Bridging Physiological Accuracy and Worldwide Reproducibility: The EpiVaginal™ Model Integrated with Simulated Vaginal Fluid

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Abstract

Background and Purpose: The EpiVaginal model (Mattek), a 3D vaginal tissue system derived from normal human ectocervical epithelial cells, has been developed as an *in vitro* alternative to the Rabbit Vaginal Irritation (RVI) assay to evaluate the safety of vaginal products. This highly differentiated 3D tissue model mimics the structural, physiological, and functional features of native vaginal mucosa and has been successfully applied in studies of vaginal irritation, inflammation, drug delivery, and microbicides. As a primary human cell-based system, EpiVaginal aligns with the goals of New Approach Methodologies (NAMs) by providing a physiologically relevant, reliable, and reproducible assay for specific context of use for hazard and risk assessment of feminine products such as lubricants, hygiene products, microbicides, spermicides, and medical devices. While commercially available in the U.S. for years, the model was only recently introduced in Europe. In this study, we assessed reproducibility and robustness of manufacturing the tissue model by comparing ET-50 values, the exposure time required to reduce tissue viability by 50%, across two production sites (U.S. and Europe). ET-50 values serve as a standardized metric for batch-to-batch and site-to-site comparisons was used to evaluate the model's reliability and reproducibility. To further evaluate prediction power, we tested a panel of widely used vaginal ingredients, in the presence or absence of Simulated Vaginal Fluid (SVF), to mimic the physiological condition..

Methods: EpiVaginal tissues were produced simultaneously by Mattek US (Ashland, MA). and Mattek Slovakia (Bratislava, Slovakia) using normal human vaginal ectocervical (VEC) cells obtained from a healthy donor undergoing hysterectomy for benign conditions. Cells were cultured on 0.6 cm² inserts in a serum-free medium for 2 weeks, under air liquid interphase (ALI) condition to form multilayered, highly differentiated 3D model that recapitulate native vaginal epithelial structure. Each batch underwent QC testing via topical exposure to 1% Triton X-100 (positive control) for 0.5, 1, and 2 h to determine ET-50 values. Ultrapure water exposure for 1 h served as a negative control. ET-50 values between 0.70-1.70 h were used as acceptance criteria. We evaluated batch reproducibility for lots produced in 2024 in the U.S. (n=20) and 2024-2025 in Slovakia (n=31). Additional, experiments performed at Mattek were exposed in two sites (Mattek Slovakia) and the National Institute of Public Health (Prague) by adding 100 µL of two concentrations of five feminine care ingredients topically for 1, 4, and 18.. Endpoint measurements included viability assay (MTT) and barrier integrity (TEER). ET-50 assays were conducted following exposure cytotoxicity profiles (ET 50 values) were compared for test article exposed tissues with and without SVF. Irritation potential confirmed by histology.

Results: ET-50 values for all 51 production lots (U.S. n=20; Slovakia n=31passed the QC criteria (0.70 < ET-50 < 1.70 h) showing high degree of reproducibility. Following 24 h exposure to feminine care ingredients, the coefficient of variation for MTT viability test across eight test articles was 7.2%, with only two exceeding 10%. The coefficient of variation for %TEER was 6.4%, with two exceeding 10%. Toxicity profiles of Boric acid (6%), benzalkonium chloride (0.06%), and benzocaine (5%) showed ET-50 >18 h with minor SVF-related shifts (e.g., BZK: early TEER dip with partial recovery; BEN: gradual TEER decline). Miconazole (2%) exhibited ET-50 between 17.0 and 17.8 h. Nonoxynol-9 (2%) caused severe loss of viability (MTT) and barrier integrity (TEER) regardless of SVF, whereas lactic acid (2%) protected in the presence of SVF (ET-50 >18 h vs <1 h) with higher MTT and partial TEER rescue. Overall, SVF buffered acidity-driven effects but not surfactant-type injury.
Conclusions: The study showed reproducible results between the two laboratories confirming lot-to-lot and site-to-site consistency. Furthermore, SVF improved physiological relevance by mitigating acidity effects (e.g., lactic acid), highlighting its importance in irritation testing. The findings support EpiVaginal as a reliable non-animal alternative for screening feminine care products.

Methods

Tissue Structure: EpiVaginal™ (VEC-100) tissues were simultaneously produced by Mattek US and Mattek Slovakia. Tissues are engineered from normal human vaginal ectocervical (VEC) cells obtained from healthy donors undergoing hysterectomy for benign conditions. Cells are cultured on 0.6 cm² inserts in serum-free medium for ~2 weeks, forming multilayered, highly differentiated 3D models that recapitulate native vaginal epithelium.

Quality Control (ET-50): N=2 tissues from each production lot was treated with 1% Triton X-100 (positive control) for 0.5, 1, and 2 h. Tissues exposed to ultrapure water (negative control) for 1 h were used as negative control. Viability is assessed using the MTT assay: *% Viability = OD(sample)/OD(negative control) × 100*

ET-50 values between 0.70–1.70 h, was used as a QC criteria to qualify tissue lots. In this study, we a total of 51 tissue lots (US (n=20) and in (Slovakia (n=31) were used.

Test Article (TA) Exposure and Endpoints: Tissues produced at Mattek Slovakia were exposed to 100 µL of two concentrations of five feminine care ingredients for 24 h at both Mattek Slovakia and the National Institute of Public Health (Prague). Viability (MTT assay) and barrier integrity (TEER) were assessed and normalized to water-treated controls. % TEER was calculated as: *% TEER = TEER (TA) / TEER (NC) × 100*

Additionally, ET-50 were calculated for selected TAs dosed for 1, 4, and 18, allowing comparison of cytotoxicity profiles in the presence and absence of SVF, and providing further validation of the model's performance under more physiologically relevant conditions. Irritation potential was evaluated based on MTT viability, TEER, and confirmed by histology.

Results

Tissue Structure: The tissue models, produced at Mattek US and Mattek Slovakia, exhibit *in vivo*-like morphological and growth characteristics which are uniform and highly reproducible (**Figure 1A-1B**). QC Testing: For the 51 EpiVaginal lots analyzed in the US (n=20) and Slovakia (n=31), were reproducible with ET-50 value ranging from 0.70 < ET-50 < 1.70 h which matches a historical data collected at MatTek. QC results for tissue batches are summarized in Table 1.

Testing of Feminine care ingredients: A comparison of the tissue viability in 2 labs for time to toxicity was monitored following 24-hour exposure time to the feminine care ingredients (Figure 2). The average difference for the %tissue viability for the 8 TAs was 7.2%; only 2 TAs had a tissue viability difference >10%. The average difference for the %barrier integrity (%TEER) was 6.4%; only 2 TAs had a difference >10%.

Boric acid (BOR, 6%), benzalkonium chloride (BZK, 0.06%), and benzocaine (BEN, 5%) showed ET-50 >18 h with only minor shifts in samples containing SVF (BZK: early TEER dip with partial recovery; BEN: gradual TEER decline), while Miconazole (MIC, 2%) had a modest ET-50 right-shift (~17.0→~17.8 h). Nonoxynol-9 (N-9, 2%) caused early, severe loss of MTT/TEER regardless of SVF, whereas lactic acid (LA, 2%) was markedly protected by the presence of SVF (ET-50 >18 h vs <1 h) with higher MTT, Figure 3. Overall, SVF appears to buffer acidity-driven effects but not surfactant-type injury. ET-50 and SVF: Regarding the ET-50 assays performed at 1, 4, and 18 h (±SVF), integrated with MTT, TEER, and histology, the data suggest a selective, context-dependent modulation of lactic acid effect by SVF (Table 2).

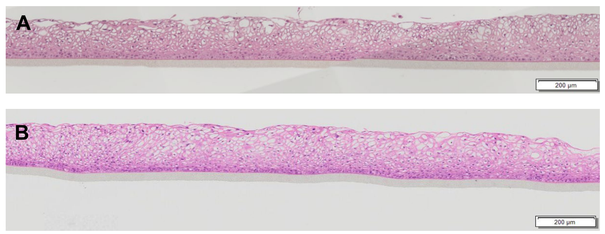

Figure 1: H&E-stained histological cross-sections of EpiVaginal tissue cultured at (**A**) Mattek US and (**B**) Mattek Slovakia

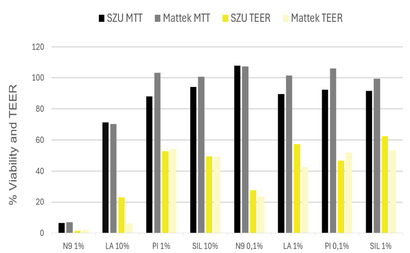

Figure 2: Comparison of tissue viability (MTT) and barrier function (TEER) results for feminine care ingredients tested at 2 concentrations in two testing sites. Test articles include: Nonoxynol-9 (N9), Lactic Acid (LA), Povidone Iodine (PI), Polydimethylsiloxane (SIL). Excellent interlab reproducibility was demonstrated based on the MTT viability and TEER values.

Location	Year	Avg ET-50 (h)	St Dev (h)	Avg Exp CV(%)	# of tissue lots
US	2024	1.0	8.2	8.2	20.0
Slovakia	2024-2025	1.4	9.3	9.3	31.0
US-historical	2006-2007	1.1	9.1	9.1	46.0

Table 1: Reproducibility of EpiVaginal tissue lots produced at Mattek US in 2024 and at Mattek Slovakia during 2024-2025. Results were compared to the historical QC data available from 2006-2007.The average experimental coefficient of variation (Avg Exp CV), a measure of intralot variability of the tissues, is low (<10%).

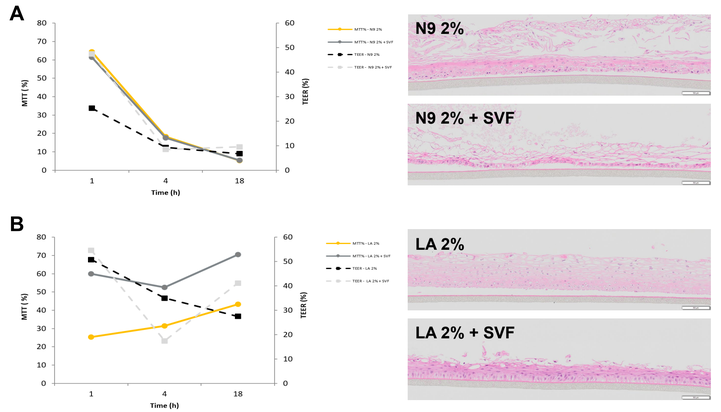

Figure 3: EpiVaginal (VEC-100) response to A) N-9 (2%) and B) lactic acid (2%) ±SVF. Left panels: dual-axis plots of MTT (solid, left axis) and TEER (dashed, right axis) at 1, 4, and 18 h; Yellow solid = MTT (−SVF); Gray solid = MTT (+SVF); Black dashed = TEER (−SVF); Light-gray dashed = TEER (+SVF). Right panels: representative H&E at 18 h. N-9 showed severe loss of viability and barrier integrity in both conditions—SVF does not protect. SVF prevented Lactic acid effect as shown by higher MTT viability; histology also confirmed the rescue effect of SVF for lactic acid exposed tissues. Scale bars as shown.

Material	SVF	ET-50 (h)	MTT% Trend	TEER% Trend
N9 2%	–	2.0	Rapid drop (≤18 h)	Sharp loss within 1–4 h
	+	1.8	Similar to N9	No significant protection
BEN 5%	–	>18	Stable up to 18 h	Preserved
	+	~10	Moderate reduction	TEER gradually reduced
BZK 0.06%	–	>18	Stable up to 18 h	Preserved
	+	>18	Stable up to 18 h	Drop at 4 h with partial rebound
MIC 2%	–	17	Progressive reduction	Maintained until 4 h, drop at 18 h
	+	17.7	Slower reduction	Higher preservation with SVF
BOR 6%	–	>18	Stable up to 18 h	Preserved
	+	>18	Stable up to 18 h	Preserved
LA 2%	–	<1	Immediate drop	Early TEER loss
	+	>18	Partial protection	Partial protection by SVF

Table 2: EpiVaginal (VEC-100) responses to test materials with and without simulated vaginal fluid (SVF) over an 18-h exposure window. ET-50 (h) = time to 50% MTT reduction (>18 = no 50% effect within test window; ~ = approximate value). MTT% Trend and TEER% Trend summarize viability and barrier kinetics, respectively. SVF: "–" = without SVF; "+" = with SVF.

Conclusions

- **Reproducibility:** ET-50 QC met across 51 lots; intralot ET-50 dose–response CV <10%; interlab MTT/TEER agreement (mean differences ≈7.2%/6.4%).
- **Model utility:** Consistent lot-to-lot and lab-to-lab performance across geographies supports EpiVaginal (VEC-100) as a reliable non-animal alternative for screening feminine-care products.
- **SVF effect:** MTT/TEER (with ET-50) indicate SVF buffers acidity/osmolarity-driven effects but not surfactant type injury.

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