Commentary on assessment of an ex-vivo irritation test performed on human skin explants and comparison of its results with those of a 24/48-hours human patch test for the evaluation of cosmetics.

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Introduction
It is essential to address the irritation potential of compounds, or mixtures of compounds, that come in contact with human skin. The Draize test was developed in the 1940s for this purpose, relying on the apparition of visible alterations on the skin of albino rabbits [1]. It served as a reference for decades [2]. Still, over the years, it has been increasingly criticized due to the subjectivity of its visual grading, low predictivity towards mild irritants and the real accuracy in the extrapolation of results to human. Concomitantly with the rise of public awareness of animal welfare, several studies highlighted its limitations [3-5]. As a result, worldwide legislations endorsed the introduction of alternative test methods.

In vitro Skin Irritation Tests on Reconstructed Human Epidermis
Among the different strategies that were explored, in vitro irritation tests taking advantage of Reconstructed Human Epidermis (RHE) became the new standard [6]. RHE are produced from epidermal cells that are allowed to proliferate and differentiate, on a basal inert substrate and at the air-liquid interface [7]. The procedure leads to structures closely resembling real epidermis, with stratification and a stratum corneum. Several features make RHE a system of choice. The stratum corneum has a crucial barrier function. The keratinocytes are instrumental in triggering the inflammatory responses upon stimulation. Being grown in a serum-free medium, they allow accurate detection of inflammation induced by topically applied substances. Results were admitted to be reproducible. Finally, detection of irritation relies on a rapid and easy method: quantification of cell viability upon enzymatic conversion of MTT into a precipitate that is measured by optical density [8].

Some Limits of Irritation Testing using Reconstructed Human Epidermis
The few RHE systems formally validated by the OECD TG439 are commercial [6]. Relying on a few tissue equivalents, their advantages must be balanced with the limits of the system. There can be an interruption in sourcing due to production problems or commercial strategies of suppliers. RHE are expensive and the cost can even be prohibitive as some countries impose high customs barriers to the import of living human tissues. They can also be quality loss due to long shipment. Therefore, the OECD encourages the development of alternatives methods. This led to the development of “open source” reconstructed epidermis that provides greater autonomy as well as enabling better control of culture parameters [9-12].

Despite the opening these “in house” skin equivalents brought, there are technical limits inherent to the use of reconstructed epidermis themselves. Despite highly standardised production process and rigorous quality controls, RHE present inter-batch variability. Their stratum corneum is also not fully mature [13-16], hindering the test of some galenic formulations, such as alcohol-based and pasty products. Finally, scoring of irritation with the MTT assay only gives an “Irritant”/“Non-irritant” type of answer providing very little insight into the irritation potential of the compound tested.

Ex vivo Irritation Tests using Skin Explants
In a recently published article [17], we report on an ex vivo irritation test performed on cosmetics, comparing results to those of 24/48-hours human patch tests. There are two originalities in our approach: the use of human skin explants instead of RHE and the quantification of irritation by histological analysis to provide deeper insights into the irritation potential of the compound tested.

Despite this untraditional approach, results show that the test accurately and repeatable detects known irritants. Also, when testing 120 non-alcoholic cosmetics of any galenic form, comparison of data between the ex vivo irritation tests and those of a 24/48-hours human patch test reveals accurate prediction of irritancy with a 10% false-positive rate, a situation putting the test on the safe side. If we have no data on the sensitivity of the ex vivo irritation test; its specificity the percentage of non-irritants correctly identified is 89.9% and its accuracy the overall percentage of the correct classification of irritants and non-irritant is 89.1%. When testing 49 alcoholic cosmetics, results are similar with a slightly higher false positive rate. Taken together, these values exceed the minimum requirements of high quality standards of OECD TG439.

One of the expected drawbacks of using skin explants is the variability of the irritation response. This is expected due to their diverse origin. Still, the meticulous and thorough scoring of minute histological alterations, as well as the rigorous correspondence scale used to grade irritation, are certainly instrumental in faithfully detecting individual diverse response to irritants. Indeed, results from 52 different skin explants subjected to 0%, 10% or 20% SDS do show variability in the type of histological alterations that were scored. Nevertheless, the resulting irritation score was highly reproducible.
Using human skin explants to perform irritation tests offers several advantages. The most obvious is that, being plastic surgery wastes, they are easily available and very affordable. Skin explants are also closer to skin physiology than RHE. They are also more robust and have a fully mature stratum corneum with its full barrier function, a feature that enabled us to successfully test cosmetics encompassing all galenic formulation of cosmetics, including alcohol based cosmetics, pasty products, and solid ones like nail polishes, something that is hardly possible using RHE.

**Discussion and Conclusion**

The *ex vivo* irritation test suffers some drawback. Grading of histological alterations is time consuming and requires highly trained histological experts. In our case, two who grade independently, randomly and blindly and who confront their observation to avoid deviation from the initial scoring grid. Besides, like any *in vitro* system, the timespan during which a compound can be tested depends on the time the skin explant can be maintained in culture without losing its characteristics. These limitations still do not enable testing chronicle exposure or long-term effects.

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**References**


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