



Automation of Caco-2 Cell Preparation, Drug Permeation and Transport Assays on the Biomek® 3000 Laboratory Automation Workstation

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Abstract

Incorporating predictive ADME (absorption, distribution, metabolism and elimination) assays in earlier stages of drug discovery can help in rejecting candidate molecules that lack necessary pharmacological properties. A human colorectal adenocarcinoma cell line, Caco-2, can be used to assess absorption, permeation and efflux transport properties of a candidate drug. A high-throughput Caco-2 assay can provide useful information for lead optimization in the drug discovery industry. This poster describes the use of Beckman Coulter's Biomek 3000 Laboratory Automation Workstation to automate Caco-2 cell preparation and differentiation in a BD Falcon® HTS 96-Multiwell Insert System. Additionally, Beckman Coulter's Biomek 3000 Laboratory Automation Workstation was used to automate the compound permeability, efflux testing and sample collection. We have demonstrated intact and functional Caco-2 monolayers after 21 days of culture manipulation by the Biomek 3000 Laboratory Automation Workstation. The Caco-2 monolayer provided a selective barrier for transcellular and carrier-mediated efflux transport of different drugs. The permeability ranking of drug standards using the Caco-2 assay system matched well with the Potential Internal Standards suggested by the FDA. The bi-directional transport studies verified functional P-glycoprotein efflux pump activities. The Biomek 3000 Laboratory Automation Workstation facilitated Caco-2 assay implementation, reduced the chance of contamination and minimized the intensive requirement of sterile skills. The automated Caco-2 assay can be used for high-throughput screening of drug candidates for absorption properties.

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Introduction

Incorporating predictive ADME (Absorption, Distribution, Metabolism and Elimination) assays in earlier stages of drug discovery can help in rejecting molecules that lack necessary pharmacological properties. Drug bioavailability is influenced by factors including absorption, and metabolism. The United States Food and Drug Administration issued the Biopharmaceutics Classification System, which is the guidance for using *in vitro* models assessing drug bioavailability. Caco-2 is a human colorectal adenocarcinoma cell line that forms monolayers of differentiated epithelial cells joined by intercellular tight junctions. It provides a selective barrier in the BD Falcon HTS 96-Multiwell Insert System (BD Biosciences, Bedford, MA) for both transcellular (from the apical to the basolateral chamber) and carrier-mediated efflux transport (from the basolateral to the apical chamber) mechanisms.

P-glycoprotein is an ATP-dependent, substrate-specific active carrier-mediated (efflux) transporter that is responsible for the active transporting of a large number of drugs. Substrates of P-glycoprotein can be transported via the efflux system and resulted in decreased intracellular drug concentration. The function of P-glycoprotein affects drug absorption, pharmacokinetics and drug-drug interactions. Differentiated Caco-2 expresses high level of P-glycoprotein. It is an excellent model for assessing drug permeability through transcellular transport, as well as screening P-glycoprotein substrates through the efflux transport mechanisms. A high-throughput Caco-2 assay is needed for implementing this cell model in drug discovery.

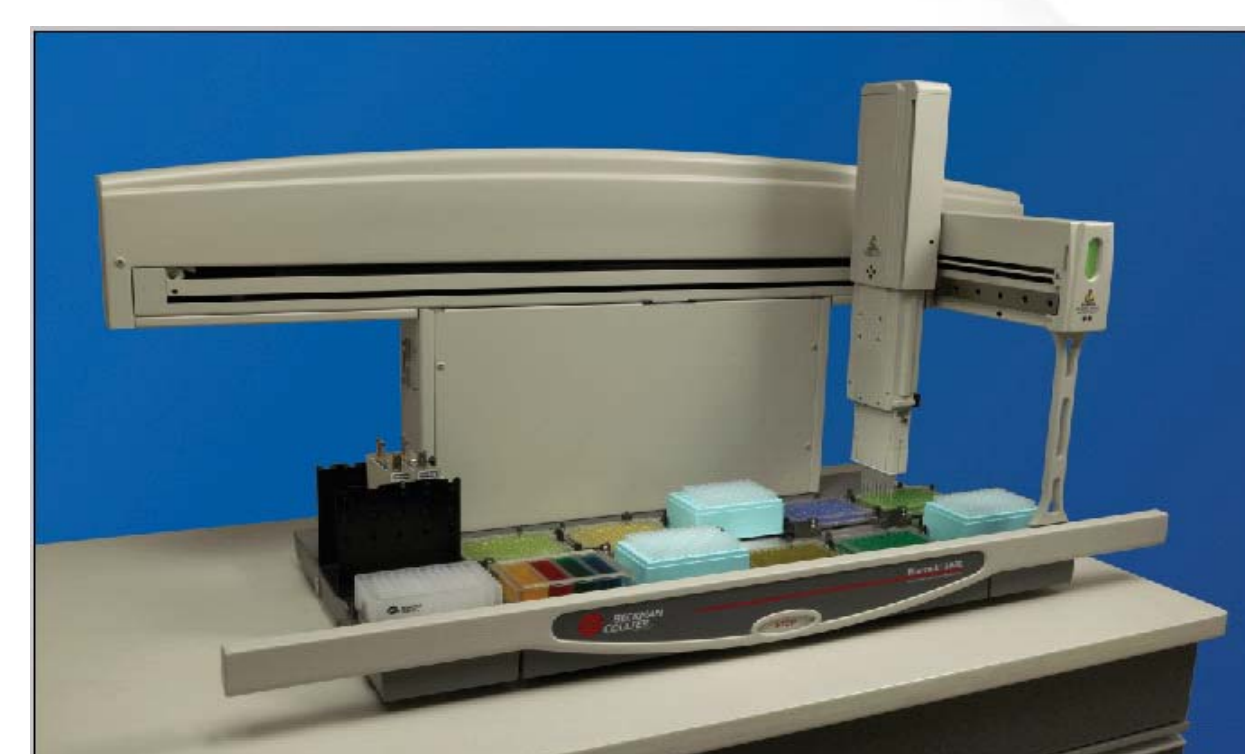
The Biomek 3000 Laboratory Automation Workstation was used to automate Caco-2 cell preparation and differentiation in the BD Falcon HTS 96-Multiwell Insert System. Additionally, the Biomek 3000 Laboratory Automation Workstation was used to automate the permeation and bi-directional transport assays. Using the Biomek 3000 Laboratory Automation Workstation for cell preparation, permeation and transport assays facilitated Caco-2 assay implementation, reduced the chance of contamination and minimized the intensive requirement of sterile skills. The automated Caco-2 assays can be used for high-throughput screening of drug candidates for absorption properties.

Materials and Methods

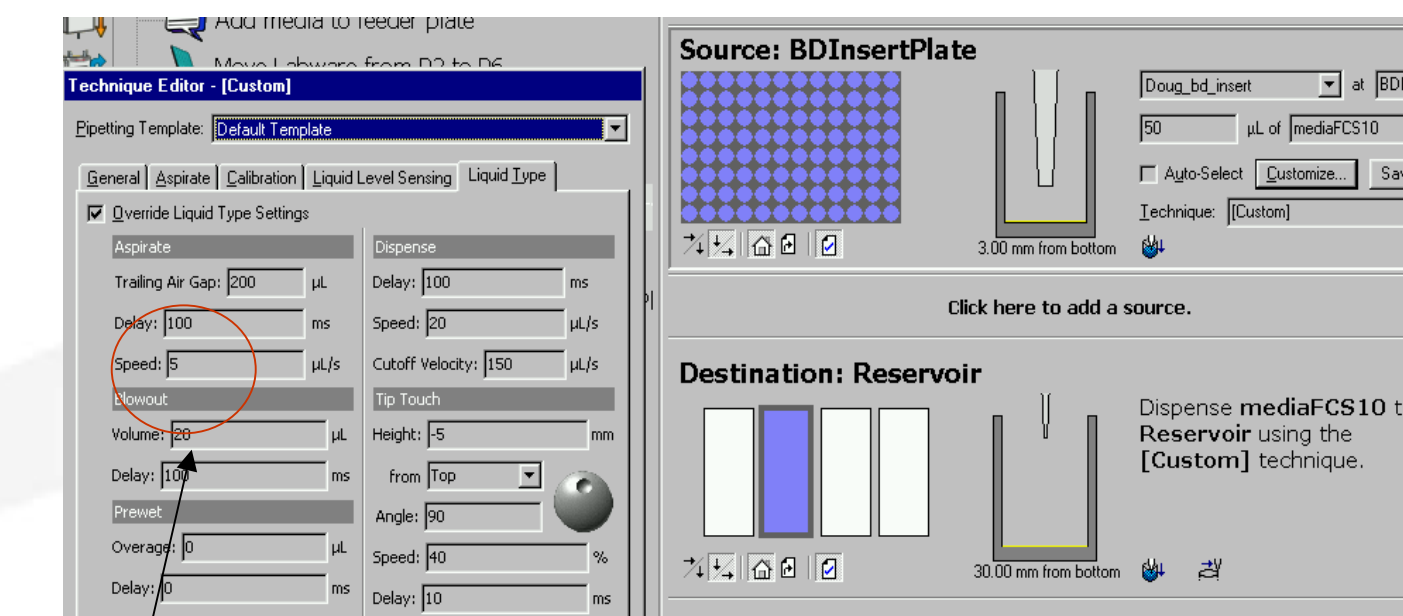
Caco-2 Culture Preparation using the Biomek 3000 Laboratory Automation Workstation

- Biomek 3000 Laboratory Automation Workstation in a biosafety hood from the Baker Company
- Caco-2 human colon carcinoma cell line (ATCC) passage number between 20 and 30
- DMEM with 10% fetal bovine serum (Invitrogen Corporation)
- BD Falcon HTS 96-Multiwell Insert System (BD Biosciences)
- 1x10⁴ cells in 70 µL of media dispensed into the insert wells
- 35 mL of media dispensed into the feeder tray for the BD Falcon HTS 96-Multiwell Insert System
- Cells incubated at 37°C and 5% CO₂ for 21 days
- Media in insert wells and feeder tray or receiver wells changed every other day
- Biomek 3000 tools including the MP200 tool and the gripper tool

Biomek® 3000 Laboratory Automation Workstation

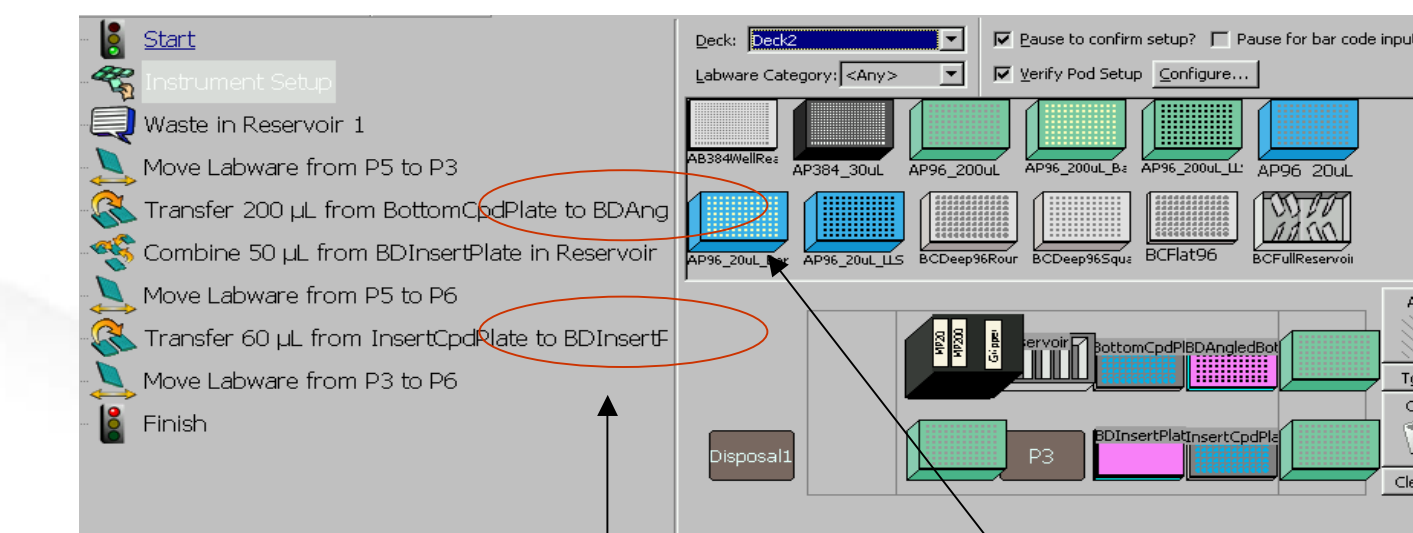


Biomek 3000 Software Screen Showing Parameters for Cell Seeding in Microplates



- A slow dispense rate was used to ensure intact monolayer differentiation.
- 1x10⁴ cells in 70 µL of media from reservoir were dispensed into the insert wells.
- Media were changed every 2 days in both insert plates and feeder trays.

Biomek 3000 Software Screen Showing Parameters for Caco-2 Permeation and Transport Assay using the BD Falcon HTS 96-Multiwell Insert System



- Insert wells with differentiated Caco-2 cells were washed twice with Hanks buffer.
- Test compounds or Hanks buffer were added to insert plate from insert sample plate.
- Insert plate was transferred onto angled bottom receiver plate with Test compounds or Hanks buffer.

Caco-2 Drug Permeation Assay using Biomek 3000 Laboratory Automation Workstation

- Test compounds listed in the Biopharmaceutics Classification System by the U.S. FDA as validation standards (Sigma-Aldrich)
 - Lucifer yellow 100 µM
 - Carbamazepine 200 µM
 - Metaprolol 200 µM
 - Hydrochlorothiazide 200 µM
 - Propranolol 200 µM
 - Atenolol 200 µM
 - Ketoprofen 200 µM
 - Furosemide 200 µM
- Test compound in 50 µL of Hanks buffer (Invitrogen Corporation) in insert wells
- 200 µL of Hanks buffer in receiver wells
- Incubate plate at 37°C and 5% CO₂ for 2 hours, collect sample from the receiver wells for permeation coefficient analysis
- Compound quantitation: SPECTRAMax® PLUS³⁸⁴ Microplate Spectrophotometer (Molecular Devices Corporation)
- Lucifer yellow detection: Affinity® Multi-Mode Plate Reader (Cambridge Research Institute) green laser

Caco-2 Drug Bi-directional Transport Assay using Biomek 3000 Laboratory Automation Workstation

- Test compounds: Rhodamine 123 and Paclitaxel at 10 µM concentration
- Apical to basolateral chamber transport assay
 - 50 µL of Test compound in insert wells
 - 200 µL of Hanks buffer in receiver wells
- Basolateral to apical chamber transport assay
 - 200 µL of Test compound in receiver wells
 - 50 µL of Hanks buffer in insert wells
- Incubate plate at 37°C and 5% CO₂ for 2 hours, collect sample from the both insert wells and receiver wells for permeation coefficient analysis
- Compound quantitation: SPECTRAMax PLUS³⁸⁴ Microplate Spectrophotometer (Molecular Devices Corporation)
- Rhodamine 123 detection: Affinity Multi-Mode Plate Reader (Cambridge Research Institute) green laser

Bi-Directional Transport Assay

	Apical to Basolateral chamber transport (A to B)	Basolateral to Apical chamber transport (B to A)
Apical chamber Insert wells	Test compounds	Hanks buffer
Basolateral chamber Receiver wells	Hanks buffer	Test compounds

Internal Standards for Establishing Permeability Rank Order Using Caco-2 Permeation Assay

Drug	Permeability Class	Solubility Class
Carbamazepine	High	Low
Ketoprofen	High	Low
Metoprolol	High	High
Propranolol	High	High
Atenolol	Low	High
Furosemide	Low	Low
Hydrochlorothiazide	Low	Low

Table 1: Caco-2 Cell Monolayer as a Barrier Prevents Leakage of Lucifer Yellow in BD Falcon HTS 96-Multiwell Insert System

	BD Falcon HTS 96-Multiwell Insert System
With Caco-2 cell barrier	3.1 ± 0.02 ¹
Without Caco-2 cell barrier	140.5 ± 17.2 ¹

¹ P_{app} A to B (x10⁻⁶ cm/sec)

Figure 1: Permeability Rank Order of Drug Standards Generated from Caco-2 System using Biomek 3000 Laboratory Automation Workstation

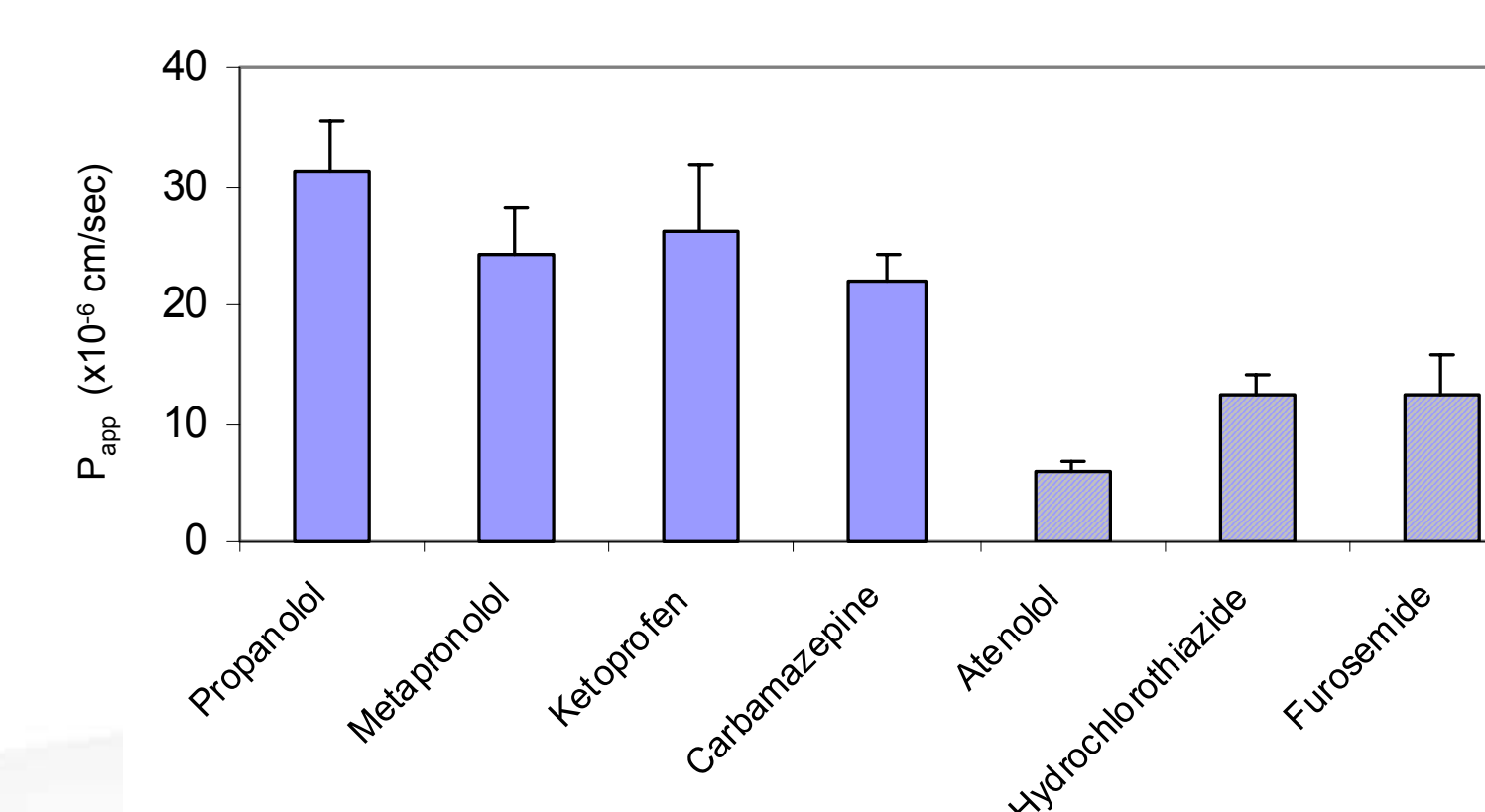


Table 2: Permeability Coefficient Rank Order of Drug Standards in BD Falcon HTS 96-Multiwell Insert System

	Automated Biomek 3000 methods	Automated Biomek 2000 and FX methods
Propranolol	31.6 ± 4.2 ¹	16.6 ± 2.7 ¹
Metapronolol	24.3 ± 3.8 ¹	11.1 ± 1.1 ¹
Ketoprofen	26.1 ± 5.8 ¹	10.8 ± 2.2 ¹
Carbamazepine	21.9 ± 2.2 ¹	12.4 ± 1.0 ¹
Atenolol	5.8 ± 1.0 ¹	2.7 ± 1.4 ¹
Hydrochlorothiazide	12.3 ± 1.7 ¹	6.9 ± 1.0 ¹
Furosemide	12.5 ± 3.2 ¹	6.5 ± 0.7 ¹

¹ P_{app} A to B (x10⁻⁶ cm/sec)

Table 3: Permeability Coefficient Rank Order of Drug Standards in BD Falcon HTS 96-Multiwell Insert System

	Automated Biomek 3000 methods	BD Bioscience Euro-biotech 2000/1
Propranolol	31.6 ± 4.2 ¹	43.0 ± 3.8 ¹
Metapronolol	24.3 ± 3.8 ¹	16.1 ± 1.9 ¹
Ketoprofen	26.1 ± 5.8 ¹	14.8 ± 3.8 ¹
Carbamazepine	21.9 ± 2.2 ¹	18.8 ± 3.8 ¹
Atenolol	5.8 ± 1.0 ¹	0.1 ± 0.07 ¹
Hydrochlorothiazide	12.3 ± 1.7 ¹	6.6 ± 1.9 ¹
Furosemid	12.5 ± 3.2 ¹	10.7 ± 0.01 ¹

¹ P_{app} A to B (x10⁻⁶ cm/sec)

Table 4: Active Efflux Transport of Paclitaxel and Rhodamine 123 through Caco-2 Monolayer in BD Falcon HTS 96-Multiwell Insert System

	Paclitaxel	Rhodamine 123
A to B	18.0 ± 1.9 ¹	1.3 ± 0.01 ¹
B to A	108.0 ± 8.1 ¹	23.7 ± 4.1 ¹
B to A / A to B	6.1	19.0

The B to A / A to B ratio of permeation coefficient greater than 1.5 indicated Active Efflux Transport through the monolayer.

Conclusions

- Differentiated Caco-2 monolayer integrity in insert wells in the BD Falcon HTS 96-Multiwell Insert System was demonstrated by lack of lucifer yellow paracellular transport from insert to receiver wells.
- The permeability coefficient of the 7 test compounds matched with known *in vivo* permeability and solubility properties listed in the Biopharmaceutics Classification System provided by the U.S. FDA.
- The ratio of efflux to transcellular transport of known reference rhodamine 123 were both greater than 1.5.
- The permeability coefficient, rank order and ratio of efflux to transcellular transport of paclitaxel matched with published data.
- The automated cell preparation, permeation and transport assays using the Biomek 3000 Laboratory Automation Workstation facilitated Caco-2 assay implementation, reduced the chance of contamination and minimized the intensive requirement of sterile skills.