An Automated System for HTS of Cell-based Flux Assays

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I. Abstract

The current trend of HTS systems has been to increase the level of automation and miniaturization. This trend has a sole objective of improving efficiency while minimizing the set up time and involvement of manual steps in HTS assays. Aurora Biomed Inc has developed an automated assay system for screening CHO/hERG to meet HTS requirements. The IC50 values for Astemizole and Terfenadine generated by the automated assay system is very comparable to values obtained from manually performed assays. The IC50 values have also proven to be reproducible over replicates performed at different times. A Z factor of 0.768 was determined for the automated assay system.

II. Introduction

Although various technologies and assays are being developed for the high throughput screening of voltage-gated ion channels, there remains a great need for HTS technology with greater accuracy and cost effectiveness. The future for managing the cost and speed of drug discovery lays in miniaturization, and automation[1]. Miniaturization addresses the pharmaceutical industry's three basic needs of lower cost, faster turnaround time, and limited space while automation allows for continuous 24-hour operation and data tracking, thereby enabling larger number of samples to be processed per day at a much lower cost. Initial automation efforts begun with the integration of sample storage directly onto the screening system with devices such as plate readers with stacking devices. The storage of samples on-line allowed for the stacking and removal of plates in an automated fashion thereby reducing manual labor, and therefore costs. However, since then automation systems have evolved, in the early 1990's, to integrated systems and workstation approaches[1,2] which have taken automation to another level, allowing for the full automation of assay preparation.

In recent years, cell-based flux assays have gained wide spread popularity as a tool for drug discovery and hence there has been a need for automation technology that can effectively perform these assays. However, such assays differ from traditional enzyme- or antibody based screening assays in that live cells are used in the assay and any automation developed has to take this fact into consideration. Aurora Biomed has developed an integrated automation system to perform the hERG assay [3] in 96-well format so as to meet the HTS capacity of the ICR series. The data is presented here showing the signal to noise ratio and IC 50 determinations of some standard blockers. The data is highly accurate and reproducible.

III. Materials and Methods

Automation was developed to carryout Aurora Biomed's optimized rubidium protocol. A stable CHO cell line expressing hERG was grown in Ham's F-12 (Gibco) supplemented with 10% FCS (Gibco), 50 mg/L Pen/Strep (Invitrogen) at 37°C, in 5% CO_2. Cells were plated at a density of 50,000 cells/well in 96-well microplates and incubated at 37°C, 5% CO_2 until 80-90% confluent. The cells were washed with 200 µl of Rb^+ Load buffer containing 5.4 mM RbCl (Aurora Biomed Inc.) and incubated with 200 µl Rb^+ Load Buffer for 3 hours at 37°C, 5% CO_2. After loading, the cells were washed twice with 200 µl of Rb^+ Wash Buffer (Aurora Biomed Inc.) followed by compound addition at 2 µl/well from a 100X stock solution (1% DMSO in Rb^+ Wash Buffer). Incubation with the compounds was for 10 minutes at 37°C, 5% CO_2. The wash buffer was then replaced with 200 µl of Channel Open Buffer (Aurora Biomed Inc.) containing 60 mM KCl and 2 µl of compound from 100 X stock solution (1% DMSO, diluted in Channel Open Buffer) for 6 minutes at room temperature. A 200 µl extracellular sample was collected from the supernatant and stored in 96-well microplates. Intracellular samples were obtained by whole cell lysis with the application of 200 µl Lysis Buffer (Aurora Biomed Inc.). The levels of Rb^+ in both the intracellular and the extracellular samples (100 µl samples) were measured by Aurora Biomed's ICR 8000.

Automation was developed to carryout Aurora Biomed's optimized rubidium flux protocol. A stable window of detection was determined by activating the channel with KCl in the absence of any blocker. The basal and activated efflux was plotted to show the window of detection. Z factor was calculated for the automated assay system according to Zhang et al[4].

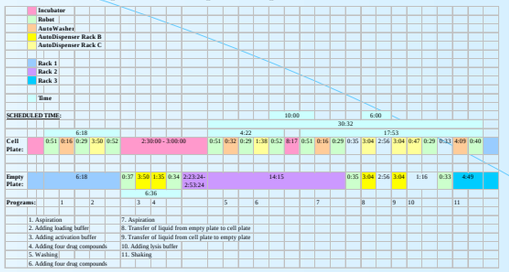
Automated Assay System:

The Automated Assay System is an integrated system of instruments designed to perform flux assays (Figure 1). It can be connected to the Ion Channel Reader to completely automate the ion channel screening process. Each component of the system is Automated Microplate Handler – Compatible with almost any liquid handling deck due to its telescoping arm and rotating wrist joint.

1. **Automated Incubator** – A top-of-the-line automated incubator maintains extremely stable conditions for all microplates.
2. **Automated Microplate Washer** – Patented dual action manifold for precise washing and overflow protection.
3. **Automated Microplate Liquid Handler** – Unique free-floating syringes guarantees reliable tip pickup and separate manifold for continuous rapid dispensing of reagents from reservoir to microplates.
4. **Customized Computer System** – One software controls the entire system, making the Automated Assay System simple to operate.
5. **Ion Channel Reader** – Precisely measures ion channel activity with flame atomic absorption spectroscopy.

Figure 1. Aurora Biomed's Automated Assay System

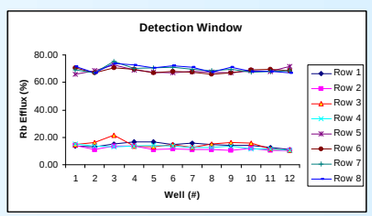
Table 1. Represents automation steps. The train of steps is shown for both the cell plate and the supernatant plate.



IV. Results

The assay provides an appreciable window of detection for screening compounds against CHO/hERG as the signal to noise ratio is more than five folds. The level of activation and basal efflux are 69.21 and 13.26, respectively (Figure 2). The Z factor value of 0.768 from the assay performed by the automation system is similar to the value obtained in the manual assay. This value of Z factor designates the assay as excellent assay.

Figure 2. Detection Window on automated assays system

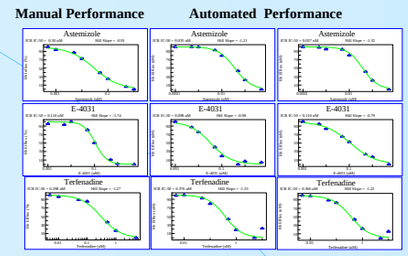


The automated assay system was used to screen three positive blockers, astemizole, E-4031 and terfenadine, to IC_{50} values. These values have been compared with those from manually performed assays and found reproducible (Table 2, Figure 3).

Table 2. The IC_{50} values for astemizole, E-4031 and terfenadine obtained at different times

Drug	Manual Perfori	Automated Perfromance	
Astemizole	30	35	37
E-4031	110	98	110
Terfenadine	298	376	366

Figure 3. The IC_{50} curves and Hill slope for astemizole, E-4031 and terfenadine generated from the Automated Assay System is compared to those from manually performed assays.



VI. Conclusion

The high throughput screening (HTS) is currently relying upon increase in the automation and miniaturization. The improved reliability and efficiency of automation has lead to an increased desire to integrate automation of assays, on line sample storage with automated screening systems. Aurora Biomed's ICR technology is commonly used for assessing hERG channel QT liability and is a powerful technique that results in generating an accurate drug rank order. The data presented here identifies the hERG assay automation as highly reproducible. The Z Factor correlate to an excellent assay giving a large window, allowing for a range of drug potencies to be known. Thus a close integration of biology, chemistry, and engineering may be deciding the future of modern drug discovery.

VII. References

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