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

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Introduction

Abstract
Liquid handling is an important part of automation for HTS especially as the demand for standardization and efficiency increase. With cell based assays, liquid handling instrumentation 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 liquid handling instrument and a table top LiCONiC® STX40 CO_2 incubator to demonstrate that complex, multiple step cell based assays could be optimized in a fully automated system.

Cell proliferation, drug transport and chemotaxis migration assays were seeded, incubated, washed and assayed using the Sciclone ALH 3000 instrument and LiCONiC STX40 incubator. 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 (Papp). Chemotaxis migration assays were evaluated by the percent migration of HT-1080 cells using serum as a chemoattractant.

Our results demonstrate that, with assay optimization, multiple step cell based assays can be fully automated to yield highly reproducible results.

Figure 1: Caliper's Sciclone® ALH 3000 liquid handling instrument and LiCONiC® STX40 CO_2 incubator

Materials & Methods

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 37º and 5% CO_2.
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 30 minute incubation plates were read using a SPECTRAmax spectrophotometer plate reader (Molecular Devices).
Drug Transport Assay: The assay was set up following the Corning HTS Transwell 96 Permeable Support Protocol for Drug Transport. Briefly, HTS Transwell®-96 Systems for Drug Transport and Permeability (Corning #3391 and 3392) were seeded with MDCK (ATCC #CCL-34) and MDCKII/ MDR1(kind gift of Dr. Piet Borst, Netherlands Cancer Institute) at 15,000 cells/well and allowed to grow for 5 days with a full media change 24 hr prior to assay. On day of 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 integrity of cell monolayer 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.
Chemotaxis/ Migration Assay: The assay was set up following the Corning Cell Migration, Chemotaxis, and Invasion Assay Protocol. Briefly, HT-1080 stocks were serum starved 24 hours prior to plating using serum free media. HTS Transwell®-96 Plates for Cell Migration (Corning #3384) were then plated with HT-1080 cells 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 Calcien Am solution (Molecular Probes). Fluorescent signal was read on the LJL Analyst. All experiments were repeated at least three separate times.

Results

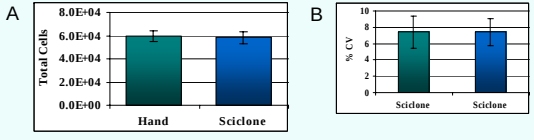

Figure 2: Evaluation of Seeding Efficiency and Cell Proliferation Using MTS Assay. HT-1080 cell proliferation and %CV for Hand vs. Sciclone seeded plates. Data is the average ± S.D from 144 wells/study for each condition from three independent studies.

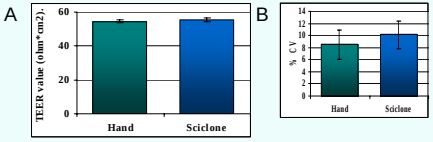

Figure 3: Evaluation of Cell Monolayer Integrity for Drug Transport Assay. TEER and %CV for Hand vs. Sciclone seeded plates. Data is the average ± S.D from 32 wells/condition from three independent studies for MDCKII/ MDR1 cells.

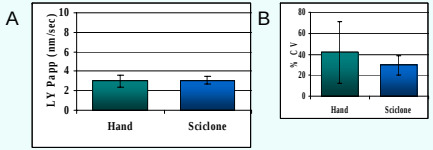

Figure 4: Evaluation of Monolayer Integrity After Washing. LY permeability and %CV for Hand vs. Sciclone handled plates. Data is the average ± S.E from 7 wells/condition from three independent studies for MDCKII/ MDR1 cells.

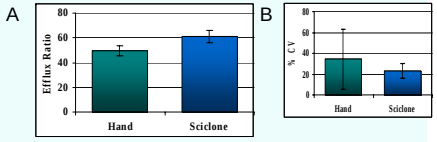

Figure 5: Evaluation of Cellular Function for Drug Transport Assay. Rh 123 Efflux ratio and %CV for Hand vs. Sciclone handled plates. Data is the average ± S.D from 8 wells/condition from three independent studies for MDCKII/ MDR1 cells.

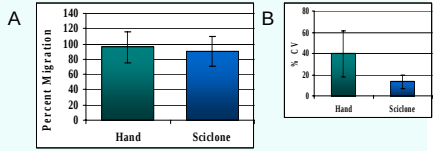

Figure 6: Assessment of Chemotaxis Response. HT-1080 % Migration and % CV for Hand vs. Sciclone handled plates. Data is the average ± S.E from 23 wells/condition from three independent studies.

Summary & Conclusions

- **Full automation of complex cell based assays can decrease processing time with lower plate to plate variation as compared to manual handling.**
- **Complex cell based assays can be fully automated with Caliper's Sciclone® ALH 3000 liquid handling instrument and LiCONiC® STX40 CO2 incubator.**
- Based on MTS proliferation results, seeding efficiency and cell viability of fully automated plates 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 plates were comparable to those done manually, with reduced plate to plate variability for the fully automated system (Figures 3, 4 and 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 fully automated system (Figure 6)