A Critical Evaluation of the Mosquito: A Tool for Drug Discovery

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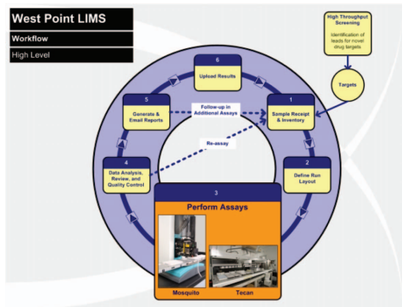
Abstract

The Mosquito is a low volume (0.05 to 1.2 µL) pipettor that uses positive displacement with disposable tips. This instrument, made by TTP LabTech (http://www.ttplabtech.com/mosquito), has a number of unique features and applications for the drug discovery process. It can facilitate the miniaturization of assays by allowing for serial dilutions on a microliter scale and the creation of assay ready drug plates. This poster discusses the uses of this instrumentation in the drug discovery workflow and provides a critical evaluation of the Mosquito.

Introduction

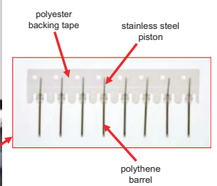
The incorporation of the Mosquito into our drug discovery process has made a direct impact on workflow efficiency and productivity in our laboratory. This low volume liquid handler has given us the ability to remove unnecessary work, reduce the volumes of costly reagents and waste, conserve on valuable compound, lower DMSO concentrations, provide low volume assay ready plates and maintain the accuracy and precision of our dose inhibition curves.

Fitting new technologies like the mosquito into our drug discovery process has been key to keeping pace with the shear number of assays and novel chemical entities being developed.



What is the Mosquito?

- Automated multi-channel pipettor capable of dispensing liquid from 50nl – 1200nl
- 96, 384, and 1536 well compatible
- Plate Types and Well Types Used
  - Nunc, Costar, Remp, Falcon, Greiner
  - Flat,V, Conical, U, Round Bottom



How does the Mosquito work?

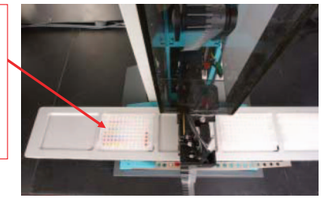
Uses miniature, disposable, positive-displacement micropipettes mounted on a reel, each containing its own piston

- $0.058/tip
- 26,000 tips/roll

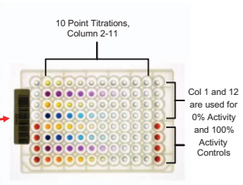
- 8 micropipettes are fed into the head
- Five Position Puck, slides horizontally under head to move plates into pipetting position
- Tray shifts in or out to accommodate 384 and 1536 well plates
- Head moves vertically into pipetting position
- Used tips are fed out of the front of the instrument

Programmed volume of liquid is aspirated from the source plate and dispensed into the destination plate (s).

Program can be set up to do 10, 3-fold serial dilutions across a 96 well conical plate using one set of 8 tips.



Uses of the Tecan Freedom Liquid Handler



- Up to 9 Assay Plates can be set up in one Run (96 Well, Deep and or Shallow Conical Bottom Plates). Up to 576 compounds can be accessed per run.
- 8 compounds per plate, up to 72 compounds per Assay Run.
- Ten point dose titrations are done by the Tecan (3-fold serial dilutions are performed (10 µL in 20 µL)).
- Reagents can be added by Tecan Genesis or a 96 well Pipetting Station or titration plates can be handed off to the Mosquito to generate other types of assay plates.

Uses of the Mosquito

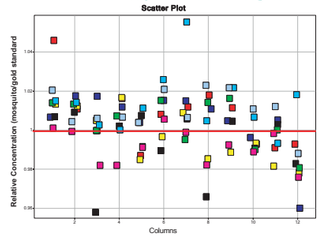
Tecan Freedom created titration plate (20 µL per well) is placed on the deck of the Mosquito.

The Mosquito can spawn up to four daughter plates using a range of volumes from 0.05 µL to 1.2 µL

- Produce Low Volume assay ready plates and **daughter plates**.

  Types of Assays that are being supported
  - FLIPR, BLA, Luciferase, cAMP, Ion Works, Patch Express, Viral assays.
  - Radio-ligand, Enzyme, SPA-based, TRF assays.
- Transfer from 96 well to 384 well plates or 384 to 384.
  - e.g., Four 96 well plates plated into one 384 well plate to support FLIPR assays.
- Two point screens. (e.g., 0.1 µL and 1 µL)
- Columns can be re-formatted to fit other desired plate layouts.
- **Ten Point Titrations (3-fold serial dilutions across a 96 well plate).**

Examination of the Mosquito Generated Daughter Plates



Conclusions

Accuracy @ 500 nL is within 2%

Standard Deviation @ 500 nL is < 2%

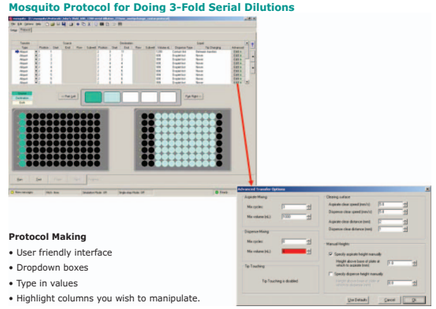
Method

- Measure absorbance of Fluorescein Dye in a 96-Well Plate by plotting the absorbance of the **Mosquito** plated Fluorescein dye and normalize to the "gold" standard for all 96 wells.
- "Gold" Standard: 100ul of straight fluorescein dye diluted 400-fold in buffer
- Mosquito: 500nl of straight fluorescein dye pipetted by the Mosquito and diluted 400-fold in buffer

Serial Dilution By the Mosquito on a 1.2 µL Level

- 3-fold Serial dilutions in 100% DMSO were done by the **Mosquito** (0.6 µL into 1.2 µL) and **Tecan Freedom** (10 µL into 20 µL).
- Each protocol uses the same 8 tips across the plate from column 2 to column 11.
- 10 mM Fluorescein Dye was made in 100% DMSO to mimic typical drug dilution.
- 20 mM HEPES pH 7.5 was added to the serial dilutions to generate Fluorescent signal.
- Signal was measured by the Spectra Max Gemini XS (Excitation 485 nm and Emission of 520 nm).
- Ratios were made by dividing the Mosquito generated fluorescence readings by the average Tecan readings to yield relative concentrations.
- The Ratio of 1 demonstrates 100% accuracy.
- Scatter graphs and box plots were generated to show accuracy (ratio of 1) and reproducibility (variation in each column).
- A similar graph of the Tecans ability to do 3-fold serial dilutions is included.

Mosquito Protocol for Doing 3-Fold Serial Dilutions



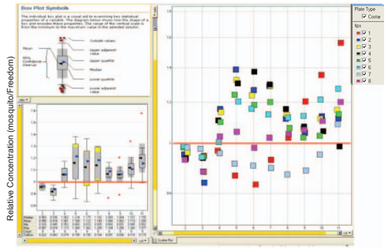
Protocol Making

- User friendly interface
- Dropdown boxes
- Type in values
- Highlight columns you wish to manipulate.
- Copy and Cut functions

Key Factors in Doing 3-fold Serial Dilutions

- Mix cycle
- Aspiration height above the base of plate
  - Base height can vary across plates
- Dispense by droplet first

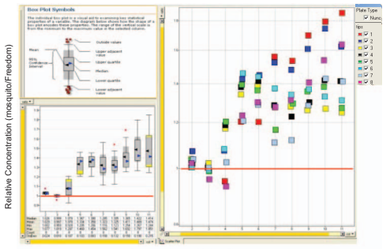
Relative Concentration Derived from a *Mosquito* Generated, 3-fold Serial Dilution (0.6 µL into 1.2 µL) using a Costar Plate.



Conclusion for 3-fold serial dilution examined by Fluorescein Dye (Costar)

- Reproducibility (%CV) is < 17%.
- Accuracy (mean value) is within 20%.

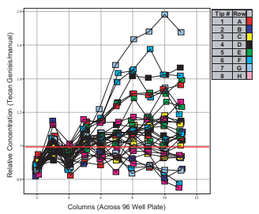
Relative Concentration Derived from a *Mosquito* Generated, 3-fold Serial Dilution (0.6 µL into 1.2 µL) using a Nunc Plate.



Conclusion for 3-fold serial dilution examined by Fluorescein Dye (Nunc)

- Reproducibility (%CV) is < 15%.
- Accuracy (mean value) is within 50%.
- Noticed that in the Nunc plate more Flourescein dye was being pipetted as one diluted across the plate. The advanced pipetting parameters for the mosquito needs to be modified for the Nunc plate.

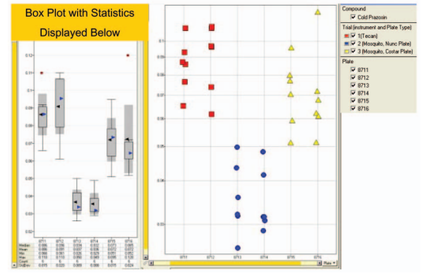
Ratio of Fluorescence Signal Derived from Four, *Tecan* Generated, 3-fold Serial Dilutions (10 µL into 20 µL).



Assessment of the Serial Dilution by the Mosquito using a Radioligand Binding Assay

- 3-fold Serial dilutions in 100% DMSO were done by the **Mosquito** (0.6 µL into 1.2 µL) and **Tecan Genesis** (10 µL into 20 µL).
- Two types of 96 well conical, v-bottom plates (Nunc #249946 and Costar #3357) were used by the Mosquito to do the dilutions.
- Illustrative radio ligand binding assay was run to judge the mosquito's ability to generate Ki values similar to those generated by the Tecan Genesis.
- Preparation of membranes, buffers, and ligand ([^{3}H]-Prazosin) is as described in O'Malley 1998.
- The KI values were determined using a KD = 0.18 nM of the radiolabel [^{3}H]-Prazosin and a nominal [Radiolabel] = 0.4 nM as described in Mosser 2003.
- Determined Ki values were plotted on a Scatter Graph and Box Plot.

Ki Values of Cold Prazosin Derived from 10 Point Dose Titration Curves Made by the Mosquito and the Tecan Freedom. 6 Titration Curves Per Plate.



Conclusion for 3-fold serial dilution examined by the Radioligand Binding Assay

- The Mosquito (costar plate) demonstrates a Ki values within 20% of the Tecan Freedom.
- The lower Ki values observed with the **Nunc plate** are consistent with the trend observed with fluorescein dye (see above). The advanced pipetting parameters for the mosquito needs to be modified for the Nunc plate.

Summary

The Mosquito has become an integral part of our drug discovery workflow. Its reproducibility and accuracy in transferring nano-liter volumes from all types and styles of 96 and 384 well plates has given the biologist a better way to answer the high demand for running multiple screens within any given project. The user friendly interface makes it easy for even the novice programmer to make usable protocols that will benefit all types of assays. Not having to do any manual pipetting with small volumes has reduced pipetting errors and has brought great satisfaction to the scientists who are doing the work.

Up until now, we have been using the Tecan Freedom to make our serial dilutions (10 µL into 20 µL). We depend upon the mosquito to transfer these 10 point, serial dilutions into other plates for further use. Now, the mosquito has demonstrated that it is highly capable of doing these same serial dilutions on a nano-liter level (600 nL into 1200 nL). The mosquito is able to do these serial dilutions in roughly three minutes per plate as compared to the Tecan Freedom which takes roughly 5 minutes per plate. Utilizing the mosquito in this fashion will decrease the volume of compound by 16 fold as well as decreasing the volume of DMSO waste that is needed to perform the serial dilutions. Using such low volumes, allows for the miniaturization of reaction sizes, thus saving on resources such as buffers, assay kits, radioligands, enzymes, substrates, membranes and more. In summary, the Mosquito will allow one to build greater flexibility into their workflow and improve their drug discovery effort.

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