

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

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Overview

Cell-based drug transport assays, such as Caco-2 and MDCK, are an essential component of ADME/Tox testing for lead compounds. The permeability and transport data they provide can determine whether or not a compound is carried forward in the drug discovery process. Current methods that use 24-well plates, run in a manual format, are no longer viable based upon the need to generate absorption data on an increasing number of compounds. By incorporating 96-well HTS Transwell® permeable supports, along with an automated process using proper instrumentation, data can be generated that is more consistent when compared to manual methods, and allows research staff to perform more important, higher level functions.

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 metrics 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 assay is 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.

BioTek Instrumentation

BioTek Liquid Handling

The EL406™ Combination Washer Dispenser offers fast, accurate media removal and plate washing capabilities through its Dual-Action™ Manifold. It also offers reagent dispensing capabilities through the use of its peristaltic or syringe pumps, with volumes ranging from 1 to 3000 µL/well. The instrument was used for cell dispensing, media exchange and removal, as well as dispensing of buffer and reagents to the Transwell permeable supports. The small footprint of the instrument allows for easy insertion into existing laminar flow hoods to ensure sterile manipulations.

The Precision™ Microplate Pipetting System combines an 8-channel pipetting head and an 8-channel bulk reagent dispenser in one instrument. The instrument was used to transfer pre-incubation and post-incubation samples from the Transwell permeable supports to the black fluorescent plates.

BioTek Detection

The Synergy™ H4 Hybrid Multi-Mode Microplate Reader combines a filter-based and monochromator-based detection system in the same unit. The filter-based system was used to read the fluorescent Lucifer Yellow signal using a 485/20 nm excitation filter, 530/25 nm emission filter, and 510 nm cutoff dichroic mirror and Rhodamine 123 signal using a 530/25 nm excitation filter, 590/35 nm emission filter, and 550 nm cutoff dichroic mirror.

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 inserts to be handled as a single unit making it an ideal tool for automated high throughput studies.

Drug Absorption Assay Protocols

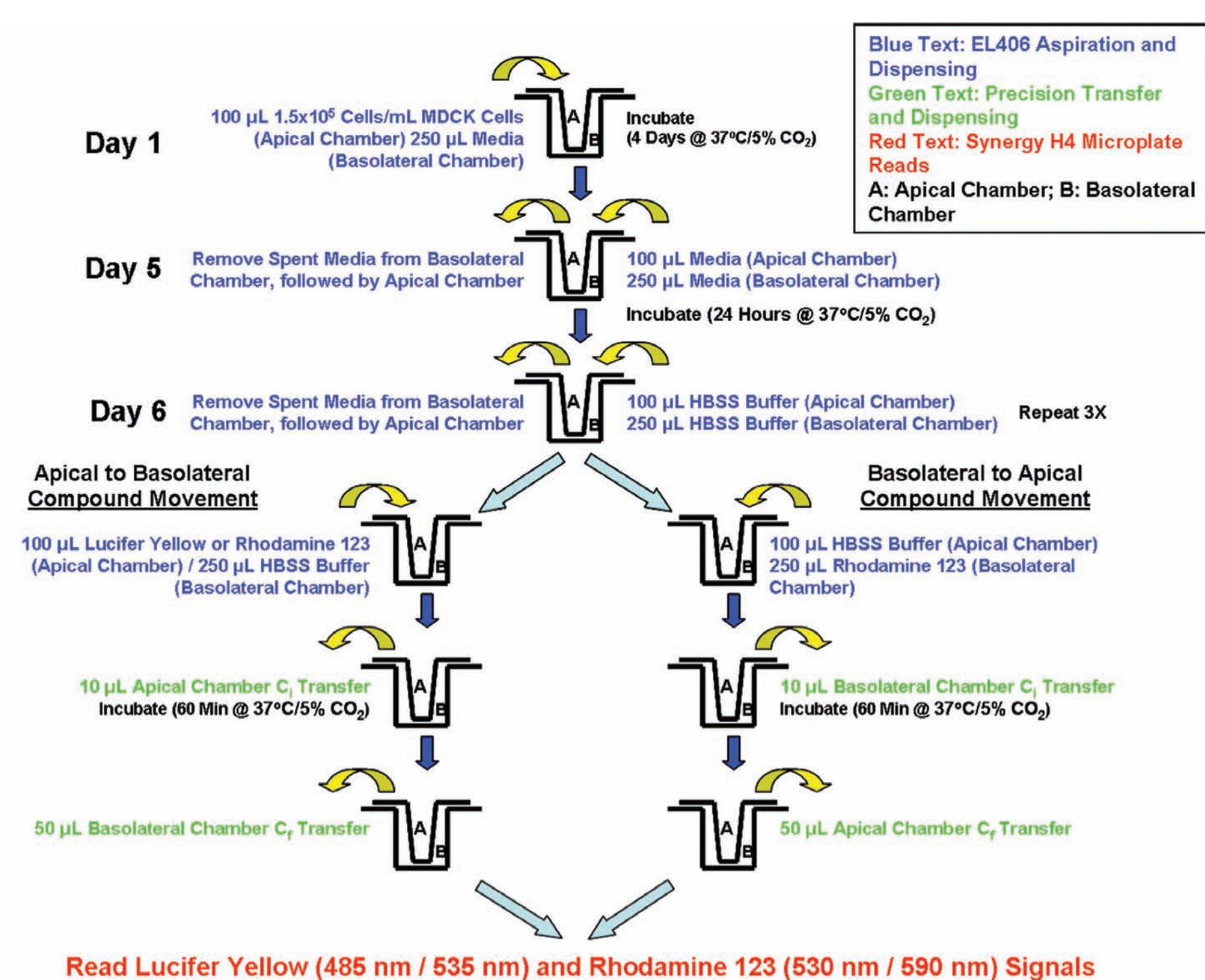


Figure 1 – MDCK Cell Preparation and Assay Protocol.

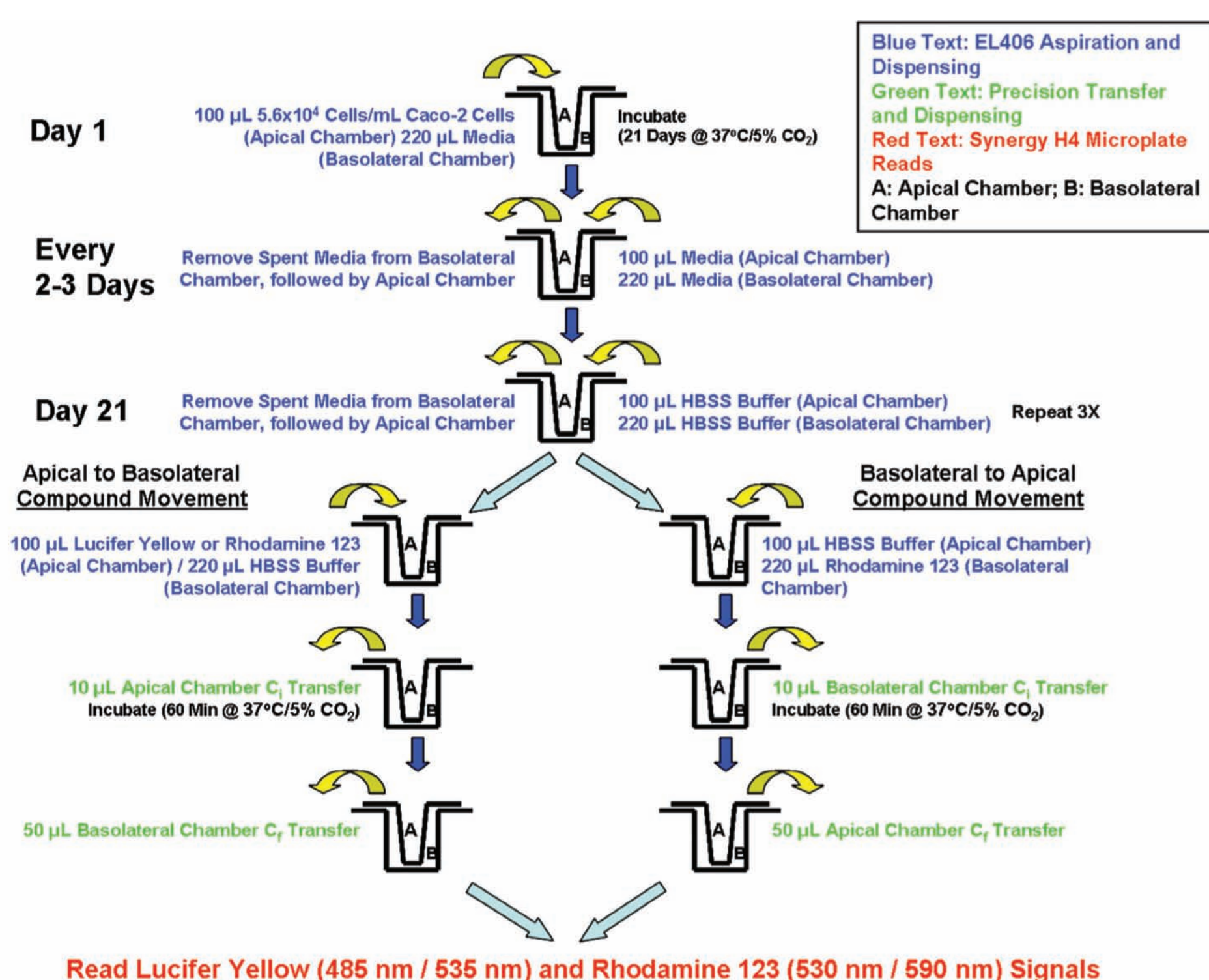


Figure 2 – Caco-2 Cell Preparation and Assay Protocol.

MDCKII/MDR1 Automated Assay Validation

Cell Monolayer Integrity Measurements

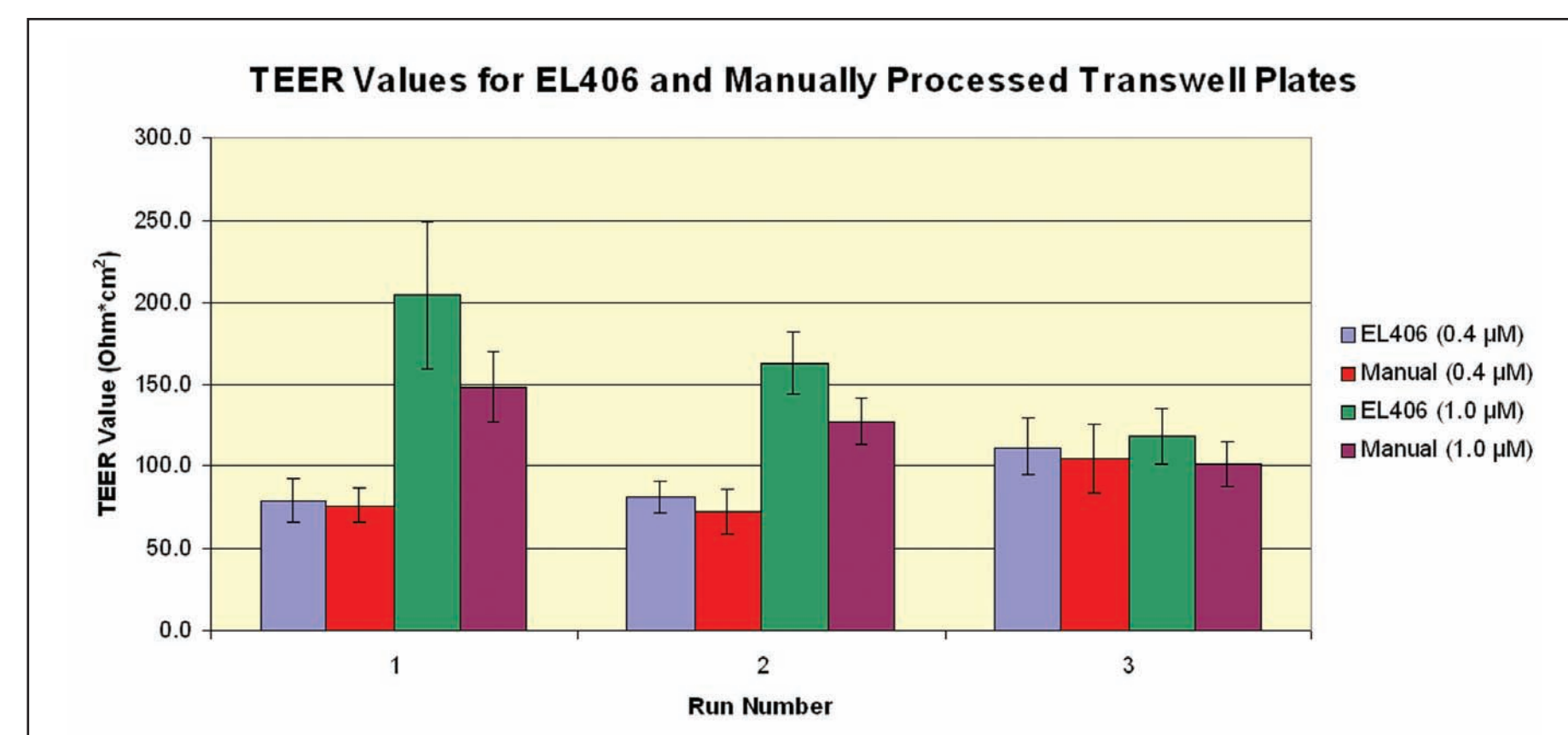


Figure 3 – Average TEER values across three runs for 0.4 and 1.0 µM Transwell permeable supports processed using EL406 or manual method (n=120).

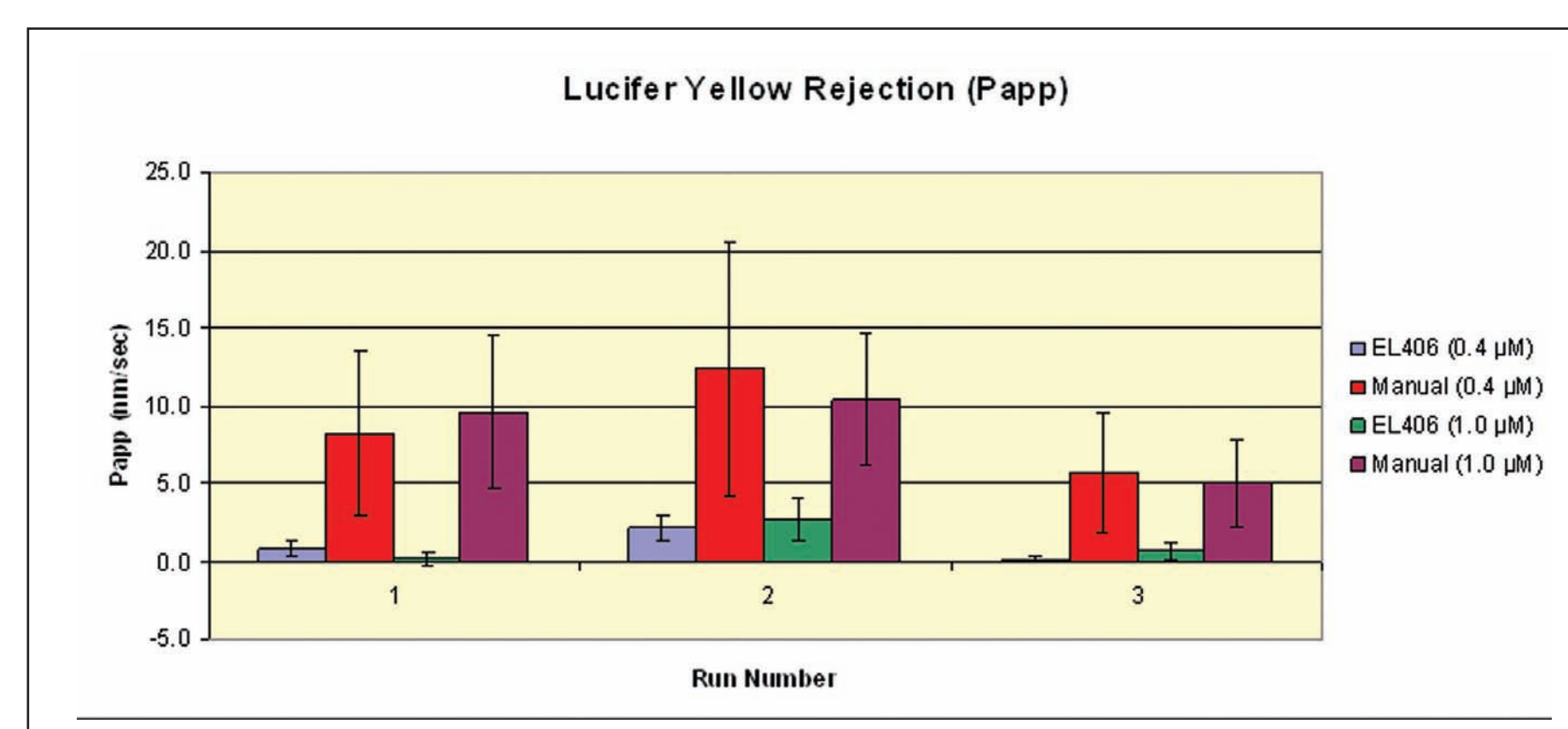


Figure 4 – Average apparent permeability values (Papp) for Lucifer Yellow across three runs for 0.4 and 1.0 µM Transwell permeable supports processed using EL406 or manual method (n=40).

TEER values for the automated method were equivalent or higher than values from manually processed plates. Papp values for Lucifer Yellow were also significantly lower, and showed less variation, indicating a tighter, more consistent cell monolayer for plates processed using the EL406.

Drug Transport Analysis

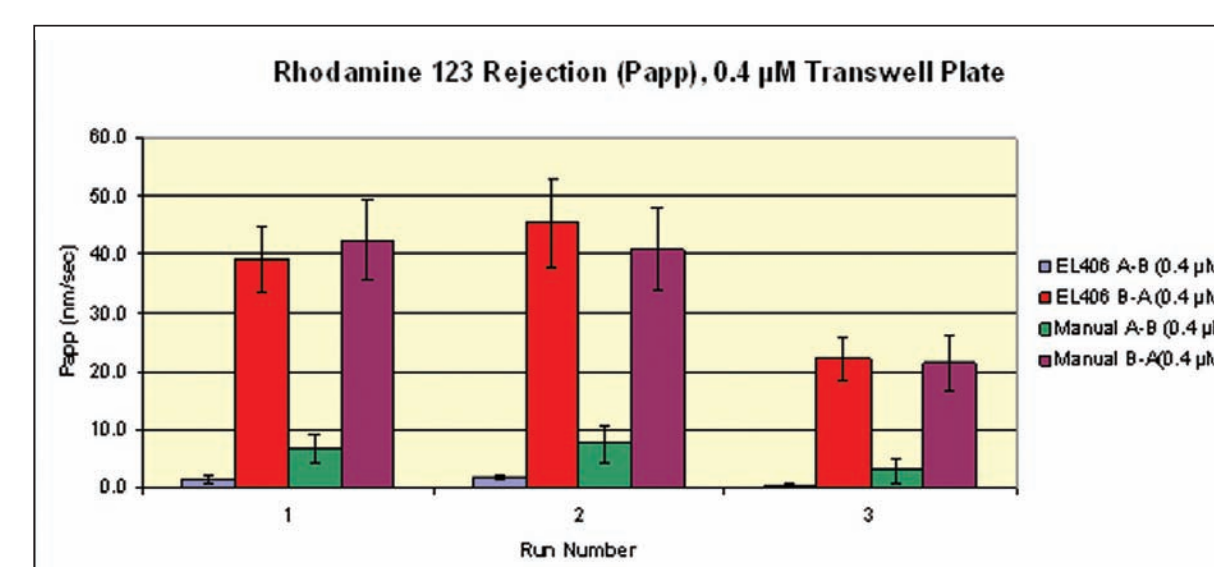


Figure 5 – Average A-B and B-A Papp values for Rhodamine 123 across three runs of 0.4 and 1.0 µM Transwell permeable supports processed using EL406 or manual method (n=40).

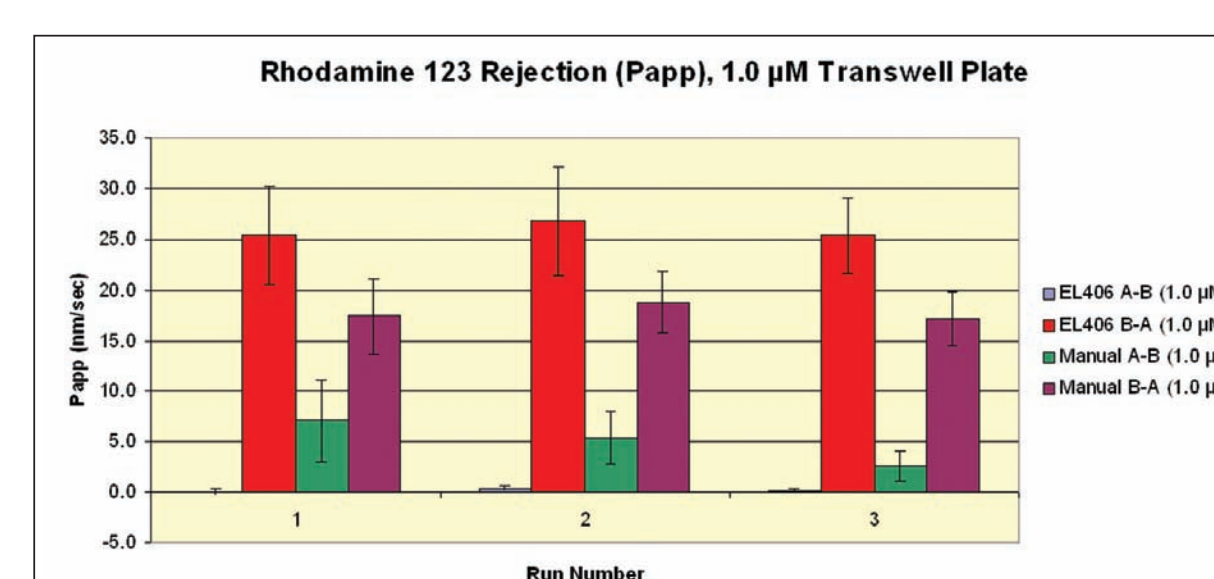


Figure 6 – Average calculated efflux values for Rhodamine 123 across three runs of 0.4 and 1.0 µM Transwell permeable supports processed using EL406 or manual method (n=40).

Lower Rhodamine 123 apical-basolateral (A-B) values and higher basolateral-apical (B-A) values for EL406 processed plates demonstrate a more intact cell layer and higher functioning P-glycoprotein, respectively, when compared to manually processed plates.

Caco-2 Automated Assay Validation

Cell Monolayer Integrity Measurements

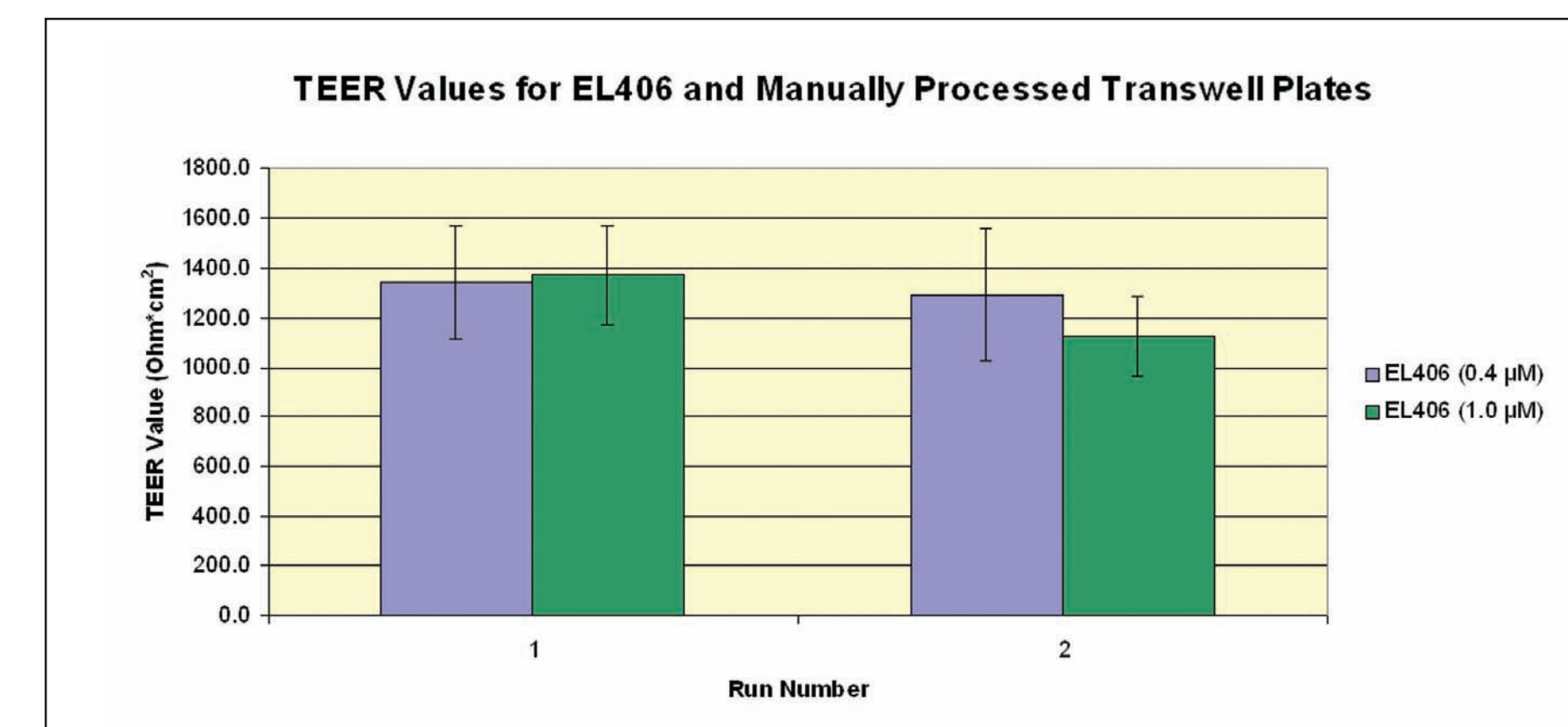


Figure 7 – Average TEER values across two runs for 0.4 and 1.0 µM Transwell permeable supports processed using EL406 (n=80).

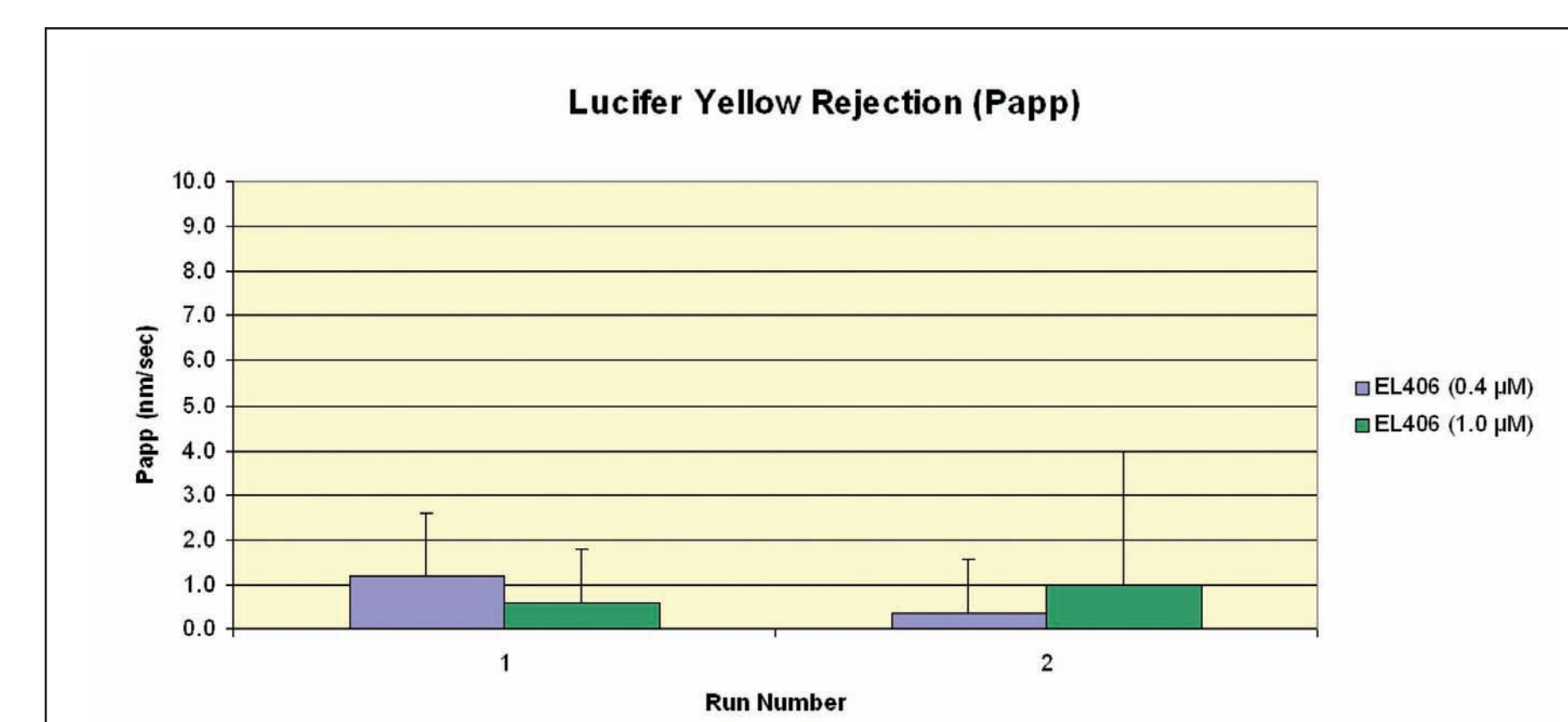


Figure 8 – Average apparent permeability values (Papp) for Lucifer Yellow across two runs for 0.4 and 1.0 µM Transwell permeable supports processed using EL406 (n=40).

TEER values for the automated method were agreeable with data shown previously with Transwell plates. Papp values for Lucifer Yellow were also low, and in line with data from MDCK cell plates processed using the EL406, indicating that the instrument could plate and process cells in the Transwell plates over the 21-day incubation time.

Drug Transport Analysis

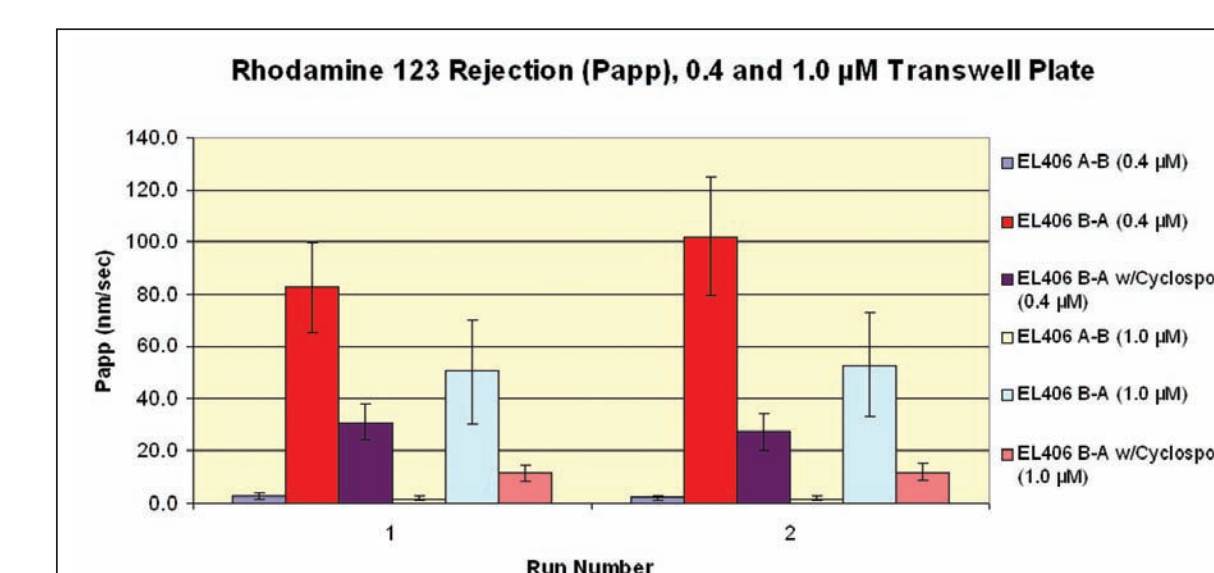
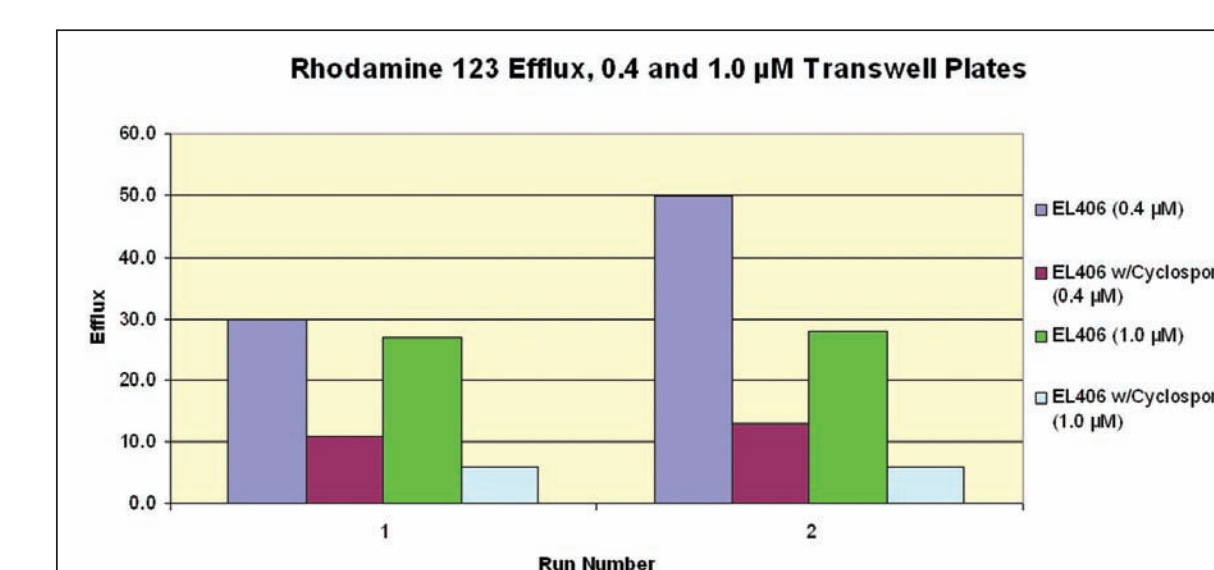


Figure 9 – Average A-B and B-A Papp values, and efflux values for uninhibited and inhibited Rhodamine 123 across two runs of 0.4 and 1.0 µM Transwell permeable supports processed using EL406 (n=40). Uninhibited apical wells for B-A transfer contained HBSS buffer, while inhibited apical wells contained 10 µM Cyclosporin A in HBSS buffer.



Low Rhodamine 123 A-B values and high B-A values further indicate that cell layers are intact after the 21-day incubation, and 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 perform 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 permeable 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.