



Integration of an Automated Workstation for Cell-Based Assays on Permeable Support Systems

Abstract

Liquid handling is an important part of automation for HTS especially as the demand for standardization and efficiency increases. With cell-based assays, automation is being used to seed, wash, and assay plates faster and with less variation than customary manual methods. In this study, we used Corning® HTS Transwell® 96 permeable support plates in conjunction with Caliper's Sciclone ALH 3000 and a table top LiCONiC® STX40 CO₂ incubator to demonstrate that complex, multiple step cell based assays could be optimized in a fully automated system. Our results demonstrate that, with assay optimization, multiple step cell based assays can be fully automated to yield highly reproducible results.

Introduction

The goal of this work was to design, implement and evaluate the feasibility of an integrated system for working with cell-based assays on Corning HTS Transwell 96 plates permeable support systems. For the automated liquid handling we used the Sciclone ALH 3000. However, in addition to liquid handling, incubation of the cells was required, so we directly integrated a Liconic STX 40 incubator. The direct integration was accomplished by positioning the STX 40 incubator equipped with a Liconic slide station to the right of the Sciclone ALH 3000, on the same side as the Sciclone's gripper. This allowed the Sciclone's gripper to pick up the plate and move it onto the deck. The entire system was controlled using Caliper's iLink Pro software. Another option would have been to use a Twister II robot to move the plates from the incubator to the deck, but this increases the equipment cost of the overall system, and is not as efficient.

The permeable support system allowed us to run a series of assays, including: cell proliferation, drug transport and chemotaxis migration assays. For each of these assays, plates were seeded, incubated, and washed. Seeding efficiency and cell viability were evaluated using an MTS cell proliferation assay. Drug transport assays were evaluated through TransEpithelial Electrical Resistance (TEER) measurements and Lucifer Yellow (LY) and Rhodamine 123 (Rh 123) permeability (P_{app}). Chemotaxis migration assays were evaluated by the percent migration of HT-1080 cells using serum as a chemoattractant.

For all assays, automated assay results were compared to side-by-side manual assays.

Materials

Equipment

- Sciclone ALH 3000 (Caliper Life Sciences)
- STX 40 incubator equipped with slide station (Liconic Instruments)
- SPECTRAmax spectrophotometer plate reader (Molecular Devices)
- LIL Analyst (Molecular Devices)

Consumables

- 96 well clear TCT plates - P/N 3585 (Corning)
- HTS Transwell-96 plates - P/Ns 3384, 3391 and 3392 (Corning)

Cell Lines

- HT-1080 cells - P/N CCL-121 (ATCC)
- MDCK and MDCKII/MDR1¹ - P/N CCL-34 (ATCC)

Reagents and Kits:

- IMDM (Cambrex)
- Fetal bovine serum (Invitrogen)
- ITS solution (Invitrogen)
- Lucifer Yellow (Sigma)
- Rhodamine 123 (Sigma)
- Calciem Am solution (Molecular Probes)
- 96[®]AQueous One Cell Proliferation Assay (Promega)



Figure 1. Sciclone ALH 3000 and Liconic STX 40 with slide

Methods and Results

Media

IMDM (Cambrex) was used as basal media. Fetal bovine serum (Invitrogen) at 10% and ITS solution (Invitrogen) at 1% were used to supplement serum free media. Cells were grown in humidity controlled incubators set to 37 °C and 5% CO₂.

Cell Proliferation Assay

Cell viability and seeding concentrations were evaluated following Promega's Cell Titer 96 AQueous One Cell Proliferation Assay (MTS assay). Briefly, 96 well clear TCT plates (Corning #3585) were seeded with HT-1080 cells (ATCC #CCL-121) at 20,000 cells/well. After a 24-hour incubation, the AQueous One Reagent (Promega) was added to cells and after a 30 minute incubation, plates were read using a SPECTRAmax spectrophotometer plate reader (Molecular Devices). Results can be seen in Figure 2.

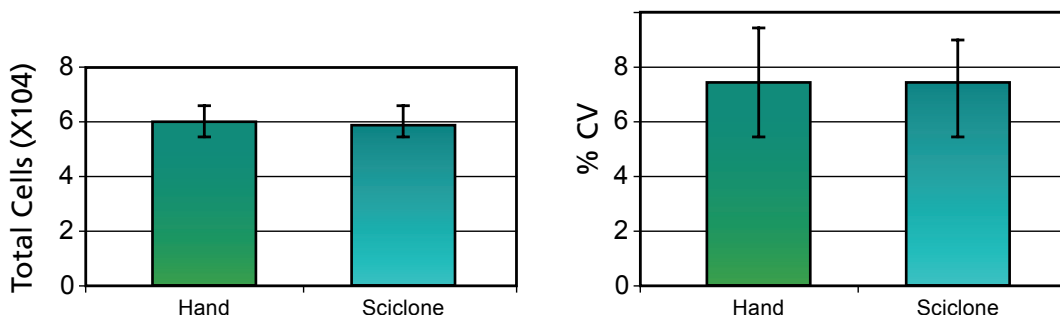


Figure 2. Evaluation of Seeding Efficiency and Cell Proliferation Using MTS Assay. HT-1080 cell proliferation and %CV for Hand vs. Sciclone seeded plates. Data are the average \pm S.D. from 144 wells/study for each condition from three independent studies.

Drug Transport Assay

The assay was set up following the Corning HTS Transwell 96 Permeable Support Protocol for Drug Transport. Briefly, HTS Transwells-96 Systems for Drug Transport and Permeability (Corning #3391 and 3392) were seeded with MDCK (ATCC #CCL-34) and MDCKII/MDR1 at 15,000 cells/well and allowed to grow for 5 days with a full media change 24 hours prior to assay. On the day of the assay, the integrity of the cell monolayer prior to washing was evaluated through TEER measurements. The plates were then washed twice and reagents were added. Lucifer Yellow (Sigma) permeability was used to evaluate cell monolayer integrity after the wash steps. Transport of Rhodamine 123 (Sigma) was used to evaluate cell function. The LJL Analyst (Molecular Devices) was used to read fluorescent signal. Results can be seen in Figures 3-5.

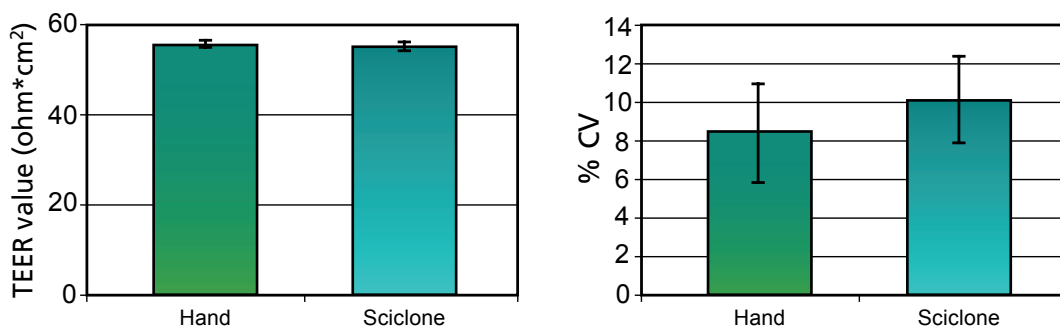


Figure 3. Evaluation of Cell Monolayer Integrity for Drug Transport Assay. TEER and %CV for Hand vs. Sciclone seeded plates. Data are the average \pm S.D. from 32 wells/condition from three independent studies for MDCKII/MDR1 cells.

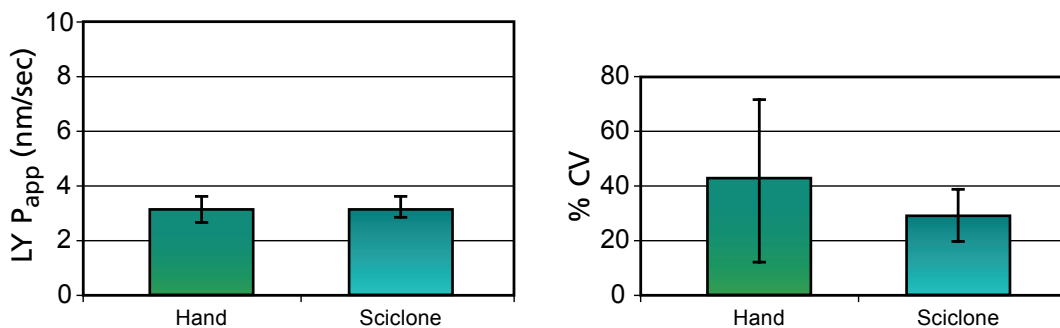


Figure 4. Evaluation of Monolayer Integrity After Washing. LY permeability and %CV for Hand vs. Sciclone handled plates. Data are the average \pm S.D. from 7 wells/condition from three independent studies for MDCKII/MDR1 cells.

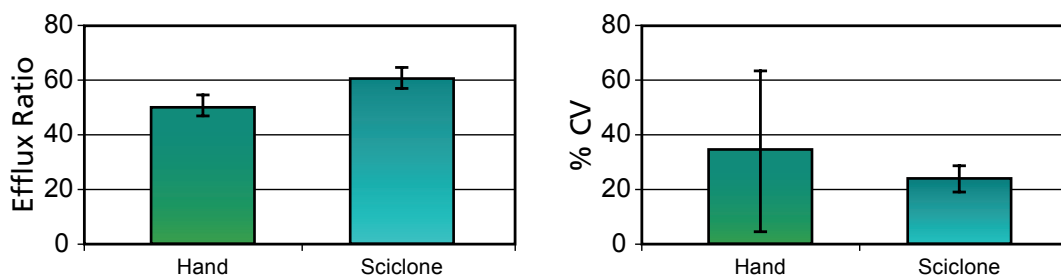


Figure 5. Evaluation of Cellular Function for Drug Transport Assay. Rh 123 Efflux ratio and %CV for Hand vs. Sciclone handled plates. Data are the average \pm S.D. from 8 wells/condition from three independent studies for MDCKII/MDR1 cells.

Chemotaxis/Migration Assay

The assay was set up following the Corning Cell Migration, Chemotaxis, and Invasion Assay Protocol. Briefly, HT-1080 cultures were serum starved 24 hours prior to plating using serum free media. HTS Transwell-96 Plates for Cell Migration (Corning #3384) were then seeded at 50,000 cells/well and receiver plates were filled with media containing 10% FBS to act as a chemoattractant. Plates were incubated overnight. After incubation, plates were washed. Migrating cells were dissociated and stained using Calcein-AM solution (Molecular Probes), and fluorescent signal was read on the LJL Analyst (Figure 6). All experiments were repeated at least three separate times.

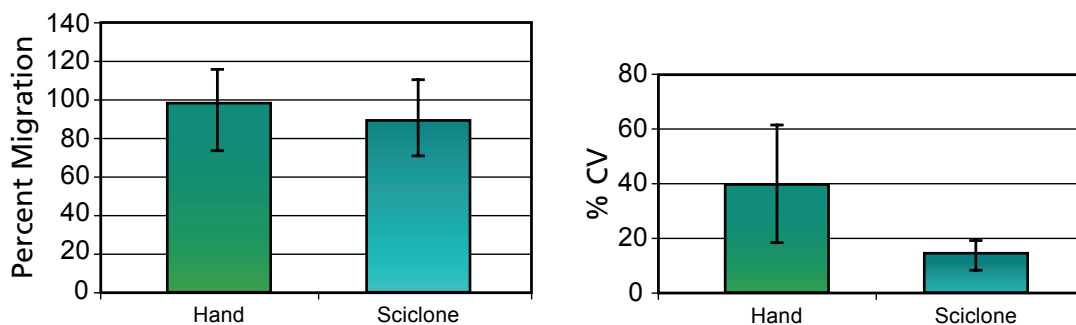


Figure 6. Assessment of Chemotaxis Response. HT-1080 % Migration and % CV for Hand vs. Sciclone handled plates. Data are the average \pm S.D. from 23 wells/condition from three independent studies.

Conclusions

- Complex cell based assays can be fully automated with Caliper's Sciclone ALH 3000 liquid handling instrument and Liconic STX40 CO₂ incubator.
- The automation of complex cell-based assays can decrease processing time with lower plate to plate variation as compared to manual handling.
- Based on MTS proliferation results, seeding efficiency and cell viability of the fully-automated assays were similar to manually handled plates (Figure 2).
- Based on TEER, LY and Rh 123 evaluations, the integrity of the cell monolayer and cell function of fully-automated assays were comparable to those done manually, with reduced plate-to-plate variability for the automated system (Figures 3-5).
- Based on chemotaxis response, seeding efficiency and cell migration were comparable to those done manually with a substantially lower plate-to-plate variability for the automated system (Figure 6).

¹ A kind gift of Dr. Piet Borst, Netherlands Cancer Institute.



68 Elm Street
Hopkinton, MA 01748-1668
Tel: 1.508.435.9500
Email: cust.support@caliperLS.com

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