Method Suitability of Caco-2 Cell Models for Drug Permeability Classification

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DISCLAIMER

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Outline

• Method Suitability
• Model Drugs
• Reference Standards
• Acceptance Criteria
• Use of Permeability Assay
Method Suitability

- Generalized approach to standardize and validate a drug permeability model
- Establishes a correlation between experimental permeability values and human intestinal absorption ($f_a$)
- Allows for the use of different permeability assays within a laboratory whether they involve human studies, intact animals, intestinal tissue, or epithelial cells
- Accounts for intralaboratory variability
- Relies on assay standardization and validation, reference standards, and acceptance criteria to improve the consistency of experimental data to predict a drug’s intestinal permeability

Volpe AAPS J. 2010; 12:670-678
Method Suitability Components

Method Development
• Establish assay protocol
• Optimize and standardize assay parameters
• Set acceptance criteria

Assay Suitability
• Rank order relationship for model drugs between experimental permeability values and human intestinal absorption
• Define the high-permeability internal standard (HP-IS)

Permeability Classification
• Reference standard to demonstrate assay reproducibility
• Molecular markers for cell monolayer integrity
• Utilize HP-IS to classify new compound

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Model Drugs

• For *in vitro* cell culture methods, at least 5 model drugs per group are recommended to establish method suitability
• Model drugs have known intestinal absorption in humans and are passively absorbed
• Model drugs should represent a range of *in vivo* human intestinal absorption:
  - “Low” permeability ($f_a < 50\%$)
  - “Moderate” permeability ($f_a = 50\text{-}84\%$)
  - “High” permeability ($f_a \geq 85\%$)

M9 BCS Guidance, 2021. [https://www.fda.gov/media/148472/download]
Reference Standards

• Use of reference standards in permeability experiments:
  – High (HP) and low (LP) permeability standards to monitor experimental variations
  – Zero-permeability standard to monitor cell monolayer integrity (e.g., FITC-dextran, PEG-4000, Lucifer yellow, inulin)
  – HP internal standard (HP-IS) to classify drugs

• Permeability values of the standards should not differ significantly between different experiments, including those conducted to demonstrate method suitability

• Experimental options:
  – measure test drug together with reference standards
  – evaluate standards in the same monolayers after experiment with the test drug
  – use additional cell monolayers for standards
Acceptance Criteria

- Acceptance criteria are defined for selected reference standards and measurements for the permeability assays:
  - Measure of monolayer integrity
  - Apparent permeability ($P_{\text{app}}$) of zero, HP ($f_a \geq 85\%$) and LP ($f_a < 85\%$) permeability standards
  - Efflux of probe substrate compound
- Demonstrate functionality of assay
- Reference standards ensure reproducibility
Efflux

• Functional expression of efflux mechanism demonstrated in bidirectional experiments with a probe transporter substrate
• Efflux revealed by higher rate of substrate’s permeability in BL-to-AP (secretion) direction as compared to AP-to-BL (absorption) direction
• A drug with an efflux ratio (ER) ≥ 2 is considered as an efflux transporter substrate
  \[
  \text{ER} = \frac{P_{\text{app,BL-AP}}}{P_{\text{app,AP-BL}}}
  \]
• Rhodamine 123, digoxin, vinblastine, paclitaxel or quinidine can be used as probe substrates for demonstrating presence of P-gp transporter

Volpe. AAPS PharmSci. 6 (2):1-6, 2004
Use of Permeability Method

• Maintain same protocol from method suitability experiments to classify drug substance permeability
• Reference standards for classification and reproducibility
• Demonstrate passive transport of test drug:
  – Evaluate test drug at several concentrations (*e.g.*, 0.01×, 0.1× and 1× the highest strength in 250 mL), and
  – Measure bidirectional permeability of test drug (*i.e.*, AP-to-BL vs. BL-to-AP)
• Classify a test drug as highly permeable when its $P_{\text{app}}$ is equal to or greater than that of the HP-IS

M9 BCS Guidance, 2021. [www.fda.gov/media/148472/download]
Example

- Caco-2 cells in 12-well plate format
- 18- to 22-day monolayer age
- HBSS pH 6.8 in AP chamber and pH 7.4 in BL chamber
- Initial drug concentration based on highest dose strength in 250 mL
- LP/HP boundary drug = Labetalol

Example

- Caco-2 cells in a 24-well plate format
- 21-day monolayer age
- HBSS pH 7.4 in AP and BL chambers
- Initial drug concentration based on maximal clinical dose in 250 mL
- LP/HP boundary drug = Metoprolol

Example

- Caco-2 cells in 12-well plate format
- 22- to 24-day monolayer age
- HBSS pH 6.8 in AP chamber and pH 7.4 in BL chamber
- Initial drug concentration = 10 μM
- LP/HP boundary drug = Minoxidil

Permeability Classification


Volpe DA. AAPS PharmSci. 6 (2):1-6, 2004
Summary

• Method suitability for intestinal permeability assays is comprised of three phases (method development, assay suitability, permeability classification)

• Each laboratory should set its own acceptance criteria for cell measurements and reference standards used in the initial experiments with the model drugs and those to classify drug permeability

• Method suitability, with its reliance on assay standardization and validation, reference standards, and acceptance criteria, enhances the consistency of experimental data to predict a drug’s intestinal permeability