Utility of Automated Drug Transport Assays in 96-Well Format, using Permeable Support Systems

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Introduction

Drug transport assays play an important part in determining how a compound is absorbed into the body. Therefore, the performance of these assays is essential to help determine the ADME/Tox profile of a new drug entity (NDE). Typically, these assays have been carried out using colorectal carcinoma (Caco-2) cells, or Madin-Darby Canine Kidney (MDCK) cells in 24-well plates. However, due to the fact that ADME/Tox testing is now moving further upstream in the drug discovery process, a greater number of lead compounds are now being tested in an effort to fail NDEs with negative profiles earlier and in a more cost-effective manner. To meet the demands for higher throughput and reduced processing time, we present an automated drug transport assay using either Caco-2 or MDCKII/MDR1 cells in 96-well Permeable Supports. The entire assay process was automated, including cell dispensing, media exchanges, and compound addition and removal, using simple, yet robust robotic instrumentation. A two-part permeable support system, incorporating an insert plate, and receiver plate, was used in order for manipulations to be performed without the need to separate the parts of the system. The matrices used to validate the automated process were Transepithelial Electrical Resistance (TEER), and Lucifer Yellow and Rhodamine 123 permeability. All automated methods were done in parallel to manual methods for comparison. Results show that the automated methods were able to deliver results that are equal to, or more consistent, than manual processing, while reducing the overall experimental time. Thus, by automating the drug transport assay, one increases efficiency without the loss of data quality or integrity.

Transwell Permeable Support System

Corning HTS Transwell-96 permeable supports are designed for high-throughput applications to examine cell polarization, drug transport, toxicity or chemotaxis in vitro. The HTS format allows for all 96 wells to be handled as a single unit making it an ideal tool for automated high throughput studies.

Drug Transport Analysis

Low Rhodamine 123 A-B values and high B-A values further indicate that cell layers are intact after the 21-day incubation, and that P-glycoprotein is functioning properly in the Caco-2 cells. Decreases in efflux values in wells containing Cyclosporin A, a known inhibitor of P-glycoprotein, indicate that inhibitor studies are able to be carried out using the automated method.

Conclusions

1. The EL406 is able to dispense MDCK and Caco-2 cells, and performs media transfers easily and efficiently without disturbing cell monolayers, as evidenced by TEER and Lucifer Yellow results.
2. Papp values, with less variability among replicates, lead to more appropriate P-glycoprotein efflux ratios and conclusions.
3. Corning HTS Transwell-96 supports provide an easy to use, and flexible method for studying drug transport.
4. The combination of BioTek’s instrumentation, and Corning’s Transwell plates, create an ideal solution for performing high-density, automated drug transport assays.