Advances in Transport Receiver Plate Capabilities

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ABSTRACT

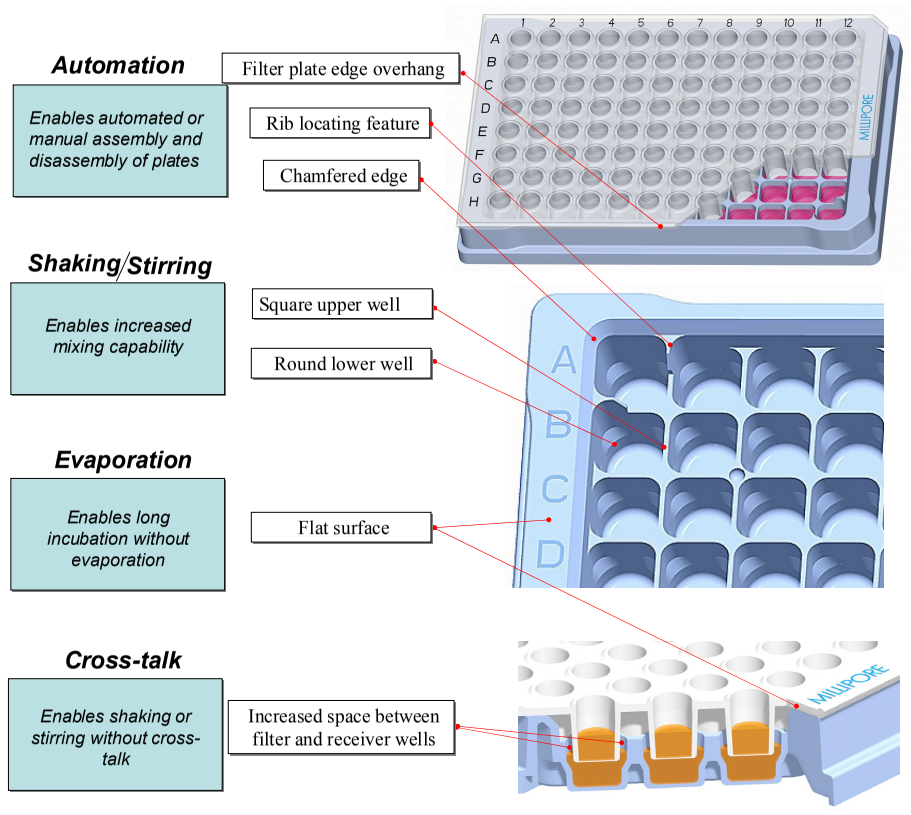
Absorption is a key predictive test for determining bioavailability. PAMPA[1](parallel artificial membrane permeability assay) is a non-cell based *in vitro* method for predicting the passive absorption of new chemical entities (NCE's). To further enable a high throughput, reproducible and automated PAMPA assay using the MultiScreen® 96-well Transport Receiver Plate (TRP) has been developed. The optimized design enables automation and robust, high speed shaking or stirring while preventing cross-talk. Additionally the design minimizes evaporation allowing for long incubations without the need for humidity controlled incubators.

To demonstrate the benefits of the TRP, consistent permeability rankings and rates of compounds from each BCS (biopharmaceutical classification system) category are shown under static, rigorous shaking and stirring conditions. High compound recovery and low evaporation are also demonstrated. The new TRP enables users to easily optimize and automate absorption assays for reproducible compound permeability.

INTRODUCTION

Many pharmaceutical organizations have implemented the PAMPA method and investigated variations using alternative conditions[3]. Conditions such as automated assembly and disassembly, shaking and stirring require optimized design of PAMPA assembly components. To illustrate the enabling features of the new transport receiver plate, the PAMPA assay was performed either manually or robotically in static, shake and stir modes. Transport receiver plates were evaluated for cross-talk, evaporation and automation compatibility. PAMPA features rapid turnaround time, low cost and a single permeability mechanism (passive diffusion) that is of great value in drug discovery[2].

TRANSPORT RECEIVER PLATE DESIGN FEATURES



TEST METHODS

Automation Cross-talk, evaporation and shaking tests used Packard MultiProbe II HT, Tecan Freedom EVO and Beckman Biomek FX robots for all dispensing, assembly and disassembly operations.

Cross-talk Well to well cross contamination was evaluated after plate assembly, 16 hour static incubation at ambient temperature and disassembly, by spiking 48 of 96 wells in a checkerboard pattern with 0.01mg/ml fluorescein and measuring fluorescence in blank wells.

Stirring Same as cross-talk test except plates were stirred for 1 hour at 300rpm with a Rotary Tumble Stirrer (V&P Scientific, VP710C1) using parylene coated stir discs (VP721F-1). Membrane integrity was also evaluated in a separate test by measuring 0.1mg/ml lucifer yellow retention in the receiver plate.

Shaking Same as cross-talk test except plates were shaken at 1200-1500rpm[4,5] for 16 hours using a MicroMix5 shaker (DPC) with form 22 and amplitude 5.

Evaporation Evaporation was determined gravimetrically on plates incubated overnight at room temperature while shaking or in static mode. The same plates were evaluated for percent evaporation in discrete wells by measuring liquid pathlength before and after incubation using the Molecular Devices SpectraMax Plus (Sunnyvale, CA).

Permeability Propranolol, ketoprofen, atenolol and furosemide permeability was measured using static, shaking and stirring conditions at pH 3.0, 5.0, 7.4 and 9.0. See Millipore Protocol Note PC040EN00[6] for method details. A DOPC lipid mixture was used (Synthetic Phospholipid Blend I, #790787 Avanti Polar Lipids Inc.).

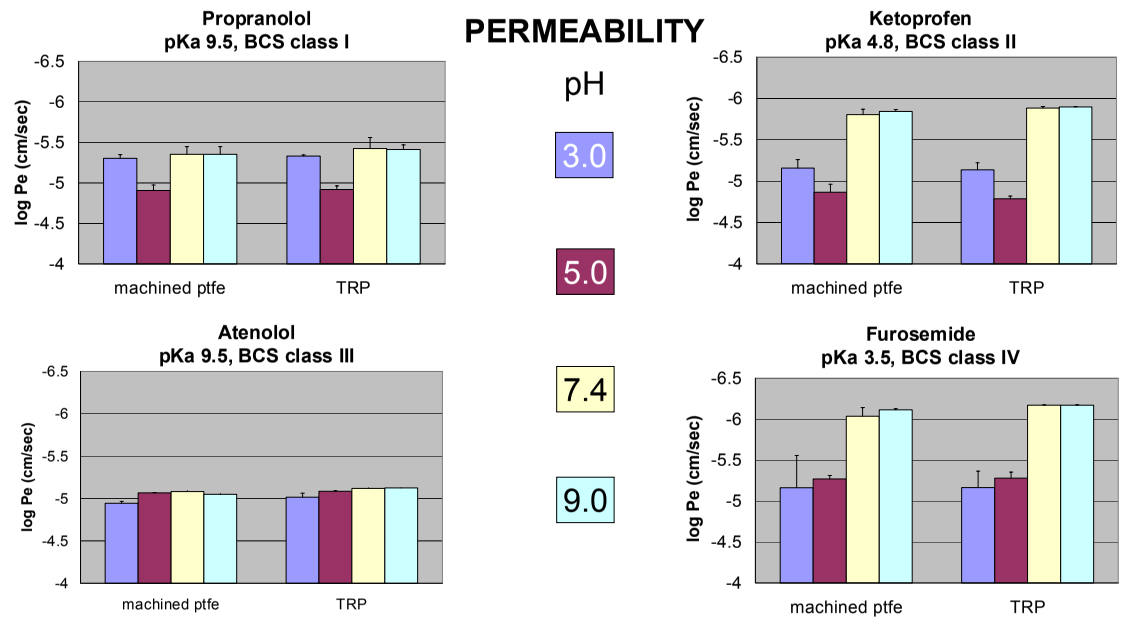
Note: All evaluations were done for the receiver plate of the PAMPA sandwich, using either the transport receiver plate or a machined PTFE receiver plate. No evaluations of filter plate data are reported.

CROSS-TALK

	Static			Shaking 1200-1500rpm			Stirring 300rpm		
	Average per plate								
Plate	Number of plates	Cross-talk ≥ 5%	Trapped air	Number of plates	Cross-talk ≥ 5%	Trapped air	Number of plates	Cross-talk ≥ 5%	Trapped air
TRP	18	0	1.3	6	0	0.4	6	0	NA
PTFE	3	22	12.0	*Not tested in shake mode*			*Not tested in shake mode*		

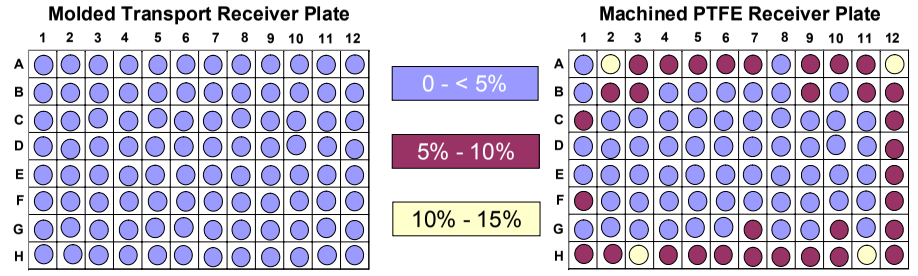
In addition to cross-talk measurements, filter plate membrane integrity was determined in stirring evaluation by measuring lucifer yellow migration from filter wells into receiver wells. All filter wells were spiked with lucifer yellow fluorophore at 0.1mg/ml and allowed to stir for 1 hour. Non-spiked receiver wells were evaluated using a Wallac Victor reader at 485/535nm to determine lucifer yellow passage. > 99% retention was measured for six plates.

PERMEABILITY



Permeability was determined for 4 compounds at 4 different pH conditions in static mode. Starting concentration for each compound was at the limit of solubility. Evaluations were done using either the molded TRP or the machined PTFE plate. N=6 for each condition shown. All conditions tested (shaking, stirring and static) showed the same permeability ranking for each compound (data not shown).

EVAPORATION



Ratio of center wells to perimeter wells for the same data set. N=6 plates.	
Molded TRP	**Machined PTFE**
1.007	**0.304**

Using the Tecan Evo, 6 assemblies were set up with 150ul buffer in filter wells and 300ul in receiver wells, (molded TRP plate or machined PTFE), assembled and lidded. Plates were left on the robotic deck overnight for 16 hours. Edge effects from evaporation are the most significant observation when using the machined PTFE plate. The new molded TRP design minimizes evaporation to <5%/well even after incubation at room temperature for 16 hours. Data shown is an average of 6 plates. Similar results were obtained using a Beckman Biomek FX (data not shown).

CONCLUSIONS

The molded Transport Receiver Plate performed the same or better when compared to a machined PTFE plate in the following areas:

- Automation compatible with several common platforms
- Cross-talk was eliminated with static or mixing conditions
- Shake and stir compatible if required
- Evaporation was reduced during overnight room temperature incubation (without humidity control)
- High compound recovery was seen when evaluated via Millipore Technical Note PS1234EN00[7] (data not shown)

REFERENCES

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