Development & Validation of an Automated Workstation for HTS Flux Assays

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I. Abstract

Advances in genomics, proteomics, and combinatorial chemistries have dramatically increased the need for automation of biological assays and microarrays. Automated liquid handling systems for carrying high-throughput screening (HTS) assays have become an invaluable tool for the drug discovery and development industry. Therefore, the lack of such systems has been a major bottleneck in the drug development process. In the recent years, the demand for higher throughput in the biotech and pharmaceutical sectors has initiated the development of versatile workstations from simple semi-automated bench-top liquid handlers to fully-automated integratable workstations.

With the objective of improving efficiency and to increase the level of automation and miniaturization, Aurora Biomed has developed a series of automated workstations, the VERSA Series This system can automate a range of applications in the fields of genomics, proteomics, drug discovery, and analytical applications. We describe the validation of this fully- automated workstation to run cell-based assays using cells expressing an ion channel of interest. A panel of positive inhibitors of the ion flux were applied to determine their IC_{50} values using the automated assay system. The IC_{50} values were then compared to those obtained from the manually- performed assay. The SEM was used to measure the variability among the replicates. The Z' factor values for both the automated system and manual performance were also compared.

II. Introduction

Need for automations of liquid handling processes in labs: As drug discovery processes demand high productivity and low cost, companies are always looking for rapid, sensitive, robust and inexpensive technology to miniaturize assays and carry precise automated analyte delivery. To meet this demand, assay technologies have evolved rapidly over the past 5 to10 years[1,2].

Issues associated with automation of cell based assays: In order to automate cell-based assays, specific technology and universal issues associated with cell-based assays need to be considered[3]. Such issues include simplification of the assay parameters and steps, user friendly software controlling the automation systems, quality control and standardization of procedures for automated large-scale screening, and the stability of the plated cells during wash steps etc.

Solution to these issues: To provide solution to these demands of HTS and to address these issues of automation, Aurora Biomed Inc, Vancouver, Canada has developed the VERSA series of workstations. In the present validation studies, the VERSA 1000 was used for drug dilution and automating the cell-based nonradioactive rubidium flux assay for screening compounds against human ether-a-go-go (hERG) ion channel.

Why automate the cell-based assay? Automating cell-based assays brings the challenge of ensuring the integrity of the plated cell layer; therefore, a cell-based assay (hERG assay) was chosen for the validation of the VERSA series. hERG channel activity is one of the most important factors in drug cardiac toxicity[4]. Thus, in order to quickly eliminate cardiac-liable compounds, increase chances of clinical success, and decrease development time, regulatory agencies require Pharma companies to screen all compounds for activity against this key ion channel.

However, manual performance of the hERG assay is labor-intensive and low throughput. As the throughput and precision of a robotic system is higher than the manual technique, we built a small, automated system to carry out this assay.

III. Materials & Methods

A. Which steps and conditions in the assay are being automated?

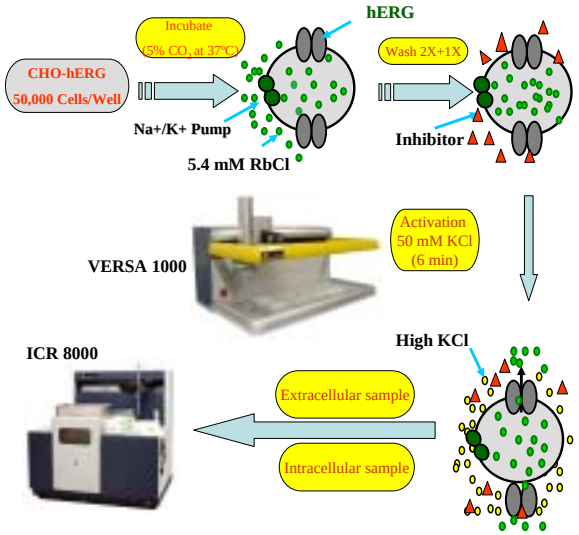

Figure 1. Flow diagram of the HTS hERG assay protocol showing different steps, durations and conditions involving liquid handling. Validation of the hERG assay was carried out in 96 well-format using the VERSA 1000.

B. What parameter of the HTS process were validated using the VERSA 1000?

The following parameters were validated for hERG HTS assay:

- Signal-to-noise ratio (window of detection)
- Standard error of the mean (SEM) among replicates
- IC_{50} determinations of some standard blockers
- Z' factor

IV. Results

C. Was the liquid handling performance of VERSA 1000 verified before validation of the hERG assay?

Table 1. Liquid handling performance of VERSA 1000

Liquid Handling Specifications			
Module	**Volume Range**	**Volume**	**CV%**
Nanopipettor	30nL – 300µL	40.0 nL	< 8%
		1.0 µL	< 5%
		10.0 µL	< 2%
ReagentDrop	1 - 1000µL	2.0 µL	<5%
		20.0 µL	<2%
Syringe Pipette	Various Sizes (300µL – 5mL)	10.0 µL	<5%
		100.0 µL	<3%
		300.0 µL	<2%

D. Was the drug dilution and liquid handling performance of the VERSA 1000 in the presence of DMSO/water verified before validation of the hERG assay?

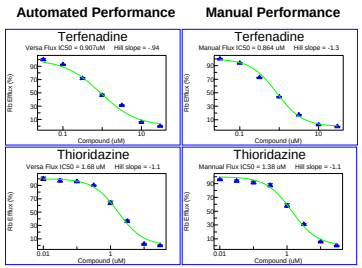

Figure 2. Liquid handling performance in the presence of DMSO was verified using terfenadine, a DMSO soluble drug and a known blocker of hERG dissolved in 100% DMSO at 100X. Thioridazine, a water soluble drug dissolved in water at 100X, was also used in these studies. Both the drugs were diluted to 8 different concentrations from the source plate, resulting 1X concentration of terfenadine along with DMSO diluted to 1% and thioridazine to 1X in assay buffers. Rest of the assay steps were performed manually. The results indicated comparable IC_{50} values of 0.907 µM (automated performance) and 0.864 µM (manual performance) for terfenadine. Similarly, IC_{50} values of 1.68 µM (automated performance) and 1.38 µM (manual performance) for thioridazine were obtained. The SEM values were very insignificant.

E What values of the assay parameters (i.e., Z', window of detection, and IC_{50} of standard blockers) were achieved upon automating all the steps of the HTS assay?

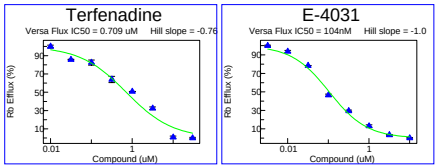

Figure 3. A window of detection showing activated and basal efflux of 69 and 19%, respectively was achieved while performing all the steps of the assay with the VERSA 1000. This automated procedure resulted in a high Z' factor value of 0.883 which suggests high robustness and low variability in the assay. The IC_{50} value for two drugs, terfenadine and E-4031, carried out using the VERSA 1000 resulted an IC_{50} values of 0.709 µM and 0.104 µM, respectively. These values were comparable to routine manual performances with insignificant SEM values.

V. Conclusion

Automation of hERG flux assay using VERSA 1000 resulted in comparable parameters to that of an assay performed manually (i.e., Z', window of detection, and IC_{50} of standard blockers. This suggests that the fully-automated VERSA 1000 can increase the throughput of applications such as IC_{50} dilution schemes and cell-based assays.

VI. Acknowledgements

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VII. References

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