

Framework-Guided Qualification of Non-Animal Methods: Case Study for Assessing Vagina Irritation Potential

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Advancing 21st Century Toxicology

Replacing animal tests

- The European Commission will publish its roadmap to phase out animal testing in chemical safety assessments—including those for medical devices and human-use medicinal products—in **April 2026**.
- Accelerating the transition to animal test-free regulations requires **validating and qualifying reliable non-animal methods** for efficacy and safety testing, including ***in vitro* assays** and **computational tools** that generate robust, human-relevant data.
- Creating **safe spaces for dialogue, collaboration, and data sharing** will be an essential early step in enabling this shift.
- Medicinal products are assessed under internationally harmonised frameworks, for example:
 - ICH guidelines**, which define expectations for quality, safety, and efficacy
 - European Pharmacopoeia**, which sets legally binding standards for test methods, specifications, and reference materials
 - ISO standards**, which outline requirements for biocompatibility, risk management, and performance evaluation
- ISO standards are the primary global reference for medical devices.
- Regulatory authorities such as the **European Medicines Agency**, the **US Food and Drug Administration**, and the **Pharmaceuticals and Medical Devices Agency** issue additional guidance documents that clarify local expectations for study design, conduct, and reporting.
- Many *in vivo* tests included in these international standards were never comprehensively evaluated for **human predictivity or reproducibility**.
- New methods—particularly **non-animal approaches**—must undergo **robust confidence-building** before they can be accepted for regulatory use.

Building scientific confidence in non-animal methods

- Validation builds scientific confidence in a method's **reliability, biological relevance, and context of use**, supported by independent review and transparent communication.
- This poster applies a published framework for building scientific confidence in non-animal methods (Figure 1)¹ that is also relevant to the qualification of methods.

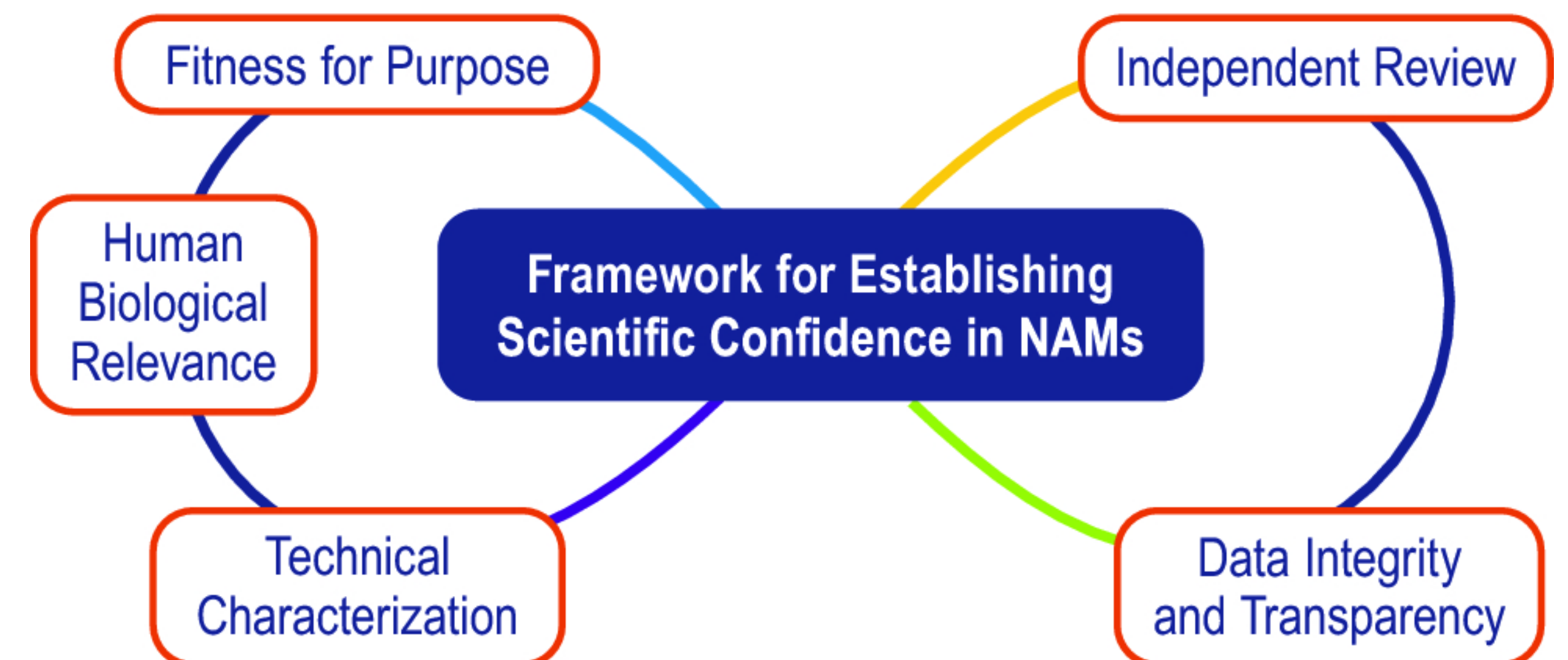


Figure 1. Framework for establishing scientific confidence in NAMs (from van der Zalm et al. 2022). Following these five criteria will increase scientific confidence in, and thus use and regulatory acceptance of, non-animal methods.

- A recent proof-of-concept study demonstrates how this framework can support the regulatory qualification of non-animal methods by showing that the rabbit vaginal irritation test can be replaced with an *in vitro* reconstructed human vaginal epithelium (RHVE) method for assessing the irritation potential of water-based personal lubricants.
- In several countries, including the **United States**, personal lubricants are regulated as **medical devices**, making this an important example of regulatory innovation.

Proof-of-concept study

A reconstructed human vaginal epithelium model to assess irritation

- The EpiVaginal model was used to evaluate five water-based products (three personal lubricants, two vaginal contraceptives containing a known vaginal irritant) and a mock lubricant chassis formulation prepared specifically for this study.
- This *in vitro* method can rank products according to their vaginal irritation potential.
- For further details, please see the poster: Using Reconstructed Human Vaginal Epithelium to Assess the Potential Irritation of Personal Lubricants.

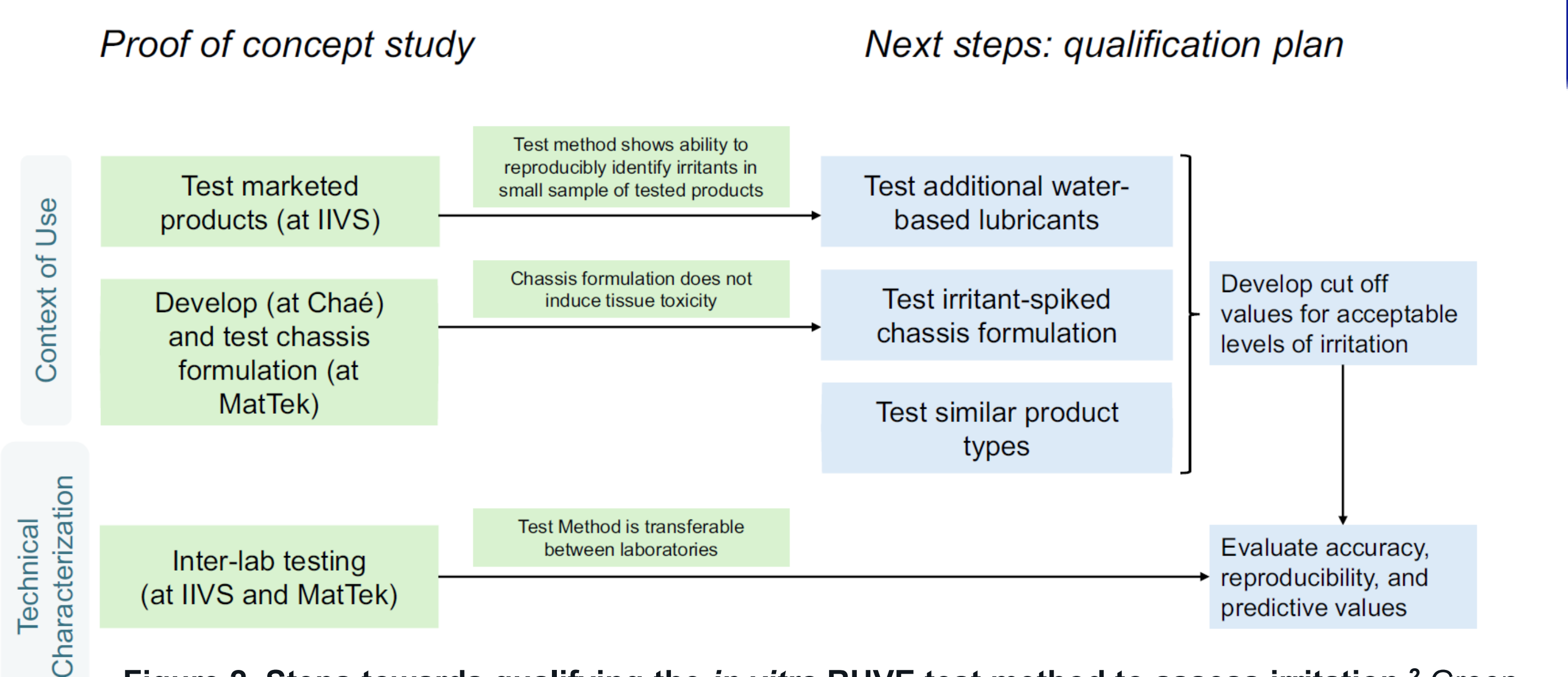


Figure 2. Steps towards qualifying the *in vitro* RHVE test method to assess irritation.² Green rectangles correspond to the published proof-of-concept studies, and blue rectangles indicate the next steps. IIVS: Institute for *In Vitro* Sciences.

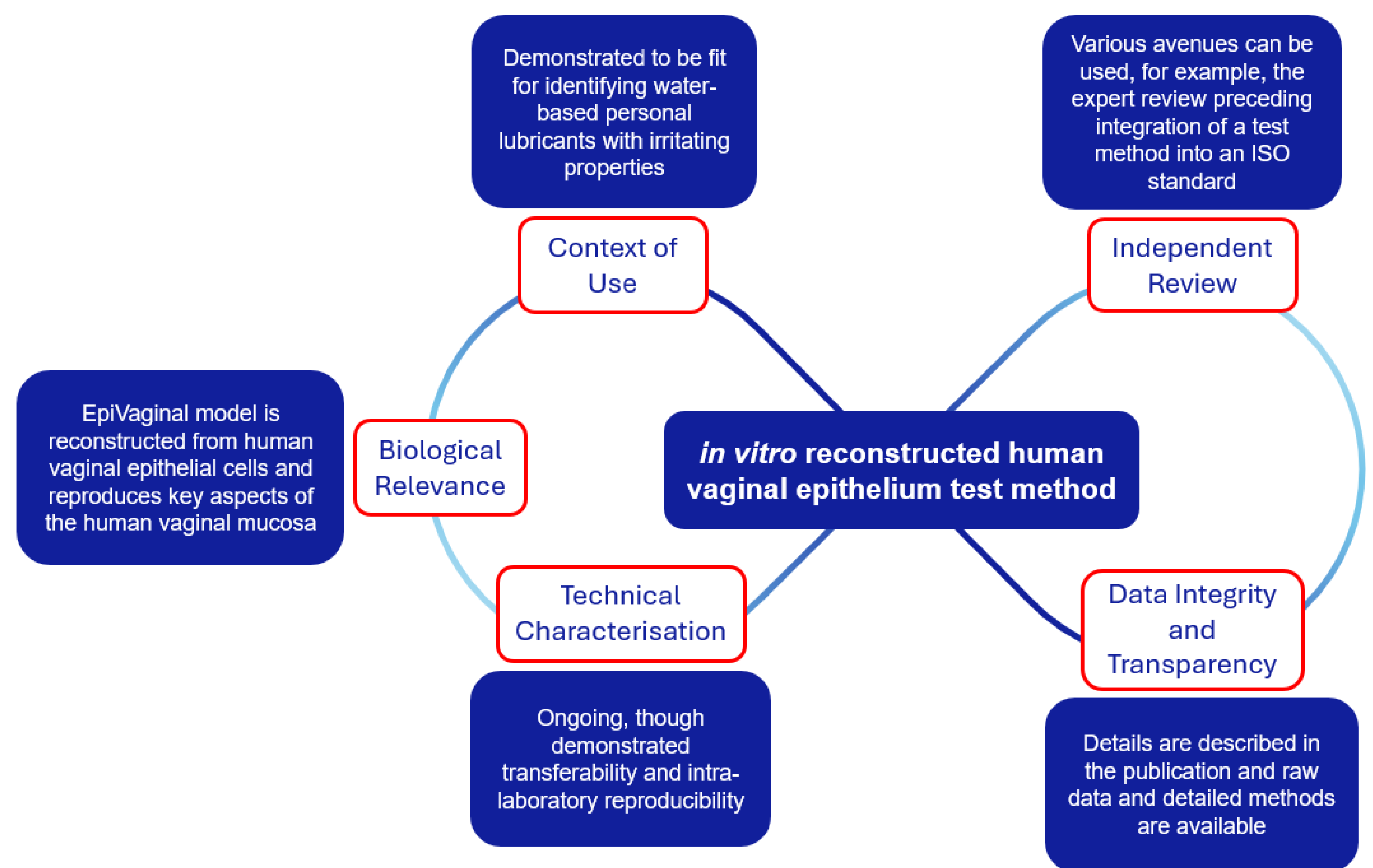


Figure 3. The *in vitro* test method based on the human tissue-derived EpiVaginal model is evaluated following the five criteria of the framework to establish scientific confidence in non-animal methods.

Safe spaces for discussion

- A key enabler for applying this framework across regulatory contexts is the creation of **“safe spaces”** for discussing and sharing data from non-animal testing strategies.
- In the pharmaceutical sector, the **EMA provides structured pathways** that allow scientists and companies to present data from non-animal methods and receive regulatory feedback.
- The **voluntary data submission (“safe harbour”) procedure** separates the qualification of new testing strategies from marketing application processes that still rely on animal tests.
- This procedure is currently **under revision** and could help strengthen scientific confidence in data generated from non-animal methods and guide the development of **qualification criteria**

Broad application of the framework

This framework has been and continues to be applied to non-animal methods across regulatory sectors:

- In vitro* and *ex vivo* methods used in defined approaches to assess eye corrosion/irritation potential of agrochemical formulations³
- In vitro* reconstructed human respiratory epithelium to assess respiratory tissue irritation
- In vitro* monocyte activation test to meet pyrogen testing requirements for medical devices

References

- van der Zalm, A., Barroso, J., Browne, P., et al. (2022). A framework for establishing scientific confidence in new approach methodologies. *Archives of Toxicology*, 96(11):2865–2879. DOI: 10.1007/s00204-022-03365-4.
- Perrin, J., Costin, G.E., Ayehunie, S., et al. (2026). Using a reconstructed human vaginal epithelium model to assess irritation: A proof-of-concept study supporting regulatory qualification of the method for use with personal lubricants. *Toxicology In Vitro*. DOI:10.1016/j.tiv.2026.106198
- van der Zalm AJ, Daniel AB, Raabe HA, et al. Defined approaches to classify agrochemical formulations into EPA hazard categories developed using EpiOcular™ reconstructed human corneal epithelium and bovine corneal opacity and permeability assays. *Cutaneous and Ocular Toxicology*. 2024;43(1):58-68. doi:10.1080/15569527.2023.2275029