Strengths and Limitations of Permeability Assays: a CRO Perspective

Intelligent Screening Symposium

Covance Laboratories Limited

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03 October 2018

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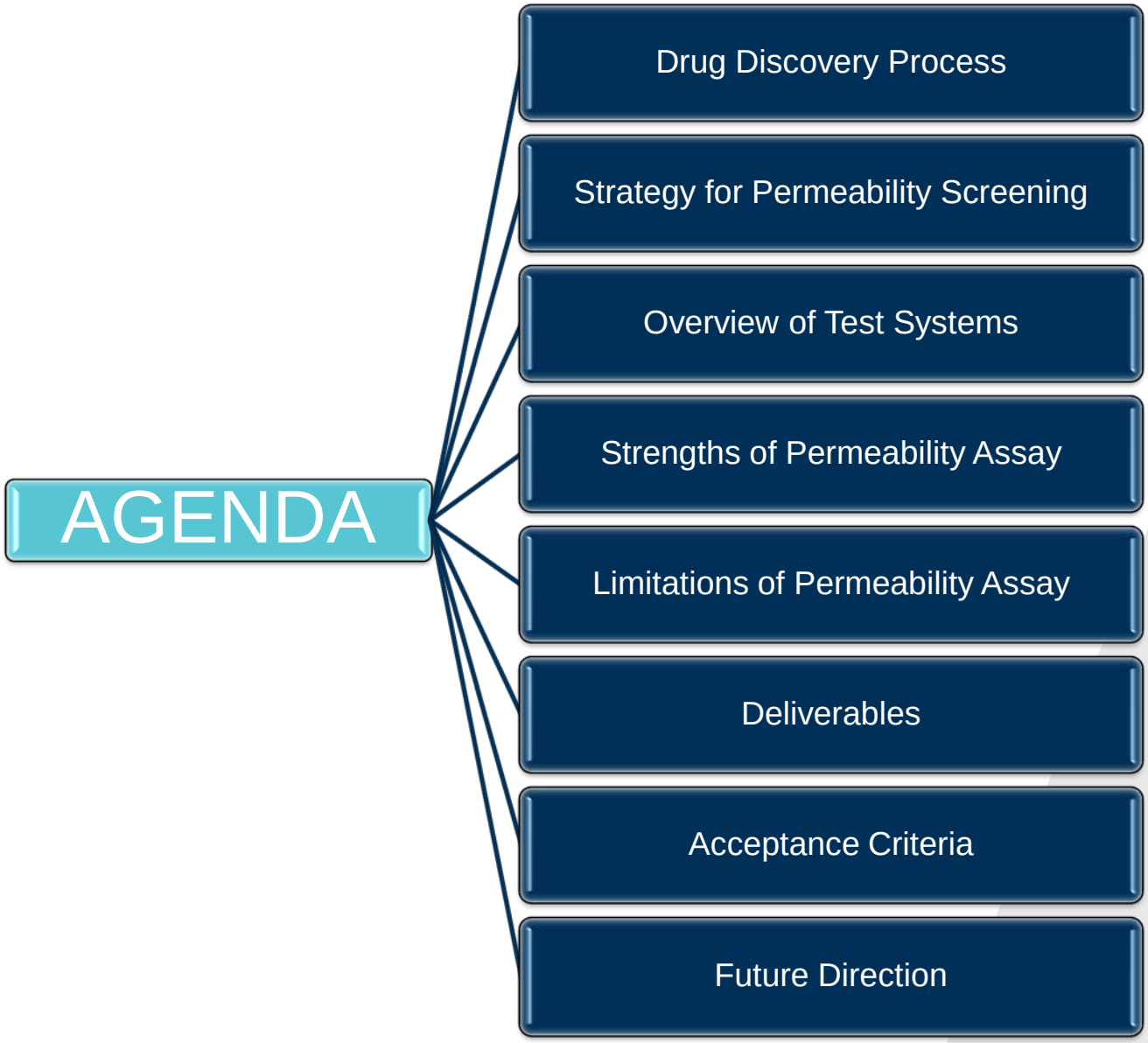
AGENDA



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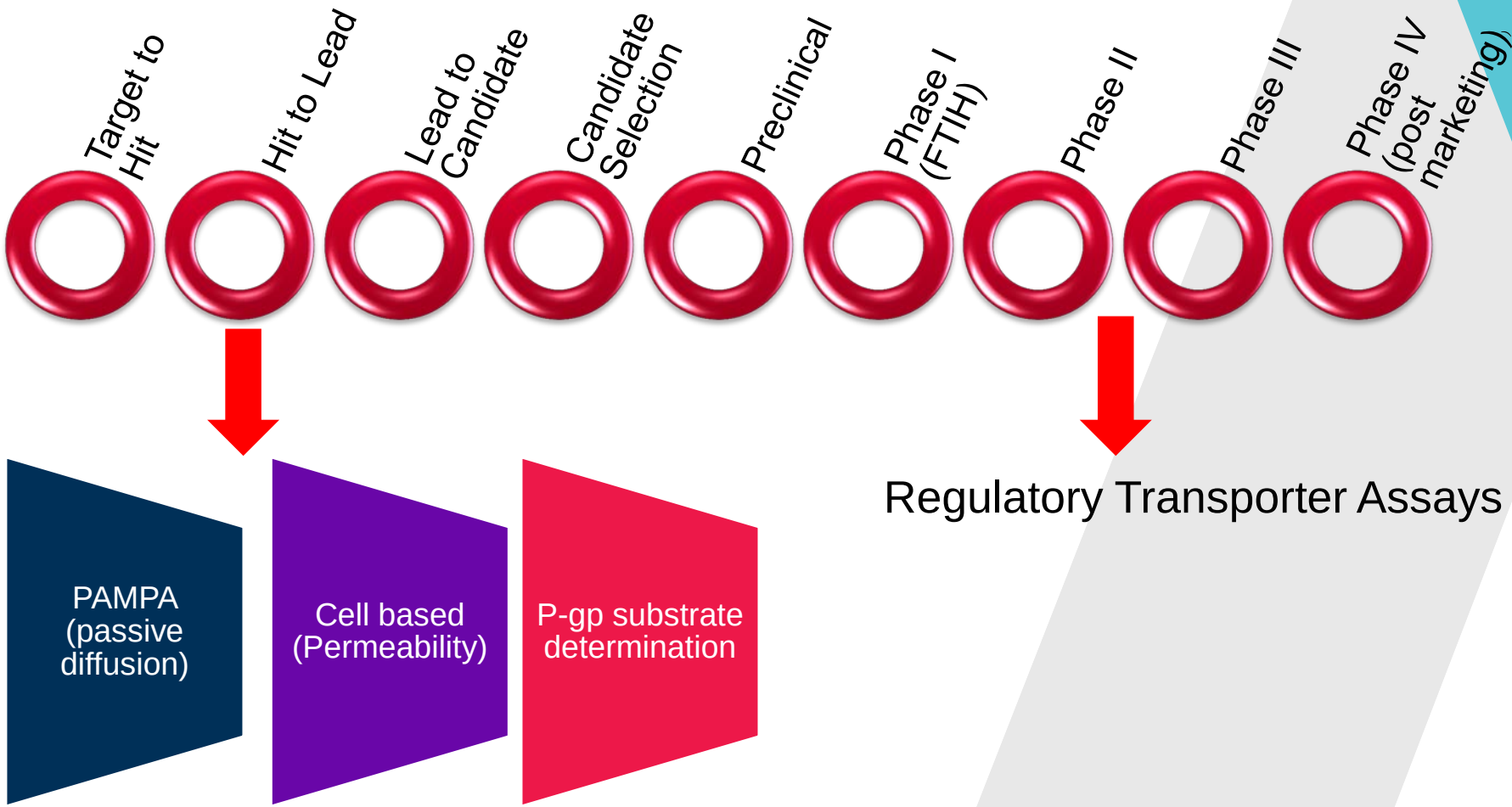
Drug Discovery Process



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Drug Absorption

- Oral drugs most desirable
- Develop drugs with good oral bioavailability
- Sufficient permeability through intestinal membranes can promote oral absorption
- Absorption is influenced by solubility, membrane partitioning, metabolism and transporters
- Several mechanisms of intestinal drug absorption
- *In vitro* models are high throughput but less predictive of intestinal permeability
- *In vivo* models are low throughput but more predictive
- *In vitro* models are utilised for screening molecules for intestinal permeability, drug absorption, transporter functions
- Cell lines have evolved as tools for evaluating the permeability characteristics of lead candidate drugs

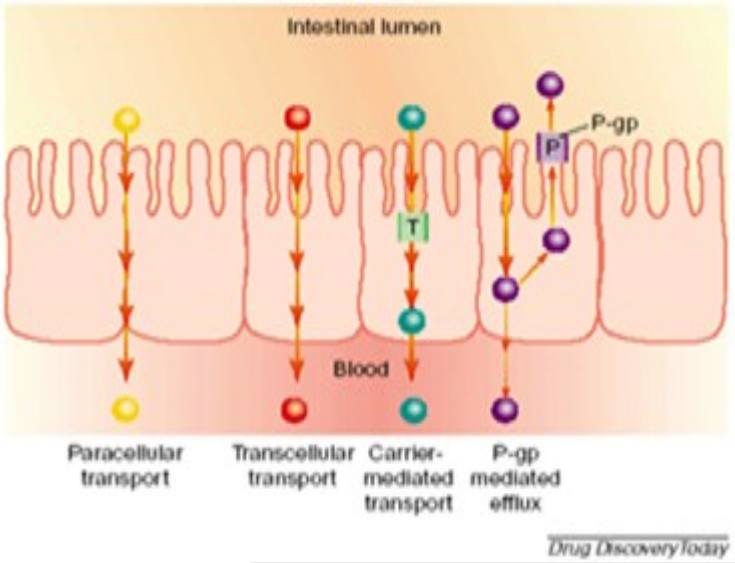

Figure from Albert Li, DDT Vol. 6, No. 7 April 2001

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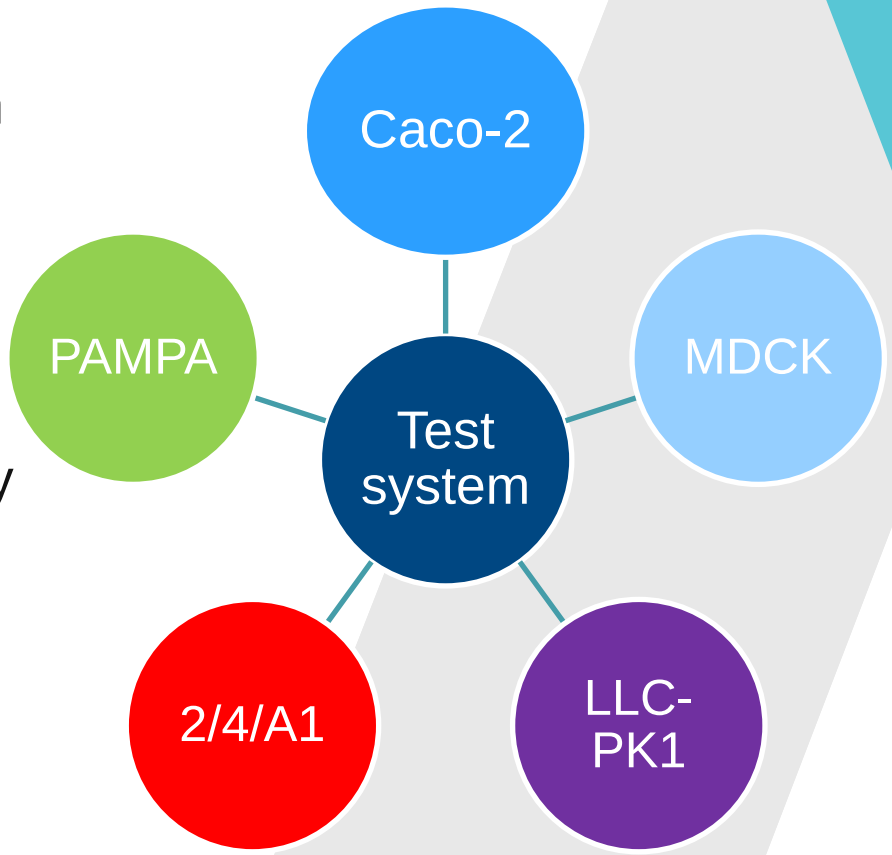
Permeability Screening Strategy

- Requirement for accurate, reliable, low-cost, and fast turnaround HTS techniques
- Tiered approach utilised:
  - ❖ High throughput permeability assessment
  - ❖ Lower throughput secondary screening and mechanistic studies
- Early screening of drug candidates for their potential to interact with P-gp
- Permeability and P-gp substrate assessment routinely outsourced

- Bidirectional permeability assay gold standard for P-gp substrates
- Additional transporters (if required)
- Challenging to use a single model to predict *in vivo* permeability
- Multiple test systems utilised (PAMPA and Caco-2)
  - PAMPA –tier 1 screen
  - Pre-screening tool for permeability ranking
  - Cell models –tier 2 screen
  - In-depth mechanistic studies

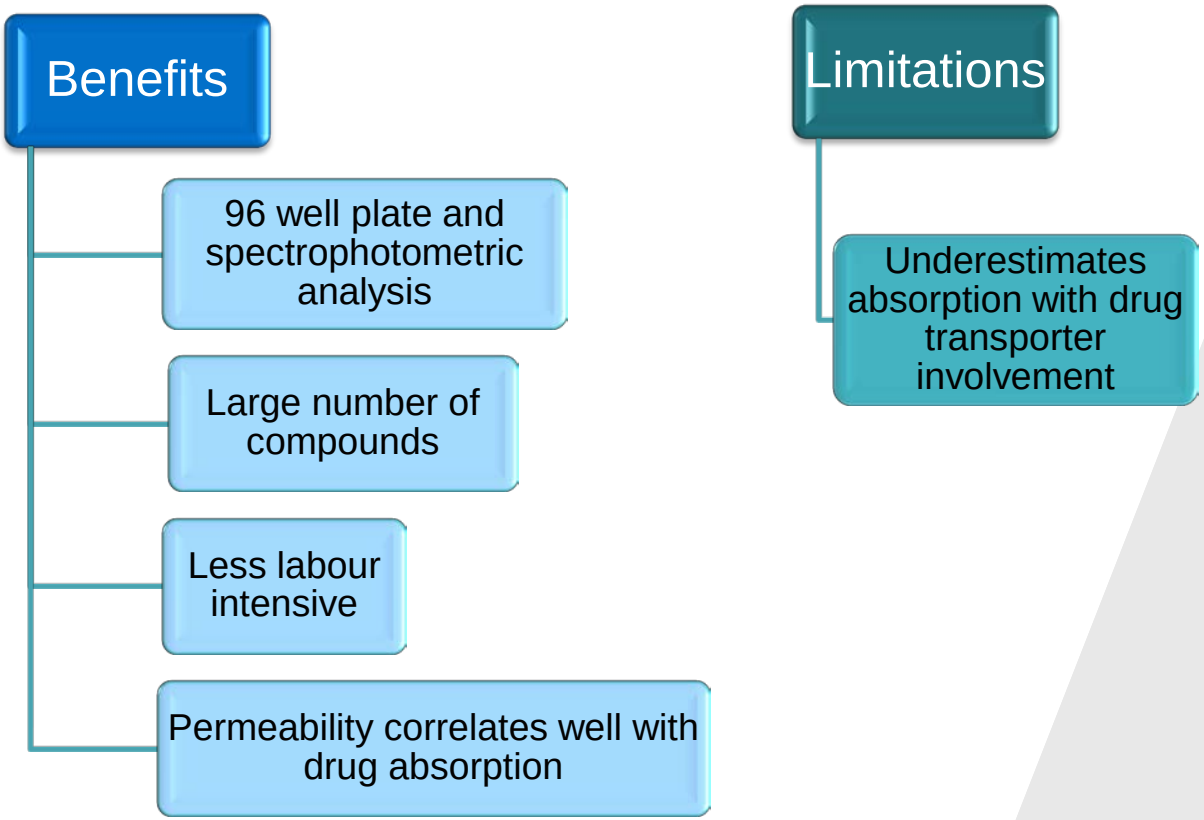
Test Systems for Permeability Screening

- Several models used for evaluation of drug permeability and absorption potential
- High throughput cell based models or artificial membranes to rank or filter compounds
- PAMPA and Caco-2 most frequently used models
- Good correlation between Caco-2 and PAMPA models
- Differences attributed to transporters



PAMPA

- Parallel Artificial Membrane Permeability Assay
- Test compound across an artificial hexadecane membrane quantified by LC-MS/MS



Benefits of Caco-2 Cell Model

- Human colon adenocarcinoma cell line
- Mimic human intestinal epithelial cells
- Polarize and form tight junctions
- Contains enzymes associated with the intestinal brush border epithelium
- Expression of multiple transporters (BCRP, P-gp, MRP2)
- Investigate interplay among different transport systems
- Relative contributions from passive and active transport mechanisms to overall permeability across the human GI tract.
- P_{app} values obtained from Caco-2 studies correlate to human intestinal absorption
- Useful tool for screening assays and for mechanistic studies of drug absorption

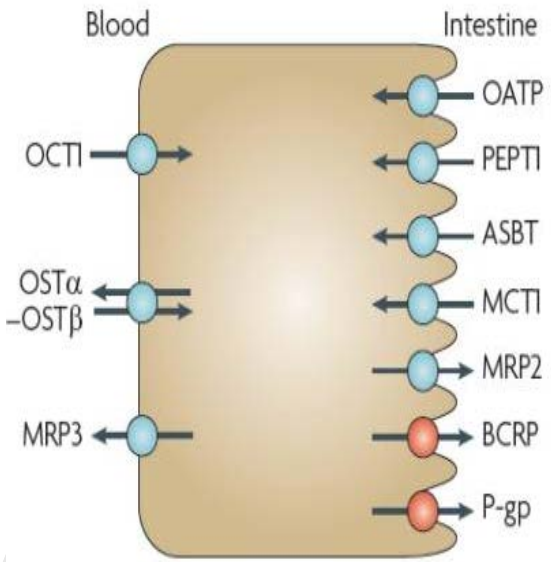


Figure from International Transporter Consortium; *Nature Reviews Drug Discovery* **volume 9**, pages 215–236 (2010)

Limitations of Caco 2 Cell Model

- 21 day culture period with multiple cell feeding occasions
- Labour intensive cell culture process (rate limiting factor)
- Additional tissue culture time and cost
- Contamination risks impact on turnaround time
- Inconsistent P-gp functional expression with passage number
- Altered expression of metabolizing enzymes and transport proteins relative to healthy small intestine
- Under prediction of paracellular absorption due to tight junctions
- Rejection of drug candidates via paracellular route
- Most suitable for high permeability compounds

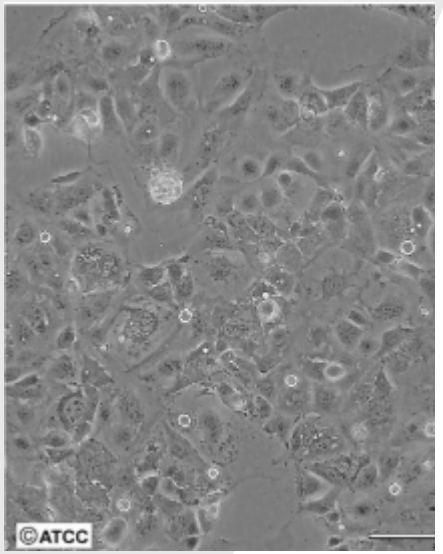


Image used from ATCC website

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Experiences of Caco-2 cells

- Impact of contamination/cell issues on timelines
- Choice of Caco-2 subclone
  - ❖ Cacoready™ - pre-plated model
  - ❖ Plateable – Plate directly onto transwell
  - ❖ Traditional culturing in flasks
- Licensing of cells (exception of pre-plated)
- In house validation to optimise cell line (seeding density, culture time, passage number)
- Optimal passage number
- Pitfalls of relying on one Caco-2 cell line in house
- Problems with decreased expression of P-gp over time
- Recent issues with global shortage of polycarbonate transwells
- Back-up cell model (C2BBe1) to minimise disruption (polyester plates)
- 7 day Caco 2 model for optimization of screening assays
- F9 subclone (aCELLerate) currently being explored at Covance

Benefits of MDCK Cell Model

- Madin-Darby canine kidney
- Differentiate into epithelial cells and form tight junctions
- Low Metabolic activity
- Low transporter expression (low P-gp expression)
- Human MDR1 transfected cell line to determine P-gp efflux
- Reach full differentiation within 3 to 7 days
- Easy cell maintenance
- Increased throughput
- Lower risk of contamination to impact on deliverables
- Low cell culture costs

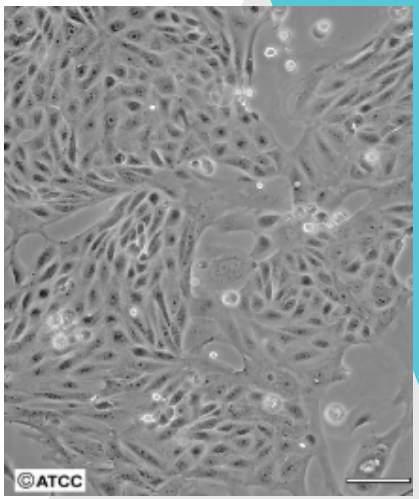


Image used from ATCC website

Limitations of MDCK cell models

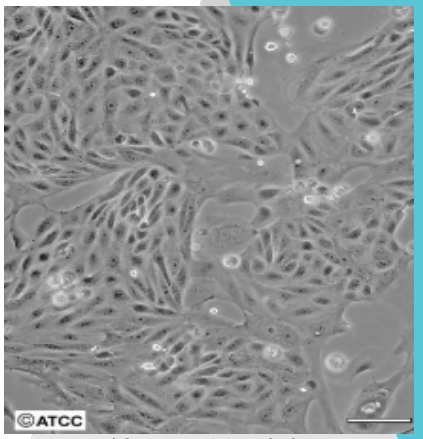


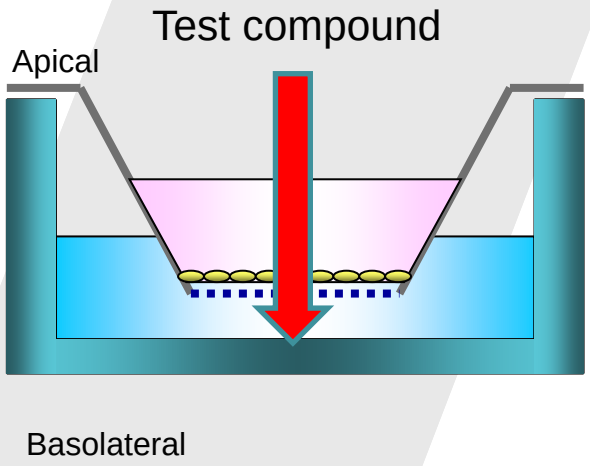
Image used from ATCC website

- Canine cell line – endogenous transporters
- Expression levels and substrate specificity of transporters differ from *in vivo* situation in humans
- Commonly used for permeability evaluation by passive transcellular diffusion mechanism
- Not suitable for accurately predicting permeability of compounds involving active uptake and efflux mechanisms
- Contribution of transporters using transfected MDCK cells (P-gp)
- Good correlation between MDCK and Caco-2 cells P_{app} values
- P_{app} correlation greatest for high permeability compounds

Bidirectional Permeability Assay

Obtained from www.corning.com

- Gold standard for permeability assays
- Cells are cultured on transwell support filters
- 96 well plates (increase throughput and minimise compound usage)
- Compound provided as DMSO solution (1 or 10 mM stock)
- Compound added to donor side in low micromolar range (1-10 µM)
- A-B and B-A direction (P-gp)
- Number of concentrations required (nominally 1)
- Number of replicates required (2-3)
- Single timepoint (2 hours)
- Standardised conditions (37°C, 5% CO_2)



Assay Requirements

Control Compounds

- Low Permeability Marker
- High Permeability Marker
- P-gp probe substrate
- P-gp probe inhibitor

Monolayer Integrity markers

- TEER
- Lucifer Yellow

Bioanalysis

- Internal standard
- Peak Area Ratio
- Quantification curve
- Cassette analysis

Assay Limitations- NSB

www.corning.com

- Non-specific binding
  - ❖ Binding to plastic surfaces
  - ❖ Binding to cells (Cacophilicity)

- Makes data interpretation challenging
- May lead to underestimation of permeability
- “False negatives”
- Addition of serum proteins (BSA) to the receiver compartment in cell assays
- Improves recovery of highly bound and lipophilic compounds
- Addition of surfactants in PAMPA
- Miniaturisation to 96 well plates may exacerbate NSB (SA to drug ratio)

Assay Limitations - Solubility

Obtained from www.corning.com

- Transport studies conducted in HBSS
- Many compounds have poor aqueous solubility
- Addition of co-solvent
- Solvents problematic for cell based models
- >1% cell tight junction is compromised
- PAMPA permeability consistent with using 10% DMSO
- Solvent can improve mass-balance recovery
- pH important variable for absorption
- Permeability studies conducted at single pH
- Cannot mimic dynamic pH environment in intestine
- Effect of pH used in assay compared to *in vivo*
- Solubility should be considered during review of results

Limitations of HTS screening

- Caco-2 cell permeability varies considerably between labs
- Challenging for a CRO to mimic a sponsors in house data
- Limitations of solubility and non-specific binding to data interpretation
- Caco 2 cells have tighter junctions compared to human intestine
- Caco-2 cells can under predict permeability of drugs absorbed via paracellular pathway
- Compounds with low permeability cannot be ruled out as poorly absorbed in humans

Data Delivery and Acceptance Criteria

- Repeat analysis criteria
  - Assay/analytical based
  - Compound related (low mass balance, cell toxicity)
- Excel spreadsheet
  - Cell line parameters
  - Concentration
  - Monolayer integrity result
  - Papp (A-B and B-A), Efflux ratio
  - Recovery
  - Control data
- Data Delivery (2 weeks form compound receipt)
- Acceptance criteria
  - Historical control data
  - Monolayer integrity control
  - Sensitivity (LLOQ)
  - Mass balance
  - Variability

Future of Screening

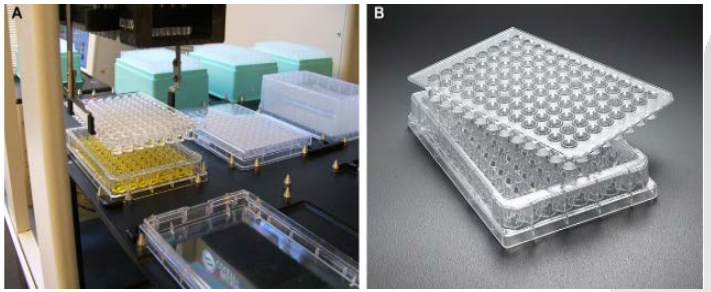
- Updated FDA drug interaction guidelines released in 2017
- Emphasis on drug transporter inhibition and substrate assessment
- Recommended to study NCE as inhibitor and substrate of P-gp, BCRP, OAT 1, OAT 3, OATP1B1, OATP1B3, OCT 2, MATE 1 and MATE 2K
- Discussions regarding earlier regulatory transporter studies following FDA 2017 guidelines
- Select compounds with less DDI liability
- Should screening be more complex?
- Negate need to repeat studies after initial screens

Alternative Transporter Investigations

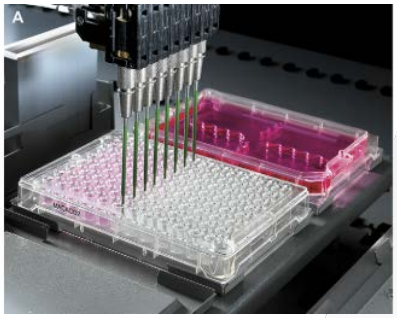
- Should additional transporters be considered?
- Transporters with severe DDI's and adverse effects (OATP1B1, BSEP) to identify clinical safety issues in discovery
- Transporter conscious drug-design to improve bioavailability and for disease targeting
- Corning® HEK293 TransportoCells™
- Thaw and use model
- Results within 48 hours of plating permits rapid data delivery
- Good signal to background ratio
- Good option for HTS

Automation at Covance

- Exploring options for automation at Covance
- Increase throughput
- Minimise replicate variability
- Automation of Caco-2
- Automation of cell culture (sterile environment)
- Media refresh
- Consistency in procedures and sterility
- Automation of permeability assay – liquid handler
- Liquid handling workstation with incubator
- Software considerations



Beckman Biomek



Tecan

Technical Brief, Libby Kellard and Marcy Engelstein Millipore Corporation, Danvers, MA. Millipore

Hamilton, Image Covance

Future Direction

Emulate Announces Strategic Collaboration with Covance to Integrate Organs-on-Chips Technology in Drug Evaluation

- Organ on a chip rapidly emerging technology
- Lack of accurate *in vitro* predictive cell culture models
- Covance-Emulate collaboration
- Kidney-chip for assessing drug-transporter interactions
- Timelines for development -1 year
- Preclinical development
- Reduction of animal testing

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Kidney on a chip

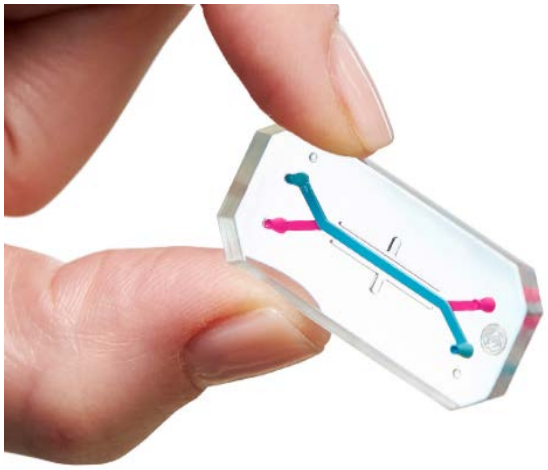
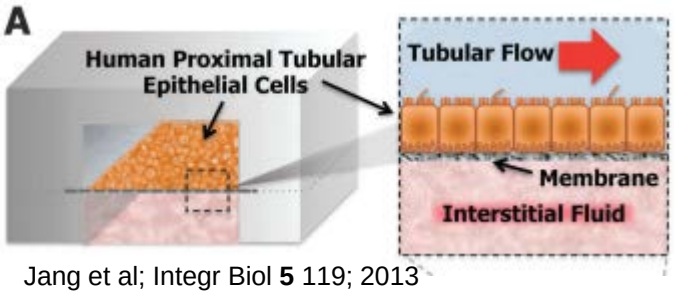


Image courtesy of Emulate website



Jang et al; Integr Biol **5** 119; 2013

- Three Dimensional microfluidic cell culture systems
- Cell cultured on ECM membrane
- PTC exposed to fluidic flow
- Two adjacent channels
- Mimics renal tubular architecture
- Supports key tubular functions (reabsorption, secretion)

- Future role in drug candidate permeability screening?
- Microfluidic cell culture of Caco-2 cells could provide real time analysis of intestinal permeability
- More predictive data earlier in drug discovery process
- Costs, materials, data output
- Automation, ease of handling
- Large scale manufacturing and throughput for chips

Summary

- Caco-2 and PAMPA valuable tools for screening for absorption and P-gp interaction potential.
- Cell models provide valuable information for lead optimisation in drug discovery
- Caco-2 cells remain the most widely used intestinal cell model for permeability screening.
- Studies indicate MDCK P_{app} values correlated well with Caco-2 when applied as a general absorption screen
- Standardisation of experimental variables important step in HTS
- Data carefully interpreted
- Can more predictive *in vitro* models be used as a platform for screening strategies
- organs-on-chips have the potential to play a transformative role across drug discovery and development.

Thank you for your attention

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