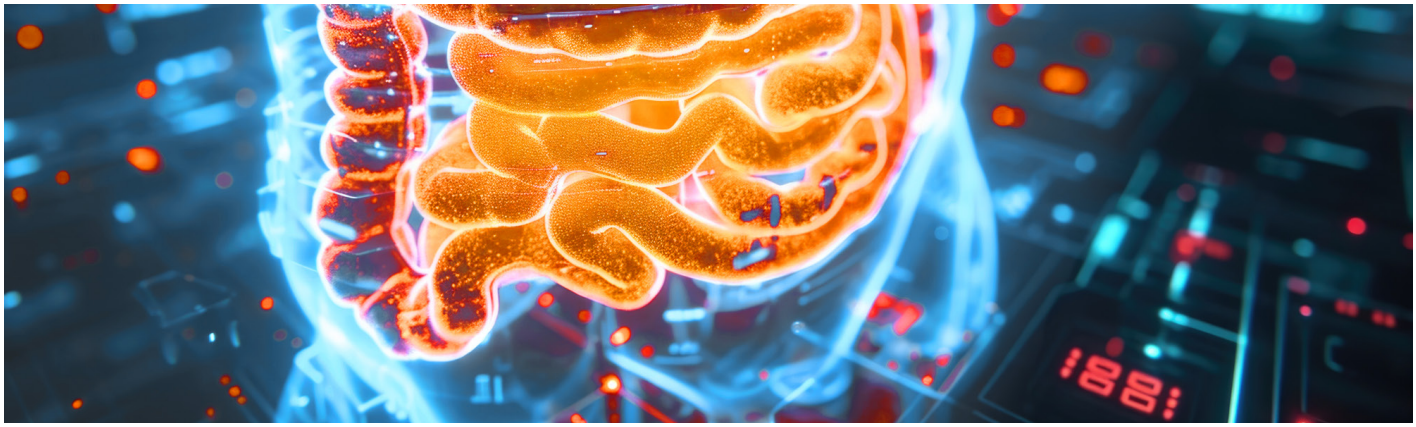
APPLICATION NOTE

Utilizing EVOM™ Auto

For High-Throughput Automated TEER Analysis: Rapid & Reliable Approach for Therapeutic Screening & Diarrhea Risk Assessment Using *In Vitro* Human Intestinal Model



ABSTRACT

The ability to predict adverse effects of drugs in early stages of drug screening is critical for effective development of safe and effective drugs. Diarrhea induced by gastrointestinal toxicities (GIT) remains the most common adverse effect (AE) leading to significant dose limitations that often reduce the efficacy of a drug and lead to delays or failures in the clinic. The risk of GIT has historically been determined in preclinical animal models, yet many therapeutics still demonstrate profound AEs in clinical trials, despite being safe in animal models. GIT is characterized by a reduction in gastrointestinal barrier integrity and transepithelial electrical resistance (TEER) has been demonstrated to be a rapid and reliable measure of barrier function of the gastrointestinal tract. A major advancement in the ability to use TEER measurements for *in vitro* screening of drugs that cause GIT is the commercial availability of World Precision Instrument's EVOM™ Auto, a high-throughput TEER instrument that enables measurement of a 96-well plate in under 3.5 minutes. In this study, we demonstrate that the RepliGut® Planar model created by Altis, which integrates proliferative cell populations derived from human intestinal crypts can be used as a platform for screening of therapeutics for GIT using TEER measurements on the EVOM™ Auto. This study reports highly accurate predictions of diarrhea potential using TEER measurement across a group of human donors and a reference panel of 30 drugs. The need for more accurate models to predict adverse effects, coupled with efforts by regulatory agencies including Europe's EMA and the United States' FDA to replace animal models with more predictive and reliable methods, make this study highly impactful, as we have established a rapid, low cost, accurate, sensitive and specific assay for risk assessment in early stages of drug discovery, facilitating rapid and cost-effective screening for GITs. Early identification of AEs and toxicities enables preemptive reformulation of therapeutics or alternative dosing schedules to mitigate GIT and ultimately reduce the risk of dose limitations in clinical trials that impair the efficacy of drugs. Implementation of alternative models like Altis's RepliGut® Planar coupled with fast and easy measurements like TEER that give a quantitative readout of a critical cellular functionality like barrier integrity and makes for an ideal screening tool to identify safe and effective drugs that will improve the lives of patients.

INTRODUCTION

Transepithelial/transendothelial electrical resistance (TEER) is commonly used to verify endothelial or epithelial barrier and tissue function. The epithelial voltmeter (EVOM™) is considered the gold standard of TEER measurement with thousands of literature citations and TEER values reported for a variety of different cellular models over the last three decades. TEER is measured by applying a very small alternating current (<10 µA) at 12.5 Hz frequency using EVOM™ instrumentation. TEER is routinely used because it offers significant benefits over other techniques to monitor barrier function of a monolayer. In particular, TEER measurements can be taken rapidly (in less than 10 seconds), and are non-invasive, meaning the measurement does not harm the cells and can be taken routinely before, during, and after screening cells with therapeutics. The same samples, used for TEER measurement by EVOM™, can be further used for other quantitative analysis, such as fluorescence microscopy and permeability assay since cells are grown onto semipermeable membranes of standard cell culture plates (transwell plates or inserts).

Recently, the ability to rapidly screen 24- and 96-well plates by TEER was enabled by World Precision Instrument's EVOM™ Auto. The EVOM™ Auto offers additional benefits over previous instruments by allowing fast and consistent TEER measurement, with 96 samples being read in under 3.5 minutes. Further, by enabling robotic (hands-free) measurements, the EVOM™ Auto minimizes any potential errors associated with human handling of the electrode and enables consistent and repeated electrode disinfection to minimize chances of sample cross-contamination. The quantitative nature of TEER measurement gives researchers instant insight into the barrier function and permeability of a cell layer that does not require the time-consuming processes of staining cells for immunofluorescence imaging or performing fluorescent molecule permeability assays that render cells unusable after the assay. Thus, automated TEER measurement by EVOM™ Auto is used as a reliable high throughput screening tool in drug discovery and development.

Gastrointestinal toxicities (GITs) are the most common clinical adverse effects (AEs) in the development of clinical therapeutic drugs and often result in dose-limitations that significantly reduce therapeutic efficacy and delay development in clinical trials. Today, there is a lack of effective early GIT screening methods which could predict AEs, before preclinical animal studies or human clinical trials. Early identification of GITs is critical to identify safe and effective therapies, optimize dose regimens, preventing potential harm to patients, and could save millions of dollars for each drug developed. In particular, oncology drugs face a record low success rate, with nearly 95% of this class of drug failing in clinical trials, with the most common reason for failure being severe diarrhea during treatment. Animal models have been used to test safety and efficacy preclinically; however, the high clinical trial failure rate demonstrates the inability of these models to consistently identify therapies with significant side effects. Recently, new alternative models including organoids, organ-on-chip, and 2D/3D cell culture models have evolved to create effective platforms to test GITs. These *in vitro* models are cost effective, have high throughput screening capabilities, and are simple to establish and assess in the laboratory.

Chemotherapeutic drugs target rapidly dividing cells, which include stem cells and progenitor cells. *In vivo*, the stem cells within intestinal crypts are responsible for replenishment of the normal tissue after tissue damage or during normal tissue turn over. The model system RepliGut® Planar, by Altis Biosystems, is a 2D human stem cell-derived platform in which cells are grown on 96-well semi-permeable membranes and the cells undergo sequential proliferation and differentiation to replicate *in vivo* process. The 96-well platform used to grow these cells is compatible with World Precision Instrument's EVOM™ Auto high-throughput, automated TEER system.

OBJECTIVE

Chemotherapeutics, which exhibit high rates of clinical GIT, frequently target mitotic cells. The objective of this study is to use a new model system for early detection of GIT. Here, we describe the conditions for toxicity prediction in the RepliGut® Planar model using automated TEER measurements and application of these to prediction of clinical diarrhea potential.

MATERIALS AND METHODS

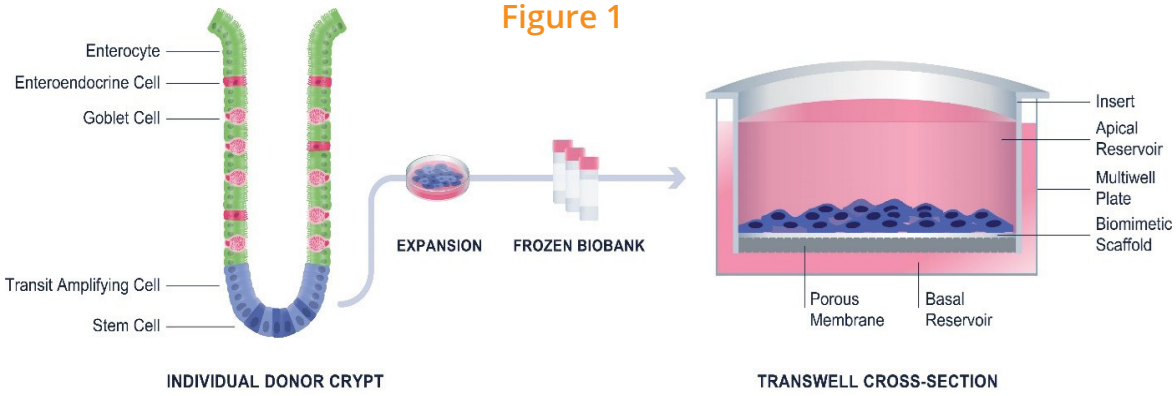
RepliGut® cells form a confluent monolayer in four-to-five days and form a matured and differentiated polarized layer in another two days. The RepliGut® platform offers a number of benefits for high-throughput therapeutic screening:

1. Easy and fast cell culture model establishment
2. Cell growth on semi-permeable membrane allows drugs to be tested on apical and basolateral (luminal) sides of cellular layers
3. Drugs can be tested on cells that are in the proliferative or fully differentiated stages
4. Cells are grown onto 96-well plate, allowing for high-throughput screening on instruments such as EVOM™ Auto which enables rapid and reproducible TEER measurements, a quantitative measure of barrier integrity of the gastrointestinal tract.

As reported previously (Pike et al. 2023), the RepliGut® platform is developed by isolating cells from post-mortem gastrointestinal crypt tissue, followed by stem cell and progenitor cell expansion, biobanking, and seeding cells onto biomimetic scaffolds in transwells/ cell culture inserts, as shown below:

After cell seeding, the RepliGut® platform forms a monolayer of cells on a semi-permeable membrane that demonstrates the

Figure 1



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UK: +44 1462 424700
Germany: +49 6031 67708-0
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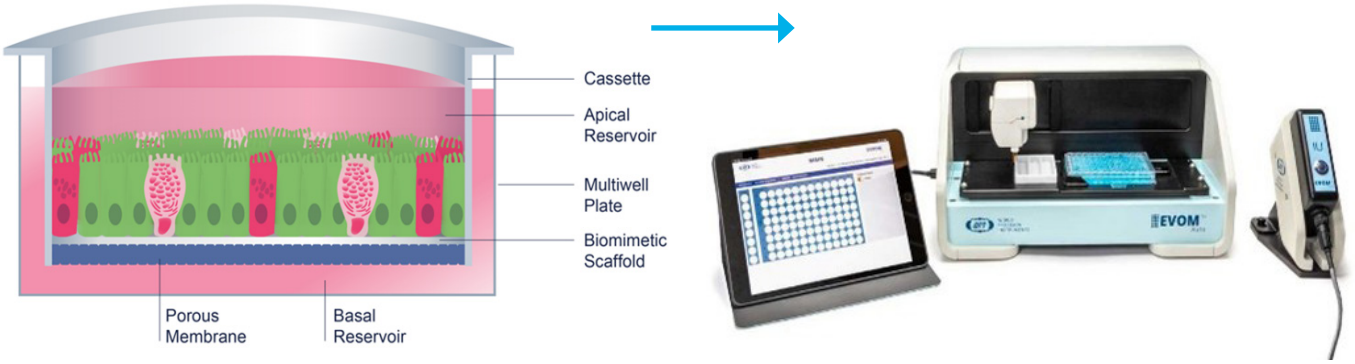
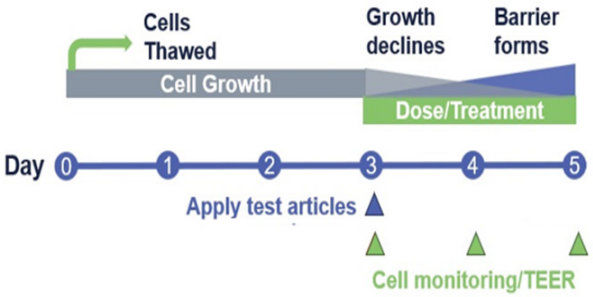
morphological and functional features of mature intestinal epithelial cells that can be used to measure TEER on the EVOM™ Auto platform. The semi-permeable membrane that the cells are grown on allows therapeutics to access both the apical and luminal sides of the cellular layer. The epithelial barrier function of the RepliGut® platform grown onto 96-well high-throughput screening plates can be confirmed by TEER values of greater than 300 Ω-cm2, representative of mature and differentiated cells (Pike et al. 2024). A 96-well plate can be read in under 3.5 minutes on the EVOM™ Auto, demonstrating the utility of this platform for high-throughput therapeutic screening:

Figure 2



Once cells are thawed, they are plated and allowed to grow for three days before therapeutic dosing. On day three, drug is given to the cells and cells undergo daily TEER measurement, which is non-invasive and non-destructive, allowing repeated measurements from the same sample over the time course for evaluation of both short-term and long-term effects of the drug (Pike et al. 2024). The workflow for drug screening and TEER monitoring is shown below:

Figure 3



A collection of 30 reference drugs with known clinical diarrhea incidence were tested in three human donors and evaluated to determine whether TEER could serve as a predictive correlation parameter for clinical diarrhea (Pike et al..2024). 96-well plates were set up to evaluate a six-point dose-response curve. The EVOM™ Auto enables rapid screening of the assays in a 96-well format, with each plate being read in under 3.5 minutes, significantly reducing the time and costs of this screen. The plate below shows an example plate set up where four compounds were evaluated over the course of six therapeutic concentrations with the appropriate controls:

Figure 4

	1	2	3	4	5	6	7	8	9	10	11	12	
A	Vehicle						Vehicle						Vehicle Controls
B	0.0001	0.0001	0.001	0.001	0.01	0.01	0.1	0.1	1	1	10	10	Pos Control (Bortez.)
C	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	Compounds
D	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	
E	1	1	1	1	1	1	1	1	1	1	1	1	
F	10	10	10	10	10	10	10	10	10	10	10	10	
G	30	30	30	30	30	30	30	30	30	30	30	30	
H	100	100	100	100	100	100	100	100	100	100	100	100	
	Compound #1			Compound #2			Compound #3			Compound #4			

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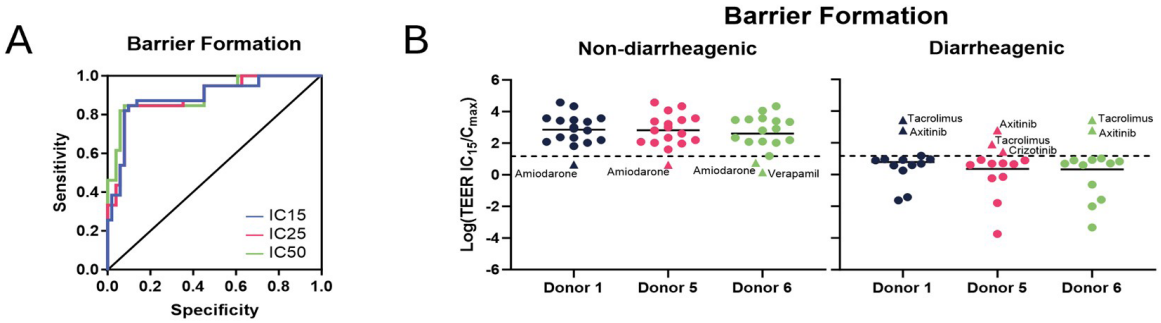
RESULTS

TEER measurements were collected for all three donor cell lines using the panel of 30 reference drugs (Pike et al. 2024). The diagnostic accuracy of TEER was 88%, with 82% sensitivity and 92% specificity. Below the TEER data is shown in (A) as the receiver-operator curved of IC15, IC25, and IC50 and in (B) as the response to the 30-drug reference panel plotted as IC15/Cmax ratios. Dashed line indicates the threshold for a diarrheagenic or non-diarrheagenic outcome. Black lines indicate mean. n = 3 technical replicates:

Figure 5



CONCLUSION

TEER measurement can be used as a functional parameter to determine altered epithelial barrier function, which correlates with GIT and the potential of drugs to cause diarrhea, a potential dose-limiting AE. Thus, TEER measurement by EVOM™ Auto provides an efficient, high-throughput screening tool to predict GITs by chemotherapeutic drugs and has the potential to be useful for predicting safety and efficacy on many other platforms for many other types of therapeutics.

REFERENCES

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