WORLD PRECISION INSTRUMENTS

Application Note

Common Applications of the EVOM™ Auto System for High Throughput Screening of TEER Measurement

Improved User Experience

EVOM™ Auto with 24 and 96 capability is the latest generation of WPI's automated transepithelial or transendothelial electrical resistance (TEER) measurement system to analyze samples in high throughput screening (HTS) 24 and 96 transwell plates. Using the same proven technology in the EVOM™ Manual and REMS, combined with a new multi-electrode array, software interface and control system, it delivers our fastest workflow solution while improving TEER measurement accuracy:

- Faster throughput – Read a 96 transwell plate in under 3.5 minutes. (A 2-rinse cycle can be completed in 7 minutes, almost halving the time compared to a REMS.)
- Easy switch and measurement in different HTS plate types (24 or 96) by swapping electrode array and plate adapters using the same system
- The 96 transwell plate electrode array is compatible with Corning, Millipore, and MatTek 96 HTS plates. The 24C electrode array is used for Corning 24 HTS transwell plates and 24M electrode array is used for Millipore 24 HTS transwell plates.
- Automatic sample averaging improves accuracy and stability
- Compact size for easy set up and operation inside in a cell culture hood or incubator
- Wireless control of the autosampler
- Smart user interface and web browser-based software for easy sample analysis and data storage and access
- Wider resistance range compared to the REMS (The upper range increased from 20 kΩ to 100 kΩ.)
- Continuous data recording capability at user-defined time intervals

EVOM™ Auto, with advanced product features, is designed for a better user experience to analyze samples and increase sample reading throughput while acquiring more accurate TEER data.

In Vitro Cell Culture Models

The *in vitro* cell culture models of human endothelial and epithelial monolayers are considered as reliable models of the *in vivo* environments and as such are used for drug toxicity and transport studies. Findings of the *in vitro* models are also translated to ascertain the metabolic and physiological functions of a particular pharmacological entity. The most commonly used endothelial/epithelilal *in vitro* models are:

- Blood-brain barrier (BBB) model
- Gastrointestinal tract (GIT) model
- Pulmonary models (including viral infection model, such as COVID-19)

These are used for understanding the absorption and transport of drugs, as well as associated cytotoxicities in the organs like the central nervous system, intestine, and lungs. These models are either comprised of primary cells or established cell lines. For utilization of these models, the most important feature is to ascertain the capability of cells to form necessary intercellular junctions. Before proceeding with particular drug cytotoxicity or transport, the formation of cellular junctions in these *in vitro* models is confirmed through a variety of methodologies. This includes

the permeability of the established barrier to compounds like sucrose having radio-labeled carbon, and lower molecular weight paracellular tracers including inulin, mannitol, and albumin. Specific studies have also reported the uses of non-radioactive, fluorescence-labeled marker proteins like dextran labeled with fluorescein isothiocyanate (FITC) and Evans blue dye/biotin-albumin. All these techniques for confirming the integrity of endothelial/epithelial barriers are cumbersome, lacking absolute specificity and the overall impact of these externally added compounds might be influencing the physiological functioning of the barrier established *in vitro*.[1]

Besides all the above described methodologies for determining the integrity of an *in vitro* endothelial/epithelial barriers, transepithelial/transendothelial electrical resistance (TEER) measurements are considered more precise and highly quantitative in nature.[2] More importantly, the TEER measurements are faster, inflicting minimal to no damage to the cells and with nothing being added externally, as the addition of any tracer or radio-labeled compound can potentially affect cellular physiology of monolayers.

The EVOM™ Auto autosampler by WPI is a unique TEER measurement system that is considered highly reliable for evaluating the integrity of *in vitro* epithelial barrier models, including the blood-brain barrier, gastrointestinal tract, and pulmonary model.[3]

TEER Measurement with EVOM™ Auto (HTS TEER Measurement System)

The TEER methods for measuring the integrity of endothelial/epithelial barrier is based on assessing the electrical resistance of the cellular barrier growing in the form of a monolayer. The technique relies on applying the AC-like (DC square wave with switching polarity) signals through electrodes which are inserted across the cellular monolayer and detects voltage change and calculates the electrical resistivity of the layer.

The EVOM™ Auto, offered by WPI, measures the electrical resistance of transepithelial/transendothelial cells grown to confluence on semi-permeable microporous filters of high throughput screening (HTS) 24 and 96 transwell microplates. The system is automated, which minimizes errors associated with a user's manual handling, generating highly reliable data and enhanced reproducibility. The system operation is controlled with an iPad® tablet and a local wireless network. Automated measurement of tissue resistance in high throughput (HTS) transwell plates provides the critical advantages of speed, precision, decreased chances of introducing contamination, and the instant availability of measured resistance or TEER data. These measurements are useful in applications, such as evaluating drug toxicity, and bioavailability studies.

The electrode array positions eight pairs of electrodes precisely in the wells and measures a column at a time, each well sequentially. As measurements are made, they appear on the iPad screen in real time.

The EVOM™ Auto is used for TEER measurements of cells grown in 24 and 96 transwell HTS plates. This equipment can capture *in vitro* tissue resistance measurements up to 100 kΩ. This system has been used to measure TEER in the following epithelial barrier systems.

The EVA-EL-03-01 electrode array has 8 pair of (1.25mm Φ) electrodes, which are specially designed to fit precisely in 96 transwell plates, ensuring consistent placement. It performs resistance measurements directly in the plate, common or divided, reducing the possibility of contamination and mechanical damage to your cultured cells.

EVA-EL-03-02 (Corning 24 HTS) and EVA-EL-03-03 (Millipore 24 HTS) have 4 pair of (1.2mm Φ) electrodes to use with 24 transwell HTS plates.

Blood Brain Barrier (BBB)

There are several *in vitro* models of the human BBB. This biological barrier separates the central nervous system from the systemic circulations. [4] The *in vitro* setup is established by growing brain endothelial cells in conjunction with astrocytes that helps in mimicking the barrier. A variety of pharmacological agents can penetrate into the brain exerting neurotoxicity besides their cytotoxicity on the barrier itself. The *in vitro* BBB models help to understand the effects of drugs on the central nervous system and barrier properties in the cellular level.

Gastrointestinal Tract (GIT) Model

The epithelial layer of the gastric mucosa (comprised of an epithelial layer, lamina propria, and muscularis mucosa) limits the drug permeation into the systemic circulation. Therefore, the *in vitro* model of GIT is used for studying the passage of drugs and the penetration of toxic elements. The Caco-2 cell line derived from the human colorectal adenocarcinoma is a widely used cell line as an *in vitro* model of the GI tract for evaluating pharmacological passage.[5] Human primary GI tract cells isolated from patients' samples have even been used to establish a GI epithelial barrier model.

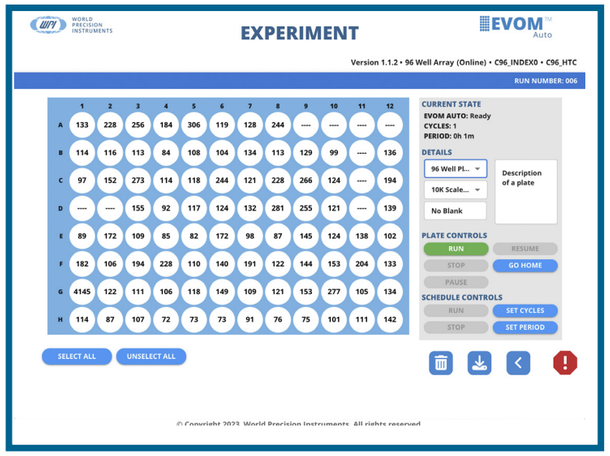
Pulmonary Models

The pulmonary barrier models are used for understanding the direct toxicity of pharmaceutical compounds, as well as transport of nutrients and pharmacological molecules into the lungs. The lungs are covered with a continuous epithelium comprised of the airway and alveolar epithelia. Both the airway and alveolar *in vitro* epithelial models (including viral infection model like, COVID-19) have been reported in the literature.[6, 8]

Drug Discovery/Toxicology

The epithelial membranous structures separate individual compartments of the human body, besides shielding the organs (like, brain, intestine, and lungs). They also play a crucial role in tissue homeostasis. The toxic effect of newly developed/already existing drug formulations on these biological barriers is an active area of research.[7] The *in vitro* barrier models are providing a wealth of information for evaluating the distribution of pharmacological agents and their relevant toxicity. To assess the barrier properties of cultured cells, it is critical to establish intercellular junctions similar to *in vivo* tissues.

The TEER method is considered fast, accurate and non-invasive, the prime choice of all the available methods. Notably, for evaluating pharmacological transport across these barriers, this method does not add any additional molecule or chemical in the system. The EVOM™ Auto has the capability to measure the electrical resistance of transepithelial/transendothelial cellular layers growing to confluence on microporous filters. For high throughput screening (HTS), the instrument has the versatility for use in 24 and 96 transwell HTS plates.



From the iPad, you can remotely control the autosampler, and on the Experiment screen, the readings display in real time.

Conclusions

The TEER measurements in the *in vitro* endothelial/epithelial barrier model (like the blood-brain barrier models, gastrointestinal tract models, and pulmonary models) can be efficiently used to evaluate pharmacological toxicities, as well as have applications in the drug discovery process. WPI's new EVOM™ Auto design includes wireless autosampler control, smart user interface, compact autosampler size, and advanced measurement electronics. EVOM™ Auto, with advanced product features, simplifies and improves throughput and measurement accuracy of automated TEER screening of the *in vitro* tissue models.

System Specifications

AutoSampler Dimensions (W×D×H)	16×10×8.4"
AutoSampler Weight	15 lbs.
CE Certified	Yes
Compatibility	Corning , Millipore, MatTek 96 Transwell HTS Plates with 96 electrode array Corning 24 HTS Plates with 24C electrode array Millipore 24 HTS Plates with 24M electrode array
Resistance Range	10KΩ, 50KΩ, 100KΩ

Number of Rinse Stations	3
Electrode Array for 96 HTS Plate	Electrode array 8 pair of (1.2 mm Φ) Electrodes: 96 well array Array 4 pair of (1.2 mm Φ) Electrodes: 24 well array
Minimum Sample Reading Time	1 Second
Control Device for Running Software	Tablet/iPad, Laptop, Desktop with Wi-Fi adapter
Output Data	CSV/Microsoft® Excel

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