

Automation of Nanoliter Volume Transfers: Doing More With Less.

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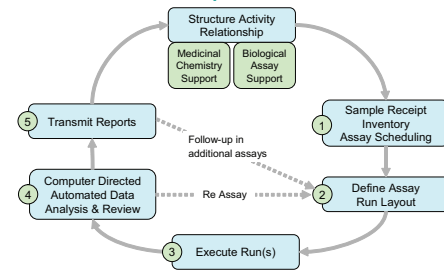
ABSTRACT

To facilitate the discovery and refinement of lead compounds, our laboratory known as the Facility for Automation and Screening Technology (FAST) serves as a site hub for processing DMSO solutions into custom assay ready plates for Basic Research project teams. FAST has integrated an in-house developed Laboratory Information Management System (WP FAST LIMS) with several technology platforms ensuring appropriate compound concentrations and plate formatting while reducing sample processing at the individual level. When used in conjunction with Tecan's Freedom™ or Hamilton's MICROLAB® STAR pipetting instrument, operations such as cherry-picking from multiple source containers, single-point dilution and dose titrations are routinely performed. This presentation describes how transforming technologies, such as the incorporation of robotics into a mature nanoliter plate replication and re-formatting process and acoustic droplet ejection (ADE) technology, have improved conservation of precious compounds, increased reliable walk-away time, and enhanced productivity in the lead optimization phase of drug discovery.

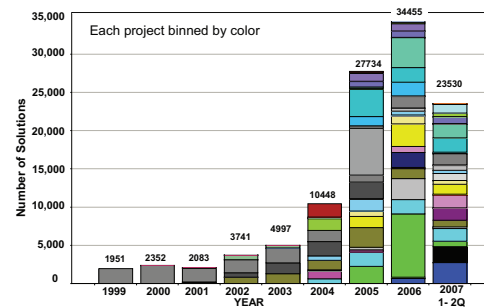
INTRODUCTION

Effective management of limited quantities of valuable compounds is an important part of drug discovery productivity. There is an increasing number of compounds being sent to and from the WP FAST group. The ability to integrate robust automated processes is a requirement to upgrade the efficiency of our facility. Nanoliter liquid handling technologies improve our capability to miniaturize assays, reduced the turn-around time from compound synthesis to assay ready plates in a variety of formats, and enhanced our ability to support various primary, secondary, and pharmacokinetic (PK) assays from a single compound submission. Two examples of nanoliter handling instruments available in our facility are TTP LabTech's mosquito™ (Royston, UK) and the Labcyte® Echo™ 555 (Labcyte Inc., Sunnyvale, CA). The mosquito™ is a positive displacement pipette that uses syringe-like disposable tips with a 50 – 1200 nL range. The Labcyte® Echo™ 555 uses focused acoustic energy to propel a 2.5 nL droplet/pulse from a compound containing source plate upward to an inverted destination plate. In this study, the ARTEL Multichannel Verification System (Artel Inc., Westbrook, ME) and a cell based assay were used to evaluate the liquid handling performance of these nanoliter liquid handling instruments.

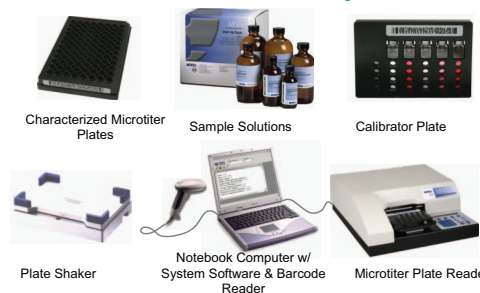
WP FAST: Assay Management for Lead Optimization



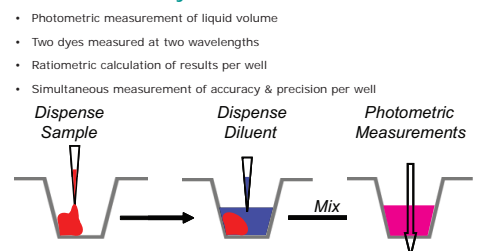
Site Compound Management: Solutions received by WP FAST



ARTEL Multichannel Verification System (MVS®)

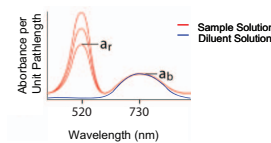


ARTEL Multichannel Verification System (MVS®) Dual-Dye Photometric Method



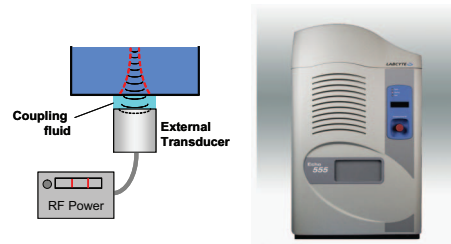
ARTEL Multichannel Verification System (MVS®) Dual-Dye Photometric Method

Mixtures of any ratio of test solution to diluent will result in the same concentration of blue dye, but a varying concentration of red dye. Thus, the blue dye is used as an internal standard.



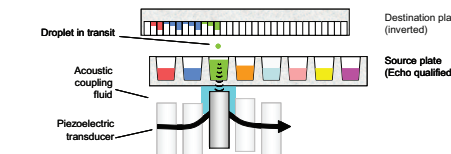
Labcyte® Echo™ 555: ADE technology used to perform direct sample transfers

- Completely "touchless"
- Auditing provides DMSO%, speed of sound and fill volume
- Automatic focus at fluid surface
- Single access door to load source/destination plates (ex. robotic integration)
- Instrument cost ~ \$350K



Labcyte® Echo™ 555: ADE technology used to perform direct sample transfers

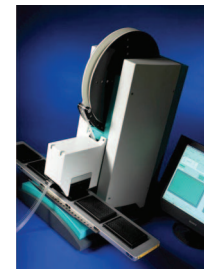
- Transfer 2.5-1000 nL compounds in DMSO (70 – 100% using our current calibration)
- Direct plate-to-plate transfer (insert required for each source plate)
- Any well to any well, at any volume (ex. cherry picking, dose-response curves and assay miniaturization)
- No tips or wash fluid needed, however coupling fluid maintenance is required based on usage rate (~ weekly)



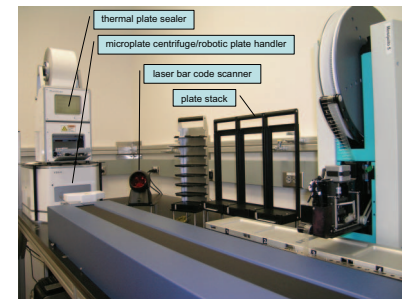
TTP LabTech's mosquito™

- Disposable positive displacement tip technology used to perform direct sample transfers (i.e., contact dispensing).

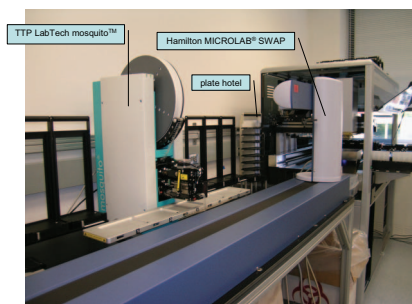
- Good conservation of compound, low dead volume when used in conjunction with conical, or pyramidal well bottom source plates.
- Compatible with 96 – 1536 standard, high, low base source plates.
- 5-position deck capacity
- Easy programming and assay protocol set up.
- Tip cost ~ \$0.06, 26000 tips/reel
- Instrument cost ~ \$100K
- Has been successfully integrated with 1400 mm track for routine plate cloning, and compression of 3 fold, 10 point titrations from 96-well to 384-well format.



mosquito™ integrated with Velocity11 PlateLoc™, VSpin™ and Access2®

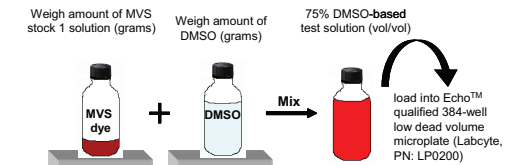


mosquito™ integrated with Hamilton MICROLAB® SWAP



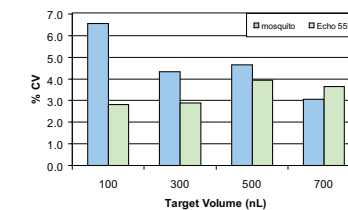
METHOD

To evaluate the nanoliter liquid handling performance of TTP LabTech's mosquito™ and the Labcyte® Echo™ 555 for accuracy and precision, an alternative dual-dye solution was prepared using Artel's MVS® Data Manager Advanced feature (version 2.2.0.19). Stock solution 1 (Lot: T10122070601) was gravimetrically combined with DMSO to prepare the 75% DMSO (vol/vol) dual-dye test solution. This solution was transferred at various submicroliter volumes by the liquid handlers, photometrically measured, and performance reports generated.

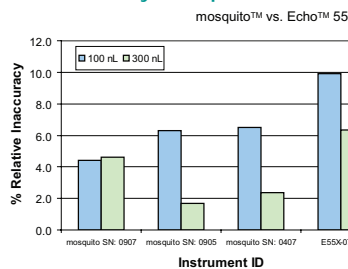


Transfer Precision: mosquito™ vs. Echo™ 555

MVS® alternative solution (dual-dye in 75% DMSO) was utilized to compare the transfer accuracy and precision in 384 well format between Echo™ 555 and the mosquito™ low volume liquid handler. At target volumes of 100, 300, 500, and 700 nL, both instrument platforms measured CVs less than 7%. Transfer accuracy was measured between three (3) mosquito™ instruments, and Echo™ 555. The liquid transfer inaccuracy was less than 10% average deviation from all target volume evaluated.



Transfer Accuracy: mosquito™ vs. Echo™ 555



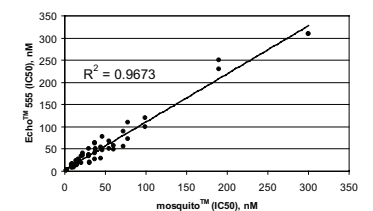
Target Volume (nL)	Transfer Accuracy (Mean volume (nL) ± SD)	
	mosquito	Echo 555
100	94.3 ± 6.2	109.9 ± 3.1
300	295 ± 12.8	319 ± 6.3
500	493 ± 23	531 ± 21
700	688 ± 21	741 ± 27

FLIPR METHOD (200 nL compound transfer, 384-well format)

1. 20 x 10³ CHO cells transfected with a human GPCR are plated in 384 well plates overnight in 100 uL of medium.
2. Plates are washed with Hank's BSS medium supplemented with probenidol then loaded with fluo-4 dye and incubated at 37°C for 60 minutes.
3. 50 uL of HBSS medium is added to 200 nL of test compound in DMSO in 384 well plates.
4. 384 well plates of agonist at 4X concentrations are prepared.
5. Cell plates are washed once again to remove all extra-cellular dye, leaving 30 uL HBSS/well.
6. The FLIPR instrument adds 15 uL of test compound/well, mixes and incubates for five minutes.
7. The FLIPR adds 15 uL of agonist/well, quantifies the fluorescent response and saves file in an Excel compatible format.

Comparison between mosquito™ vs. Echo™ 555

mosquito™ vs. Echo™ 555 correlation
200 nL compound transfer



SUMMARY

Nanoliter plate replication and reformatting into higher density plates is an important and routine process in our facility. With over 150 protocols initiated per month on TTP LabTech's mosquito™ instrumentation, the ability to replace human labor with machine labor was a critical initiative. Hamilton's MICROLAB® SWAP (swivel arm plate handler) has been used in the automation of this nanoliter liquid handler and is fully integrated with Velocity11 PlateLoc™, VSpin™ and Access2®.

To ensure that the mean-time-to-repair (MTTR) rate is well understood for our liquid handlers, and volume transfer accuracy and precision performance could be rapidly assessed we have installed Artel's Multichannel Verification System (MVS®) into our processes.

With the receipt of over 20,000 compounds/year to support various primary, secondary, and pharmacokinetic (PK) assays our ability to make significant reductions in sample volume has an important impact on our valuable compound store. Nanoliter liquid handlers, TTP LabTech's mosquito™ and the Labcyte® Echo™ 555 when evaluated with the MVS® (75% DMSO dual-dye) measured CVs less than 7% and liquid transfer inaccuracy was less than 10% average deviation from all target volumes.