Low volume automated liquid handling for studying autophagy and cellular transduction in cancer cells

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introduction

Drug discovery is a complex and involved process, utilizing a multi-disciplinary approach to identify efficient small molecules or biologics against drug targets. Millions of potential drugs are screened in order to obtain a marketable drug.

High-Throughput Screening (HTS) approaches are used to collect a large amount of experimental data in a relatively short amount of time with miniaturized amounts of the expensive libraries. Measurements of compounds against targets must be standardized using concentration-dependent curves and determining IC_{50} values.

Drawbacks of traditional serial dilution of compounds include: limited to microlitre volumes, limited data accuracy, poor reproducibility, high consumption of consumables.

Here, we describe two examples of HTS approaches and follow up concentration-dependent curve studies and demonstrate how TTP Labtech's mosquito® HTS liquid handler can overcome many of the challenges associated with manual serial dilutions and high-throughput pipetting.

1. accurate and reproducible high-throughput liquid handling

mosquito® HTS (25 nL – 1.2 µL) and **mosquito HV** (0.5 – 5 µL) are automated 8- or 16-channel liquid handlers that are compatible with 96-, 384- and 1536-well SBS format plates (Fig1a).

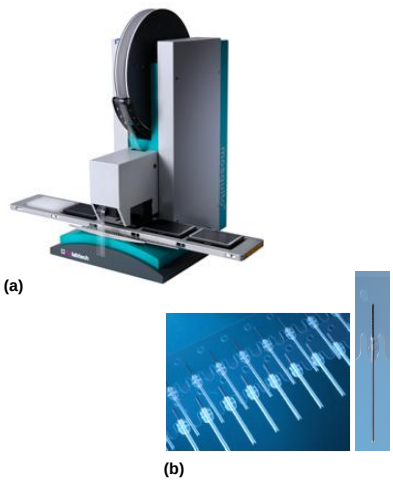
mosquito's true positive-displacement technology provides:

- fast and accurate low volume pipetting
- high reproducibility across a large dynamic range
- extremely low dead volumes

mosquito's unique **disposable tips** are stored on a spool of 26,000 or 36,000 tips (Fig 1b). Each pipette tip has its own stainless steel piston – not an air gap or liquid – offering true positive-displacement pipetting.

mosquito tips enable:

- efficient handling of any liquid including solvents and viscous solutions
- multi-aspirate/ multi-dispense pipetting
- optimal mixing
- piercing of foil plate seals
- pipetting without clogging



(a)

(b)

Fig 1. (a) mosquito HTS liquid handler, (b) mosquito tips, with true-positive displacement pistons

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2. drug modulation of autophagy in cancer cell lines

Scientists at the Sanford Burnham Prebys (SBP) Medical Discovery Institute (La Jolla, CA, USA) undertook an extensive study to better understand the modulation of the autophagic pathway in 10 different cancer cell lines. The study examined the response to different nutrient and oxygen conditions, as well as chemical stimulus. An initial step was required to serial dilute the compounds and transfer to assay ready plates.

methods

8 point serial dilutions of 6 different chemical compounds (in DMSO and aqueous) were created, in triplicate in a 384-well plate by mosquito HTS. The total volume of each dilution was 1.8 µL. The accuracy of these dilutions was analysed using a fluorescent-based assay. 60 nL of each dilution point was transferred to the assay plates, containing 40 µL of media using mosquito HTS.

The %CV between triplicates was calculated for each compound.

Baflomycin was used as internal control for the autophagic assay. The compound was delivered accurately by mosquito HTS and phenotypic expression was observed quantified.

results

mosquito HTS liquid handler performed well in serially diluting the compounds and in wet dispensing the compounds into the media.
For each dilution the average %CV was calculated across triplicates and then the average of these values across compounds was presented in the table below (Table 1).

The results demonstrated that mosquito HTS performed the pipetting of the serial dilution in DMSO well and was consistent across replicates (Fig 2).

The autophagic assay demonstrated increased autophagy over time with the addition of 25 nM bafilomycin (Fig 3).

Dilution	%CV
1 (highest conc.)	4.5
2	10.5
3	8.9
4	11.2
5	7.2
6	5.9
7	4.1
8 (lowest conc.)	1.1

Table 1. Average %CV across replicates and compounds.

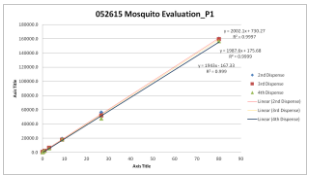

Fig 2. Concentration-dependent curve for one compound in triplicate.

mosquito HTS was selected for this assay due to its accurate and consistent transfer of nanoliter volumes, and the low dead volume (<500 nL) that remains in the source plate.

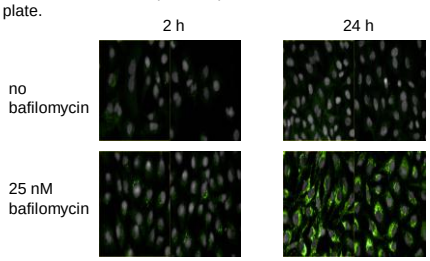

Fig 3. The autophagic effect of bafilomycin on cancer cell lines over a 24 h period.

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3. drug concentration effects on lung cancer cell line

A separate group at SBP set up a time-dependent concentration-dependent assay to determine if constituent luciferase levels could be reduced in a specific human lung cancer cell line when exposed to various stimuli.

methods

Bright-Glo™ luciferase assays (Promega, USA) were run to determine drug concentration effects on constituent luciferase levels in a human cancer cell line over an 8 h time period.

CellTiter-Glo® luminescent cell viability assays (Promega, USA) were performed to verify that any decrease in luciferase signal was due to a drug affect.

Using mosquito HTS, 150 nL of a drug compound at each concentration (total 10 concentrations) was dispensed into 25 µL of reagent in a 384-well plate (Fig 4).

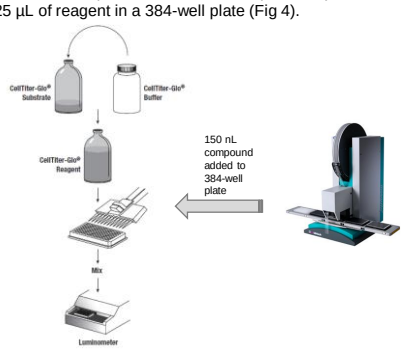

Fig 4. The use of mosquito for CellTiter-Glo® luminescent cell viability assays

results

Data demonstrated that wells with the same concentration and same volume of the drug produced the same number of colony forming units (CFUs). This demonstrated that mosquito HTS was highly accurate and precise in pipetting providing reproducible results for every well independent of the position on the plate.

Plate effects were seen when dispensing with an alternative low volume plate dispenser, or by hand, resulting in poor CVs for *in vitro* assays, and therefore delaying the *in vivo* studies.

mosquito HTS has enabled validation of the *in vitro* assays which are now progressing into *in vivo* assays. If the results from the *in vitro* and *in vivo* assays are paralleled this could become a valuable tool in determining effectiveness of cancer treatments.

conclusion

High-throughput drug screening requires rapid and accurate liquid handling especially when creating concentration-dependent curves.

Many of the limitations associated with serial dilution of drugs can be overcome by TTP Labtech's mosquito HTS due to its ability to miniaturize assays, reduce costs and improve reproducibility.

The examples presented in this poster validate mosquito HTS as an extremely effective tool in enabling high-throughput, low volume assays in drug discovery.

mosquito HTS's benefits include:

- accuracy to perform serial dilutions well
- low dead vol in source plate reduces vol of compound needed
- ability to perform wet dispensing accurately
- high precision and accuracy independent of position on the plate
- consistent reproducibility

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