FULLY AUTOMATED 24-WELL MADIN-DARBY CANINE KIDNEY (MDCK) PERMEABILITY ASSAY UTILIZING THE HAMILTON MICROLAB® STAR LIQUID HANDLING PLATFORM

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Figure 1: The Hamilton MICROLAB STAR Automated Pipetting Workstation

Abstract:

Permeability assays are used to elucidate the structure-activity relationships in the hit-to-lead stage, rank order compounds for *in vivo* pharmacokinetics studies, and predict the potential for oral absorption. It is important to have reliability, accuracy and precision for such assays especially when used in mid-high throughput screening. This application note describes the fully automated 24-well Madin-Darby Canine Kidney (MDCK) permeability assay using the MICROLAB STAR liquid handling workstation.

Introduction:

A robust permeability assay is an essential tool in the drug discovery process. MDCK cells are a common model for studying drug transport mechanisms in distal renal epithelia. Like Caco-2 cells, MDCK cells differentiate into columnar epithelium and form tight junctions when cultured on semi-permeable membranes. Primarily for assessing passively absorbed compounds, drug permeability data from MDCK cell assays has been shown to be similar to permeability data from Caco-2 cell assays.[1] Permeability data is also an important input parameter for *in silico* software programs which can be used to model oral bioavailability and other ADME (Absorption Distribution Metabolism Excretion) parameters. MDCK cells grown to confluence on permeable membranes are widely used to examine passive permeability and active transport. These assays have typically been run manually or have been partially automated requiring an operator's presence at all times. We have successfully developed a fully automated protocol utilizing a Hamilton MICROLAB STAR integrated with select third party peripheral

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devices to suit the assay requirements.

The method was validated using 23 marketed drugs with diverse physico-chemical properties and a wide range of published apparent permeabilities (Papp). The validation set also included subsets of drugs required for GastroPlus and SimCyp ADME simulation software allowing the simultaneous validation of the method while providing required data for *in silico* modeling. The automated method was also tested against a 12-well manual method using 13 marketed drugs and eight Genentech compounds to ensure the rank order of low, moderate, and high permeability classifications were similar between the different methods.

Hamilton Robotics provides world-class automation solutions for a variety of applications with increased robustness, flexibility, precision and accuracy using its unique CO-RE technology. This application note demonstrates the automation capability of the Hamilton MICROLAB STAR and how it can be used to fully automate the MDCK permeability assay quickly and reliably.

Materials Overview:

Hamilton MICROLAB STAR

Automated Pipetting Workstation

The Hamilton MICROLAB STAR Workstation is a small, flexible, automated robotic workstation that can be adapted to perform a variety of applications. In this application note, we demonstrate the ability of the Hamilton MICROLAB STAR to fully automate the MDCK permeability assay from sample to data in a robust, precise and most accurate way.

It is also possible to integrate on- or off-deck peripheral devices such as microplate heating/cooling modules, shakers, and vacuum systems as needed by the application. The STAR platform uses air displacement technology, which offers increased pipetting accuracy and repeatability while eliminating sample contamination or dilution effects commonly associated with fluid-based systems.

The STAR workstation can be configured with multiple arms, each arm housing multiple pipetting channels or labware gripping options. Pipetting channels and labware grippers move independently for increased efficiency and support the use of a wide range of labware. An autoload option provides real-time barcode tracking of samples, labware, racks and carriers as they are loaded onto the deck.

All workstation functions and integrated third-party devices can be controlled by Hamilton's VENUS One software. The STAR platform also features a host of unique capabilities to facilitate a more robust automated process, including Anti-Droplet Control, TADM and CO-RE technologies, capacitance and pressure-based liquid level detection, among others.

Some of these features are described below.

CO-RE Technology

Many robotic applications require highly precise and accurate tip positioning. To meet these requirements, Hamilton employs novel CO-RE (Compressed O-Ring Expansion) tip attachment technology, which uses a stable lock-and-key mechanism to facilitate pick up of disposable or washable steel tips. This proprietary means of affixing tips to the pipetting channels produces a positional precision of +/- 0.1 mm in all axes.

CO-RE Gripper

CO-RE technology also allows access to a variety of labware handling tools. CO-RE grippers are a set of small paddles that can be picked up by two independent liquid channels, used to grip and move microplates or tip racks to virtually any position on the STAR deck. They represent a low cost alternative for transporting labware, compared to the more traditional style of gripper arm.

Liquid Level Detection

All STAR pipetting channels are equipped with capacitative and pressure-based liquid level detection (cLLD and pLLD) and recording functions, used to check or confirm liquid levels in tubes or microplate wells before, during and after a pipetting routine. pLLD allows for sensing of non-conducting fluids and volatiles.

Scheduler Software

The STAR system used for this application was equipped with VENUS One instrument control software and VENUS Dynamic Scheduler software. The scheduler software provides for efficient allocation of available tools and resources, facilitating parallel processing and increased throughput.

Multiflex Tilt Module

Mounted on the flexible Multiflex carrier, the Tilt Module allows for selective tilting from 0°-10°, maximizing the volume of media or reagents that can be aspirated from a microplate well.

12-Channel Configuration

The STAR used for this application featured a 12-independent liquid channel configuration to enable flexible processing of 12, 24 and 36-well microplate formats.

Description	Part Number
MICROLAB STAR Autoloading Liquid Handling Workstation with 12 independent channels, iSWAP, CO-RE96	Hamilton 173000-027
CO-RE Gripper	Hamilton 184089
VENUS ONE, VECTOR 4.1	Hamilton 911004-V1, 911004
DWP Carrier (5 SBS positions) x 1 PLT_CAR_L5AC	Hamilton 182090
MTP Carrier (5 SBS positions) x 3 PLT_CAR_L5MD	Hamilton 182365
Tip Carrier (5X96 Tips) x 3 TIP_CAR_480	Hamilton 182085
Multiflex Carrier Base (Landscape Orientation)	Hamilton 188039
Multiflex Tip Module	Hamilton 188160
Multiflex Tilt module	Hamilton 188061APE
Multiflex DWP Module x 3	Hamilton 188042

Labware Required	Part Number
50 uL CO-RE disposable tips without filters (black conductive)	Hamilton 235966
300 uL CO-RE disposable tips without filters (black conductive)	Hamilton 235902
1000 uL CO-RE disposable tips without filters (black conductive)	Hamilton 235904

User Supplied Materials	Part Number
Millicell 24-well Cell Culture Plate	PSRP010R5
BD Falcon Flat Bottom UV Plates w/lid	353293
E&K Scientific 12 Column Reservoirs, Deep Well, Pyramid Bottom	EK-2033
Axygen 12 Column Reservoirs, Low Profile, Partitioned	Axygen RES-MW-12-LP
Seahorse Bioscience Custom 36-well Deep Well Plate	S30052
Thermo Scientific Matrix 2D Barcoded ScrewTop Storage Tubes & Caps: 500 µl 2D Barcoded, V Bottom ScrewTop Tubes w/ Caps, Sterile	3744

The Hamilton MICROLAB STAR automated workstation uses the deck layout as shown in Fig 3 for the fully automated permeability assay.

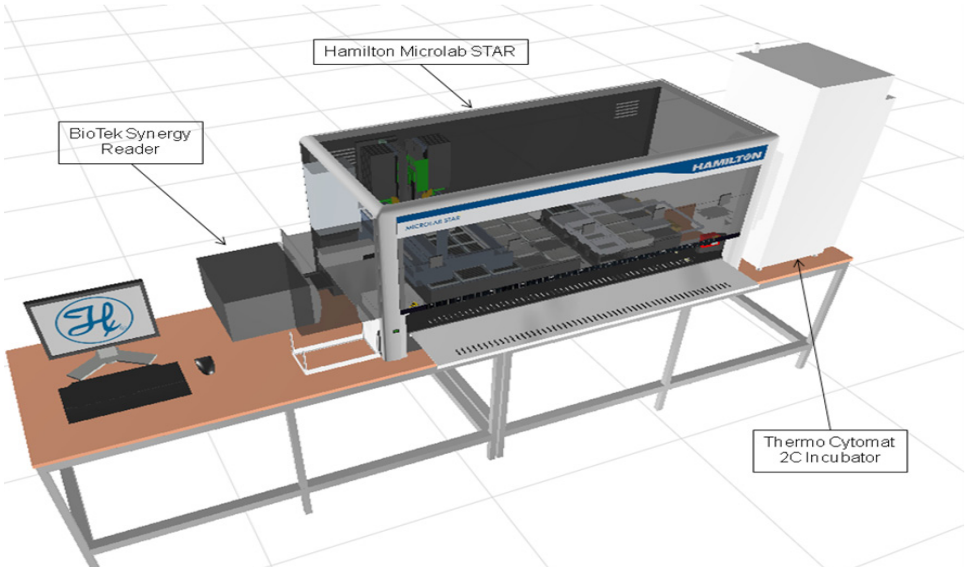

Figure 2: 3-D rendering of deck layout of ML-STAR

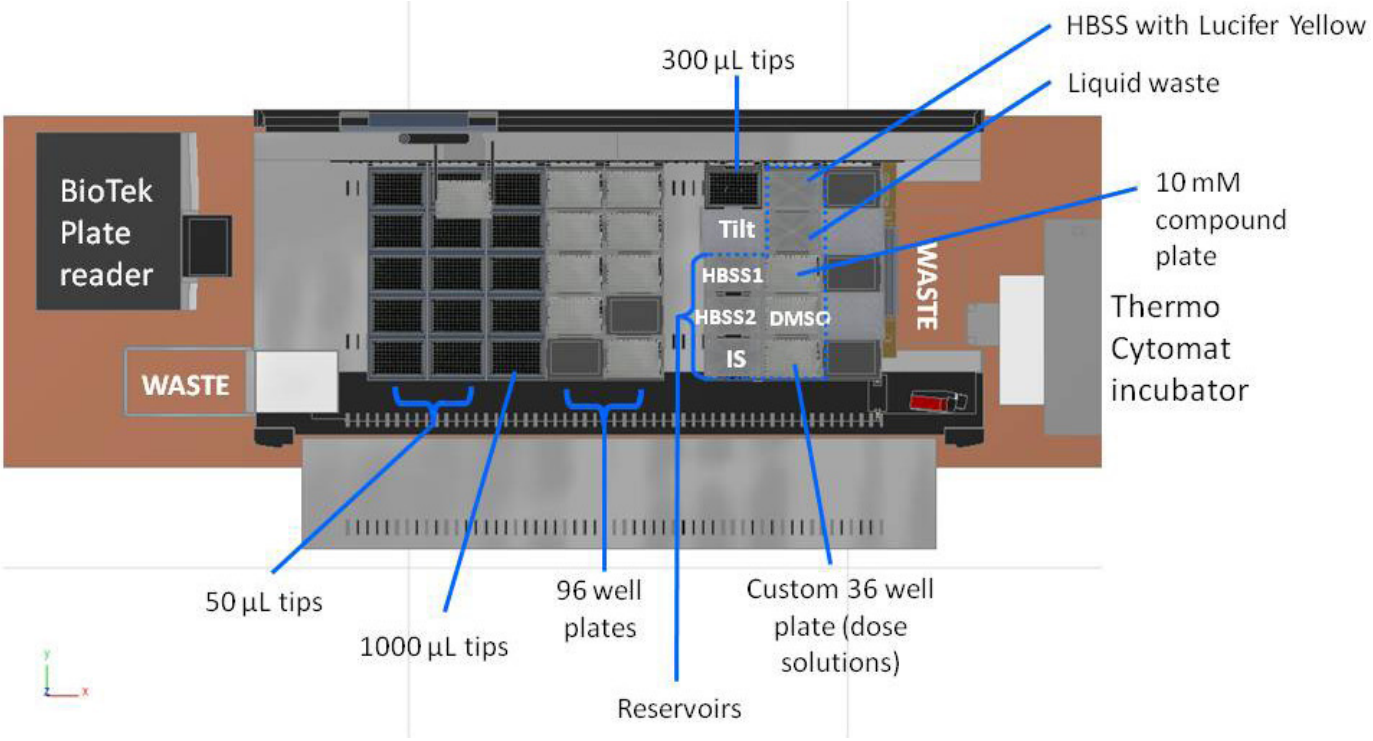

Figure 3: Deck layout on the MICROLAB STAR workstation showing the layout at the start of the method

Method Overview:

The method used 23 different generic drugs and a range of published apparent permeabilities, tested on MDCK cells grown in 12-well and 24-well plates. Figure 4 below shows the workflow of the method.

Steps involved in the method

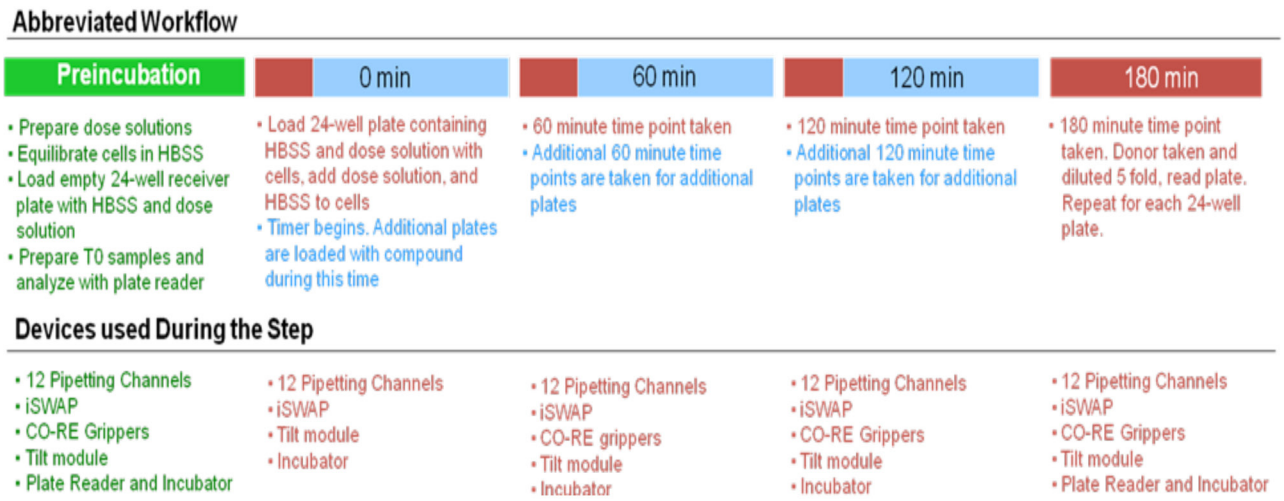

Figure 4: An abbreviated workflow of the method and list of devices used during the process steps

Results:

Permeability data and ranked order of the compounds tested is presented in Table 1. The permeability data correlates well with published results. The rank order classification resulted in similar class separation of compounds as reported in the literature, with the exception of one compound that shifted by one category.
The study also revealed that the apparent permeability between 12 and 24-well formats is very similar for most of the

compounds when studied across 13 generics and 8 Genentech compounds (Table 2). Table 3 illustrates the acceptable Papp values between the three classes. As evidenced in Table 4, all the controls used in the experimental design behaved as expected. Figure 5 shows the correlation data of the compounds tested in a 24-well format using MDCK cells and the published Papp data. The grouping classifies the compounds into low, medium and high Papp categories and the data suggests that there is a good correlation between published data and results obtained from the MDCK permeability assay performed using Hamilton STAR automation.

This study successfully demonstrates a robust automated solution for conducting MDCK permeability assays with highly reproducible and reliable data.

Table 1: Apparent Permeability, Rank Order and Permeability Classification of the Automated Assay Compared to Reported Literature Values.

		24-well Automated Assay		Reported Literature Values[1]	
Permeability Class	**Drug**	**Rank**	**Papp (10^{-6} cm/s)**	**Rank**	**Papp (10^{-6} cm/s)**
LOW ≤1 x 10^{-6} cm/s	atenolol	1	0.05	8	1.8
	acebutolol	2	0.08	7	1.4
	nadolol	3	0.11	6	1.4
	lisinopril	4	0.11	2	0.18
	sumatriptan	5	0.2	9	1.9
	amoxicillin	6	0.21	3	0.24
	ranitidine	7	0.22	NA	not detected
	penicillin V	8	0.23	1	0.15
	cephalexin	9	0.33	4	0.48
	terbutaline	10	0.59	5	1
MODERATE 1-10 x 10^{-6} cm/s	labetalol	11	4.9	11	25
	timolol	12	7.1	15	55
	trimethoprim	13	8.8	14	52
	dexamethasone	14	8.9	10	20
	acetaminophen	15	9.9	12	35
	propranolol	16	10.5	22	170
	*pindolol**	17	11	16	59
HIGH ≥10 x 10^{-6} cm/s	antipyrine	18	11.4	19	150
	metoprolol	19	12.2	20	150
	oxyprenolol	20	12.9	17	130
	alprenolol	21	14.3	21	160
	guanabenz	22	14.5	23	190
	*warfarin**	23	17.5	13	44
	testosterone	24	19.8	18	140

* The permeability classification differed by one category between methods.

Table 2: Comparison of Apparent Permeability Generated in the 24-well Automated and 12-well Manual Assays for 13 Marketed Drugs and Eight Genentech Compounds

	24-well Automated Assay	12-well Manual Assay
Compound	**Papp** (10^{-6} cm/s)	**Papp** (10^{-6} cm/s)
GNE-001	0.01	0.01
atenolol	0.05	0.4
acebutolol	0.08	1.5
nadolol	0.11	0.2
cimetidine	0.29	1
GNE-002	0.3	0.28
cephalexin	0.33	0.7
GNE-003	0.5	0.06
terbutaline	0.59 (L)*	1.8 (M)*
GNE-004	2.6	2.4
GNE-005	4.1	1.9
labetolol	4.9	8.6
amprenavir	5.7	4.6
acetaminophen	9.9 (M)*	15.4 (H)*
propranolol	10.5	13.4
GNE-006	11.4 (H)*	8.6 (M)*
GNE-007	11.8	16.9
metoprolol	12.2	19.9
GNE-008	14.0 (H)*	7.2 (M)*
verapamil	15.2	11.2
midazolam	21.3	20.5
* The permeability classification differed by one category between methods.		

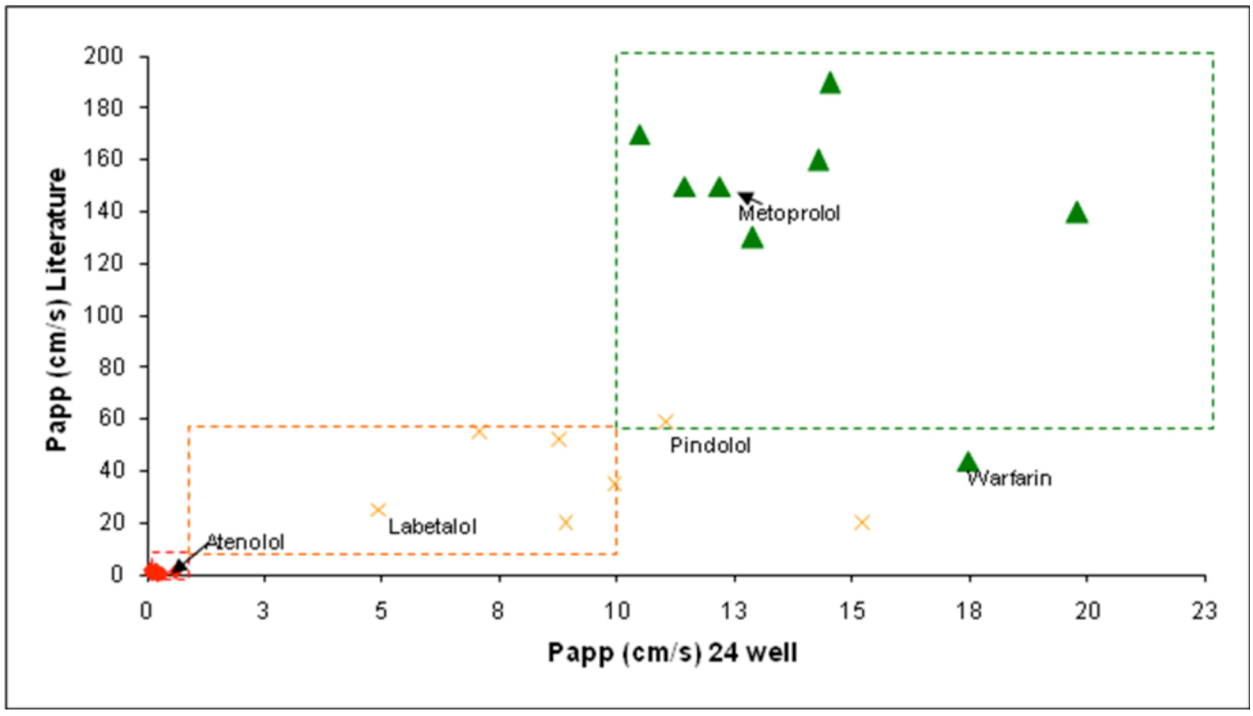

Figure 5: 24-well Automated and Reported Literature Papps Grouped into Low, Moderate, and High Permeability Categories

Table 3: Permeability Categories	
Permeability Category	**Papp (10^{-6} cm/s)**
LOW	≤ 1
MODERATE	1 - 10
HIGH	≥ 10

Table 4: 4 in 1 Cassetted Controls used in Permeability Experiments		
Compound	**Purpose**	**Papp (10-6 cm/s) AVE ± SD**
Nadolol	Low permeability marker	0.17 ± 0.16
Labetalol	Moderate permeability marker	5.8 ± 1.4
Metoprolol	High permeability marker	14.1 ± 2.7
Amprenavir	P-gp positive control	Efflux ratio: 3.8 ± 1.2

Conclusions:

1. The described automated permeability method performed on the Hamilton MICROLAB STAR workstation provides consistent results, in agreement with the previously established 12-well method and published results despite significant assay differences (membrane material, surface area, pore size, drug concentration, and analytical methods). (Spearman's rank correlation coefficients were 0.81 and 0.89, respectively).

2. The permeability categories can be determined using the boundaries: LOW ≤ 1, MODERATE = 1 – 10, and HIGH ≥ 10 (10-6 cm/s) and confirmed with permeability markers (nadolol, labetalol, metoprolol, and P-gp control amprenavir).

3. When compared to the 12-well method and reported literature values, the Hamilton Robotics' automated method correctly grouped nearly all compounds into the low, moderate, and high permeability categories.

References:

1. J. D. Irvine et al, J. Pharm. Sci. 88:28–33 (1999).

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