Miniaturized Drug Sensitivity and Resistance Testing with the Echo® Liquid Handler and Access™ Workstation

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Abstract
Recently identified associations between variants of cancer genes and drug resistance increase the value for comprehensive drug sensitivity and resistance testing in combination with molecular profiling of cancer cells. The measure of sample sensitivity or resistance to a drug requires high throughput screening of engineered cancer cell lines or samples directly from affected patients against combinations of anti-cancer therapeutics. Results are compared with genetic profiles in an attempt to determine the more effective treatment. Advancements in next generation sequencing and qPCR technologies persuade many research organizations to increase effort in these areas. In doing so, researchers immediately recognize screening efficiency as a critical factor to accurate and reproducible drug sensitivity and resistance testing. This application note discusses the implementation of miniaturized drug sensitivity and resistance testing, at the Institute of Molecular Medicine in Finland (FIMM), with assay-ready plates produced by the Echo liquid handler.
Introduction
Echo Liquid Handlers use acoustic energy to provide high throughput, non-contact, liquid handling for a range of applications. Non-contact transfer avoids the risk of cross-contamination, eliminates tip costs, and facilitates the progression to high density assay formats. Echo Liquid handlers precisely and accurately transfer 2.5 nL droplets of sample and reagent, which enable the miniaturization of biochemical screens. This application note discusses the implementation of miniaturized drug sensitivity and resistance testing, at the Institute of Molecular Medicine in Finland (FIMM), with assay-ready plates produced by the Echo liquid handler.

It has been shown that acoustic dispensing of compounds can result in a more accurate assessment of compound activity. Certain compounds, particularly those that are hydrophobic in nature, have a tendency to adhere to plastic pipette tips. This retention results in a loss of compound transferred, which in turn results in reduced activity in the assay. This is particularly relevant to any high throughput screening program where the potency of a particular compound will guide decisions on further study and possible acceptance.

A recent publication in PLoS ONE discusses the difference in the biological activity of some compounds when dispensed with acoustic-based methods versus tip-based methods. By generating computational pharmacophores from data generated with both liquid handling methods and comparing them with subsequent data sets and X-ray crystallography, pharmacophores generated from acoustic-based dispensing methods were found to be more accurately predictive of activity.

Drug Sensitivity and Resistance Testing
Drug sensitivity and resistance testing involves dosing cancer cells with compounds or combinations of compounds in 384- or 1536-well assay plates, followed by treatment with a live/dead stain or detection reagent for analysis. In the workflow used at FIMM, cancer cells from patients are treated with known cancer drugs, and then screened in live/dead assays such as CellTiter-Glo® and CellTox™ Green (Promega) to assess sensitivity or resistance. The ability to dose compounds accurately and precisely at nanoliter volumes with the Echo liquid handler is critical to enabling this workflow in a high-throughput manner.
Access® Workstation

Drug sensitivity and resistance testing can be automated with the Access workstation. One or more bulk dispensers integrated onto the system enables dispenses of cells, fixatives, stains, or detection reagents. The system automatically removes and replaces microplate lids. Incubators of varying capacity can be integrated to provide on-line incubation. Various readers can also be integrated onto the system for analysis. Additional components including plate sealers, peelers, centrifuges, and plate washers can be integrated for complete walk-away processing.

Figure 2 ▶
Access workstation, configured for drug sensitivity and resistance testing.

Integrated Components on Access Workstation

<table>
<thead>
<tr>
<th>Thermo Scientific™ Cytomat® 6000 Series Incubator</th>
<th>Labcyte Echo 550 liquid handler</th>
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<tbody>
<tr>
<td>Agilent Technologies PlateLoc Sealer</td>
<td>Lid removal station</td>
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<tr>
<td>Agilent Technologies Microplate Centrifuge</td>
<td>Barcode reader</td>
</tr>
<tr>
<td>Brooks Automation Xpeel®</td>
<td>Thermo Scientific™ Multidrop Combi dispenser</td>
</tr>
<tr>
<td>BMG Labtech PHERAstar FS plate reader</td>
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The Access workstation uses Echo Dose Response software to coordinate all liquid handling events required for the Echo system in a drug sensitivity screen. Echo Dose Response uses an interactive wizard to map transfers of samples to plated cells to create dose-response curves with varying concentration points and lengths.

Echo Software Applications enable researchers to map combinations of drugs into complex layouts commonly used for combination screening.
Figure 3 ▶
A plate map for a source plate containing samples at two different concentrations (represented by “A” and “B”).

Figure 4 ▶
Configuration of the transfer volume requirements for each sample concentration and the backfill requirements for normalization to generate the calculated final concentration points in the assay.
Drug Sensitivity and Resistance Testing at FIMM

The Institute for Molecular Medicine Finland utilizes an Echo 550 liquid handler and an Access workstation to facilitate their drug sensitivity and resistance testing. Cancer cells from patients are treated with known cancer drugs and screened for subsequent sensitivity or resistance using live/dead assays such as the Promega CellTiter-Glo® or CellTox™ Green assay. Data from these screens is used to make modifications in patient treatment – enabling precision and personalization.

Materials

- Blast cells from acute myeloid leukemia (AML) patients
- 384-well polypropylene Echo-qualified source plates (Labcyte, P-05525)
- 384-well assay plates
- CellTiter-Glo (Promega, P/N G7570 – G7573)

Methods

The Echo liquid handler is used to transfer nanoliter volumes of known oncology-relevant drugs to 384-well assay plates to create 5-point dose-response curves. 5 µL of media is dispensed to the plates using a Thermo Scientific Multidrop Combi. Blast cells taken from the bone marrow of AML patients are re-suspended in media to a concentration of 5 x 10⁵ cells/mL. 20 µL of the cell suspension is dispensed to the plates using a Thermo Scientific Multidrop Combi. The plates, containing cells and compound, are then incubated for 72 hours at 37°C.

After incubation, the plates are brought to room temperature. 25 µL of CellTiter-Glo reagent is added to the plates using a Thermo Scientific Multidrop Combi. The plates are shaken for 5 minutes, and then centrifuged for 5 minutes at 1000 rpm. They are then read on a Molecular Devices Paradigm® or BMG Labtech PHERAstar® FS reader using luminescence detection.
Results
Data is analyzed and drug sensitivity or resistance is determined by calculating a Drug Sensitivity Score (DSS). This score is calculated as a modified area under the dose response curve, and provides for better assessment of drug sensitivity and resistance than a standard IC50 value. The model for this was developed by Bhagwan Yadav, Tero Aittokallio, Tea Pemovska, and Krister Wennerberg at FIMM. Illustration of the difference between a simple IC50 calculation and the DSS calculation.

Figure 6
Comparison of 3 different dose response curves with three different cell samples against the same drug, where, taken individually, the drug has the same calculated IC50 value for each sample, even though the maximum responses are clearly different.

Figure 7
Calculation of the DSS, better illustrating the difference in the dose response between the patient sample and the control sample to the same drug.
With the Echo liquid handler, high quality, reproducible data has been generated. Comparison of DSS scores from repeated screens of treated leukemic samples shows excellent correlation between replicate data.

**Summary**

The Echo liquid handler and Access workstation offer a complete solution for miniaturized, high-throughput drug sensitivity and resistance testing. As demonstrated by the study described in the article by Ekins S., et al, the ability to acoustically dispense samples without contact can positively impact results. This benefit along with the cost savings from the elimination of pipette tip use has led research institutions such as FIMM to utilize the Echo liquid handler to screen large numbers of compounds against patient cancer cells to assess drug sensitivity and resistance. Repeated testing of samples at FIMM has shown excellent correlation of the calculated Drug Sensitivity Score across screens. This further validates use of the Echo liquid handler and Access workstation for this application.
References


2. Tea Pemovska, Mika Kontro, Bhagwan Yadav, et al., Individualized Systems Medicine (ISM) strategy to tailor treatments for patients with chemorefractory acute myeloid leukemia, Cancer Discovery Published OnlineFirst September 20, 2013.