Determining Intestinal Absorption *In Vitro*

Need

For new drugs, it is crucial to determine how much of the administered dose will reach systemic circulation and result in the desired efficacious effect. The primary factors that limit the bioavailability of a drug are:

- Accurate and reliable data for informed decision-making
- Fast turnaround times to keep your drug development on track

Reproducible high quality intestinal absorption data that can be used to develop pharmacokinetic models for predicting accurate bioavailability (%F) is an essential component of the drug development process.

Solution

LifeNet Health LifeSciences *In Vitro* Assay Service group is comprised of senior scientists who are highly experienced with the Caco-2 cell line and 3D organotypic models for understanding intestinal absorption. When these data are combined with liver metabolic stability data, which is also provided by LifeNet Health, the data becomes even stronger. The combined assays allow for a more accurate prediction of %F and systemic exposure.

Accurate & reliable data

Fast turnaround times

Unsurpassed expertise

Collaborative approach

Basic Study Design

ASSAY PARAMETERS	PROTOCOL
Test system and options	Caco-2 cell line, CacoReady, or EpiIntestinal human 3D system (MatTek Corp)
Species	Human
Test Article concentration	10 µM (can vary on request)
Direction of movement	Apical→Basolateral, Basolateral→Apical (a single direction may be selected)
Time points	0, 15, 30, 60, 120 minutes (Times are flexible)
Sample Replicates	3
Barrier Integrity	TEER, and Lucifer Yellow Dye
Solvent	DMSO at final concentration 0.2-0.5%
Stock solution test article	10 mM in DMSO
Controls (High and Moderate)	Pindolol (H) and Ranitidine (M)
Analytical method	LC/MS/MS
Data	Barrier integrity, Flux rate, Papp, %Recovered, Cytotoxicity
Report	Full written report

Note: If necessary non-specific binding, metabolism, cytotoxicity and amount of test drug retained in the cell system can be evaluated.

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Summary of Intestinal Permeability A→B (Proficiency Compounds)

Test Compound	Dose Stock (ng/mL)	Mean Conc (ng/mL) Basolateral T=2 hr	Basolateral Volume (mL)	A→B Total Flux (ng)	Time (sec)	Flux rate (ng/sec)	Co (ng/cm3)	A (cm2)	A x Co = ng/cm	Papp (cm/sec) x 10-6	Fa Class
Naproxen	557	33	0.75	24.75	7200	0.0034375	557	0.33	183.81	19.00	High
Propranolol	2590	128	0.75	96	7200	0.01333333	2590	0.33	854.70	16.00	High
Antipyrine	1882	221	0.75	165.75	7200	0.02302083	1882	0.33	621.06	37.00	High
Caffeine	1942	178	0.75	133.5	7200	0.01854167	1942	0.33	640.86	29.00	High
Minoxidil	2092	31	0.75	23.25	7200	0.00322917	2092	0.33	690.36	5.00	High
Pindolol	2483	122.5	0.75	91.875	7200	0.01276042	2483	0.33	819.39	16.00	High
Amiloride	2661	3.83	0.75	2.8725	7200	0.00039896	2661	0.33	878.13	0.50	Moderate
Hydrochlorothiazide	2950	5.3	0.75	3.975	7200	0.00055208	2950	0.33	973.50	0.60	Moderate
Ranitidine	3144	2.9	0.75	2.175	7200	0.00030208	3144	0.33	1037.52	0.30	Moderate
Famotidine	3370	1.65	0.75	1.2375	7200	0.00017188	3370	0.33	1112.10	0.20	Low
Atenolol	2663	1.028	0.75	0.771	7200	0.00010708	2663	0.33	878.79	0.12	Low
Acyclovir	2250	1.45	0.75	1.0875	7200	0.00015104	2250	0.33	742.50	0.20	Low
Lisinopril	4030	2.3	0.75	1.725	7200	0.00023958	4030	0.33	1329.90	0.18	Low
Nadolol	3090	1.3	0.75	0.975	7200	0.00013542	3090	0.33	1019.70	0.13	Low
Mannitol	1822	BLQ	0.75	ND	7200	ND	1822	0.33	601.26	< 0.30	Low
Pindolol	1753	53	0.75	39.75	7200	0.00552083	1753	0.33	578.49	9.50	High
Ranitidine	3144	3.3	0.75	2.475	7200	0.00034375	3144	0.33	1037.52	0.33	Moderate

Note: BLQ stands for below the limit of quantification, and ND stands for not determined.

Note: Pindolol and Ranitidine were run on separate Caco-2 plates to demonstrate consistency

Note: Although atenolol is classified as having moderate permeability by IHC guidelines several published studies show Papp values in a Caco-2 system that categorize it as low (Artursson P. 1989; Teksin et al. 2010)

Summary of Intestinal Absorption (P_{app}) Class

P_{app} (x 10-6 cm/sec)	Fa Class
$P_{app} < 0.3$	Low
$0.3 < P_{app} < 2.5$	Moderate
$P_{app} > 2.5$	High

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