Automated High Content Drug Transport Screening with Transwell Plates for Drug Discovery

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- The purpose of this study was to develop a method to use bidirectional intestinal permeability and drug transport to identify substrates and inhibitors of MDR1 or BCRP.
- The alteration of drug transporters potentially affects clearance and efficacy.
- Cells grown on transwell permeable membranes were washed, treated, and samples were collected with an automated liquid handler.
- Data showed the automated method as consistent with in-house historic values and within and between assays.

Introduction

Measuring bidirectional intestinal permeability and drug transport using Caco-2 and MDCKII can identify potential substrates or inhibitors of MDR1 or BCRP and identify the permeability class of potential new drugs. Two recent FDA documents, 2012's Drug Interaction draft guidance and 2015's Waiver of *In Vivo* Bioavailability and Bioequivalence, show the increasing interest and significance in transporters and permeability, particularly in MDR1 (P-gp) and BCRP. The alteration of drug transporters potentially affects clearance and efficacy. Early assessment of this potential leads to better, safer drugs and reduced development costs. A method utilizing a Tecan liquid handler and Caco-2 or MDCKII cells grown on permeable membranes (**Figure 1**) was created to measure bidirectional intestinal permeability and drug transport. The screen evaluates up to 14 compounds in one assay and measures permeability and efflux.

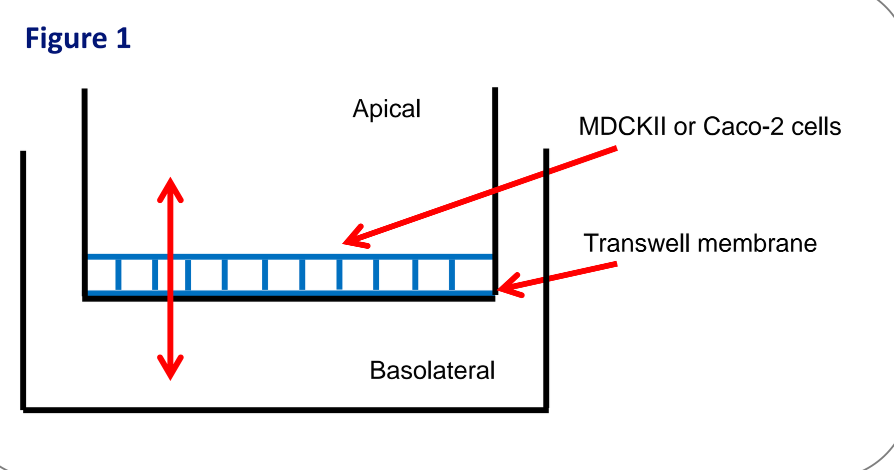


Figure 1

Materials & Methods

Chemicals and Reagents: Digoxin and valspodar were purchased from Sigma Chemical Co., St. Louis, MO. Digoxin-d3 was purchased from Toronto Research Chemicals Inc., North York, Ontario, Canada. All other chemicals and reagents were of analytical grade.

Cell Culture: MDCKII cells (Madin-Darby Canine Kidney cells) over-expressing human MDR1 (P-gp) were cultured in 96-well transwell plates (Corning; 3391) in DMEM supplemented with FBS (10% v/v) and penicillin-streptomycin (45 U/mL and 45 µg/mL, respectively). Cells were stored in a humidified culture chamber (37 ± 2 °C, 95 ± 5% relative humidity and 5 ± 1% CO2) and allowed to form a confluent monolayer with tight junctions over 4-5 days. MDCKII cells were purchased from the Netherlands Cancer Institute.

Screening Assay: This method was developed on a Tecan Freedom EVO-200 liquid handling instrument (Tecan Group Ltd., Mannëdorf, Switzerland) utilizing a Multi-Channel Arm (MCA-96) and 8 channel Liquid Handling Arm (LiHa) on the EVOware v2.5 software platform (Tecan Group Ltd., Mannëdorf, Switzerland). This method was designed to test up to 14 compounds (at a single concentration) with a positive and negative control.
Permeability incubations were carried out in transport buffer (HBSS supplemented with 25 mM HEPES and 25 mM glucose) at 37 ± 2°C. TEER (transepithelial electrical resistance) values were recorded 10 minutes after the transport buffer was added and then the cells are warmed to 37 ± 2°C for 30-60 minutes. After the preincubation, transport buffer with digoxin (10 µM final concentration) containing the solvent control, control inhibitor or the test item was added to the donor chamber. Transport buffer containing the solvent control, control inhibitor or the test item was added to the receiver chamber. Aliquots were taken from the donor chambers at the start of the incubation (time zero). Samples (50 µL) were collected from the receiver and from the donor compartment at the final time point (120 minutes). The samples were then mixed with the internal standard, Digoxin-d3 (1 µg/mL) in 50:50 v/v methanol: water, and analyzed by LC-MS/MS. P_{app} (apparent permeability), TEER and recovery (at 0 and the highest concentration of the test item) were determined for digoxin.
Following incubations, Lucifer yellow samples and 1000 fold standard curve were prepared. For the Lucifer Yellow assay, the MDCKII cells were washed with transport buffer and then Lucifer yellow (40 µg/mL) was added to the cells, apical, to test the integrity of the membranes. Lucifer yellow was measured, basolateral, at λ_{ex} =420 and λ_{em} =590. Standard curve samples were mixed with internal standards and analyzed by LC MS/MS.

Analytical Methods: Samples were analyzed by liquid chromatography (LC) with tandem mass spectrometric detection (MS/MS) on an API4000 with a Shimadzu HPLC system interfaced by electrospray ionization (ESI). Relative or absolute quantification of each analyte was performed using AB SCIEX, version 1.6.1.

Figure 3. Comparison of historical and Tecan Papp and efflux ratio in MDCKII-MDR1 (n≥6)

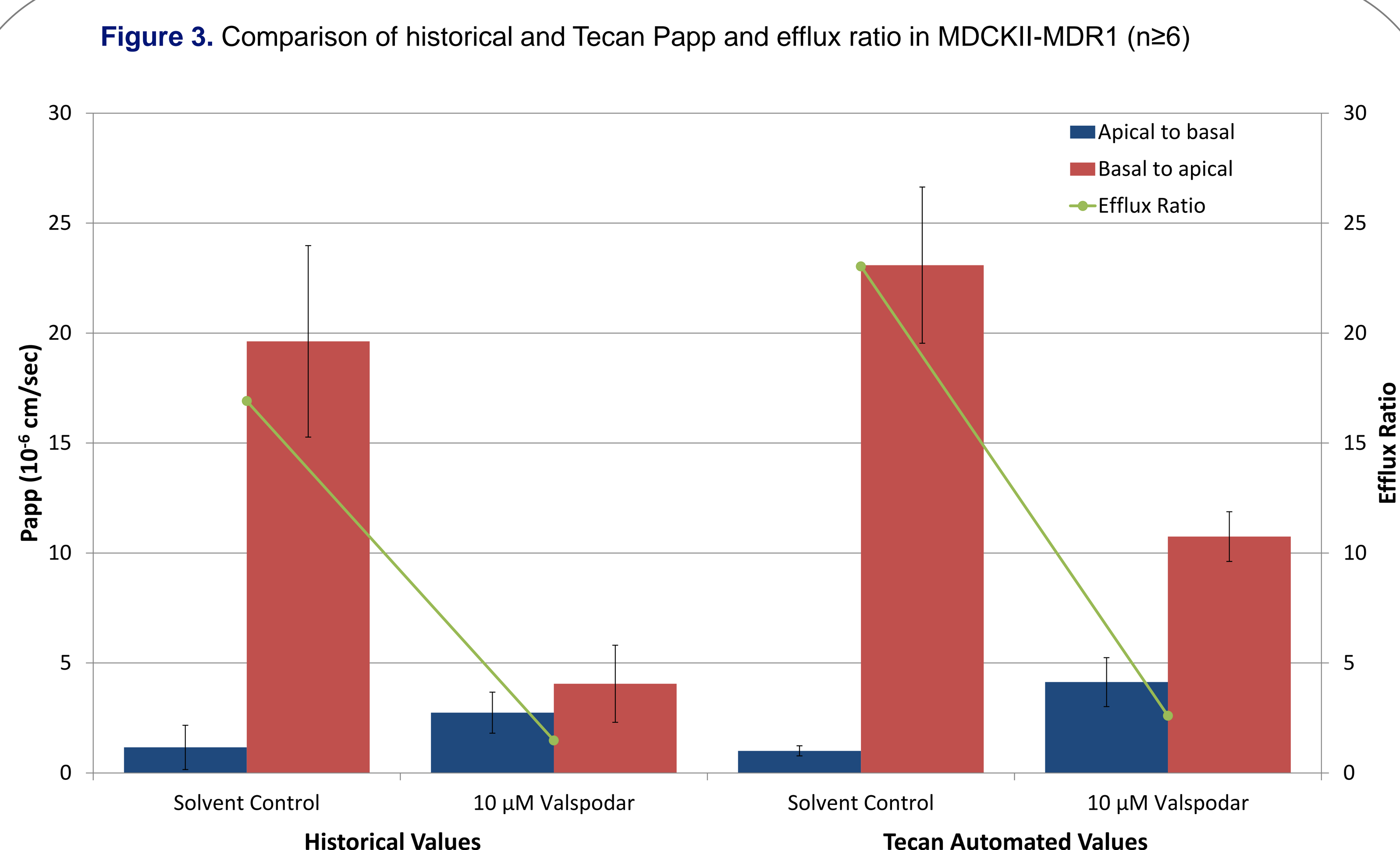
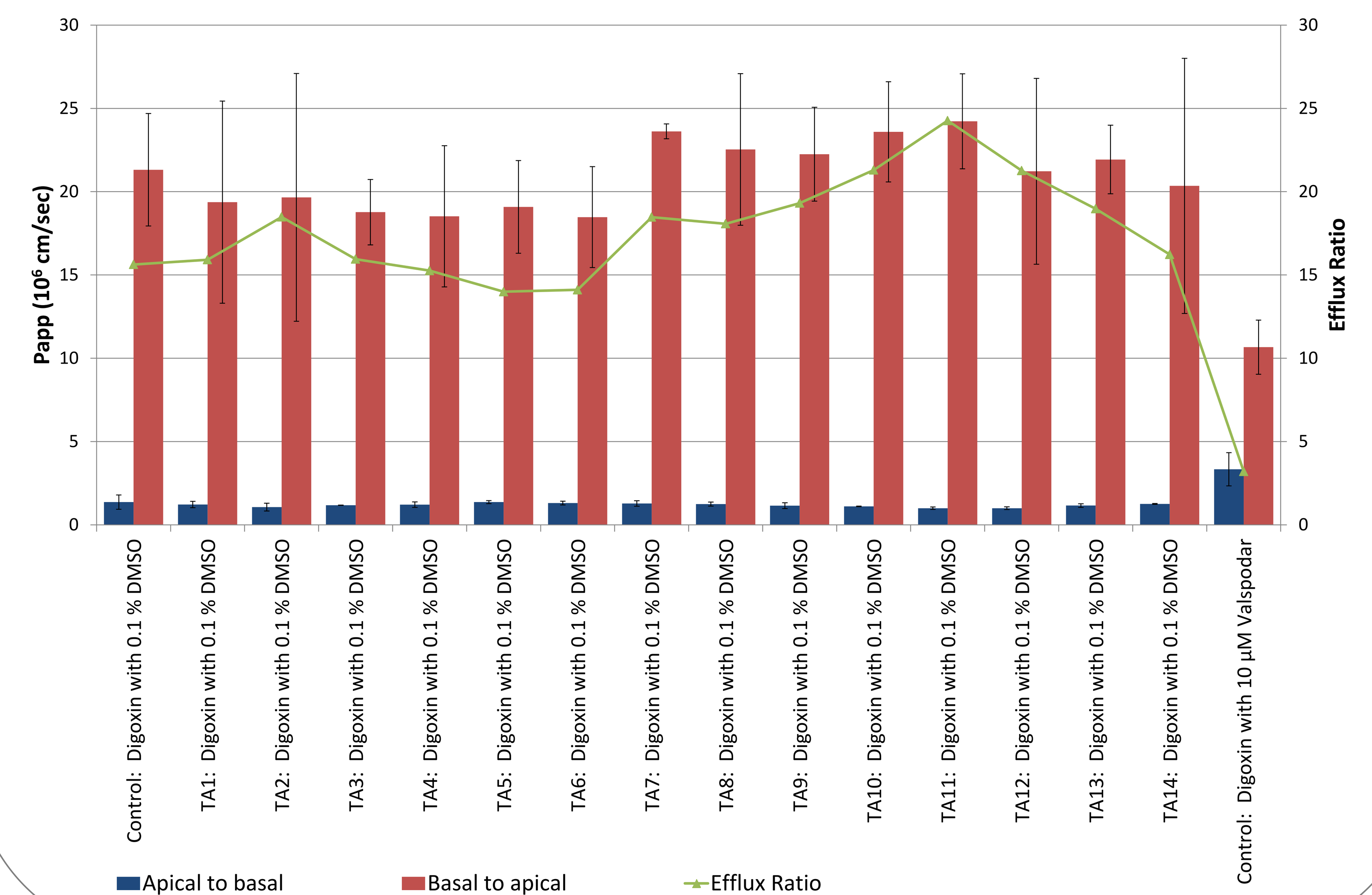


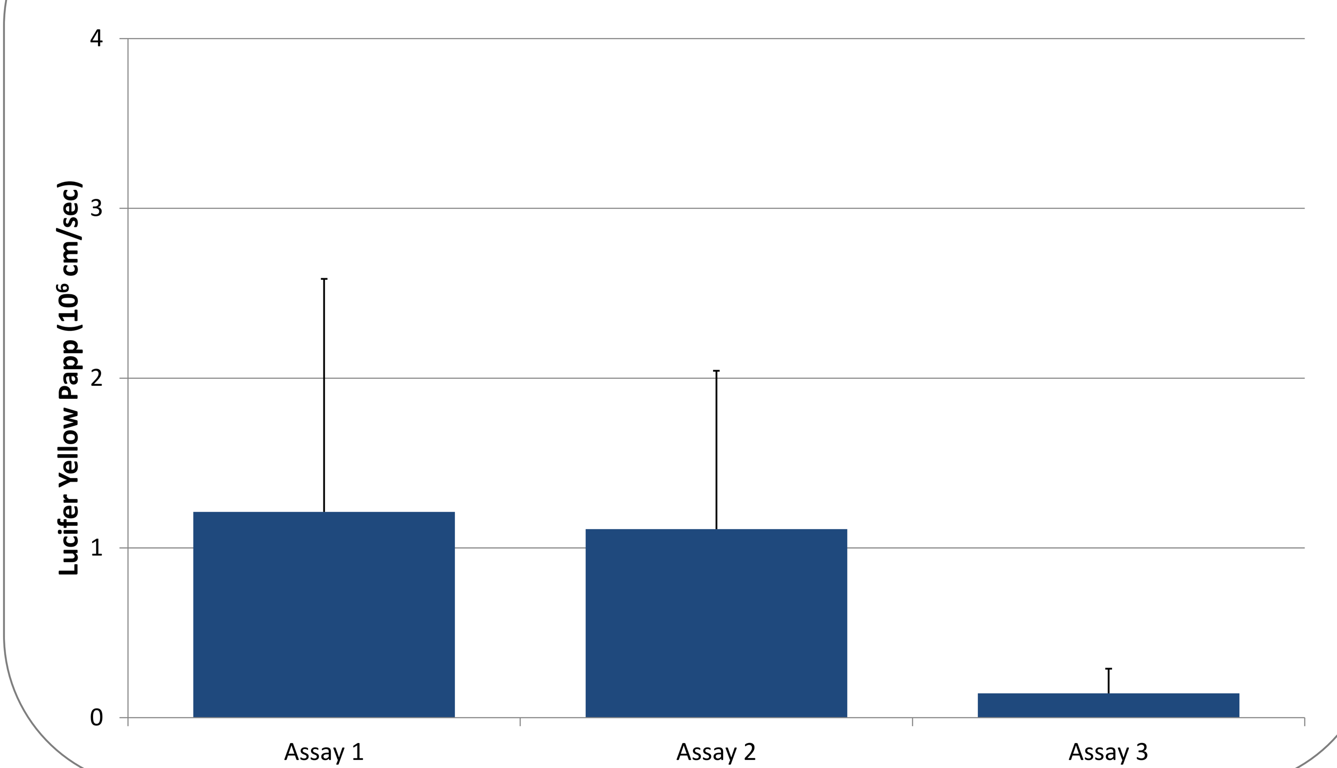
Figure 2. Individual triplicates from an assay with MDCKII-MDR1, with consistency across replicates (error bars are standard deviation)



Results

- Bidirectional permeability, apical to basolateral and basolateral to apical, of digoxin (10 µM, in 0.1 % DMSO) was measured in the presence and absence of 10 µM valspodar. Digoxin and valspodar are, respectively, a probe substrate and positive control inhibitor commonly used in permeability assays. The automated assay was performed three times with all tests in at least triplicate.
- Apparent permeability (Papp) in transfected MDCKII-MDR1 was consistent within an assay. Digoxin was used as the probe substrate and as the test article for bidirectional permeability (apical to basolateral and basolateral to apical in triplicate) for a total of 15 tests in the example assay in Figure 2. Papp and efflux ratio were consistent in the 15 measurements of digoxin; exposure of cells to digoxin and valspodar produced the expected, statistically significant inhibition.
- Papp and efflux ratios in the automated method were similar to manual historical results and produced more consistent results than manual assays (**Figure 3**), despite the change to a 96 well format for automated assays from 24 well plates for manual assays. Lucifer yellow Papp, measured post assay, was well below the required 4.0 for each of the three assays (**Figure 4**) and was equivalent or better than manual historical results (data not shown).

Figure 4. Lucifer Yellow Papp of MDCKII-MDR1 cells post-assay in each of three assays (n ≥ 24 wells)



Conclusions

Tecan automated screening demonstrated accuracy and precision while establishing repeatability and ruggedness.

- TEER and Papp show monolayer integrity as of consistently high quality post-assay.
- Monolayer integrity is of better quality post-assay than in manual assays.
- Efflux ratios are consistent within and between Tecan automated assays.