Automated In Vitro MDCKII-BCRP Monolayer Assay with Ultra-Fast LC-MS/MS

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1 INTRODUCTION

Bidirectional permeability assay:

- In vitro modeling of pharmacokinetic barriers (e.g., Intestinal epithelium, blood-brain-barrier, etc.).
- Informs us about the compounds of interest active and passive transport characteristics.
- Tight cell monolayer grown on a porous support
- **MDCKII** (Madin-Darby canine kidney strain II) cells overexpressing the BCRP (Breast Cancer Resistant Protein, ABCG2) efflux transporter in their apical membrane (MDCKII-BCRP) and acts as a B (basolateral) to A (apical) pump for its substrates.

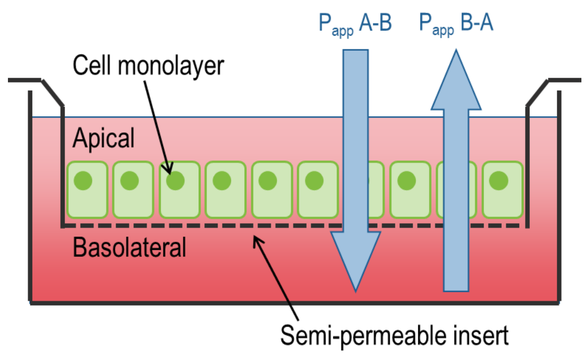


Fig 1. In vitro bidirectional permeability assay design for assessing apparent permeability (Papp) of a compound.

Challenges of a high throughput system:

- Changing from the typically used 24-well to 96 well plate format.
- High requirements for analytical instrumentation. As sample complexity increases, sensitivity and reliability of MS-based plate reading approaches decrease.

2 METHODS AND MATERIALS

Assay Setup

- Cell seeding was performed 4 days before the experiment onto Millicell-96 culture plates.
- The reagent mix containing the test articles and controls were prepared on a deep well plate (helper plate).
- Proper cell function was validated by analyzing A-B and B-A movement of radiolabeled prazosin, a known BCRP substrate (accepted if Efflux Ratio (ER) >8).
- Cell monolayer integrity was confirmed via measurements with Lucifer yellow (LY) as low permeability control (accepted if P_{app}<2x10^{-6} cm/s) in each well and metoprolol as high permeability control (n=3).

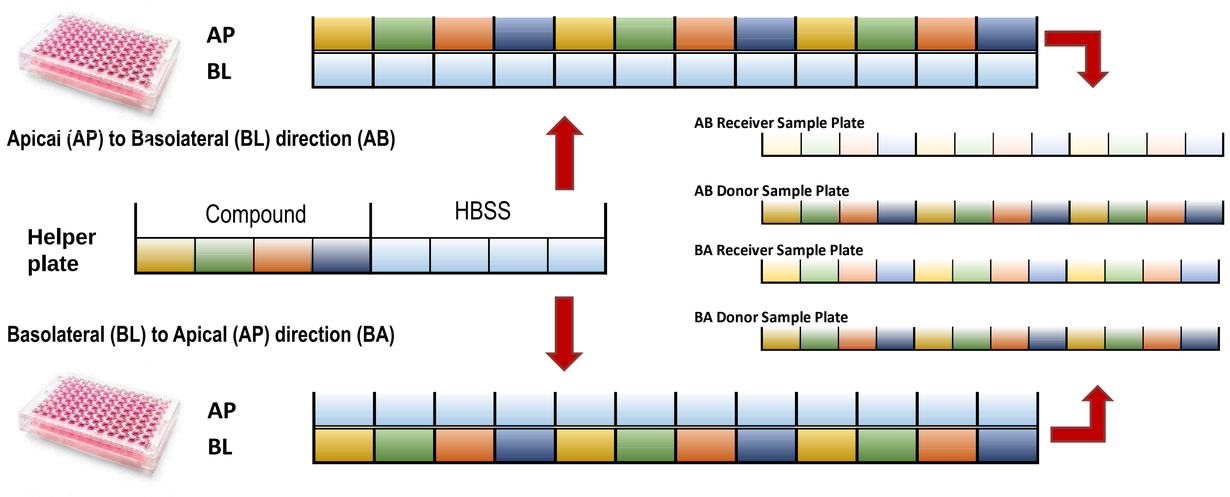


Fig 2. Bidirectional permeability assay plate layout. The apical and basolateral wells of the assay plates were filled with the reaction mixture prepared on the helper plate. After the proper incubation time the samples were collected on separate microplates.

3 WORKFLOW AND SYSTEM DESIGN

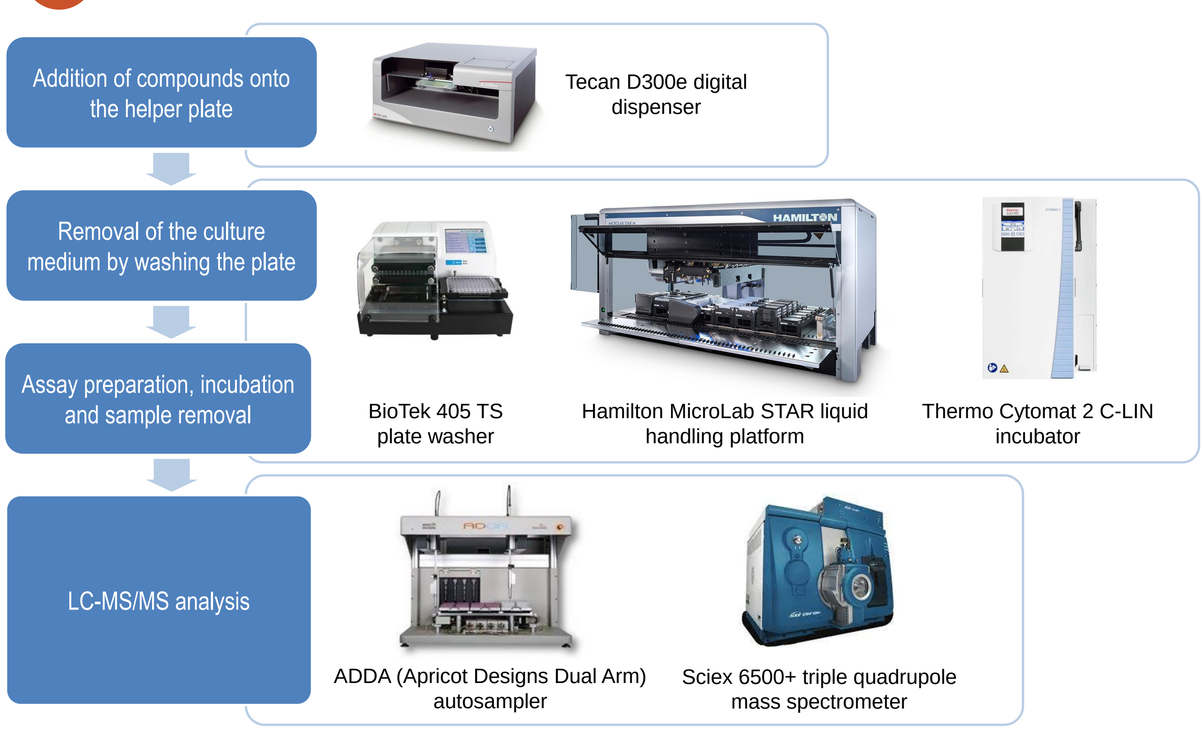


Fig 3. Steps of the experimental workflow and the related instruments.

The assay was performed in a 96-well format plate. The cell seeding and media exchanges were performed manually.

The helper plate was prepared using a **Tecan D300 digital dispenser** and a **Hamilton MicroLab STAR** automated liquid handler. The liquid handler, equipped with a 96 CO-RE Head was used for the compound dosing and sample collection. The instrument is also integrated with a **BioTek 405 TS microplate washer** for the buffer exchange, and a **Thermo Cytomat 2 C-LIN incubator**.

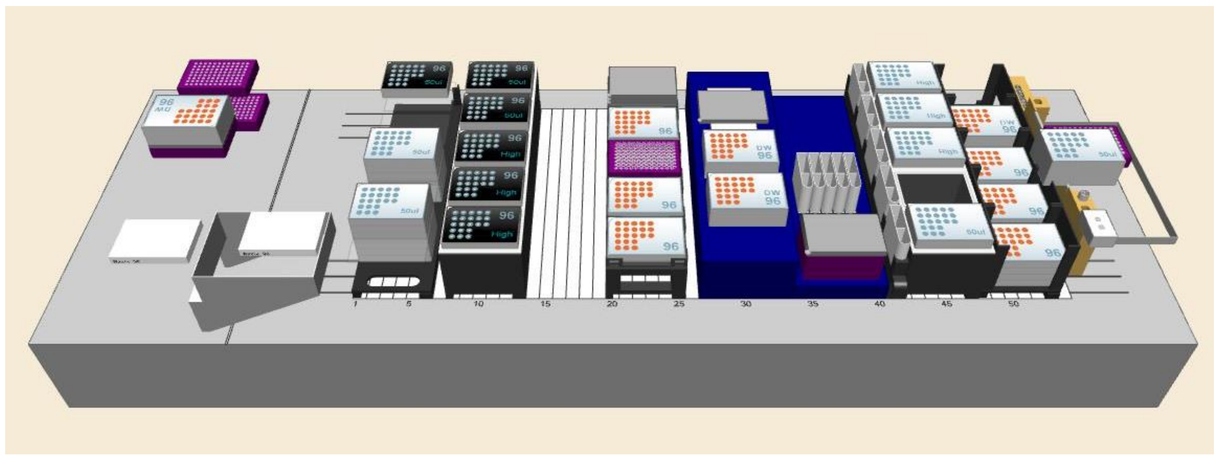


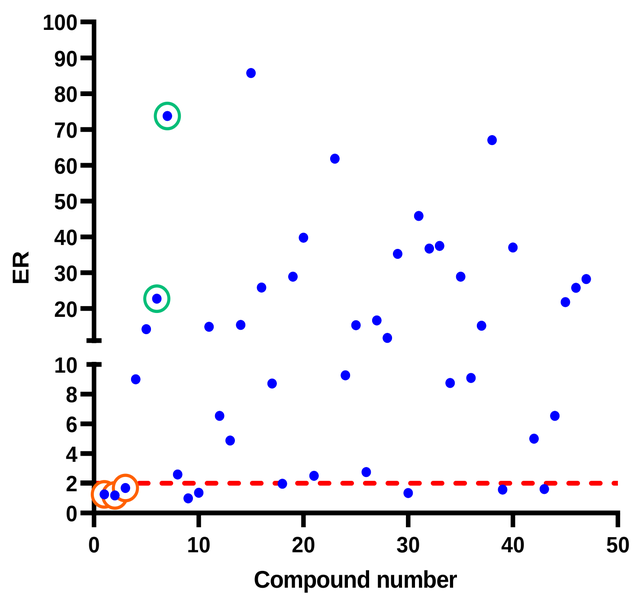
Fig 4. Schematic representation of the Hamilton MicroLab Star deck layout

LC-MS/MS analysis was performed on an ADDA (Apricot Designs Dual Arm) autosampler coupled to a Sciex 6500+ triple quadrupole mass spectrometer. Method development and sample analysis were performed using LeadScape software, designed for high throughput analysis.

4 RESULTS

	Manual workflow			Automated workflow		
	P_{app}, A-B (×10^6 cm/s)	P_{app}, B-A (×10^6 cm/s)	ER	P_{app}, A-B (×10^6 cm/s)	P_{app}, B-A (×10^6 cm/s)	ER
	Average± SEM	*Average± SEM*	*Average ± EP*	*Average± SEM*	*Average± SEM*	*Average ± EP*
Prazosin	*2.07 ± 0.29*	*67.78 ± 12.62*	*32.77 ± 7.59*	*1.77 ± 0.17*	*114.84 ± 5.52*	*64.77 ± 5.52*
Lucifer Yellow	*1.28 ± 1.97*	-	-	*0.237 ± 0.018*	*0.370 ± 0*	-
Metoprolol	*39.66 ± 24.50*	-	-	*41.99 ± 2.58*	*59.28 ± 0.47*	*1.41 ± 2.62*

Table 1. Bidirectional permeability of control compounds using the manual or automated workflow, respectively.



- ER values were calculated by dividing their apparent permeability (P_{app}) results in the B-A (basolateral to apical) and A-B (apical to basolateral) directions across the MDCKII-BCRP cell monolayer.
- Reliability of the automated protocol versus standard protocol was assessed by comparing data obtained with the appropriate controls in each measurement.
- ER>2 indicates active efflux of the compounds via the BCRP efflux pump (cut-off marked in red).
- Highlighted data points represent known substrates (green) and non-substrates (orange), where ER values reproduced literature findings [1-3].

Fig 5. Efflux ratios (ER) calculated for all compounds tested using the automated assay workflow.

5 CONCLUSIONS

Our results demonstrate that the presented automated monolayer assay system can yield bidirectional permeability data that reliably reproduces values obtained in the manually conducted assay workflow. Permeability of both the high- and low-permeability reference compounds as well as the selected BCRP probe substrate was similar using the two workflows and can serve as appropriate controls when testing unknown substances. The application of an automated assay system where possible can reduce human error, increase throughput and save time, whilst producing reliable and reproducible data.

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[3] Lukas Cerveny, Petr Pavek, Jana Malakova, Frantisek Staud, and Zdenek Fendrich. Lack of Interactions between Breast Cancer Resistance Protein (BCRP/ABCG2) and Selected Antiepileptic Agents *Epilepsia*, **47**(3):461–468, 2006

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