**Introduction**

Acoustic-based non contact droplet ejection compound transfer methods have become more widely utilized due to advantages demonstrated over traditional methods. These advantages include minimising compound usage and subsequent conservation of compound bank as well as improved data quality through removal of serial dilution cross-contamination issues, reduction in false negative rates, lower consumable costs through reduced plastic and reagent wastage and lower final assay DMSO concentration for poorly tolerant assays.

Within the Lead Identification arena we have utilised acoustic based compound transfer where a requirement for flexible operation is appropriate software to enable easy design of transfer protocols. We have evaluated and utilised the Labcyte Echo Dose Response version 1.1.0 software for a range of direct microtitre to microtitre plate applications on the nanolitre transfer technology of the Labcyte Echo® 555 liquid handler.

The Echo® dose-response software enables the user to define the layout of the source and destination plates, specify the number of points in the dose response curve, and transfer various concentrations and volumes of compounds to the destination plates. The software runs the liquid transfer as well as the DMSO back-fill, and generates a report of the survey and transfer results.

**Plate Formatting**

The Labcyte Echo® Dose Response software is versatile, flexible and user friendly, allowing users to easily create compound transfer protocols in a windows wizard format. Running through the wizard based protocol creation the user can:

- Define source plate layout (+ possible intermediate concentration source plate if required) (Fig 1a)
- Define destination plate layout and DMSO backfilling requirements (Figs 1b and c)
- Define bespoke concentration ranges e.g. Top concentration, 2-fold, half log, three fold
- Run compound transfer protocol in simulation mode to define actual compound usage

**Calibration**

Utilising the Echo® Dose-Response software to dispense fluorescein diluted in 100% DMSO (v/v) it was possible to assess the accuracy and precision of the generated concentration response curves. A 10pt concentration response curve was generated (see Fig 2a) mimicking those dilutions used for typical hit to lead compound dilutions.

Method: 20uL HBSS added to all wells on 384 black flat bottom Greiner assay plate → 50nM fluorescein in 100% DMSO dispensed from Labcyte low dead volume ECHO 384 well source plate (volumes set to 50nl) → 30uL DMSO added to all wells - plate was placed on a plate shaker for 5 min → 700rpm → Plate was spun in a plate centrifuge for 2 min @ 1000rpm → Plate read on Tecan Safire fluorescence plate reader (Ex 496, Em 518 nm)

- Calibration concentration response curves generated were within acceptable limits for accuracy and precision

**Pharmacological Compound Profiling (pA2)**

Concentration response curve generation accuracy was also determined using a biochemical enzyme assay, comparing compounds diluted manually with electronic pipette to concentration-response curves generated on the Echo® by the Dose-Response software. Briefly, the assay comprised a 384 well fluorescence quench assay format, where 20 µl of enzyme was added to each well followed by compound and finally substrate (50 µl final volume). After 60 min incubation at RT product was quantified using an Envision fluorescence plate reader (Ex 380nm Em 450nm)

- Compound pA2 data showed good correlation to manually diluted compounds

We have also utilised the Labcyte Echo® Dose Response software to create more complex protocols for pharmacological testing of compounds. For example we have studied antagonist activity through a pA2 determination experiments at a receptor expressed recombinantly.

Method: 20 µl assay media added to all wells on 384 black flat bottom Greiner assay plate → 4 concentrations of test compounds added from Labcyte low dead volume Echo® 384 well source plate (volumes set to 50nl) → Challenge against 20 µl EC50 curve added to Echo®→ 30 µl HEX/receptor X cells added per well
- Compounds tested were non-competitive antagonists demonstrating unsurmountable antagonism of known ligand stimulation
- Time to generate plate map ≈ 5 min
- Time to the compounds ≈ 4 min
- Compound usage 350nl (3x 384 well volume)

**DMSO Sensitive Assay Formats**

**DMSO sensitivity**

The Prazosin u5l-Glo Cytochrome P450 (CYP) assay was evaluated for use in high throughput screening conditions. CYPs can be sensitive to DMSO at the levels used previously to generate IC50 curves (1.25% (v/v) final). Using the Echo® Dose Response software compounds 1 were dispensed directly into assay well with final DMSO concentration of 2.5% (v/v), avoiding issues observed in the graphs shown.

**Time and Compound Savings**

Utilising Echo® Dose Response software has given us clear advantages in compound usage and time savings compared to traditional flat bed liquid handler or manual compound handling.

For a typical 10-point EC50 curve generation for 32 compounds, Echo® 555 with Dose Response software:
- Time to generate plate map = 2 min (not every assay)
- Generate source plate = 1 min ( offline)
- Compound used = 0.52 µl
- Time to transfer 32 compounds in to destination plate = 2 min
- Typical flat bed liquid handler: Time to generate and transfer dilutions = 20 min
- Compound used = 20 µl

- Compound saved versus manual dilution =16 µl compound/assay
- Time saved versus manual dilution = 5 min/32 compounds

1 mg of compound solubilised at 4 mg/ml gives 250 µl ± 12 separate 10 pt 2-12 dilutions generated on typical flat bed liquid handler or ± 80 separate tests using Echo®

**Conclusions**

Labcyte have developed Echo® Dose response version 1.0.1 software which has provided utility and benefits for acoustic compound dispensing for activities in the Lead Generation environment including:

- Good flexibility and ease to use
- Accuracy and precision
- Reduction in compound usage and time
- Reduction in final assay DMSO levels
- Less false negatives as compounds transferred direct into assay therefore no loss due to precipitation
- Back fill DMSO for DMSO sensitive assays
- Pre-experiment simulation of compound usage
- Addition of standards and controls

Note: Best assay reproducibility requires good mixing (plate shaking and centrifugation) following acoustic dispense, particularly into ‘dry’ plates. However this can be minimised by dispensing in to a ‘wet’ plate.

Application of the Labcyte Echo® has improved liquid handling precision and led to more reproducible compound inhibition curves, whilst reducing the time and cost in running a HIL screening cascade