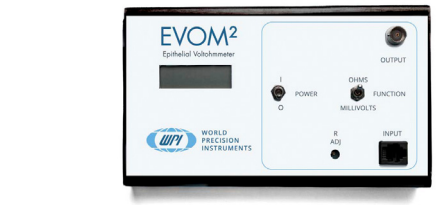
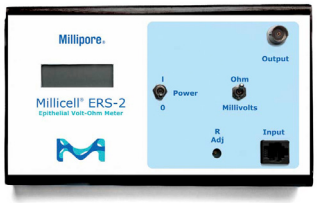
APPLICATION NOTE

EVOM™ Manual

WPI's EVOM™ Manual Shortens TEER Analysis Experimentation Time

Current EVOM™ MANUAL





Older Model TEER Meters

BACKGROUND AND INTRODUCTION

EVOM™ based TEER measurement is considered the gold standard for the transepithelial electrical resistance (TEER) measurement to evaluate cellular confluence, barrier function, permeability, and tissue function. WPI is the pioneer in the field of TEER measurement and introduced TEER measuring instruments more than 30 years ago. WPI has manufactured manual TEER measurement system (EVOM, EVOMX, EVOM2, EVOM3, Millicell ERS, andERS-2), as well as automated system like REMS. EVOM™ technology has been used in numerous studies including in blood brain barrier (BBB), gastrointestinal tract, and airway epithelial models.[1] Currently, WPI offers a manual system called EVOM™ Manual with the SMARTouch control screen, data storage capability, andother advanced features. Also, WPI currently offers EVOM™ Auto system for automated TEER measurement with wireless control, high throughput, improved measurement accuracy, andadditional advanced features.

EXPERIMENTAL COMPARISON (EVOM2 VS. EVOM™ MANUAL)

Here in this application note, we show some comparative data of cell sample analysis using an older model system: EVOM2 compared with the new model system, the EVOM™ Manual.

Part 1: Bronchial Epithelial Cell Model

Corning 3470 (6.5 mm diameter with 0.4 µm pore polyester membrane) 24 well plate Transwells (N=12) were seeded with 16HBE14o- (Human Bronchial Epithelial Cells) at a cell seeding density of 1.5×10^5 cells/well. These cells were grown with 0.2 mL apical, and 0.5 mL basolateral media volumes. The resistance values of blank Transwells (without any cell seeding) with same media volumes as samples were subtracted from sample resistance values to obtain resistance contributed by cellular layers. Measurements were taken using:

- EVOM2 with STX2 chopstick electrode, and
- EVOM™ Manual with STX4 electrode.

TEER values were calculated by multiplying resistance with membrane area (cell growth area= 0.33 cm^2).

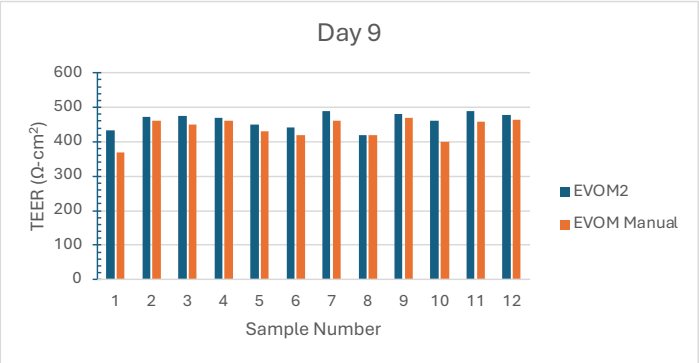


Fig. 1—TEER of 16HBE14o-cells was measured on Day 9 by EVOM2, and EVOM™ Manual.

EVOM™ Manual Shortens TEER Analysis Experimentation Time

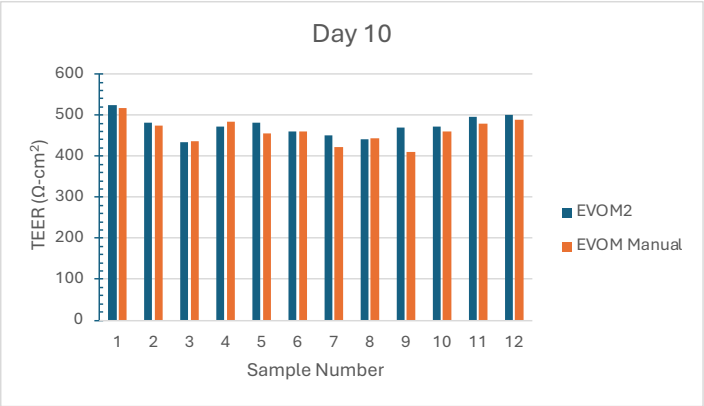


Fig. 2—TEER of 16HBE14o-cells was measured on Day 10 by EVOM2, and EVOM™ Manual.

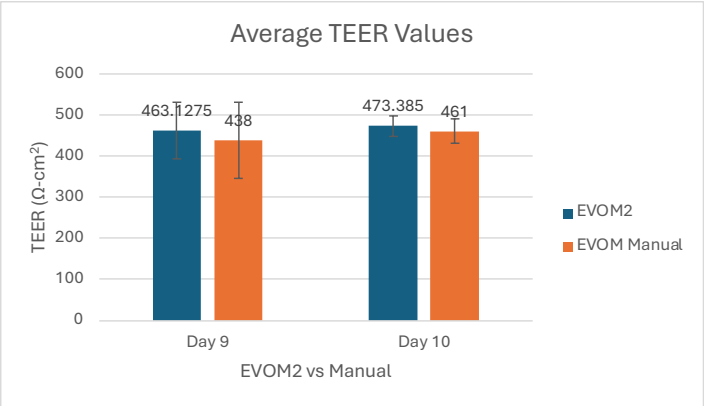


Fig. 3—Average TEER values of 16HBE14o-cells measured by EVOM2, and EVOM™ Manual.

As shown in Fig. 1, 2, and 3, the TEER values of individual samples, and average TEER values of 16HBE14o-cells were very similar when measured by old EVOM2, and new EVOM™ Manual systems.

Next, the experimentation timings were compared between the two systems: EVOM2, and new EVOM™ Manual.

Sample	System	Warm Up Time	Test with Resistor	Total Sampling time with Electrode (including electrode rinses, and data recording)	Time to complete data entry in the excel sheet or process the data in excel for EVOM2 or USB removal, and plug in for EVOM™ Manual.	Time to analyze (calculate the TEER, and calculate the average, and standard deviation)	Total Time
Plate 1 Day 9	EVOM2 with STX2	20 min.	2 min.	11 min.	2 min.	1 min.	36 min.
	EVOM™ Manual with STX4	0 min.	1 min.	6 min.	1 min.	3 min.	11 min.
Plate 1 Day 10	EVOM2 with STX2	20 min.	< 1 min.	10 min.	2 min. + 2.5 min.	< 0.5 min.	~36 min
	EVOM™ Manual with STX4	0 min.	0.5 min.	4 min.	< 0.5 min.	< 0.5 min.	6 min.

Table 1: Comparison of TEER Experimentation times for 16HBE14o-cell sample analysis using EVOM2, and EVOM™ Manual

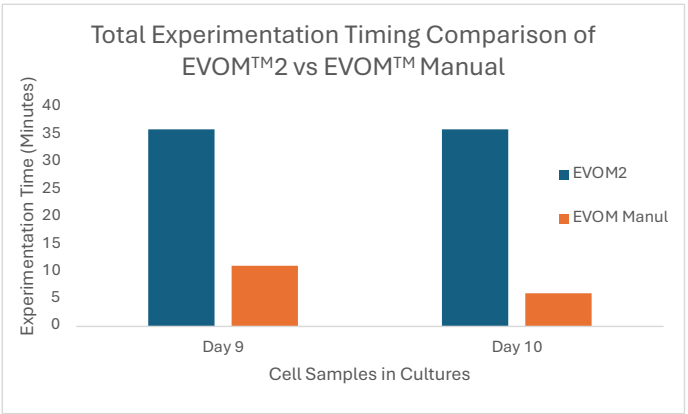


Fig. 4—Comparison of TEER Experimentation times for 16HBE14o-cell sample analysis using EVOM2, and EVOM™ Manual

As shown in Table 1, and Fig. 4, EVOM™ Manual can help users to complete a TEER experiment 3 to 6 times faster as compared to an EVOM2.

[This part 1 study with 16HBE14o-cells was performed Athena Chien Ph.D. Student in Coulter Department of Biomedical Engineering, Georgia Institute of Technology & Emory University: PI: Dr. Craig Forest. All the data shown in Fig. 1-4, and Table 1 were collected by Athena Chien]

Part 2: Caco-2 Intestinal Epithelial Model

The Caco-2 cells (HTB-37) were seeded in sixteen inserts of two 24-well Transwell plates (Corning 3470; 8 seeded inserts/plate) from passage 14 at a cell seeding density of 5.89×10^4 cells/insert with 0.1 mL of apical, and 0.6 mL basolateral media volumes. The cell line was cultured for 21 days, and transepithelial electrical resistance (TEER) values were measured on days 3, 7, 14, and 21 using:

- EVOM2 with STX2 electrode, and
- EVOM™ Manual with STX4 electrode.

Four (4) unseeded inserts per plate were assigned as blanks for the assay. The average TEER values with standard deviations are plotted.

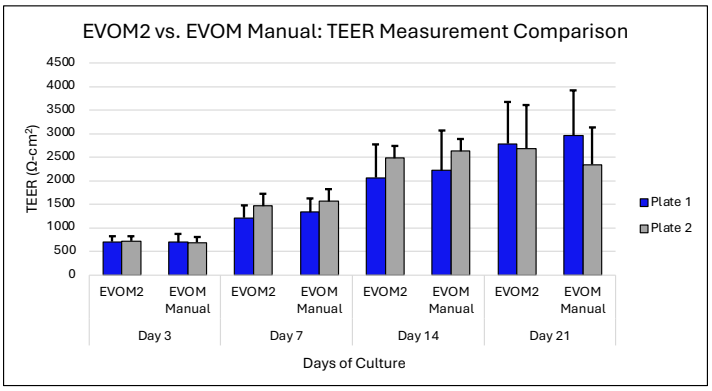


Fig. 5—TEER Measurement of Caco-2 over days in culture

As shown in Fig. 5, the average TEER values of Caco-2 were very similar when measured with EVOM2, and EVOM™ Manual.

Next, the experimentation timings were compared between the two systems: EVOM2, and new EVOM™ Manual for Caco-2 cell sample analysis.

	Instrument Warm Up (min.)	Sample Reading (min.)	Data Entry (min.)	Data Analysis (min.)	Total Time (min.)
Day 3: Plate 1: EVOM2	20	17	5.5	2.5	45
Day 3: Plate 1: EVOM Manual	0	17.25	1	1	19.25
Day 3: Plate 2: EVOM2	20	15.5	5.5	2.5	43.5
Day 3: Plate 2: EVOM Manual	0	7.5	1	1	9.5
Day 7: Plate 1: EVOM2	20	16.08	3	3	42.08
Day 7: Plate 1: EVOM Manual	0	7.47	1	3	11.47
Day 7: Plate 2: EVOM2	20	15.45	3	3	41.45
Day 7: Plate 2: EVOM Manual	0	7.73	1	3	11.73

Table 2: Comparison of TEER Experimentation times for Caco-2 cell sample analysis using EVOM2, and EVOM™ Manual

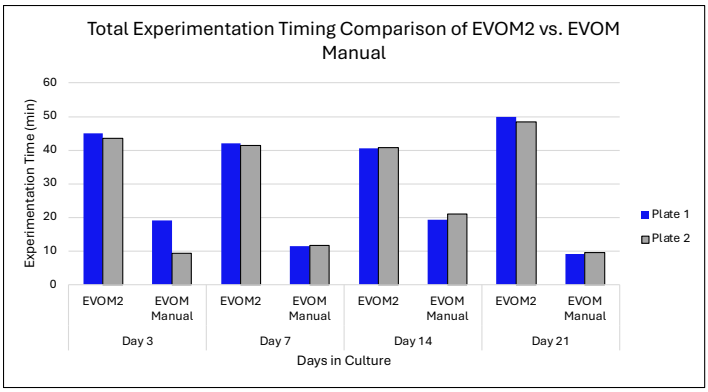


Fig. 6—Comparison of TEER Experimentation times for Caco-2 cell sample analysis using EVOM2, and EVOM™ Manual

As shown in Table 2, and Fig. 6, using an EVOM™ Manual, TEER experimentation times can be shortened to 2-5 times as compared to EVOM2.

[Part 2 study with Caco-2 cells was performed by Cristabel Gordon, and Dr. Deborah Ramsey of SynVivo Inc. (Director: Dr. Gwen Fewell), Huntsville, AL, USA. All the data shown in Fig. 5, and 6, and Table 2 were collected by Cristabel Gordon, and Dr. Deborah Ramsey]

SUMMARY OF RESULTS

As shown with two different cellular models, the TEER measurement can be very similar when measured with EVOM2, and EVOM™ Manual.

The total experimentation time can vary based on individual users, and their level of expertise to perform TEER experiments. EVOM™ Manual allows the ability to store the data in the USB flash drive in Excel/CSV format, whereas in EVOM2 users need to manually write down individual displayed data on a lab notebook, and later manually enter into an Excel data sheet. Also, in EVOM™ Manual, one can easily record data using a footswitch, in EVOM2 it was not possible. With improved internal electronics of EVOM™ Manual, it does not need 20 minutes of warm up time to achieve stable readings after powering on the system. Due to all these benefits, the EVOM™ Manual can shorten the TEER measurement experimentation time 2-6 times. Since EVOM™ Manual does not require data to be manually written down by the user, it minimizes or eliminates the chances of errors associated with manual data recording.

As evident in the data of this application note, EVOM™ Manual can be adopted by EVOM, EVOM2, ERS, , and ERS-2 users to get comparable data with the ability to complete the TEER experimentation faster, and obtain more reliable data. With EVOM™ Manual, WPI offers different types of STX chopstick, and ENDOHM electrodes matching different experimentation platforms: 6, 12, 24 removable inserts, and 24, and 96 high throughput screening plates for accurate measurements. WPI will continue to invent new electrodes, and EVOM™ based measurement systems, and solutions for new emerging fields of research, such as microfluidic chip and organ-on-a-chip (OOC).[2,3] EVOM™ based TEER measurement can be a promising solution to ensure consistent, and functional tissue formation in OOC, and other *in vitro* models.

REFERENCES

Srinivasan, Balaji, et al. "TEER measurement techniques for *in vitro* barrier model systems." *Journal of Laboratory Automation 20*.2 (2015): 107-126.

Elbrecht, Daniel H., Christopher J. Long, and James J. Hickman. "Transepithelial/endothelial Electrical Resistance (TEER) theory and applications for microfluidic body-on-a-chip devices." *Journal of Rare Diseases Research & Treatment 1.3* (2016).

Tu, Kai-Hong, et al. "Development of real-time transendothelial electrical resistance monitoring for an in vitro blood-brain barrier system." *Micromachines 12*.1 (2020): 37.