

Cloe Screen MDCK (Wild Type) Permeability

experts in **ADME**

Background Information



'When using LLC-PK1-MDR1 or MDCK-MDR1 cells for bi-directional studies, the wild type LLC-PK1 MDCK cells, respectively, should be included as negative controls.'

FDA Draft Guidance for Industry - Drug Interaction Studies - Study Design, Data Analysis, and Implications for Dosing and Labeling (September 2006)

- Madin Darby canine kidney (MDCK) cells are an epithelial cell line of canine kidney origin.
- MDCK cells have low expression of transporter proteins and low metabolic activity¹.
- MDCK cells are often transfected with transporter proteins to investigate drug efflux e.g., the MDR1-MDCK cell line which expresses human P-glycoprotein. This cell line is a useful model for the identification of P-gp substrates and inhibitors.
- As MDCK cells endogenously express other transporters such as canine P-gp², it is recommended that the compound is screened through the MDCK (Wild Type) Permeability assay to calculate a net flux ratio in order to confirm the role of human P-gp in the MDR1-MDCK studies.



Protocol

Test Compound Concentration
10 μ M (different concentrations available)

Direction
Apical to Basolateral and/or
Basolateral to Apical

Number of Replicates
2

Incubation Time
60 min

Growth Period
4 days

Compound Requirements
100 μ L of 10 mM solution

Analysis method
LC-MS/MS quantification

Data Delivery
 P_{app} ;
Efflux Ratio for
Bidirectional Assessment;
Net Flux Ratio
(if MDR1-MDCK data available)

Cloe Screen MDCK (Wild Type) Permeability assay is used to confirm the role of human P-glycoprotein in the MDR1-MDCK assay as recommended in the draft FDA guidelines.



Cloe Screen MDCK (Wild Type) Permeability

20 compounds were screened in the Cloe Screen (Wild Type) MDCK Permeability assay (pH 7.4 buffer in the apical and basolateral compartments) in quadruplicate on 3 separate occasions. These data are highly reproducible for both low and high permeability values.

Figure 1
Cloe Screen MDCK (Wild Type) Permeability validation for apical to basolateral transport.

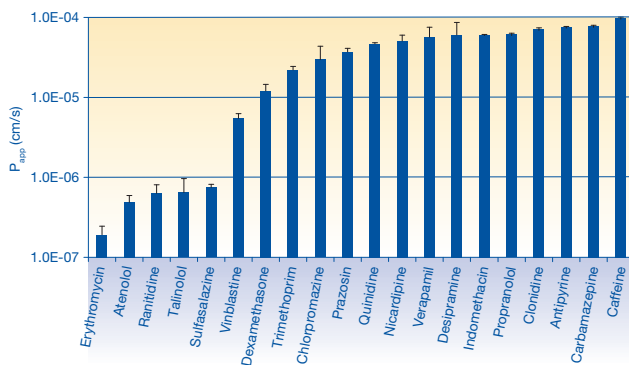


Figure 2
Comparison of efflux ratio generated in the wild type and MDR1-MDCK assays.

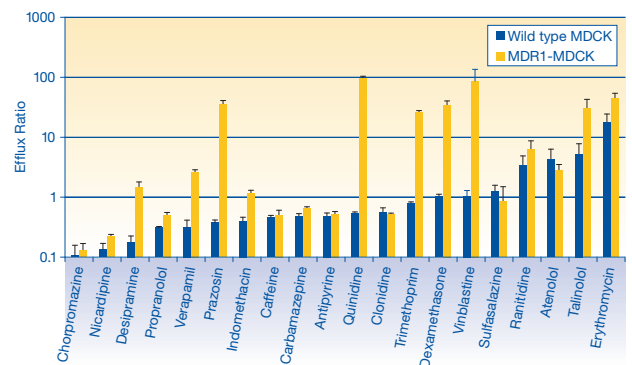
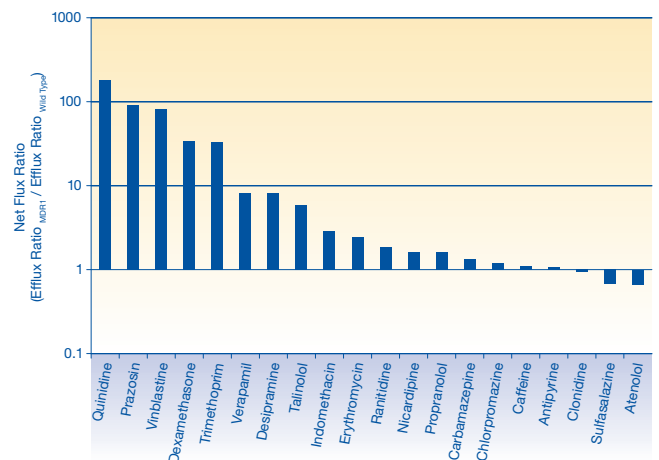


Figure 3
Net flux ratio for a set of 20 compounds (calculated using the efflux ratios of the wild type and MDR1-MDCK bidirectional assays).



References

- Braun A et al. (2000) *Eur J Pharmaceut Sci* **11**; (Suppl 2) S51-S60.
- Goh LB et al. (2002) *Biochem Pharmacol* **64**; 1569-1578.