Using Reconstructed Human Vaginal Epithelium to Assess the Potential Irritation of Personal Lubricants

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INTRODUCTION

- Personal lubricants can be defined as substances applied to the genital area to enhance comfort during sexual activity.
- Safety testing requirements vary by region and depend on whether they are classified as medical devices.
- Biocompatibility tests supporting marketing approval for medical devices are described, among others, in standards from the International Organization for Standardization (ISO).
- In the US, personal lubricants are classified as class II medical devices, and tests required by the US Food and Drug Administration (FDA) include, among other animal tests, a rabbit vaginal irritation (RVI) test, described in ISO 10993-23:2021.
- The EpiVaginal™ (MatTek Now Part of Sartorius)[1] and the SkinEthic™ Human Vaginal Epithelium (EPISKIN) models are reconstructed human vaginal epithelium (RHVE) models that can be used as *in vitro* test systems for the safety evaluation of personal lubricants.[2]
- This poster proposes a test method qualification plan for an RHVE-based test method to support regulatory decision-making as to whether water-based personal lubricants are biocompatible with the vaginal epithelium.[3]

Methods

Test model: EpiVaginal™ tissue, VEC100, topical application of 100 µL of product or controls.

Exposure time: positive control 1% Triton X-100 for 0.5, 1, or 2 hours, negative controls and products for 1, 4 or 24 hours.

Readout: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

Output: percentage viability (%) and exposure time required to reduce cell viability by 50% (ET_{50} value).

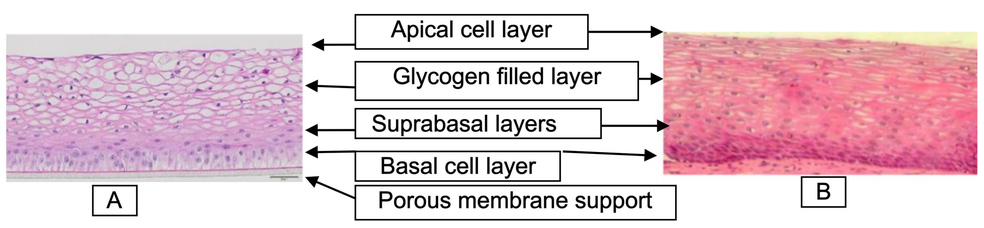

Figure 1: H&E-stained histological cross-sections of (A) EpiVaginal™, and (B) human vaginal explant

Results Proof-of-Concept: The RHVE test method ranked marketed products according to their irritation potential

- Three marketed water-based personal lubricants showed no reduction in tissue viability, with only a mild decrease observed for Personal lubricant 3, formulated with a sensorial ingredient.
- In contrast, vaginal contraceptives containing the known vaginal irritant nonoxynol-9 (N-9) caused decreased tissue viability.

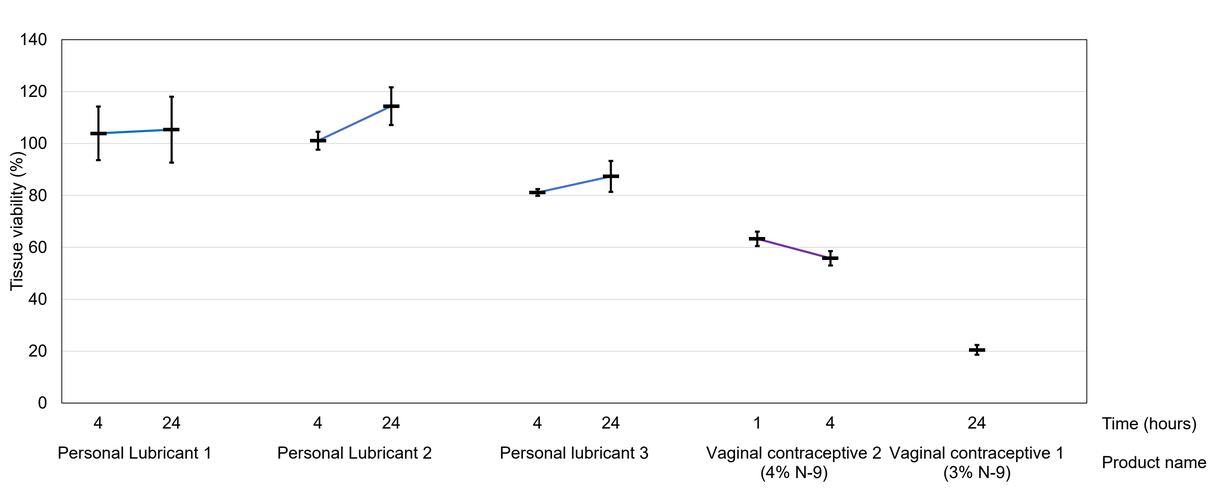

Figure 2: Mean tissue viability (n=2) as a function of time in the EpiVaginal™ model after exposure to five different products. Error bars indicate the absolute difference between the two replicates divided by two.

Table 1: Products and controls tested in the proof-of-concept study

Product or control name	Description
Positive control	1% (w/v) Triton X-100 solution in deionized, sterile water
Negative control	Sterile deionized water or untreated tissues
Personal lubricant 1	Personal lubricant without sensorial ingredients
Personal lubricant 2	Same manufacturer and product line as personal lubricant 3, without sensorial ingredients
Personal lubricant 3	Same manufacturer and product line as personal lubricant 2, with sensorial ingredients
Vaginal contraceptive 1	Product containing 3% N-9
Vaginal contraceptive 2	Product containing 4% N-9

Towards Regulatory Acceptance: Qualification Plan

- There are no well-described personal lubricants known to cause vaginal irritation.
- The proposed strategy to qualify the RHVE test method involves spiking known irritants into a water-based chassis formulation.
- The qualification plan was developed with input from an industry consortium.
- Interactions to promote regulatory integration in a non-product-specific manner:
  - ISO Technical Committee 194
  - FDA's Medical Device Development Tool program[4]

Table 2: Products for qualification plan step 1 and 2

Product	Label	Qualification step
Chassis formulation: 5% glycerin, 0.7% hydroxyethylcellulose, 0.19% potassium sorbate, 0.36% sodium benzoate, and 0.19% citric acid anhydrous	Acceptable	1
Chassis + 2% benzalkonium chloride (BZK)	Non-acceptable	1
Chassis + 0.1% BZK	Acceptable	1
Chassis + 2% sodium dodecyl sulfate (SDS)	Non-acceptable	1
Chassis + 8% Chlorhexidine gluconate	Non-acceptable	2
Chassis + 10% Phenoxyethanol	Non-acceptable	2
Chassis + 8% Cinnamaldehyde	Non-acceptable	2
Chassis + Glycolic acid pH = 2.5	Non-acceptable	2
Chassis + Lactic acid pH = 2.5	Non-acceptable	2
Chassis + 8% Nonoxynol-9	Non-acceptable	2
Chassis + 8% Cetrimonium chloride	Non-acceptable	2
Chassis + C31G	Non-acceptable	2
Marketed personal lubricant 4	Acceptable	2
Marketed personal lubricant 5	Acceptable	2

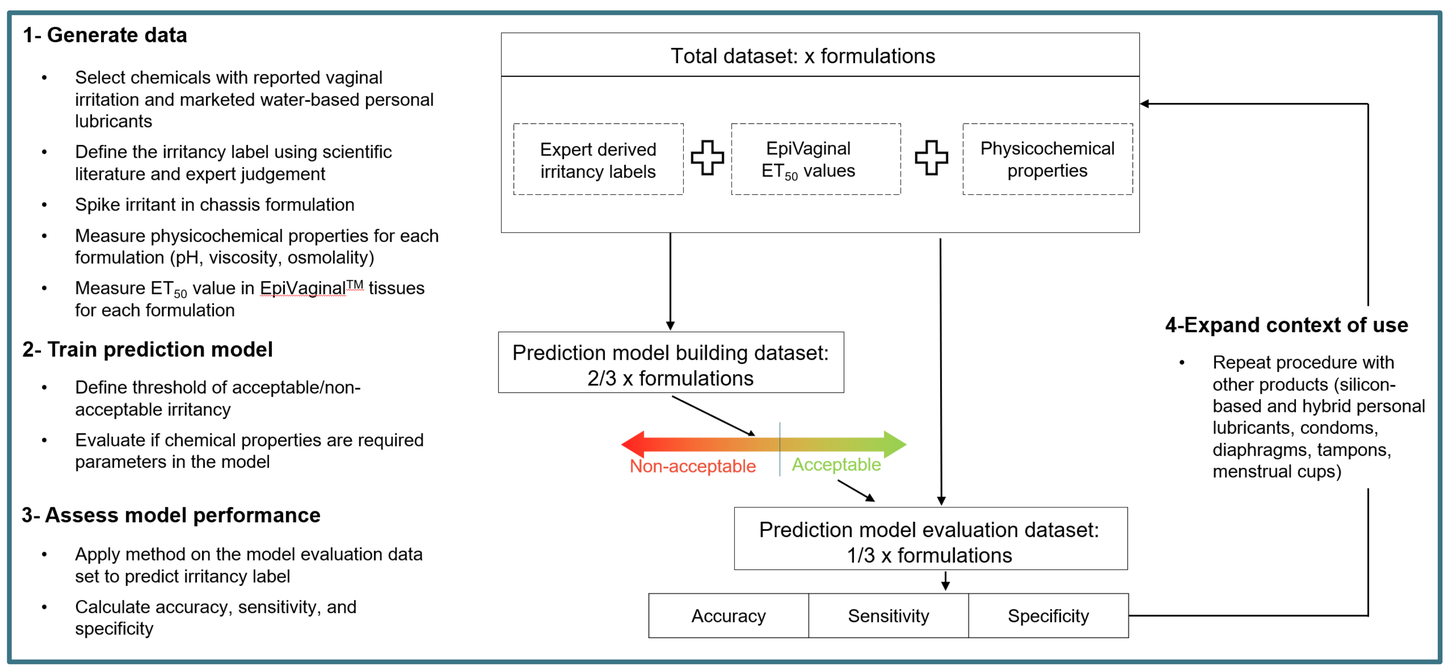

Figure 3: Qualification plan

Results Qualification Step 1: The RHVE test method ranked spiked-in products according to their irritancy label

Table 3: ET_{50} values and labels for products tested in qualification step 1

Product	Label	ET_{50} value
Chassis formulation	Acceptable	> 24 hours
Chassis + 2% BZK	Non-acceptable	0.02 hours
Chassis + 0.1% BZK	Acceptable	3.2 hours
Chassis + 2% SDS	Non-acceptable	1.3 hours

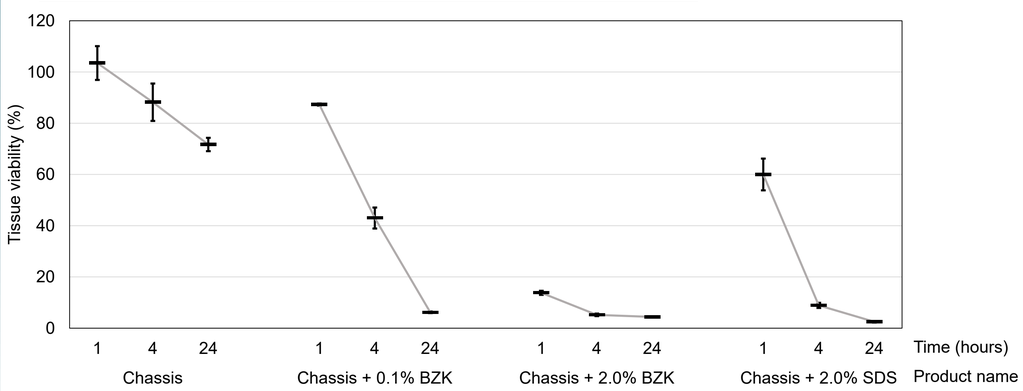

Figure 4: Mean tissue viability (n=3) as a function of time in the EpiVaginal™ model after exposure to chassis and spike-ins. Error bars indicate the standard deviation.

Conclusions and Outlook

- The RHVE test method demonstrated the ability to rank products according to vaginal irritation potential, supporting its development as a human-relevant alternative to the rabbit vaginal irritation (RVI) test used in regulatory biocompatibility assessments.
- The data generated to date provide a scientific foundation. However, formal qualification is required to support regulatory replacement of the RVI for water-based personal lubricants.
- The ongoing qualification plan is designed to generate the data needed for regulatory confidence and integration -> **see Poster "Framework-Guided Qualification of Non-Animal Methods".**
- The context of use could expand to other products contacting the vaginal mucosa, such as tampons, menstrual cups[5] or condoms.

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