
Technical Note

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Title: Automation of HDM-PAMPA using a MultiScreen® Permeability Plate on a Hamilton MICROLAB® STAR Workstation

Introduction

The HDM-PAMPA is a non-cell based assay designed to predict passive, transcellular permeability of drugs in early drug discovery¹. The assay is carried out in a 96-well MultiScreen Permeability filter plate and measures the ability of compounds to diffuse from a donor to an acceptor compartment separated by a hexadecane/hexane artificial membrane on a polycarbonate membrane support. HDM-PAMPA can be used to determine the effect of pH on compound permeability by adjusting the pH of the solutions used in the analysis. The pH permeability profiles in the permeability assay are valuable in predicting gastrointestinal absorption of ionizable drugs.

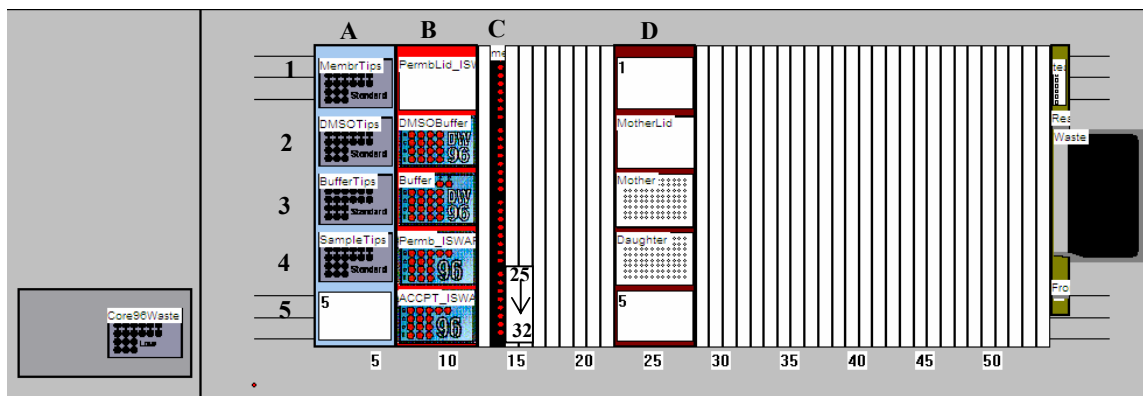
An artificial membrane is applied to the polycarbonate membrane in a 96-well filter plate (Donor plate). The Donor plate is filled with buffer solutions containing the compounds to be tested. The Donor plate is placed in a 96-well Acceptor plate filled with sufficient buffer to ensure liquid contact between the liquid in the Acceptor plate and the polycarbonate membrane. The Donor and Acceptor plates are incubated together for 5-7 hours after which time the Donor plate is removed from the Acceptor plate. Samples from the Acceptor and Donor plates are analyzed by LC/MS or transferred to a UV compatible 96 well plate and analyzed immediately in a UV/Vis spectrophotometer. At the end of the incubation time, the integrity of the artificial membrane layer can be measured using electrical resistance. Automation of HDM-PAMPA on the Hamilton MICROLAB STAR takes about 1 hour and 17 minutes (this does not include the 5 – 7 hours incubation). This time includes the formation of the artificial membrane, dilution of compounds from a mother plate, addition of compounds to the Donor plate and removal of samples for UV/Vis analysis from the Acceptor and Donor plates.

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Configuration of the MICROLAB STAR Work Surface for HDM-PAMPA (Membrane formation, drug compound addition and incubation)



Important:

- Program created using Hamilton Vector Software V 3.1.0.2160 on a MICROLAB STAR Workstation.
- Program written for a system with 96 CO-RE pipetting head, an 8- tip pipetting head, and an iSWAP.
- 8-tip pipetting head is only used for dispensing the hexadecane/hexane solution.

Prior to starting the program (*Millipore Permeability_Std.med*), make sure the deck configuration is as follows:

A: Disposable Tip Carrier (tip_car_480_a00.tml)

1. 300 μ L pipette tips (Creating membrane layer)
2. 300 μ L pipette tips (Adding DMSO buffer to Acceptor plate)
3. 300 μ L pipette tips (Preparing daughter plate)
4. 300 μ L pipette tips (Adding samples to Donor plate)
5. Empty

B: 5 Position Plate Archived Carrier (plt_car_15ac.tml)

1. Lid for MultiScreen Permeability filter plate
2. 5% DMSO in PBS buffer, pH 7.4 (Hamilton reagent trough)
3. PBS buffer, pH 7.4 (Hamilton reagent trough)
4. MultiScreen Permeability filter plate (in a single well tray)
5. PTFE Acceptor plate

C: Sample Carrier – test tubes (smp_car_32_12x75_a00.tml)

First 8 test tubes in the front (25 – 32) – 300 μ L in each of 5% hexadecane in hexane

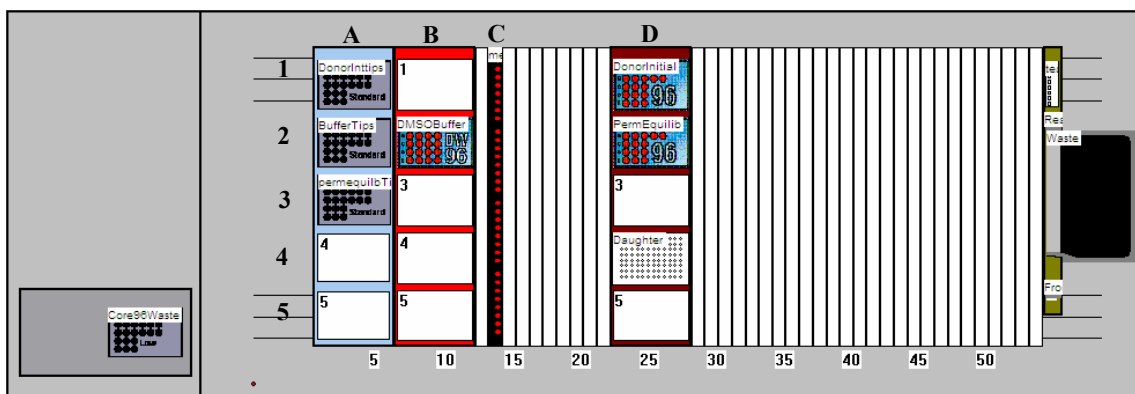
D: 5 Position Plate Medium Density Carrier (plt_car_15md_a00.tml)

1. Empty
2. Lid for mother plate
3. Mother plate (v-bottom poly-propylene plate)
4. Daughter plate (v-bottom poly-propylene plate)
5. Empty

Procedure (Millipore Permeability_Std.med):

1. Distribute 15 μL of 5% hexadecane in hexane (C1-8) to each well of the MultiScreen Permeability filter plate (B4) with the membrane tips (A1) using the 8-tip pipetting head.
2. Allow the hexadecane/hexane layer to dry without a lid on it for 60 minutes.
3. Distribute 300 μL of 5% DMSO buffer (B2) to the PTFE acceptor plate (B5) using the DMSO tips (A2).
4. Distribute 285 μL PBS buffer (B3) to the daughter plate (D4) using the buffer tips (A3).
5. Remove the cover from the mother plate using the iSWAP and place the cover at D2.
6. Transfer 15 μL of drug compound from the mother plate (D3) to the daughter plate (D4) using the sample tips (A4). Mix in daughter plate 10 times with a volume of 275 μL .
7. Transfer 150 μL from the daughter plate (D4) to the MultiScreen Permeability filter plate (B4) using the sample tips (A4).
8. Move the MultiScreen Permeability filter plate (B4) to the PTFE acceptor plate (B5) and cover with the lid (B1).
9. Incubate for 5 hours at room temperature.

Configuration of the MICROLAB STAR Work Surface for HDM-PAMPA (Analysis of Initial Donor Plate and Equilibrium Plate)



Important:

- Program created using Hamilton Vector Software V 3.1.0.2160 on a MICROLAB STAR Workstation.
- Program written for a system with 96 CO-RE pipetting head and an iSWAP.

Prior to starting either of the following programs make sure the deck is configured as below:

- *Millipore Permeability Equilb_Std.med* for Initial Donor and Equilibrium plates.

A: Disposable Tip Carrier (tip_car_480_a00.tml)

1. 300 μ L pipette tips (Initial Donor Plate)
2. 300 μ L pipette tips (Buffer addition)
3. 300 μ L pipette tips (Equilibrium Plate)
4. Empty
5. Empty

B: 5 Position Plate Archived Carrier (plt_car_15ac.tml)

1. Empty
2. 5% DMSO in PBS buffer, pH 7.4 (Hamilton reagent trough)
3. Empty
4. Empty
5. Empty

C: Sample Carrier – test tubes (smp_car_32_12x75_a00.tml) - Empty

D: 5 Position Plate Medium Density Carrier (plt_car_15md_a00.tml)

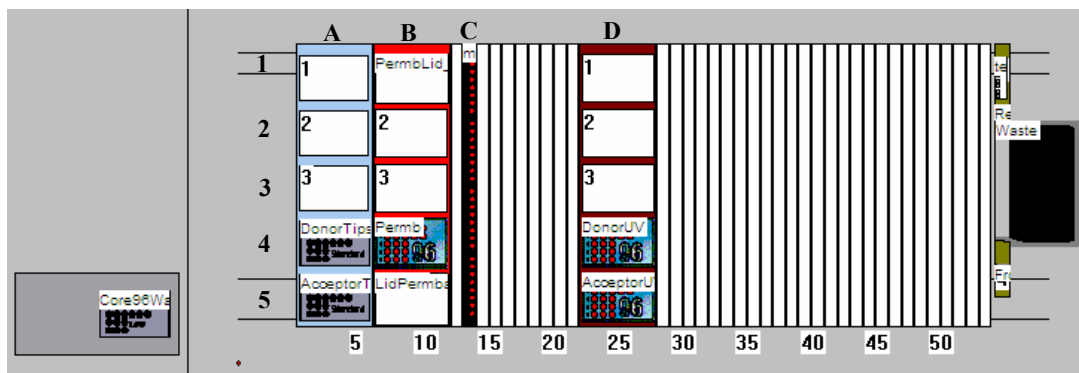
1. Initial Donor plate (UV plate)
2. Equilibrium plate (UV plate)
3. Empty
4. Daughter plate (v-bottom poly-propylene plate)
5. Empty

Procedure (Millipore Permeability Equilb_Std.med):

***Note this protocol can be run while the 5 hour incubation is occurring.**

1. Transfer 100 μL from the daughter plate (D4) to the Initial Donor plate (D1) using the DonorInt Tips (A1).
2. Analyze the Initial Donor plate on a UV/Vis microplate spectrophotometer.
3. Replace the Initial Donor plate to position D1 on the MICROLAB STAR deck.
4. Transfer 80 μL from the Initial Donor plate (D1) to the Equilibrium plate at position D2 using the Permequilb Tips (A3).
5. Distribute 170 μL 5% DMSO buffer (B2) to the Equilibrium plate (D2) using the Buffer Tips (A2). Mix 10 times with a volume of 225 μL .
6. Remove the Equilibrium plate (D2) and analyze it with a UV/Vis microplate spectrophotometer.

Configuration of the MICROLAB STAR Work Surface for HDM-PAMPA (Analysis of Final Donor and Final Acceptor)



Important:

- Program created using Hamilton Vector Software V 3.1.0.2160 on a MICROLAB STAR Workstation.
- Program written for a system with 96 CO-RE pipetting head and an iSWAP.

Prior to starting either of the following programs make sure the deck is configured as below:

- ***Millipore Permeability Analysis_Std.med* for Final Donor and Acceptor plates.**

A: Disposable Tip Carrier (tip_car_480_a00.tml)

1. Empty
2. Empty
3. Empty
4. 300 μ L pipette tips (Final Donor Plate)
5. 300 μ L pipette tips (Final Acceptor Plate)

B: 5 Position Plate Archived Carrier (plt_car_15ac.tml)

1. Lid for MultiScreen Permeability plate
2. Empty
3. Empty
4. MultiScreen Permeability filter plate
5. PTFE Acceptor plate

C: Sample Carrier – test tubes (smp_car_32_12x75_a00.tml) - Empty

D: 5 Position Plate Medium Density Carrier (plt_car_15md_a00.tml)

1. Empty
2. Empty
3. Empty
4. Final Donor plate (UV plate)
6. Final Acceptor plate (UV plate)

Procedure (Millipore Permeability Analysis_Std.med):

***Note this protocol is to be run after the 5 hour incubation.**

1. Remove the lid from the MultiScreen Permeability filter plate (B5) using the iSWAP and place it at position B1.
2. Remove the MultiScreen Permeability filter plate from the PTFE Acceptor plate (B5) using the iSWAP and place it at position B4 (on top of the single well tray).
3. Transfer 100 μ L from the MultiScreen Permeability plate (B4) to the UV Final Donor plate (D4) using the Donor tips (A4).
4. Transfer 250 μ L from the PTFE Acceptor plate (B5) to the UV Final Acceptor plate (D5) using the Acceptor tips (A5).
5. Remove the Final Donor and Acceptor plates and analyze them with a UV/Vis microplate spectrophotometer.

Assay Reproducibility

A. Manual Handling

	Carbamazepine	Furosemide	Methotrexate*	Testosterone	Warafarin	Propranolol
Day 1	-4.1 ± 0.01	-5.8 ± 0.06	$< -6.6 \pm 0.45$	-3.6 ± 0.10	-4.9 ± 0.02	-4.0 ± 0.02

B. Automated Handling

	Carbamazepine	Furosemide	Methotrexate*	Testosterone	Warafarin	Propranolol
Day 1	-4.0 ± 0.05	-5.7 ± 0.39	$< -5.3 \pm 0.46$	-4.1 ± 0.05	-4.9 ± 0.05	-3.9 ± 0.04
Day 2	-4.0 ± 0.01	-5.9 ± 0.03	$< -7.0 \pm 1.00$	-3.9 ± 0.07	-4.9 ± 0.02	-3.9 ± 0.01

Each value listed is an average Log P_e for 12 wells per drug for each plate. UV/Vis absorbance was determined for each plate using a SPECTRAMax plate reader (SpectraMax[®] Plus, Molecular Devices). For this experiment, testosterone donor concentration = 100µM. All other drugs are at 500µM.

*This drug is at the limit of detection with Uv/Vis and should be analyzed using a more sensitive method (LC/MS).

Conclusion:

The data above shows that the HDM-PAMPA protocol using Millipore's Permeability filter plate can be easily automated on a Hamilton MICROLAB STAR. The results obtained from automation are comparable to results obtained manually. The automation results also show reproducibility of the protocol from day to day. The use of the Hamilton MICROLAB STAR allows 96 samples to be processed in less than 7 hours (this includes the incubation periods).

Refer to Tech Note #'s AN1729EN00 and AN1725EN00 for more detailed information on running the HDM-PAMPA.

Millipore Ordering Information:

	Part Number	Package Size
MultiScreen Permeability Plate	MAPBMN310	10/pk
PTFE Acceptor plate	MSSACCEPTOR	each

Hamilton Accessories:

Item	Part Number
1 – Disposable Tip Carrier (TIP_CAR_480_A00)	182085/00
1 – 5 Position Archived Plate Carrier (PLT_CAR_L5AC_A00)	182090/00
1- Test Tube Rack (SMP_CAR_32_12x75_A00)	173410/00
1 – 5 Position Medium Density Carrier (PLT_CAR_L5MD_A00)	182365/00
2 - Hamilton Reagent Trough	4750-01
300 µL disposable pipetting tips	235902
96-CO-RE Pipetting Head	173084
8 Tip Pipetting Head	173081
iSWAP Gripper Mechanism	182600

Note: Hamilton accessories are U.S. part numbers and are subject to change. Please check with Hamilton prior to any purchase.

References:

¹ Wohnsland, F.; Faller, B. High-throughput Permeability pH Profile and High-throughput Alkane/Water Log P With Artificial Membranes, J. Med. Chem., 2001; 44, p. 923–930.

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