The Advantages of Acoustic Liquid Handling for Drug Sensitivity Screening

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Abstract

Recently identified associations between variants of cancer genes and drug resistance have increased the value for comprehensive drug sensitivity screening in combination with molecular profiling of cancer cells. This in turn, has led to a demand for improvements to screening throughput and efficiency. 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 enables the miniaturization of biochemical screens. This poster discusses the implementation of miniaturized drug sensitivity screening, at the Institute of Molecular Medicine in Finland (FIMM), with assay-ready plates produced by the Echo liquid handler.

The Echo 555 Liquid Handler

The Echo 555 liquid handler uses acoustic energy to transfer fluids without contact. In 2.5 nL droplet increments, the Echo liquid handler can transfer a range of fluids to produce assays in the low microliter to sub-microliter range. Hundreds of droplets are rapidly transferred into low volume, high density assay plates to achieve the requirements of a high throughput screen. True non-contact transfer additionally eliminates the risk of cross-contamination, carry-over, and the cost of consuming or repeatedly washing tips. Eliminating the risk of compound retention in tips also results in better accuracy of data from compound screening.

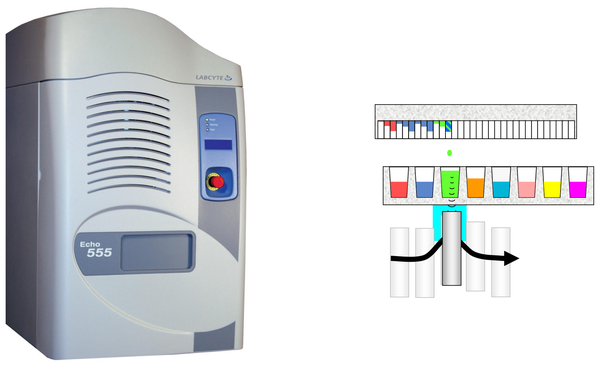


Figure 1. Echo 555 liquid handler and technology concept. An acoustic transducer moves beneath a source plate containing fluids and emits acoustic pulses to rapidly transfer 2.5 nL droplets to an inverted destination plate.

Advantages of Acoustic-Based Dispensing

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 (*Dispensing Processes Impact Apparent Biological Activity as Determined by Computational and Statistical Analyses*, Ekins S., et al) 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.

Automation with the Access® Workstation

All steps of a drug sensitivity screening assay can be fully automated on the Access workstation. This requires the integration of a bulk dispenser to dispense cells, fixatives, stains, or detection reagents, a lid removal station to de-lid plates as necessary, an incubator to provide on-line incubation, and a multimode or high content reader for analysis. The addition of a plate sealer, peeler, and centrifuge, enables complete walk-away processing.

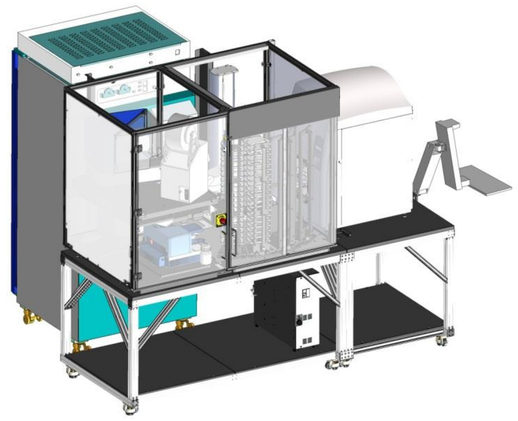
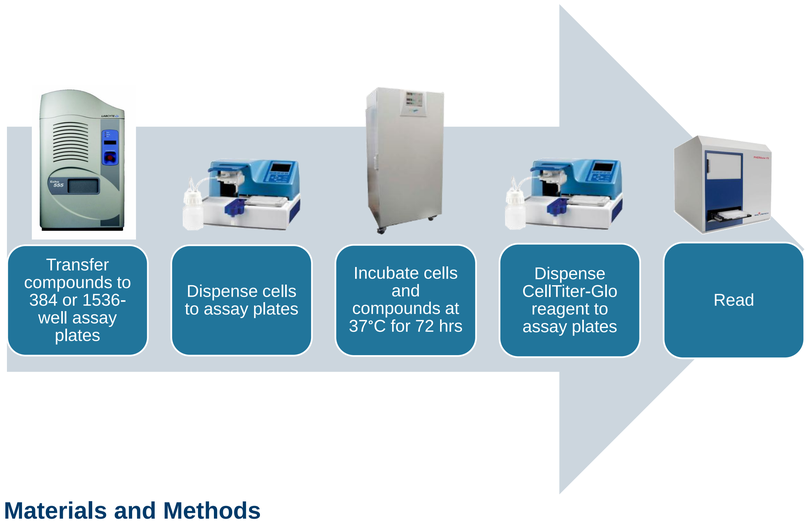


Figure 2. Access workstation integrated with an Echo 555 liquid handler, lid removal station, barcode reader, Thermo Scientific™ Multidrop Combi dispenser, Agilent Technologies PlateLoc Sealer and Microplate Centrifuge, Brooks Automation Xpeel®, Thermo Scientific Cytomat® 6000 Series Incubator, and a BMG Labtech PHERAstar FS reader.

Drug Sensitivity and Resistance Testing

Drug sensitivity screening involves dosing cancer cells with 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, 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.



Materials and Methods

- Blast cells from acute myeloid leukemia (AML) patients
- 384-well assay plates
- CellTiter-Glo

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 resuspended in media to a concentration of 5 x 10^5 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, then centrifuged for 5 minutes at 1000 rpm. They are then read on a Molecular Devices Paradigm or BMG PHERAstar FS reader using luminescence detection.

Results

Data is analyzed and drug sensitivity 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 IC_{50} value. The model for this was developed by Bhagwan Yadav, Tea Pemovska, Krister Wennerberg and Tero Aittokallio at FIMM.

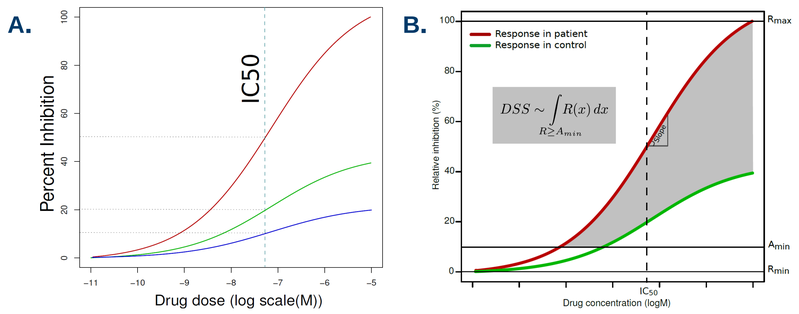


Figure 3. An illustration of the difference between a simple IC50 calculation and the DSS calculation. A) Comparison of 3 different dose response curves with three different cell samples against the same drug, where, taken individually, the drug has the same calculated IC50 value for each sample, even though the maximum responses are clearly different. B) Calculation of the DSS, better illustrating the difference in the dose response between the patient sample and the control sample to the same drug. (Adapted from Yadav et al., *Quantitative Scoring of Differential Drug Sensitivity in Anticancer Compound Testing*, submitted)

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.

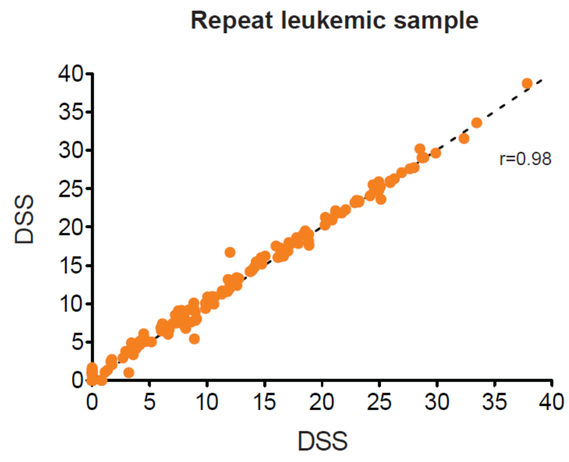


Figure 4. Correlation between repeat screens of drugs against a leukemic patient sample, with drug dosing performed by the Echo liquid handler and Access workstation.

Summary

- The ability of the Echo liquid handler to dispense volumes as low as 2.5 nL allows for miniaturization of drug sensitivity screening, increasing throughput and reducing compound and reagent use.
- Acoustic-based liquid handling has many advantages compared to traditional tip-based methods, and has been shown to produce more accurate compound data.
- With the Echo liquid handler and Access workstation, FIMM has been able to establish a high-throughput drug sensitivity and resistance testing workflow that has produced high-quality, repeatable data.