Enriched datasets from in-house compound libraries using direct dilution methods in 1536-well plates

Joe Zer*, Kristen Sebring*, Jose Quiroz*, Soheila Vaezeslami#, Joby Jenkins#

*Dart NeuroScience, 12278 Scripps Summit Drive, San Diego, CA, USA , #TTP Labtech Ltd, Melbourn Science Park, Melbourn, Hertfordshire, UK

introduction

Serial dilution of drug compounds is standard practice for preparing a dose response series to determine the potency of a compound.

1536-well plate **serial dilution** has proven less than optimal due to the compounding errors inherent in each dilution step. In addition, many liquid handlers have difficulties with achieving effective and consistent mixing within the well.

Direct dilution has been of interest, in recent years, for preparing the dose response curves. However, a narrow dynamic range and the requirement for fixed well volumes are limiting factors for performing an accurate direct dilution. Taking advantage of low volume liquid handling systems in the nanolitre range has enabled this approach to be utilized more widely.

This poster will demonstrate how TTP Labtech's automated low volume liquid handler (mosquito® HTS) can create a semi-direct dilution series into 1536-well plates. This method will be compared to the gold standard serial dilution method.

rapid and accurate creation of dose response curves in 1536-well plates using mosquito HTS

mosquito® HTS (25 nL – 1.2 µL) is an automated 8- or 16-channel liquid handler that is compatible with 96-, 384- and 1536-well SBS format plates (Fig 1a). Its true positive-displacement technology enables pipetting of any liquid type, irrespective of viscosity and environmental conditions.

mosquito's main features include:

- robust performance from nanolitre to microlitre pipetting
- highly accurate and precise
- ability to aspirate, dispense and mix
- zero cross-contamination
- minimal dead volumes
- ease-of-use

mosquito® X1 (25 nL – 1.2 µL or 0.5 – 5 µL) is an automated single channel liquid handler. Automated cherry picking of hits following dose response screening can be performed by TTP Labtech's mosquito X1 enabling the whole process to run 24 hours a day (Fig 3).

mosquito HTS'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)

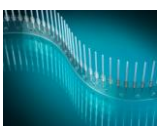


Fig 1. (a) mosquito HTS liquid handler, (b) disposable tips of mosquito liquid handlers

Dart NeuroScience

case study: high-throughput, automated direct dilution for dose response screening

A semi-direct dilution method involving an on- the-fly dilution step eliminating the need for an intermediate dilution plate, was developed and optimized in 1536-well plates using mosquito HTS by scientists at Dart NeuroScience (San Diego, CA, USA). They compared the dose response curves set up using this semi-direct dilution approach (Fig 1b) to the gold standard method of serial dilution (Fig 1a).

(a) serial dilution (gold standard)

(b) semi-direct dilution

Fig 2. schematic of (a) serial dilution - the gold standard method of diluting compounds for dose response curves and, (b) semi-direct dilution method that involves a serial dilution step* as part of the series which can be performed on-the-fly using mosquito HTS

1. study design

A. **serial dilution** - 11 point curve in a 384-well plate (gold std) created by alternative large volume liquid handler

B. **semi-direct dilution** - 11 point curve in a1536-well plate created by mosquito HTS, a low volume liquid handler

2. methods

A half-log dose response curve was set up with a starting concentration of 1 mM for both the serial dilution and the semi-direct dilution methods. The serial dilution series was set up in a 384 well plate but the semi-direct dilution was successfully set up in a 1536–well plate.

mosquito HTS was used to pipette compound volumes down to 63 nL.

For the 11 point semi-direct dilution method, mixing steps were incorporated at dose 4, 7 and 10 respectively. mosquito is able to mix and transfer the mix step on-the-fly without the need for additional plates.

FITC was used to measure the amount of florescence emitting with EnVision multilabel plate reader (Perkin Elmer, MA, USA).

Hits were then cherry picked using mosquito X1 (Fig 3) enabling automated dilution and hit picking without human intervention 24 hours a day.

Biological IMAP TR-FRET cAMP enzymatic assays were performed to verify the results.

(a) (b)

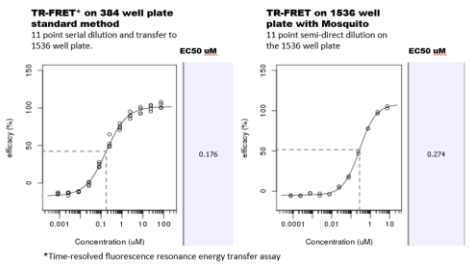
Fig 3. (a) mosquito X1 liquid handler, (b) mosquito X1 addressing a 1536-well plate

3. results

The biological assay verified that there was no differences between the semi-direct dilution method in a 1536-well plate and the serial dilution approach into a 384-well plate. In both cases, the sigmoidal curves were comparable and the EC_{50} levels were equivalent across all the compounds tested (Fig 4a and b).

The semi-direct dilution method was run across multiple test plates and a %CV under 10% was achieved for all doses.

Using 1536-well plates reduced the reagent and compound usage by 10-fold, thus reducing costs as well as improving data quality.



(a) (b)

Fig 4. comparison of 11 point dose response curves using different dilution methods for compound A (in triplicate) (a) serial dilution in a 384-well plate (gold standard) transferred to a 1536-well plate (b) semi-direct dilution in a 1536-well plate using mosquito HTS

Fig 5. Dart's automated follow-up station with 1) mosquito HTS (diluter) and 2) mosquito X1 (cherry-picker)

conclusion

- the EC_{50} results obtained using this method in 1536-well plates is comparable to the gold standard serial dilution method in 384-well plates, but in addition saves reagent, compound, cost and time
- rapid, high-throughput, low volume direct dilution of compound libraries is now possible using mosquito HTS. This will overcome many issues associated with using serial dilution dose response curves

Overall benefits of **mosquito HTS** for dose response screening:

- can perform both serial and semi-direct dilution on-the-fly
  - no intermediate source plate required
  - can mix within a well so can perform whole series in one run
  - saves time and intermediates
- accuracy at small volumes which enables accurate direct dilution
- saves on reagent and compound by at least 10-fold
- unrivalled speed – rapid set up in 1536-well plates

mosquito HTS enables dose response curves of compound libraries to be performed in high-throughput both rapidly and accurately

www.ttplabtech.com

TTP Labtech Ltd
Melbourn Science Park, Melbourn
Hertfordshire, SG8 6EE,
United Kingdom
Tel: +44 (0) 1763 262 26
Fax: +44 (0) 1763 261 964

TTP Labtech Inc
One Kendall Square, Suite B2303
Cambridge MA 02139-1594,
United States
Tel: +1 (617) 49 9794
Fax: +1 (617) 4949795

TTP Labtech India Private Limited
SRS Tower – 212, 2nd Floor
Sector 31, 14/5 Mathura Road
Faridabad (NC Delhi) 121003, India
Tel: +91 9910397385
+91 9968291292