

## Identification of Substrates and Inhibitors of P-glycoprotein (P-gp) with MDR1-MDCK Cells

### Background

Drug transporters are increasingly recognized for playing an important role in drug disposition (ADME) and potential DDIs. P-glycoprotein (P-gp/MDR1/ABCB1), expressed in a wide variety of tissues including intestine, brain, liver, and kidney, is the most extensively studied drug transporter that is known to transport structurally diverse classes of compounds. P-gp's ubiquitous physiological presence together with its ability to extrude a large number of chemicals has prompted the FDA to develop guidelines to test whether an NCE is a substrate and an inhibitor of P-gp<sup>[1]</sup>. The MDCK (Madin-Darby Canine Kidney) cell line that is stably transfected with human MDR1 gene<sup>[2]</sup> is a widely accepted model for assessing the P-gp substrate and inhibitor potential of test compounds. The ability of MDCK cells to form tight junctions in a short culture time ensures quality data generation (highly reproducible) with a fast assay turnaround. Accordingly, this assay is routinely incorporated as a part of a new drug application (NDA) submission for the regulatory review process.

### Assay Outline

Substrate Assay	Inhibition Assay
<ul style="list-style-type: none"> <li>MDR1-MDCK cells and WT-MDCK cells (parental MDCK as background)</li> <li>Apparent Permeability (<math>P_{app}</math>) in both AP→BL and BL→AP</li> <li>Efflux Ratio (BL→AP/ AP→BL) of test article (3 conc) ± P-gp inhibitor (3 time pts)</li> <li>Net Efflux Ratio (MDR1-MDCK / WT-MDCK)</li> <li>Monolayer Integrity: TEER &amp; Lucifer Yellow</li> </ul>	<ul style="list-style-type: none"> <li>MDR1-MDCK cells</li> <li>Apparent Permeability (<math>P_{app}</math>) in both AP→BL and BL→AP</li> <li>Efflux Ratio (BL→AP/ AP→BL) of [<sup>3</sup>H]-Digoxin ± test compound (6 concs &amp; 1 time pt)</li> <li>IC<sub>50</sub> of test compound with Digoxin Efflux Ratio</li> <li>Monolayer Integrity: TEER &amp; Lucifer Yellow</li> </ul>

### Typical Results

#### Substrate Assay Data:

Transport of digoxin (500 nM) and vinblastine (5 μM) in WT-MDCK and MDR1-MDCK monolayer in the presence and absence of P-gp inhibitors, Cyclosporin A (CyA) and Verapamil.

P-gp substrate		WT-MDCK			MDR1-MDCK			$R_E^c$
		$P_{app,AP→BL}^a$	$P_{app,BL→AP}$	$ER^b$	$P_{app,AP→BL}$	$P_{app,BL→AP}$	ER	
Digoxin	Control	9.3 ± 3.0	33.3 ± 8.2	3.6	3.9 ± 0.6	52.8 ± 5.9	13.5	3.8
	+ CyA <sup>d</sup>	20.1 ± 5.8	18.1 ± 7.0	0.9	14.2 ± 4.4	13.5 ± 4.4	0.9	1.1
	+ Verapamil <sup>e</sup>	15.9 ± 4.6	15.7 ± 6.3	1.0	13.0 ± 3.4	18.9 ± 4.0	1.4	1.5
Vinblastine	Control	10.4 ± 2.0	63.4 ± 20.9	6.1	2.4 ± 0.4	63.4 ± 11.2	26.0	4.3
	+ CyA	34.4 ± 5.6	32.1 ± 3.4	0.9	18.1 ± 3.1	9.7 ± 1.5	0.5	0.6
	+ Verapamil	39.8 ± 7.5	33.2 ± 6.9	0.8	23.5 ± 8.2	17.6 ± 5.2	0.7	0.9

<sup>a</sup>  $P_{app}$ s are in the unit of nm/s and presented as Mean ± SD (n ≥ 3 experiments);

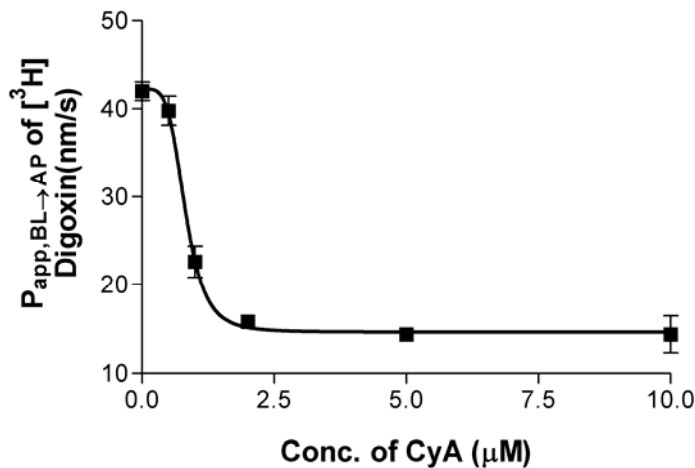
<sup>b</sup> ER = (efflux ratio) =  $P_{app,BL→AP} / P_{app,AP→BL}$ ;

<sup>c</sup>  $R_E$  (net efflux ratio) =  $ER_{MDR1-MDCK} / ER_{WT-MDCK}$

<sup>d</sup> 10 μM of CyA was used; <sup>e</sup> 200 μM of verapamil was used.

The table shows asymmetrical transport of two prototypical P-gp substrates, digoxin and vinblastine. The efflux ratios, calculated as a ratio of BL→AP/ AP→BL was higher in MDR1-MDCK (Digoxin: 13.5; Vinblastine: 26) than in WT-MDCK (Digoxin: 3.6; Vinblastine: 6.1) demonstrating P-gp mediated efflux of these prototypical substrates in the MDR1-MDCK cells. With the addition of P-gp inhibitors CyA or verapamil,  $R_E$  was significantly reduced to almost unity further demonstrating the viability of the system. These data show that our MDR1-MDCK P-gp assay system provides an excellent model for the purposes of P-gp substrate identification as outlined by the FDA draft guidance<sup>[1]</sup>. Further, the aforementioned procedure is our standard study design and can be tailored upon clients' request.

## Inhibition Assay Data:



The figure shows the apparent permeability of [<sup>3</sup>H]digoxin from the basolateral to apical direction across MDR1-MDCK cell monolayer in the presence of various concentrations of CyA, a potent P-gp inhibitor. Data are presented as mean ±SD (n = 3 experiments) and K<sub>i</sub> was estimated as 0.82 ± 0.03 µM. These data show that our MDR1-MDCK assay system is optimal for identifying P-gp inhibitors as outlined by the FDA draft guidance [1]. The aforementioned procedure is our standard study design and can be tailored upon clients' request, including a rapid throughput screening protocol.

NoAb's P-gp Substrate/Inhibitor assay allows the evaluation of the P-gp interaction of an NCE, helping to identify and avoid developing candidates with potential adverse drug interaction properties.

For drug-drug interaction studies, NoAb also offers a complementary activity based CYP450 induction or inhibition assays, to evaluate the inductive or inhibitory potential of a NCE. In addition, NoAb's proprietary DTE<sup>TM</sup> Gene Expression Analysis allows investigators to simultaneously survey drug related induction and suppression of ADME related genes (such as CYP450s, drug transporters and transcription factors) at the gene expression level, which is useful where activity based assays are unreliable or unavailable. All of these services are examples of NoAb's commitment to providing the best drug discovery tools for our clients, helping to shape drug discovery.

## References

[1] USFDA (2006) Draft Guidance for Industry: Drug Interaction Studies-Study Design, Data Analysis, and Implications for Dosing and Labeling. Available from:

<http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm072101.pdf>

[2] Pasta I, Gottesman MM, Ueda K, Lovelace E, Rutherford AV, and Willingham MC (1988) A retrovirus carrying an MDR1 cDNA confers multidrug resistance and polarized expression of P-glycoprotein in MDCK cells. Proc Natl Acad Sci USA 85:4486-90.

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