PAMPA: Novel BD Gentest™ Pre-coated PAMPA Plate System with High Caco-2 and Human Absorption Predictability

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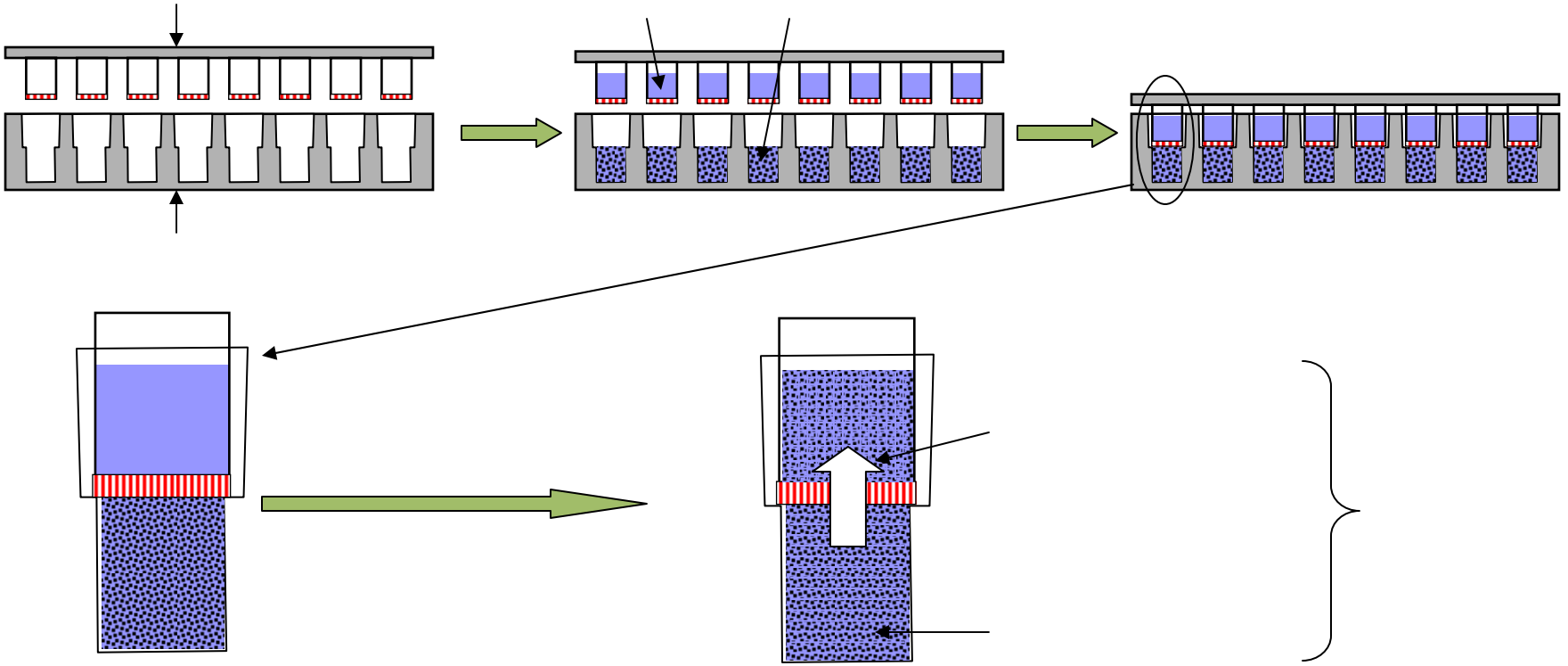
High Throughput Permeability Assay

- Drug candidates are screened for their oral-absorption potential early in the discovery and development phase, as a filter to remove poor performers and identify candidates that need to be modified.
- Most drugs are absorbed through the intestines without using cellular pumps. Therefore, passive permeability assays are useful for screening oral-absorption potential of drug candidates.
- Two permeability assays have become prevalent in recent years:
  - Cell-based (especially Caco-2) permeability assay
  - Parallel artificial membrane permeability assay (PAMPA)

Caco-2 vs. PAMPA

	Caco-2	PAMPA
Type of model	Cell monolayer	Artificial membrane
Type of Permeability	Measures the sum of passive and active permeability	Measures passive permeability in absence of transporters or efflux systems
Assay preparation	Requires cell culture (up to 21 days)	Easy, fast preparation
Cost	High	Low

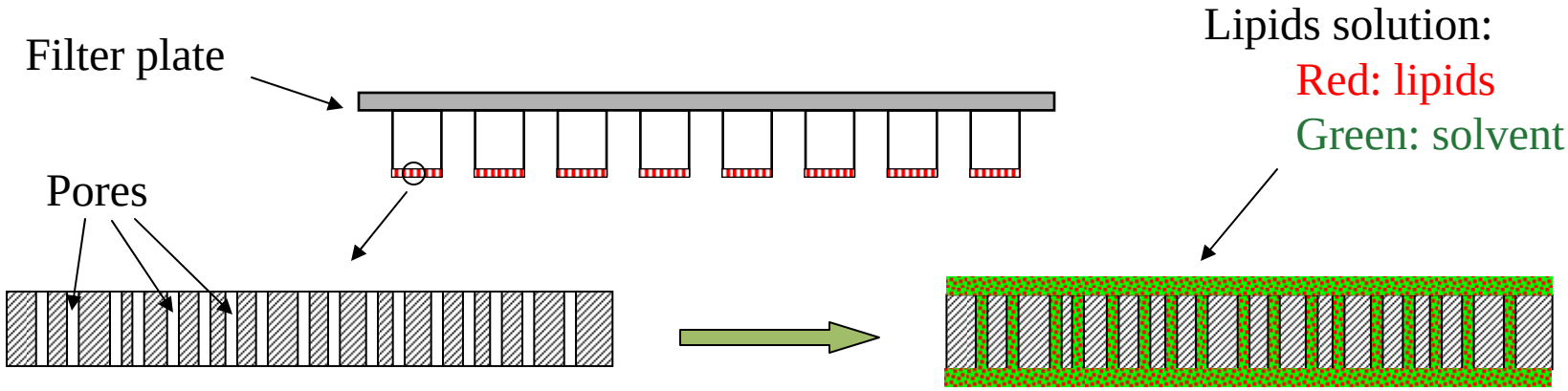
PAMPA and Caco-2 tests can be complementary tests to determine both passive and active permeability. Caco-2 tests alone measure the sum of passive and active permeability which can not be decoupled without the information obtained from PAMPA tests.



Existing PAMPA Methods

- Original PAMPA
  - The lipid solution consists of 10% lecithin in dodecane.
  - Reference: Kansy, M. *et al.* (1998) *J. Med. Chem.* 41,1007-1010.
- DOPC-PAMPA
  - The lipid solution consists of 2% DOPC in dodecane.
  - Reference: Avdeef, A. *et al.* (2001) *Eur. J. Pharm. Sci.* 14, 271-280.
- HDM-PAMPA
  - The lipid solution is 100% hexadecane.
  - Wohnsland, F. and Faller, B. (2001) *J. Med. Chem.* 44, 923-930.
- Bio-mimetic PAMPA (BM-PAMPA)
  - The lipid solution consists of a mixture of PC, PE, PS, PI and cholesterol in an organic solvent
  - Reference: Sugano, K. *et al.* (2001) *Int. J. Pharm. 228,*181-188.
- Double-Sink™ PAMPA (DS-PAMPA), available from pION Inc
  - The lipid solution consists of 20% dodecane solution of a phospholipid mixture
  - The acceptor solution contains a surfactant mixture
  - Reference: Avdeef, A. *et al.* (2005) *Eur. J. Pharm. Sci.* **24**:333-349 (2005).
- BD Gentest™ Pre-coated PAMPA Plate, available from BD Biosciences
  - A lipid-oil-lipid tri-layer structure is constructed in the pores of the porous filter.
  - Solvent contents are reduced by using volatile solvents in membrane preparation.
  - Improves correlation with human absorption and Caco-2, and reduces mass retention
  - Reference: Chen, X. *et al.*, (2008), *Pharm. Res.*, In Press

Traditional PAMPA Membrane

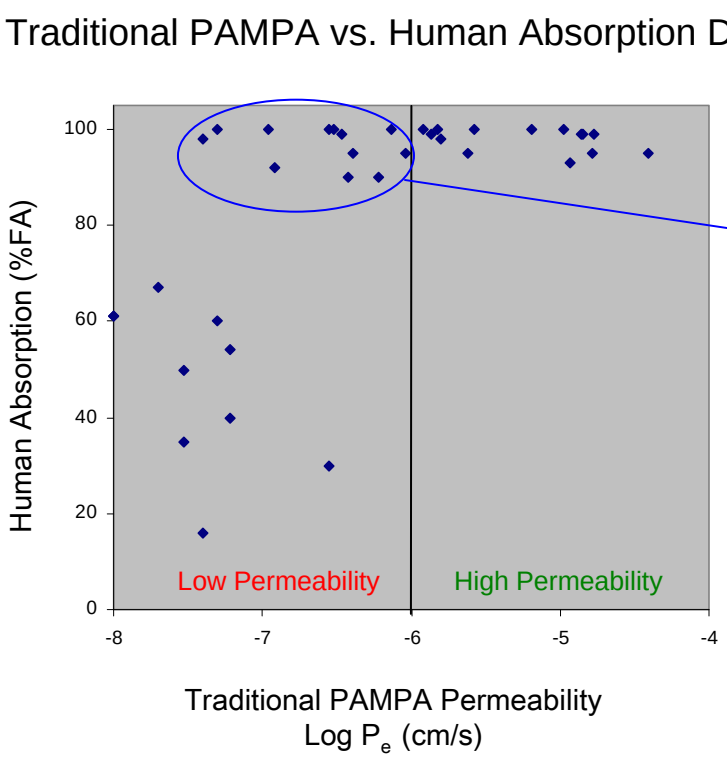


- A porous filter is soaked with a solution of lipids
- The lipids are likely dispersed in the solvent
- The PAMPA membrane is dominated by solvent, instead of lipids
- The excess amount of solvent may become an extra barrier for the compounds to permeate through

Issues of the Traditional PAMPA Membrane that We Would Like to Address

1. Incorrect prediction of a group of commercial compounds that are classified by the biopharmaceutical classification system (BCS) as high permeability compounds.
   - These compounds have high human absorption values and high Caco-2 permeability values, but are predicted by traditional PAMPA as “low permeability”.
2. Some “sticky” compounds are trapped in the artificial membrane, resulting in high mass retention values.
3. The artificial membrane needs to be prepared immediately before the assay, due to the poor stability of the artificial membrane.

Traditional PAMPA Issue 1A: Correlation with Human Absorption

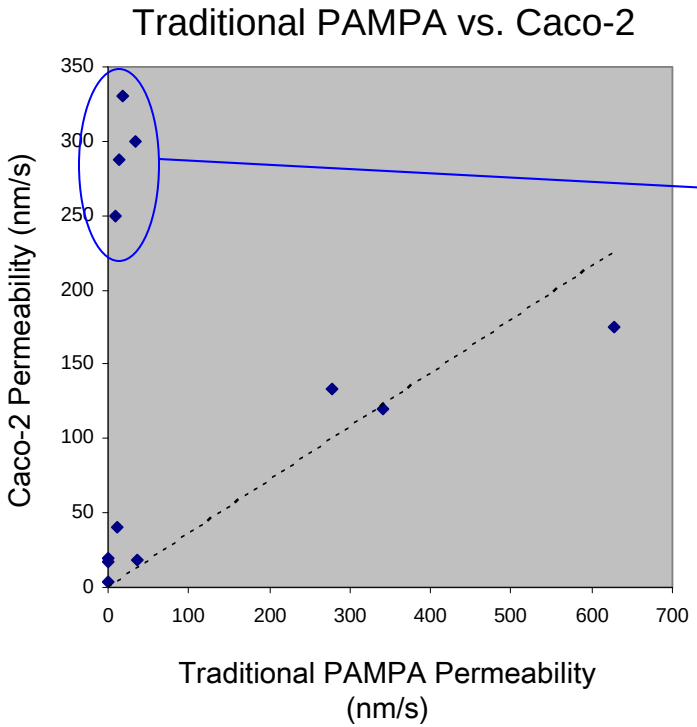


Under-predicted Compounds:

phenytoin
timolol
pindolol
metoprolol
theophyline
naproxen
acetaminophen
acetylsalicylic acid
antipyrine
indomethacin
ketoprofen

- Both traditional PAMPA (DOPC-PAMPA) permeability data and human absorption data are cited from: Ruell, J.A.; Avdeef, A.; Du, C.; Tsinman, K. A Simple PAMPA Filter for Passively Absorbed Compounds, Poster presented at 2002 ACS National Meeting, Boston, MA, USA. (available at http://www.pion-inc.com).

Traditional PAMPA Issue 1B: Correlation with Caco-2 Data



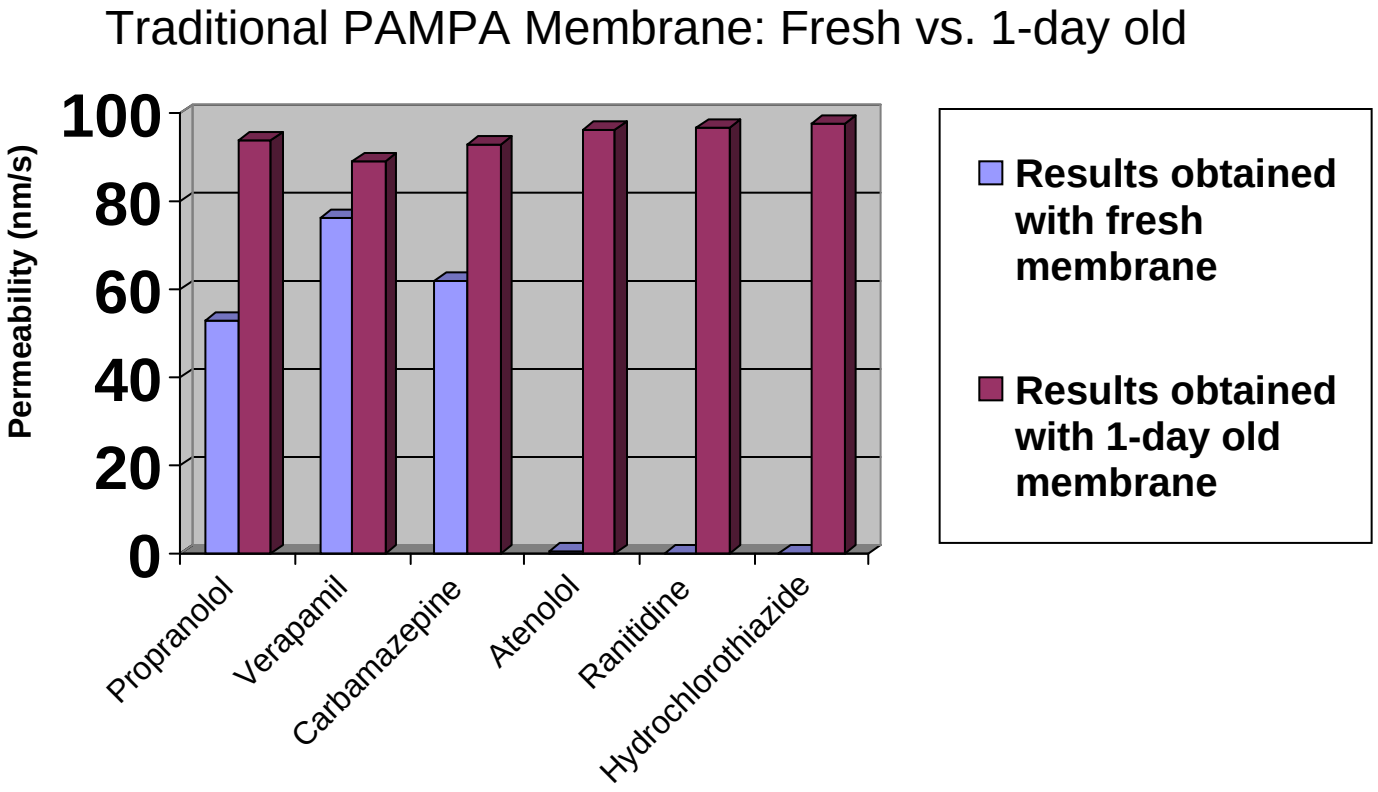
Under-predicted Compounds:
naproxen
antipyrine
caffeine
ketoprofen

- Both traditional PAMPA (Double-Sink™ PAMPA) permeability data and Caco-2 permeability data are cited from: Balimane, P.V., Han, Y-H., Chong, S. Current Industrial Practices of Assessing Permeability and P-Glycoprotein Interaction. *AAPS Journal* **8**(1):E1-E13 (2006); and other publications from the same laboratory.

Traditional PAMPA Issue 2: Mass Retention by the Membrane

- Mass retention: the percentage of the total mass of the compound lost during the permeability measurement as a result of binding to the plastic surface and being retained in the filter membrane
- Mass retention of some "sticky" compounds by the traditional PAMPA membrane (data cited from: Avdeef, A., Strafford, M., Block, E, Balogh, M., Chambliss, W., and Khan, I. *Eur. J. Pharm. Sci.* **14**:271 (2001)).
  - Amitriptyline: 53%
  - Ketoconazole: 64%
  - Phenazopyridine: 69%
- "Sticky" compounds are very common among pharmaceutical research compounds

Traditional PAMPA Issue 3: Stability of the Membrane

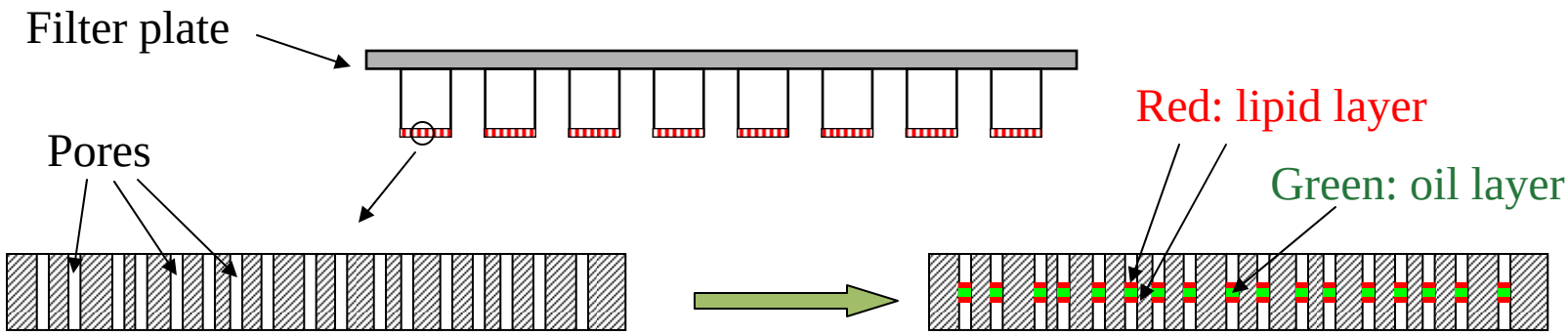


Because of this instability, traditional PAMPA membrane needs to be prepared by the user immediately before the assay.

Design of the New PAMPA Membrane

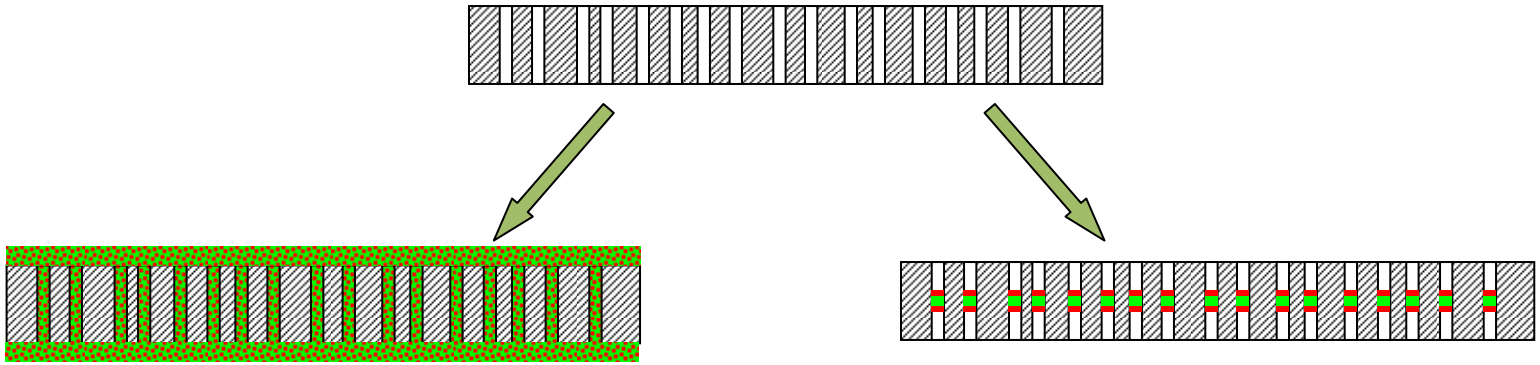
- Ideally, the PAMPA artificial membrane should be a true lipid bilayer. However, a true lipid bilayer is too fragile for use in high throughput assays.
- To create a model closer to the true lipid bilayer and robust enough for high throughput screening, a lipid-oil-lipid tri-layer structure is constructed in the pores of the porous filter.

New PAMPA Membrane



- A lipid-oil-lipid tri-layer structure is constructed in the pores of the porous filter using three consecutive coating steps
- The oil layer mimics the hydrophobic interior of the biological membrane; and the amphiphilic lipids anchoring on the oil / water interface mimic the exterior of the biological membrane.
- The oil layer is crucial for maintaining a robust and stable PAMPA membrane
- The oil layer is ultra thin to minimize compound retention and interference with the compound permeation

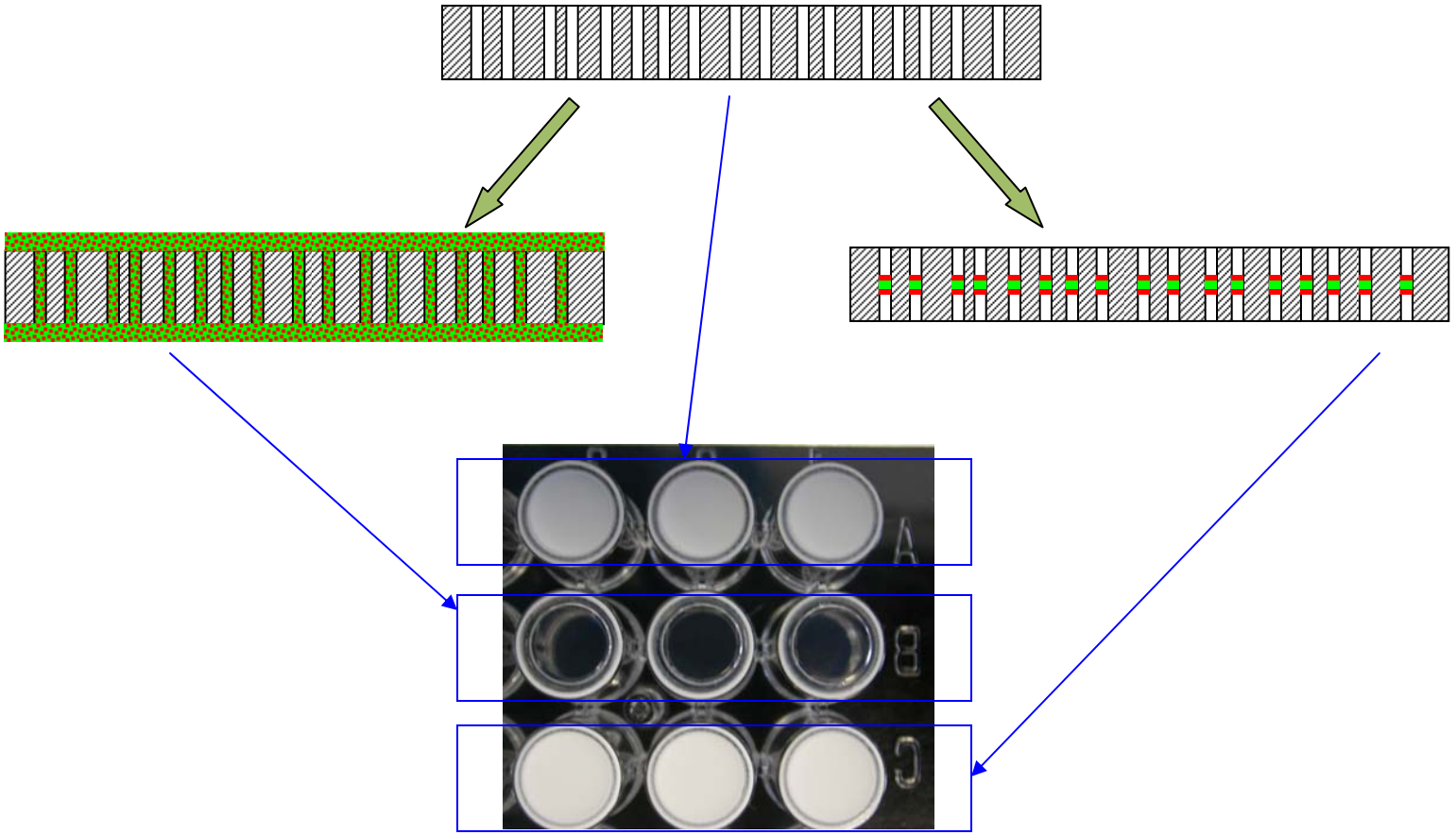
Comparison of traditional and new PAMPA membrane



- Excessive solvents
- No structured layers
- Long permeation pathway

- No excessive solvents
- Structured layers
- Short permeation pathway (closer to biological membrane)

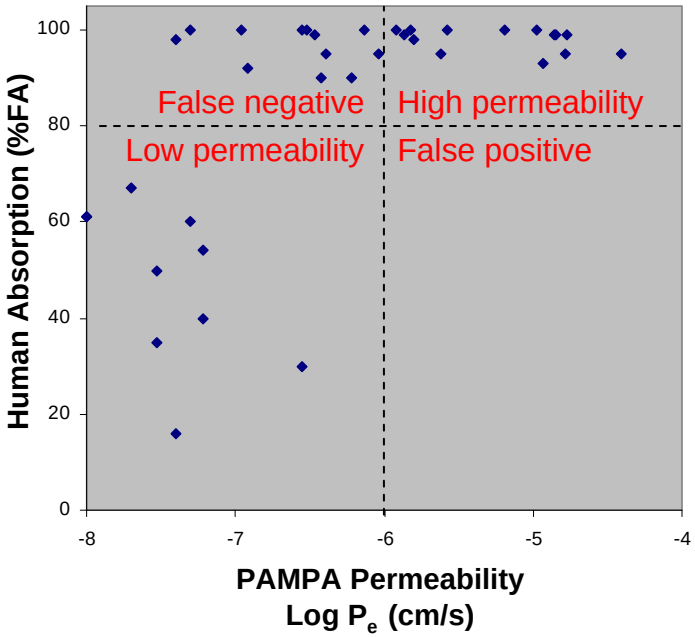
Comparison of traditional and new PAMPA membrane



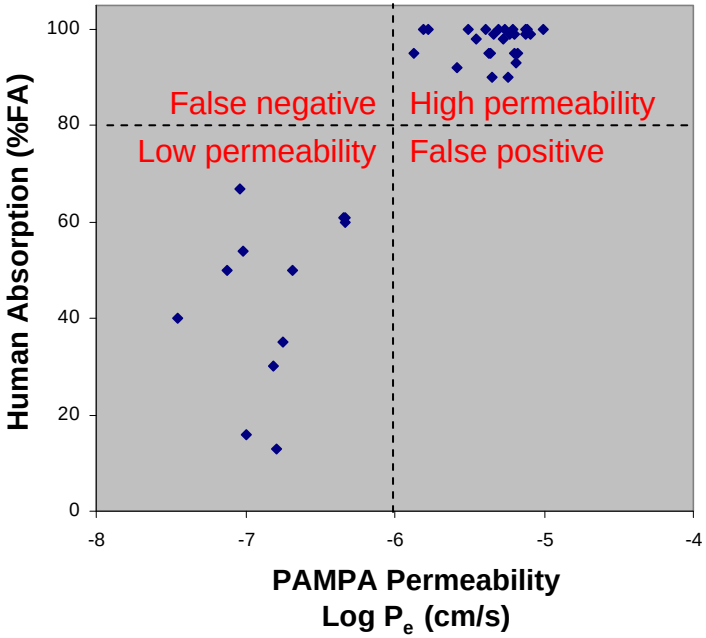
New PAMPA Membrane Improves Correlation with Human Absorption

Correlation plots using the same group of compounds

Traditional PAMPA



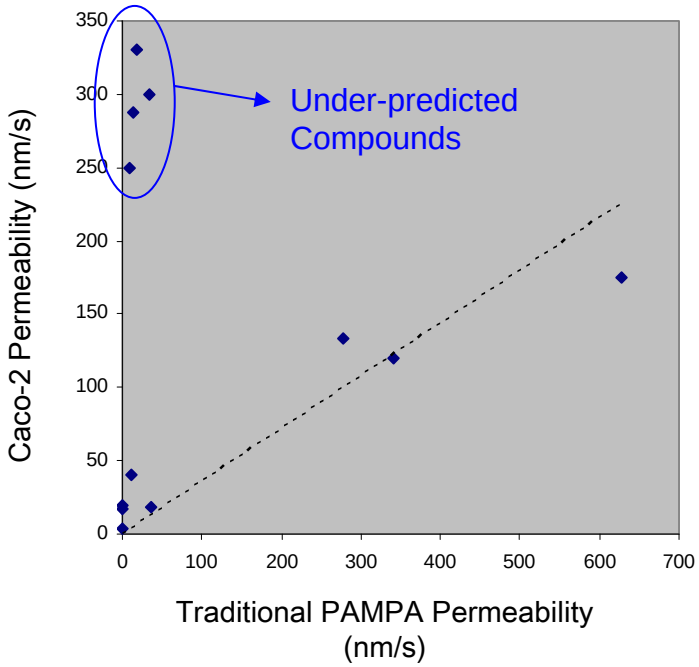
BD Gentest™ Pre-coated PAMPA



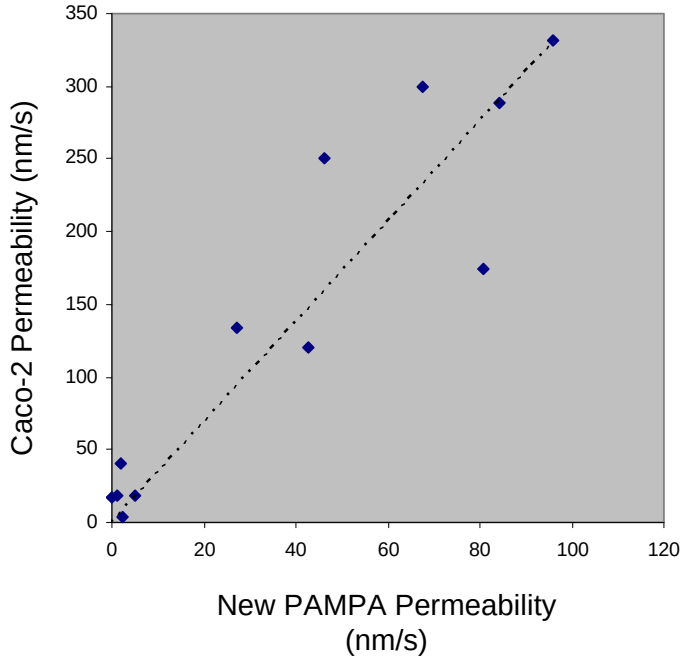
New PAMPA Membrane Improves Correlation with Caco-2 Data

Correlation plots using the same group of compounds

Traditional PAMPA

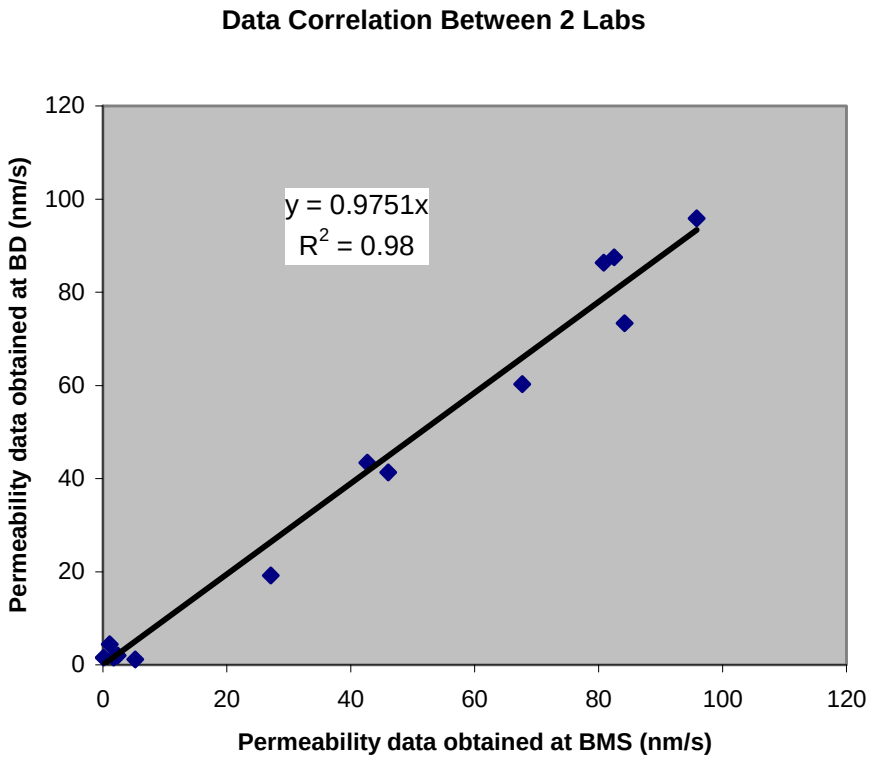


BD Gentest™ Pre-coated PAMPA



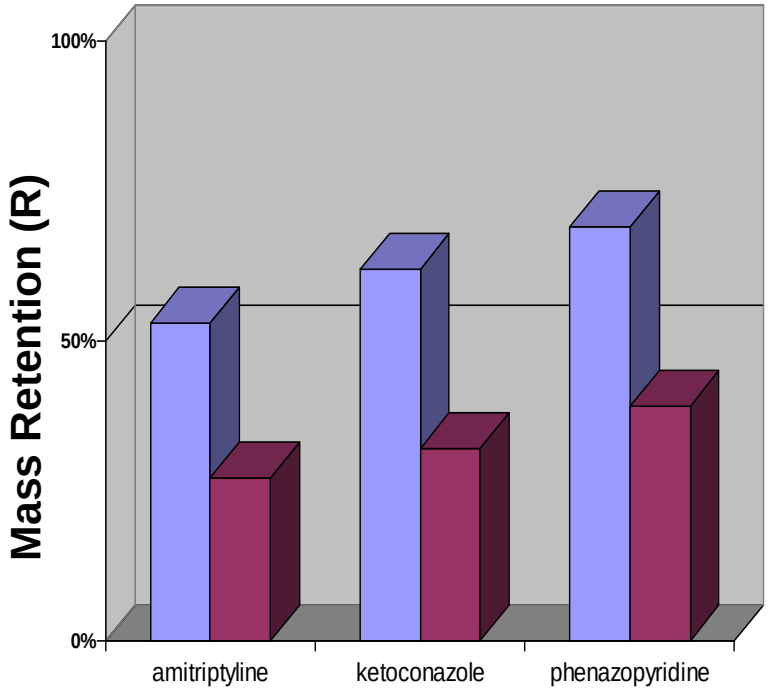
Reproducibility of the New PAMPA Membrane across 2 sites*

Compound	% Human absorption	BMS Pe (nm/sec)	BD Pe (nm/sec)
Sulfasalazine	12	2.4 ± 1.5	2.0
Nadolol	33	0.1	1.6
Norfloxacin	35	5.2 ± 4.7	1.1
Acebutolol	55	1.8 ± 0.8	1.5
Etoposide	50	1.1 ± 1.9	4.4
Ketoprofen	90	46 ± 2.9	41.3
Caffeine	100	95.8 ± 6.3	95.8
Dexamethasone	95	27.1 ± 4.1	19.2
Propranolol	90	80.8 ± 10.2	86.4
Metoprolol	95	42.7 ± 1.2	43.4
Verapamil	95	82.5 ± 6.6	87.5
Naproxen	100	67.6 ± 4.9	60.3
Antipyrine	97	84.2 ± 3.2	73.3



* Poster presented by Bristol-Myers Squibb (BMS) at 2006 AAPS Annual Meeting, San Antonio, TX, USA.

New PAMPA Membrane Reduces Mass Retention of "Sticky" Compounds



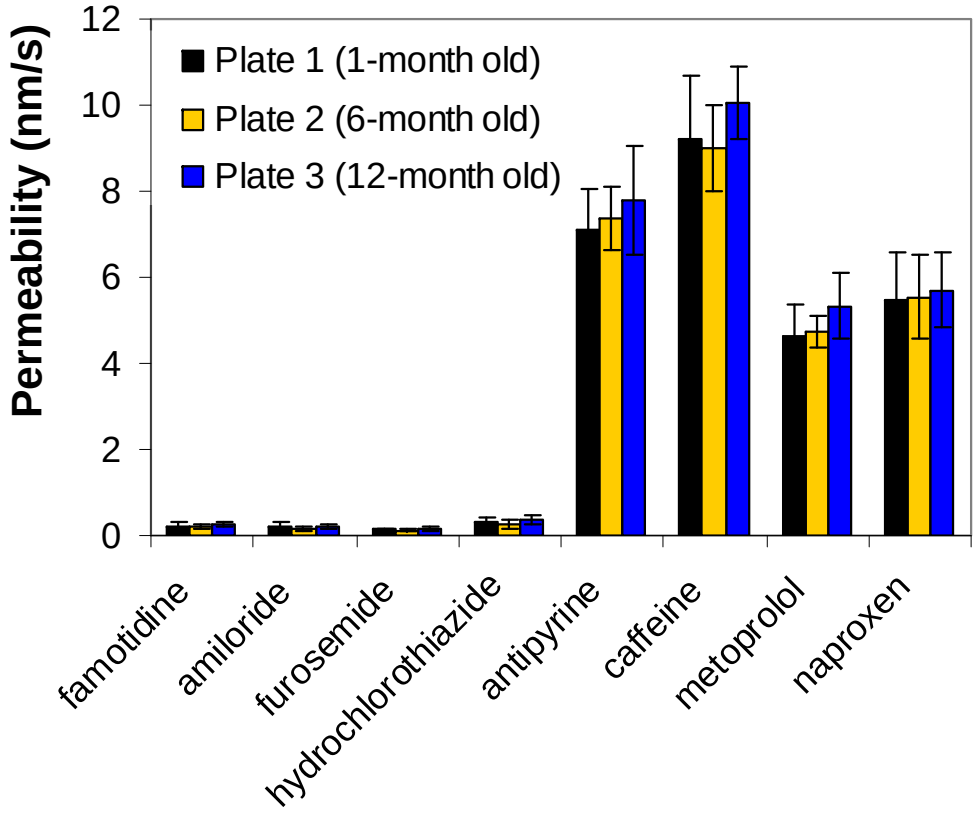
$$R = 1 - [C_D(t) * V_D + C_A(t) * V_A] / (C_0 * V_D)$$

- Traditional DOPC-PAMPA
- BD Gentest™ Pre-coated PAMPA

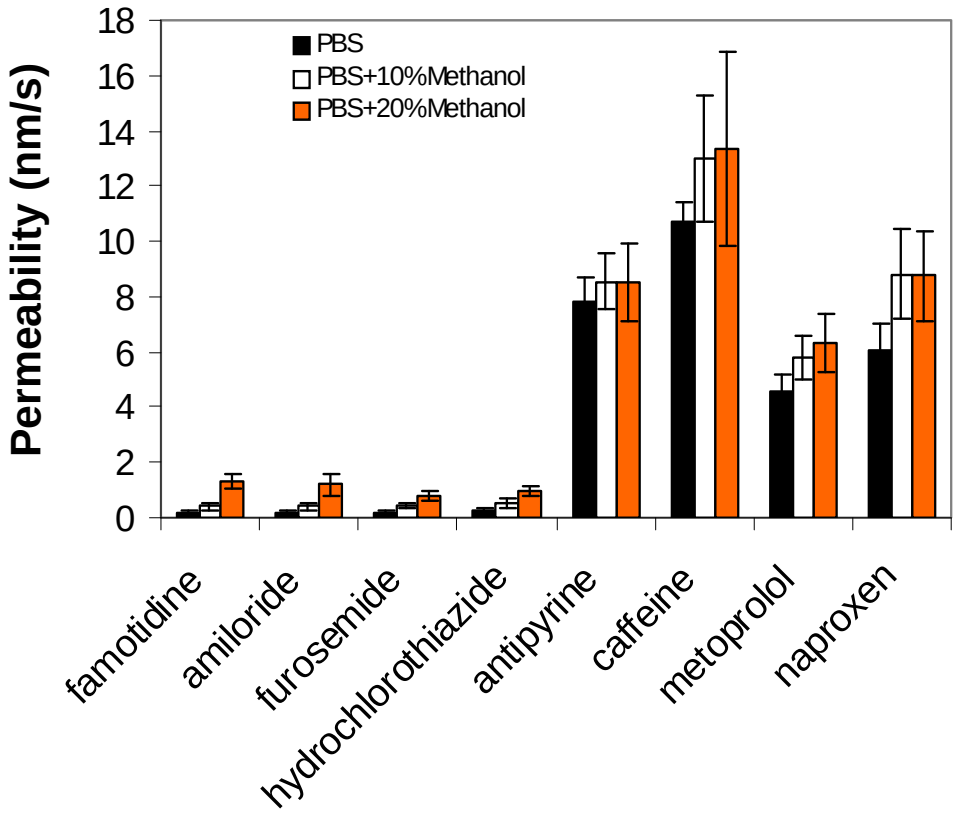
* Avdeef, A., Strafford, M., Block, E., Balogh, M., Chambliss, W., Khan, I. *Eur. J. Pharm. Sci.* **14**:271 (2001)

Pre-coated PAMPA plate is stable and highly reproducible

BD Gentest™ Pre-coated PAMPA Plate System is stable when stored at -20 ˚C

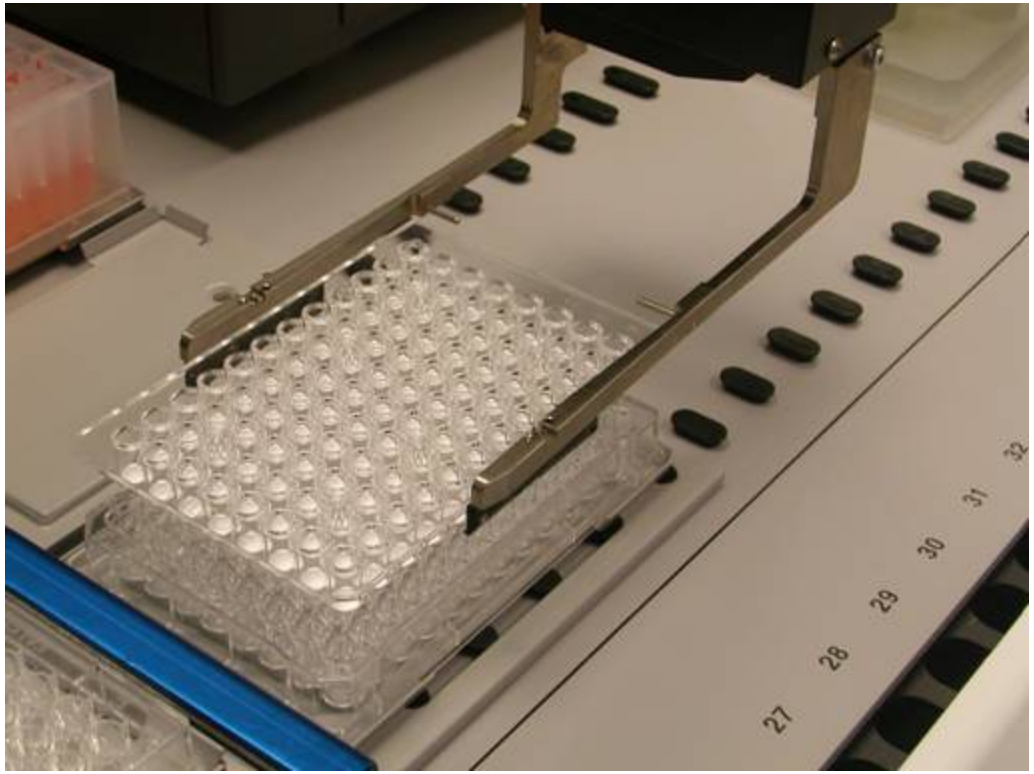


BD Gentest™ Pre-coated PAMPA Plate is Compatible with Buffers Containing Organic Solvents



Using buffers containing organic solvents helps increase the solubility of low solubility compounds.

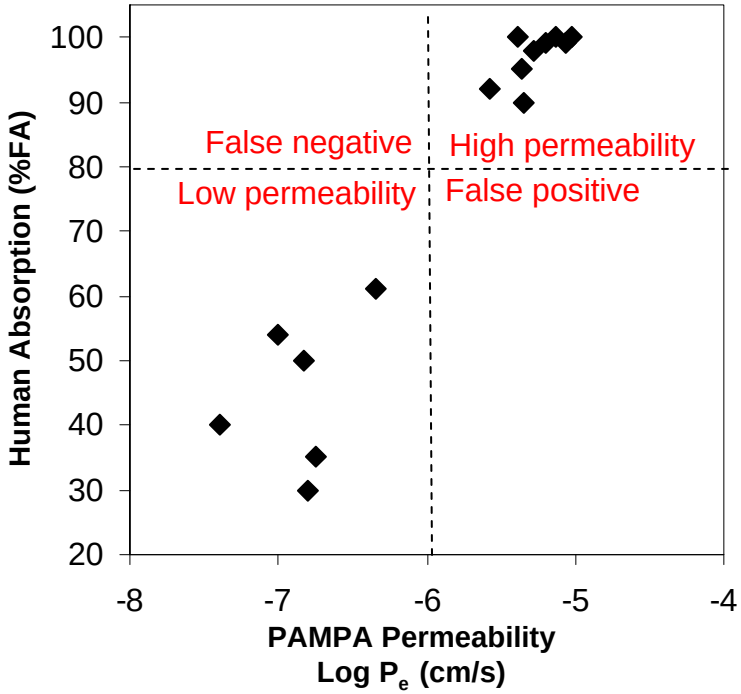
BD Gentest™ Pre-coated PAMPA Plate is Compatible with Automation Systems



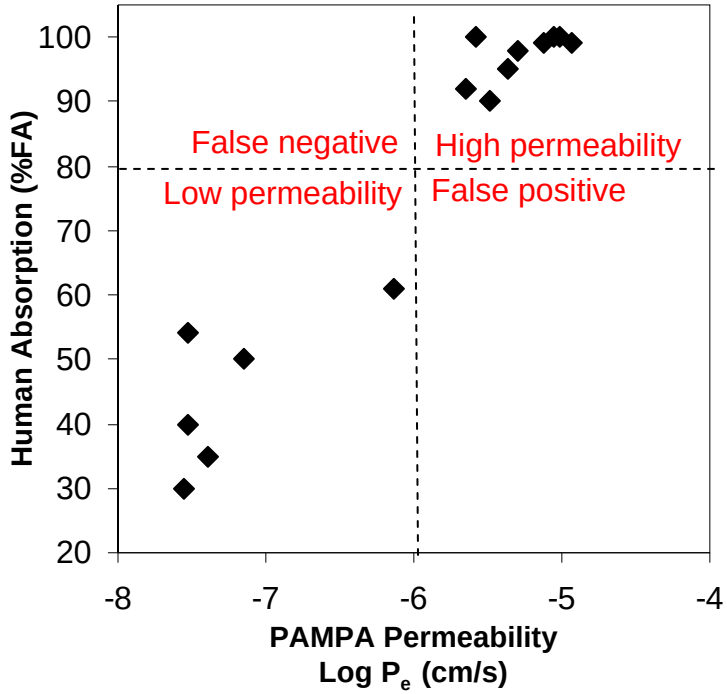
BD Gentest™ Pre-coated PAMPA Plate has been Validated using both UV Plate Reader and LC/MS Analysis

Correlation plots using the same group of compounds

Results obtained using UV Plate Reader



Results obtained using LC/MS



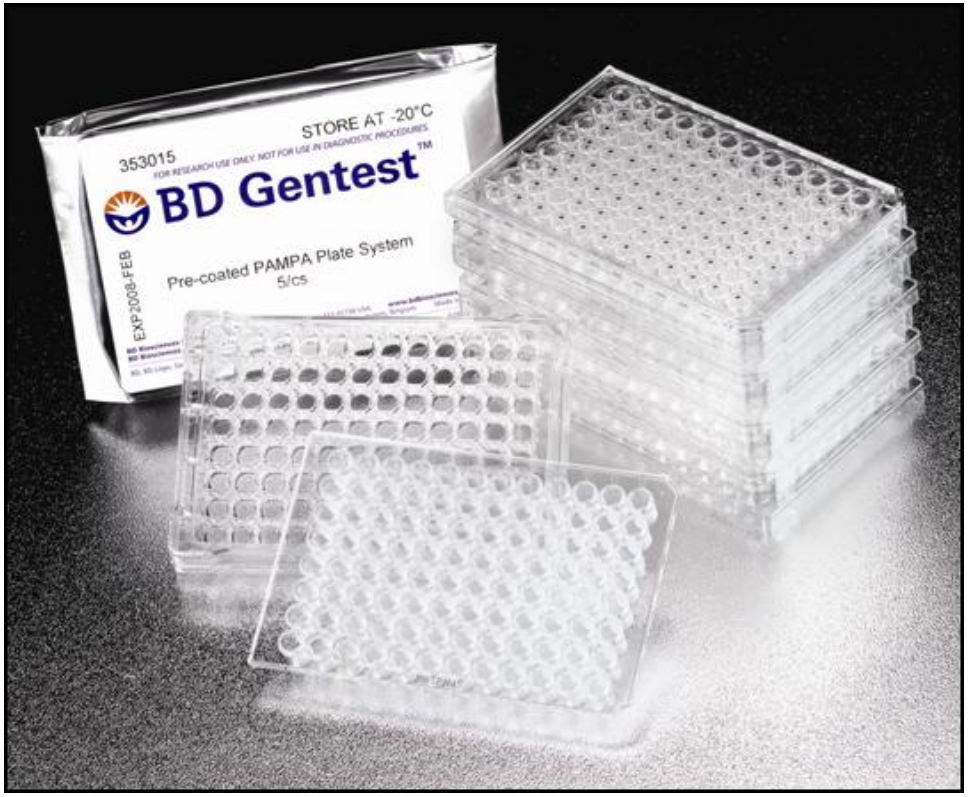
Both methods produced correct predictions for high and low permeability compounds.

Conclusions

BD Gentest™ Pre-coated PAMPA Plate System

- Improves correlation with Caco-2
- Improves correlation with human absorption
- Reduces mass retention
- Is stable and highly reproducible
- Is compatible with buffers containing organic solvents (to help low solubility compounds)
- Is compatible with automation systems
- Has been validated using both UV Plate Reader analysis and LC/MS analysis

BD Gentest™ Pre-coated PAMPA Plate System



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