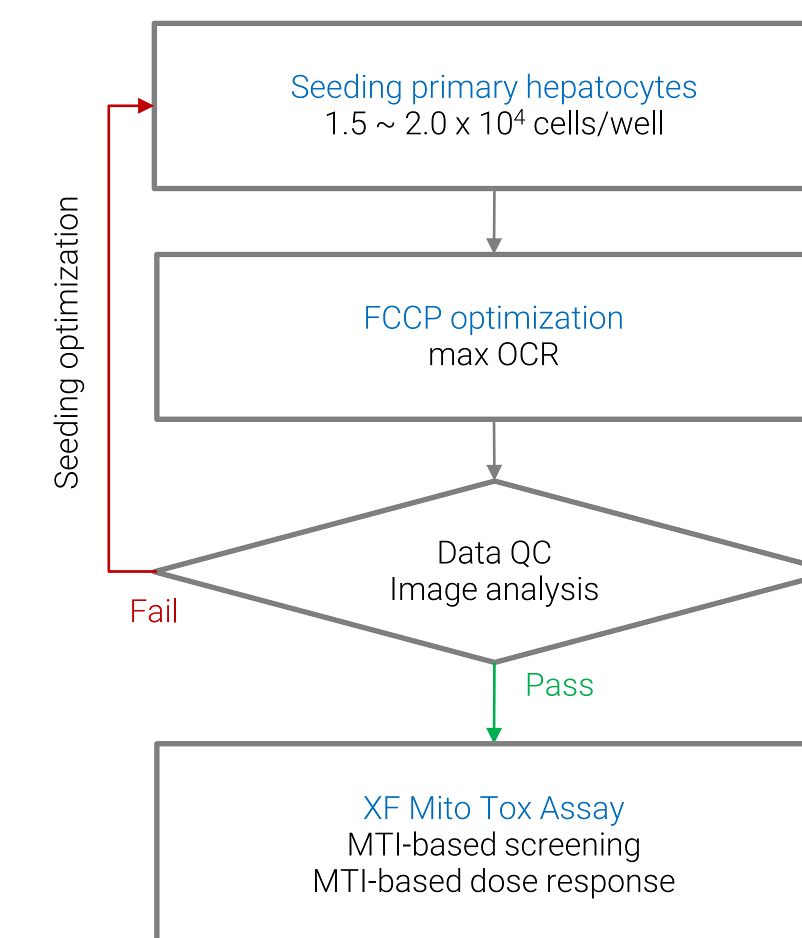

Figure 5. XF Seahorse Mito Tox Assay Workflow including the optimization steps for new cell type

References

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2. Kam *et al.* A Customized XF Workflow for Detection and Characterization of Mitochondrial Toxicity. Agilent Technologies, application note, publication number 5994-4778EN, 2022
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RA44984.2573032407

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Printed in the USA, April 4, 2023
5994-5991EN