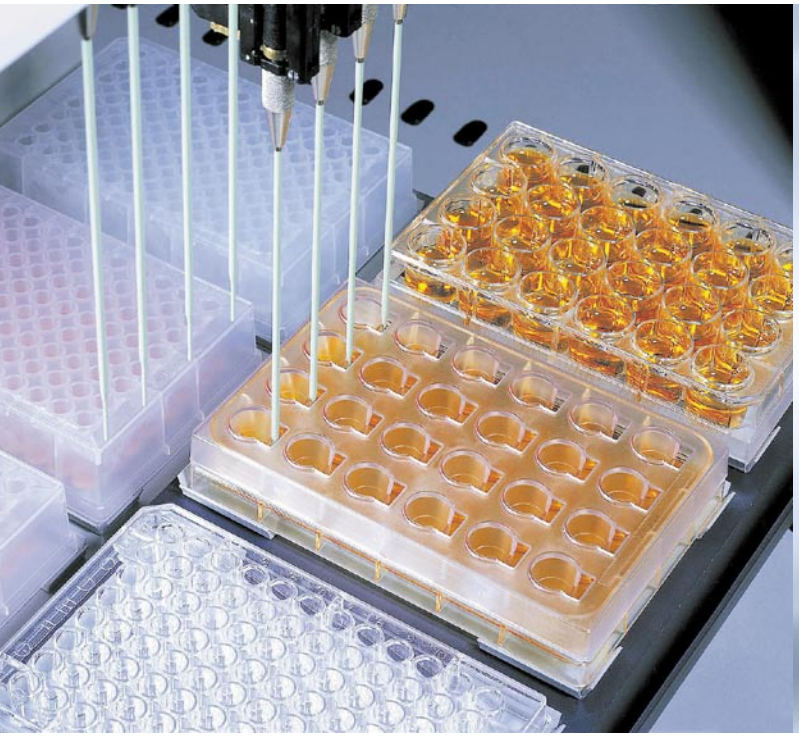
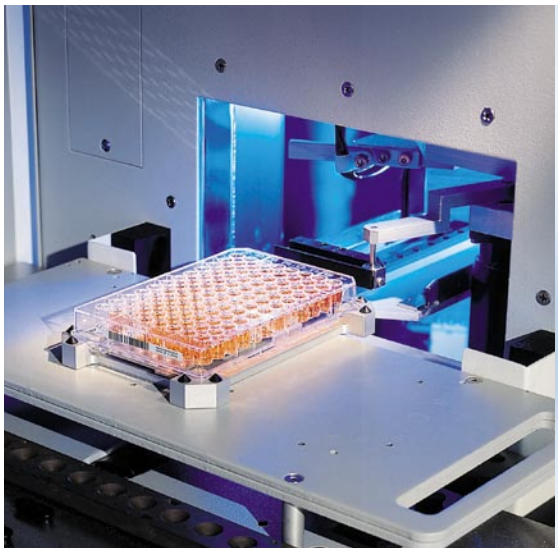
TECAN.



High throughput ADME screening systems

Enabling walkaway ADME solutions

The ADME product suite



The current trend within the drug discovery arena is to incorporate ADME (absorption, distribution, metabolism and excretion) screening in the early stages of drug discovery in order to reduce high-cost, late-stage compound failures. To achieve this, there is a growing need for higher throughput assays and automation of ADME screens to process large numbers of compounds.

Tecan has created a range of automated ADME screening systems which maximize the efficiency and throughput of these processes. These platforms are based on our flexible and scalable Freedom EVO® liquid handling platform, for automated cell permeability, compound characterization, in vitro drug metabolism and cell culturing procedures.

The Freedom EVO platforms offer four different worktable capacities, each with building-block modularity that brings precision liquid handling, easy-to-use robotics and advanced process control. Allowing every laboratory scientist, from first-time user to laboratory automation specialist to run ADME processes. Researchers can either select the best pre-configured automation solution for the laboratory, or create a customized system together with our automation specialists according to specific needs.
The building blocks include, the optional MCA™ multichannel pipetting arm to increase throughput and efficiency, the robotic manipulator (RoMa) arm option moves microplates automatically into microplate readers anywhere on the deck of the platform. The Extended Z RoMa option enables access to a reader or centrifuge placed under the deck of the Freedom EVO platform.

Any of Tecan's family of microplate readers can be easily integrated into the ADME assay platform to facilitate the full data capture process. Integrating a microplate reader such as the Safire2™ or the Infinite™ 200 series offers a full range of detection methods.

Cell Permeability

Our Cell Permeability platform has been designed to include all of the components necessary to perform compound permeability assays across either cell or artificial membrane barriers. Tecan has worked with leading assay providers to verify the performance of their kits on Tecan instruments. The Freedom EVO platform has been configured for Caco-2 or Madin-Darby canine kidney (MDCK) cell assays, used to predict the intestinal permeability potential of a compound. An example of data generated is shown in table 1. The Cell Permeability platform can also be routinely used for active transport and/or compound efflux measurements, and for artificial membrane permeability assays complimentary to the cell-based systems, for example, for running parallel artificial membrane permeability assays (PAMPAs). An example of data generated is shown in figure 1.

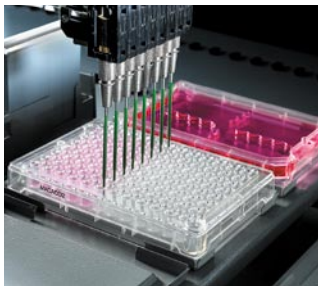


Table 1: Comparison of manual and automated compound permeability assays.

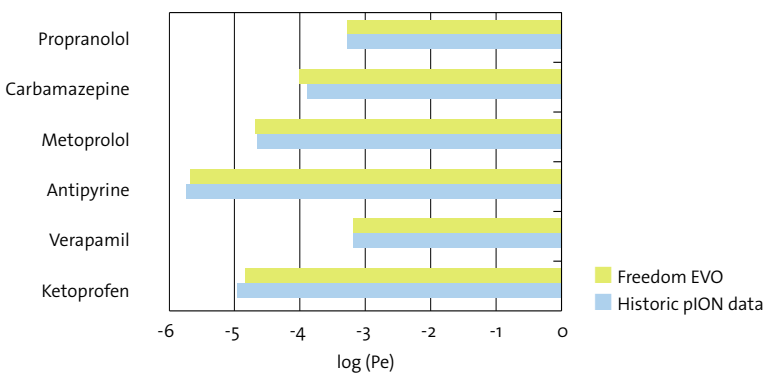
		Permeability rates (A → B) (1×10^{6} cm/s)			Efflux ratio	
		Mannitol	**Testosterone**	**Digoxin**	**Mannitol**	**Digoxin**
3-day cell growth, MDCK	Automated*	0.2 ± 0.0	15.9 ± 2.3	0.1 ± 0.0	1.3	87.1
	Manual	0.3 ± 0.0	17.4 ± 3.3	0.1 ± 0.0	0.9	92.4
7-day cell growth, MDCK	Automated*	0.3 ± 0.0	22.5 ± 0.5	0.2 ± 0.0	0.9	99.2
	Manual	0.2 ± 0.0	22.7 ± 3.2	0.2 ± 0.1	1.4	67.0
21-day cell growth, Caco-2	Automated*	1.0 ± 0.1	21.9 ± 2.0	0.8 ± 0.3	1.3	20.7
	Manual	2.1 ± 0.5	21.7 ± 0.4	1.2 ± 0.4	0.8	14.8

*Automated assays were run on a Freedom EVO platform, with Millipore Millicell® 24-well filter plates.

Permeability rates (Papp) were determined radiometrically for both Caco-2 and MDCK cell lines. Each drug was run in replicates of six wells per cell line, transepithelial electrical resistance (TEER) measurements (not shown) before drug analysis indicated an integral cell monolayer, and Lucifer yellow post-processing indicated that the cell monolayer was not disturbed during processing (all samples ≥98%, data not shown). Kellard L, Engelstein M. 2007. Journal of the Association for Laboratory Automation (JALA), 12:104–109.

Figure 1: Parallel artificial membrane permeability assay (PAMPA).

Comparison of a set of Double-Sink® PAMPA measurements on new PAMPA EvolutionPLUS system against values from pION's database. The assay conditions were pH 6.2 in donor, 30 minutes incubation and Gut-Box® stirring at 40 µm setting.
Data courtesy of pION INC, Woburn, MA, USA.



Drug Metabolism

The Drug Metabolism platform can routinely perform a variety of in vitro assays, including metabolic stability, cytochrome P450 inhibitions, isoform identification, metabolite identification and cytochrome P450 induction assays. For microsome or hepatocyte metabolic stability assays, a choice of temperature-controlled shakers that keep microsomes or cells in suspension, and temperature-controlled racks or reagent carriers to handle pre-incubation or post-incubation processing steps can be included. Example of data generated is shown in table 2 below.

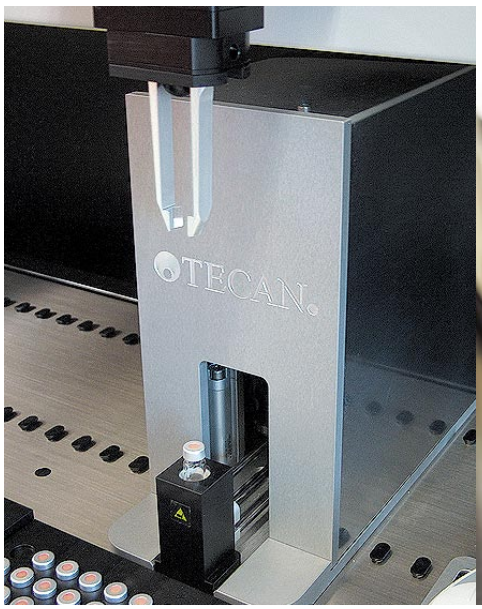
Table 2: Drug metabolizing profiling data

	CYP1A2	CYP 2C9	CYP 3A4 PPXE	CYP 3A4 (BE)	CYP 2D6	CYP 2C19	MAO A
Furafylline	0.6	>100	>100	NI	>100	NI	NI
Published values	0.67-6.0						
Sulfaphenazole	NI	0.1	>100	>100	NI	NI	NI
Published values		0.18-1.3					
Ketoconazole	>100	1.0	0.01	0.01	13.6	1.5	>100
Published values			0.083-0.17	0.083-0.17			
Quinidine	NI	>100	>100	+	0.003	>100	NI
Published values					0.009-0.18		

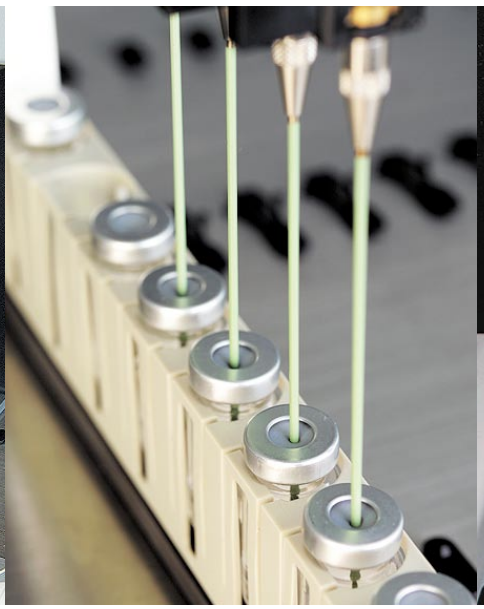
12-point dose response curve. Final dose range 100 µM – 2 nM, DMSO control. IC50 (µM) listed (unless noted otherwise).
+ = positive cooperativity (stimulation).
NI = No inhibition. >100 = inhibition noted at higher concentrations.
Data courtesy of Promega, as presented by T.Worzella at 2006 SBS, Seattle, USA.
Data generated using Promega P450-610™ and MAO-610™ Assays. A bibliography for published values can be found in:
Cali JJ, Ma D, Sobol M etal. Luminogenic cytochrome P450 assays, Exp. Op. Drug Metab. Toxicol. (2006) 2 (4): 629-645.

Compound Characterization

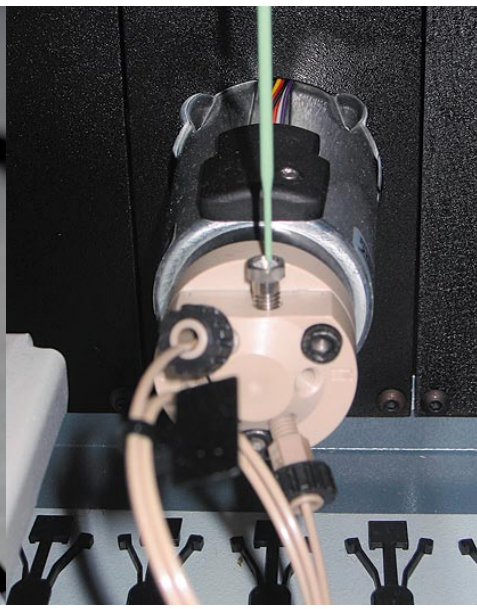
The Compound Characterization platforms are designed to help researchers better understand the biophysical nature of their compounds. These systems include an integrated Safire2 monochromator-based microplate reader that can quickly scan a compound in solution and rapidly determines the relative purity and amount of compound present. The system is ideal for determining compound solubility, logP values, and whether or not the compound possesses intrinsic optical properties that might interfere with a downstream assay. The Freedom EVO platform is capable of performing all the necessary dilutions and standard curve set-ups required for these processes. It can also be used to load and cap LCMS vials or inject directly into a GC/HPLC sample loop.



An integrated vial crimping station with automated tube handling arm.

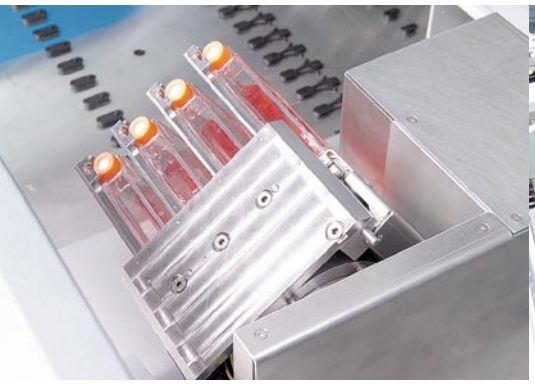


Septa piercing of vessels during aspiration or dispensation.



Direct inject into an integrated GC/HPLC sample loop on a Freedom EVO deck with the liquid handling arm.

Automated Cell Maintenance System



Routine *in vitro* ADME screening assays require a reliable supply of cells that have to be grown and maintained.

This is a considerable challenge for researchers in ADME groups, who must struggle to maintain cells that require long culture periods (Caco-2) or which are human in origin (hepatocytes), even before they can be used in a particular assay.

Tecan has designed the Automated Cell Maintenance System, Cellerity™, to make cell culture as effortless and simple as possible. Based on the Freedom EVO liquid handling platforms, this system contains an automation-compatible incubator, integrated cell counter, media storage and warming devices, and a multi-way valve to handle different media types, buffers and sterilizing agents.

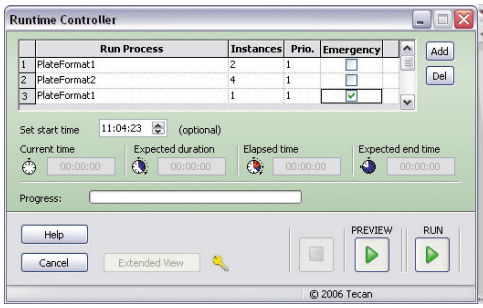
The basic liquid handling unit rests inside a custom built cell culture cabinet that maintains a HEPA-filtered airspace where cells are processed.

The Automated Cell Maintenance System is fully capable of handling typical cell culture steps for adherent cells in microplate or multiwell insert formats, and all routine cell seeding and media exchange processes.

Freedom EVOware® Standard & Freedom EVOware Plus

The Freedom EVOware software offers a single, scalable package that can be operated by all laboratory personnel. Its multilevel user management allows fixed scripts to be run by technicians and full accesses for system integrators. Scripts are defined using drag-and-drop with a consistent and intuitive wizard driven Graphical User Interface (GUI) that offers full control of all Freedom EVO platform instruments and options. It supports the pipetting of IC50 plates for running P450 dose response curves. The realistic 3D simulator allows script development without tying up instrument resources. There are device drivers for more than 30 standard options and many more third party devices including those needed for ADME application, incubators, shakers and plate readers.

The Freedom EVOware Plus software includes advanced dynamic scheduling features that ensure your platform's resources are fully utilized. Workloads vary on a day-to-day basis, with changes in the number of required assays or different sample volumes in compound plates. This can make it challenging to estimate the time for each task, so Freedom EVOware Plus automatically reschedules to make use of spare seconds, without breaking your assay's timing constraints. It allows for scheduling of tasks like media change for Caco-2 cell plates.



Freedom EVOware Plus allows different processes to be started together.

The simulator allows scripts to be developed at your desk.

The scheduler maximizes parallel operation without compromising critical times.

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