



Qualification of a non-animal vaginal irritation method admitted as nonclinical assessment model (NAM) in the Incubator Phase of the United States Food and Drug Administration (US FDA) Medical Devices Development Tool (MDDT)

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ABSTRACT

The U.S. Food and Drug Administration (FDA) Center for Devices and Radiological Health (CDRH) classifies personal lubricants as Class II medical devices. Because of this status and the nature of body contact common to personal lubricants, CDRH reviewers routinely recommend a standard biocompatibility testing battery that includes: an *in vivo* rabbit vaginal irritation (RVI) test; an *in vivo* skin sensitization test, such as the guinea pig maximization test (GPMT); and an *in vivo* acute systemic toxicity test using mice or rabbits. These tests are conducted using live animals, despite the availability of *in vitro* and other non-animal test methods that may be suitable replacements. The only test included in the biocompatibility battery currently conducted using *in vitro* assay(s) is cytotoxicity. FDA's recently launched Predictive Toxicology Roadmap calls for the optimization of non-animal methods for the safety evaluation of drugs, consumer products and medical devices. In line with these goals, a Consortium comprising the Institute for *In Vitro* Sciences, Inc. (IIVS), industry, the Consumer Healthcare Products Association (CHPA), and the PETA International Science Consortium (PETA-ISC) is qualifying the use of an *in vitro* testing method as replacement for the RVI test. Participating companies include manufacturers of personal lubricants and those interested in the advancement of non-animal approaches working collaboratively with the FDA CDRH to develop an *in vitro* testing approach that could be used in place of the RVI in pre-market submissions. Personal lubricants and vaginal moisturizers with diverse chemical and physical properties (e.g., formulation, viscosity, pH, and osmolality) in their final undiluted form will be the focus of the program. *In vitro* vaginal irritation data generated using commercially available human re-constructed vaginal tissue model(s) will be paired with existing *in vivo* RVI data and analyzed to develop a Prediction Model for the safety assessment of these products. This research plan has been accepted into the FDA CDRH Medical Device Development Tools (MDDT) program as a potential non-clinical assessment model (NAM). The proposed NAM aligns with the goals of the recently launched FDA Roadmap to integrate predictive toxicology methods into safety and risk assessment with the potential to replace or reduce the use of animal testing.

1. Introduction

Historically, the safety assessment of raw ingredients and finished products has been performed using animal-based test methods to provide whole organism responses to toxicants. The toxicologist relied upon the animal tests to closely predict human response to hazards despite the natural differences in anatomy and physiology. The animal tests are known for their lack of reproducibility and predictive accuracy of human responses and also involve subjectivity of endpoints

interpretations. For example, despite being criticized due to its subjectivity and lack of reproducibility, and based on animal welfare concerns, the Draize eye irritation test is still in use with minimal modifications since 1944 (Draize et al., 1944). A study by Adrieans et al., 2014 resampled the Draize eye test results from more than 2000 studies and showed an overall probability of at least 11% that chemicals classified as Category 1 according to the United Nations Globally Harmonized System (UN GHS) could be equally identified as Category 2 and of about 12% for Category 2 chemicals to be equally identified as

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No Category. More recently, a study by Luechtefeld et al., 2015 analyzed results of Draize experiments and related data available in European Chemical Agency (ECHA) chemical dossiers and concluded that there was a 10% chance of an eye non-irritant evaluation after a prior severe-irritant result according to GHS classification criteria. Overall, the study concluded that most reproducible outcomes were negative (94% reproducible) and severe eye irritant (73% reproducible), respectively. For the skin sensitization endpoint, a study by Kolle et al., 2013 showed that by retesting 22 LLNA (Local Lymph Node Assay) performance standards using the standard LLNA protocol, a reproducibility of only 77% was determined. Furthermore, a recent study by Hoffmann et al., 2018 showed that for hazard identification, the LLNA had a 78% concordance with itself, while the potency categorization was 63–73% (depending on the summary metric used), further emphasizing the variability of animal data.

In recent years, these types of ethical and scientific considerations have led to the modernization of predictive toxicology through the acceptance of non-animal test methods and strategies, many of which have been validated for regulatory purposes. These technologies support effective global product stewardship principles and also ensure adherence to the 3Rs, which call for the replacement, reduction and refinement of animal use to label products or to receive pre-market clearance like in the case of the specific class of personal lubricants.

As defined by the FDA CDRH, personal lubricants are products “for penile and/or vaginal application, intended to moisturize and lubricate, to enhance the ease and comfort of intimate sexual activity, and supplement the body's natural lubrication”, all of which are regulated by the Agency as Class II medical devices (FDA, 2019a). Prior to marketing a personal lubricant, Sponsors (manufacturers of the products) must receive pre-market clearance from CDRH based in part on the results of a battery of biocompatibility assays intended to assess a device's ability to function without harming living tissue. Generally speaking, CDRH reviewers recommend specific ISO testing guidelines that require the use of animals when defining these recommended biocompatibility tests, including the rabbit vaginal irritation (RVI) test per ISO 10993-10:2010 (ISO, 2010), the guinea pig maximization test (GPMT) per ISO 10993-10:2010 (ISO, 2010), and an acute systemic toxicity test using mice or rabbits per ISO 10993-11:2006 (ISO, 2006). Each of these tests has been in use for decades with little to no refinement or innovation. They are painful for the animals used, and animals may die during the test or be sacrificed to complete the test. The only *in vitro* test included in the biocompatibility battery is the assessment of cytotoxicity, which is conducted using either the MEM Elution or Agar Overlay assays in accordance to the ISO 10993-5:2009 guideline (ISO, 2009) (Table 1).

Carrying out new animal-based tests for each new product, though, is not strictly required by CDRH. As the Agency writes in its guidance on medical device biocompatibility testing, each of these testing needs can be met using approaches other than what is recommended by Agency reviewers, regulations, or guidance. Sponsors can avoid carrying out new animal tests through the use of existing data, skin testing on human volunteers, or by providing a rationale for why a specific endpoint does not require additional assessment (FDA, 2016). This flexibility creates opportunities for the use of rapidly evolving technologies that routinely lead to the development of non-animal methods that can directly replace the use of animal tests. In 2017, the

FDA co-sponsored a National Research Council report that emphasized the need to shift away from animal-based tests on the basis of their drawbacks in terms of human relevance and expense. That same year, the FDA published its Predictive Toxicology Roadmap, which notes that new technologies can simultaneously expand the capacity to ensure that new devices reach the market with improved human safety and effectiveness profiles while moving away from reliance on animal tests (FDA, 2017). Nevertheless, the process of formally validating a test method as a replacement for a historically accepted animal test can be prohibitively time and resource intensive. The Roadmap, though, notes that adequately describing how a new method will be used within a specific context of use may be a more efficient approach for the Agency to gain confidence in that method's usefulness rather than fully validating it for a wider range of possible applications.

Even though non-animal methods that can replace these tests in theory are available, Sponsors have struggled to successfully adopt them in practice. In essence, the availability of a test method that may seem to replace the need for an animal test is not a guarantee that Agency reviewers will accept or even be familiar with it. The question remains on how to increase Agency reviewers' comfort with new test methods without relying on time-consuming, expensive formal validation studies. Qualification, rather than validation, is intended to provide assurance that the FDA will accept the results of new tests; in this regard, the FDA's MDDT program is the ideal opportunity to advance the field faster and in a targeted, cohesive manner while employing partnerships between industry and regulators.

2. Implementing new approach methodologies for regulatory use

The FDA CDRH MDDT program provides a standardized pathway for the FDA to modernize testing approaches by qualifying tools that can be used in the development and evaluation of medical devices in line with the Predictive Toxicology Roadmap. In its final guidance on the MDDT program published in 2017, CDRH notes that the voluntary process of developing and proposing the use of a tool through its MDDT program will speed up medical device tool evaluation by providing an efficient and predictable way for test method developers to work with the Agency and regulated industries to collect exactly the information needed for CDRH to deem a new method qualified for use within a given context of use.

New test methods can be qualified as MDDTs if they are intended to assess features of medical devices, including safety. The qualification process establishes the scientific rigor of an MDDT for a specific use in supporting regulatory decision-making. Under the program, CDRH encourages developers to make their qualified MDDTs publicly available so that they can be used by any Sponsor of a device that falls within the defined context of use. Once the Agency has qualified an MDDT, reviewers will accept the tool without the need to reconfirm that the method can be used in a regulatory submission and decision making.

Several MDDTs have already been qualified by CDRH (FDA, 2018). They can be used for a number of purposes, including to predict the safety or *in vivo* performance characteristics of a device; specifically, CDRH's guidance on the program notes that MDDTs can replace the use of tests that use animals. In this context, IIVS initiated the development of a MDDT to replace the RVI test by an *in vitro* method based on human reconstructed tissue models. A collaborative, non-competitive

Table 1
Battery of biocompatibility tests currently conducted for personal lubricants submissions.

Biocompatibility test	ISO guideline	Test system
Rabbit Vaginal Irritation Test (RVI)	ISO 10993-10:2010	<i>in vivo</i>
Guinea Pig Maximization Sensitization Test	ISO 10993-10:2010	<i>in vivo</i>
Acute Systemic Toxicity	ISO 10993-11:2006	<i>in vivo</i>
Cytotoxicity Using a Direct Contact Method: MEM Elution or Agar Overlay	ISO 10993-5:2009	<i>in vitro</i>

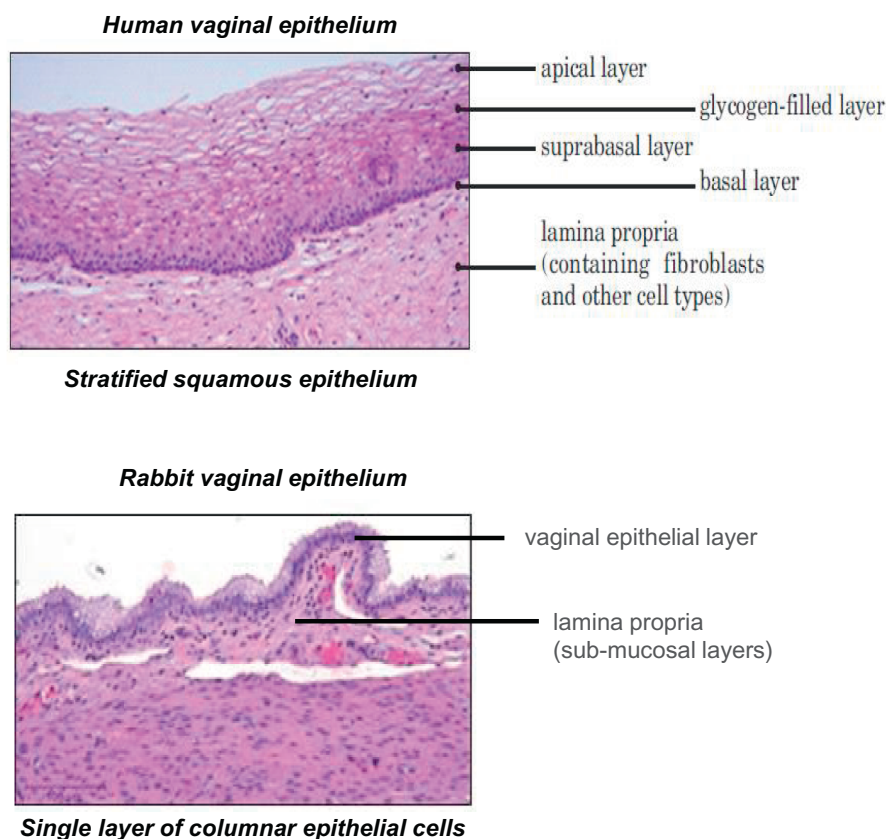


Fig. 1. A comparison between Hematoxylin & Eosin (H&E)-stained human and rabbit vaginal epithelium, respectively. Figures reproduced from Costin et al., Vaginal irritation models: the current status of available alternative and *in vitro* tests, ATLA 39, 317–337 (2011).

Consortium of leading personal lubricant manufacturers, IIVS, the PETA International Science Consortium (PETA-ISC) and the Consumer Healthcare Products Association (CHPA) has been created with the goal to work together and share the resources needed to ease the burden on individual companies that otherwise would have to demonstrate that a given test method had been evaluated through product-specific validation. IIVS, PETA-ISC, and CHPA recruited industry partners to participate in the project, and PETA-ISC held initial meetings with CDRH on the value of using the MDDT program approach to replace the RVI. As the project moves forward, industry partners will share historic testing data and contribute product samples for further testing. IIVS will serve in the program management role and as the *in vitro* testing laboratory conducting the safety assessment of the products submitted by industry partners to the program.

The proposed non-animal vaginal irritation method (described in the next section) has been accepted by FDA into the Incubator Phase of the MDDT program. This optional phase has been created by CDRH after considering the proposal of high potential public health impact that would need further development with the Agency's support. In the Incubator Phase, the MDDT platform provides a mechanism for discussing early concepts about the tool, fosters collaboration on the tool development and potentially increases its adoption and use when qualified.

3. Overview of the NAM based on an *in vitro* test method as replacement for the RVI test

To support clearance of a personal lubricant, CDRH requires an evaluation of irritation as part of the biocompatibility assessment of the device. The RVI test conducted per ISO 10993-10:2010 (ISO, 2010) might be used to meet this requirement, despite its well-established limitations. One of the shortcomings relates to the structural differences

between rabbit and human vaginal tissue (Costin et al., 2011): for example, two-thirds of the rabbit vagina is lined by columnar epithelium, which is structurally distinct from the stratified squamous epithelium (8–12 cells thick) of the human vagina (Fig. 1), and is also much more sensitive to many vaginal irritants than its human counterpart (Eckstein et al., 1969). The RVI test features an extended contact time between the tissue and the test materials applied to the vaginal mucosa, potentially exaggerating normal in-use human exposure. Moreover, rabbits lack cyclic reproductive stages, vaginal *Lactobacilli* and acidity, and cervical mucus production. Rabbits are also unresponsive to most human genital pathogens (Noguchi et al., 2003). Finally, the RVI test is based on a subjective evaluation of tissues' responses to test materials.

Scientific and ethical considerations are the main drivers of the cosmetic and personal care industry position to replace animal procedures with *in vitro* testing strategies and methodologies that are relevant to human response to Class II medical devices. One of the most promising *in vitro* methods is based on human reconstructed vaginal tissue models and represents the basis of the NAM described herein. Commercially available human reconstructed tissue models considered are: EpiVaginal™ from MatTek Corporation (Ashland, MA, USA) (Aychunnie et al., 2006; MatTek Corporation, 2019) and Human Vaginal Epithelium (HVE) from SkinEthic (Lyon, France) (Schaller et al., 2003; Schaller et al., 2005; EpiSkin, 2019) (Fig. 2). The proposed test system represents an advance in the safety assessment of final formulations that is not offered by other *in vitro* assays (Fichorova et al., 1997) due to solubility challenges posed to the test system (cell lines). The use of cell lineages of human origin for the preparation of reconstructed tissue models represents an advantage compared to the animal model that eliminates the need for inter-species extrapolations. Furthermore, the complexity of the reconstructed tissues allows the topical application of full strength lubricants (gels, creams, etc.) in much the same way that the products are used by consumers, as well as the mode of application

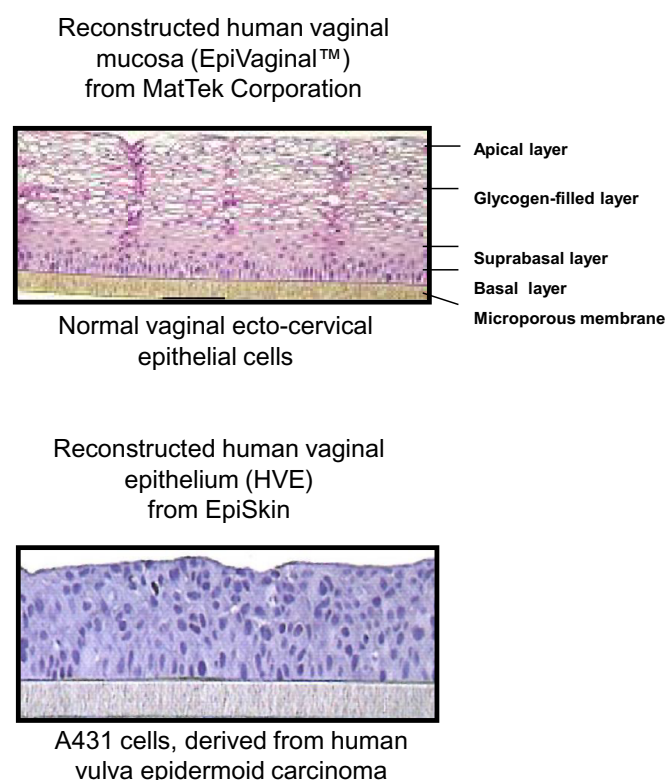


Fig. 2. A comparison between H&E -stained vaginal epithelium of: reconstructed human vaginal mucosa from MatTek Corporation (Ashland, MA, USA), and EpiSkin (Lyon, France). Images were provided by the manufacturers, and are available online at <https://www.mattek.com/products/epivaginal/> and <http://www.episkin.com/en/HVE%20Vaginal%20Epithelium>., respectively. The legend indicating the reconstructed tissue models' cell layers applies to both models, however it is displayed only once to avoid repetition.

in the RVI test.

Lubricant test materials will be selected to address a variety of chemical and physical properties (e.g., formulation, viscosity, pH, and osmolality) and will be applied topically onto the surface of the reconstructed tissues (usually undiluted if not otherwise instructed by the manufacturer/supplier of the products). The toxic effects will be assessed based on tissue viability as the main endpoint which is considered to closely mimic the safety assessment provided by the RVI test. The test materials included in the NAM will be separated into the following groups: Group 1 (Hypothesis Generating Group) will be comprised of products with historical animal data (RVI) and mostly new *in vitro* data tested un-blinded. After a data correlation analysis, a provisional Prediction Model will be generated to the best alignment of the *in vivo* and *in vitro* data sets. Group 2 (Confirmatory Group) will contain products with historical RVI and *in vitro* data conducted in a blinded

manner by IIVS. The decoding will take place after the data analysis is performed to determine if the Prediction Model correctly categorized products in Group 2 within acceptable limits (Hill et al., 2018).

The tissue viability endpoint will be expressed and analyzed as a percent of the negative control-treated tissues and/or based on ET₅₀ values representing the duration of exposure resulting in a 50% decrease in the viability of the test material-treated reconstructed tissue; the vital dye of choice will be MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) unless otherwise determined during the execution of the testing plan; histological evaluation, cytokine analysis and possibly other endpoints may be considered if determined necessary during the conduct of the research experiments.

It is anticipated that the use of this *in vitro* test system will address irritation potential by the use of a cytotoxicity assay (e.g., MTT test) based on the success of several *in vitro* assays previously validated against sets of *in vivo* data to predict human responses to toxicants for other safety endpoints (skin irritation, skin corrosion, eye irritation, etc.) (OECD, 2015; OECD, 2016; OECD, 2018). Specific cut-off values for the tissue viability endpoint have been optimized during multiple validation studies to allow accurate predictions of the animal response and separate test materials into various categories of irritation potential they may induce to humans upon exposure which is a concept transferable to the current NAM.

4. Progress report and future plans

Through its mission, IIVS employs several programs to promote the acceptance of *in vitro* methods with government agencies in the US and internationally. Most of these programs are in collaboration with industry and animal protection organizations and have as ultimate goal the assistance of regulators in learning about the advantages of non-animal methods so they may be considered in regulatory decisions. Based on the vast experience of IIVS and past successful partnerships with other players vested in the success of the 3Rs, taking the lead on the development of an alternative method to the RVI test seemed a perfect fit filling a gap in the scientific approaches to regulatory decisions and a not yet addressed need of industry regarding product stewardship and safety assessment for personal lubricants and vaginal moisturizers.

The MDDT program provides a way for the FDA to qualify tools that can be used in the development and evaluation of medical devices. The voluntary qualification process began by submitting a project proposal developed by the Consortium that provided sufficient information for CDRH to understand the tool, how it is intended to be used, and a brief overview of a plan for collecting the evidence needed to support its use. CDRH evaluated the initial submission and accepted the NAM briefly described in this manuscript as MDDT029 in the Incubator Phase. In the two years following acceptance, there have been numerous exchanges with CDRH regarding the tool and meant to decide whether it concurs with available supporting evidence that the tool produces scientifically-plausible measurements and works as intended within the specified

Table 2

Timeline and milestones of the Medical Devices Development Tool MDDT029 (NAM), Pre-Qualification Package (PQP) Q170887.

Major activities	Milestones	Completion
MDDT029	Submission to US FDA-Center for Devices and Radiological Health (CDRH)	December 2016
	Admission into the Pilot Program in the Incubator Phase	January 2017
Q170887	Receipt of written feed-back from the US FDA	January 2017
	Response submitted by IIVS	May 2017
	Review of request for pre-submission, informational meeting request	June 2017
	Initial discussion of the research plan for the validation program with the US FDA	August 2017
	Supplement 001 to PQP: Q170887 regarding testing strategy	17 May 2018
	Introducing the program to other Industry members and assess their interest in participation	Ongoing
Validation Program	<i>In vitro</i> testing	To be determined
	Data review	
Qualification Package	Final submission including validation data and proposed prediction model	To be determined

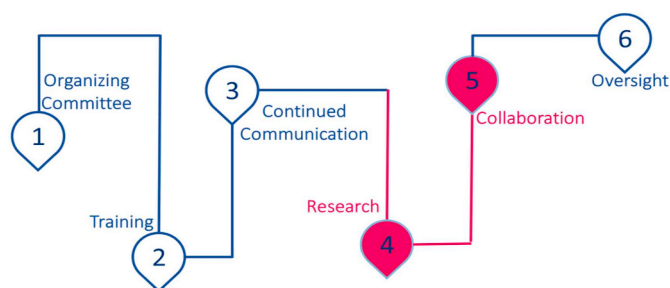


Fig. 3. Representation of the six-part US FDA's Predictive Toxicology Roadmap. Through research and collaboration with stakeholders, the proposed NAM described in this manuscript aligns with the goals to integrate *in vitro* predictive toxicology methods into safety and US FDA's Predictive Toxicology Roadmap risk assessment with the potential to replace or reduce the use of animal testing.

context of use. As submitter of the MDDT, IIVS can meet with FDA before fully developing the proposal, via the Agency's Q-Submission Program (FDA, 2019) (refer to Table 2 for the current status of activities related to the MDDT029). The project has been assigned Q170887 as identifier of its progress through the MDDT program.

When qualified, the NAM described in this manuscript will use an *in vitro* testing approach to substitute for the RVI test when biocompatibility testing for vaginal irritation is required to support: a clinical trial (IDE – Investigational Device Exemption) or a marketing application submissions (510k, PMA – Premarket Approval) or a *de novo* application for personal lubricants and vaginal moisturizers in their final, undiluted, formulations with chemical and physical properties within the boundaries of products included in the qualification package (e.g., formulation, viscosity, pH, osmolality) and that are regulated as medical devices in CDRH.

The NAM discussed herein could provide an example of how to incorporate an *in vitro* assay into regulatory review, expand predictive toxicology capabilities, contribute significantly to the reduction of animals use for testing and, importantly, to foster dialogue and feedback among all relevant stakeholders. This type of collaborative work and partnership between industry, regulators and other organizations supporting non-animal testing methodologies is the solution for implementation of modern predictive toxicology platforms that can support regulatory decisions. Through research and collaboration with stakeholders, the proposed NAM aligns with the goals of the US FDA's Predictive Toxicology Roadmap (Fig. 3) to integrate predictive toxicology methods into safety and risk assessment with the potential to replace or reduce the use of animal testing. Importantly, this collaborative effort has the potential to realize real reductions in animal use, as CDRH is the only agency worldwide that companies consider conducting the RVI test for; thus, its replacement at CDRH will mean a global elimination of this test.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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