

In vitro skin irritation test in evaluation of dermal irritation caused by cosmetics

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Introduction

In vitro skin irritation test can be utilized as an alternative in evaluating dermal irritation prior to clinical tests. It is performed on a model of the human epidermis, EpiDerm™ based on neonatal, foreskin-derived normal human epidermal keratinocytes (NHEK). EpiDerm™ allows the application of cosmetic finished products (liquid and solid) directly to the stratum corneum of this air-lifted, highly differentiated culture. The *in vitro* skin irritation test is based on the measurement of the reduction in tissue viability using MTT endpoint.

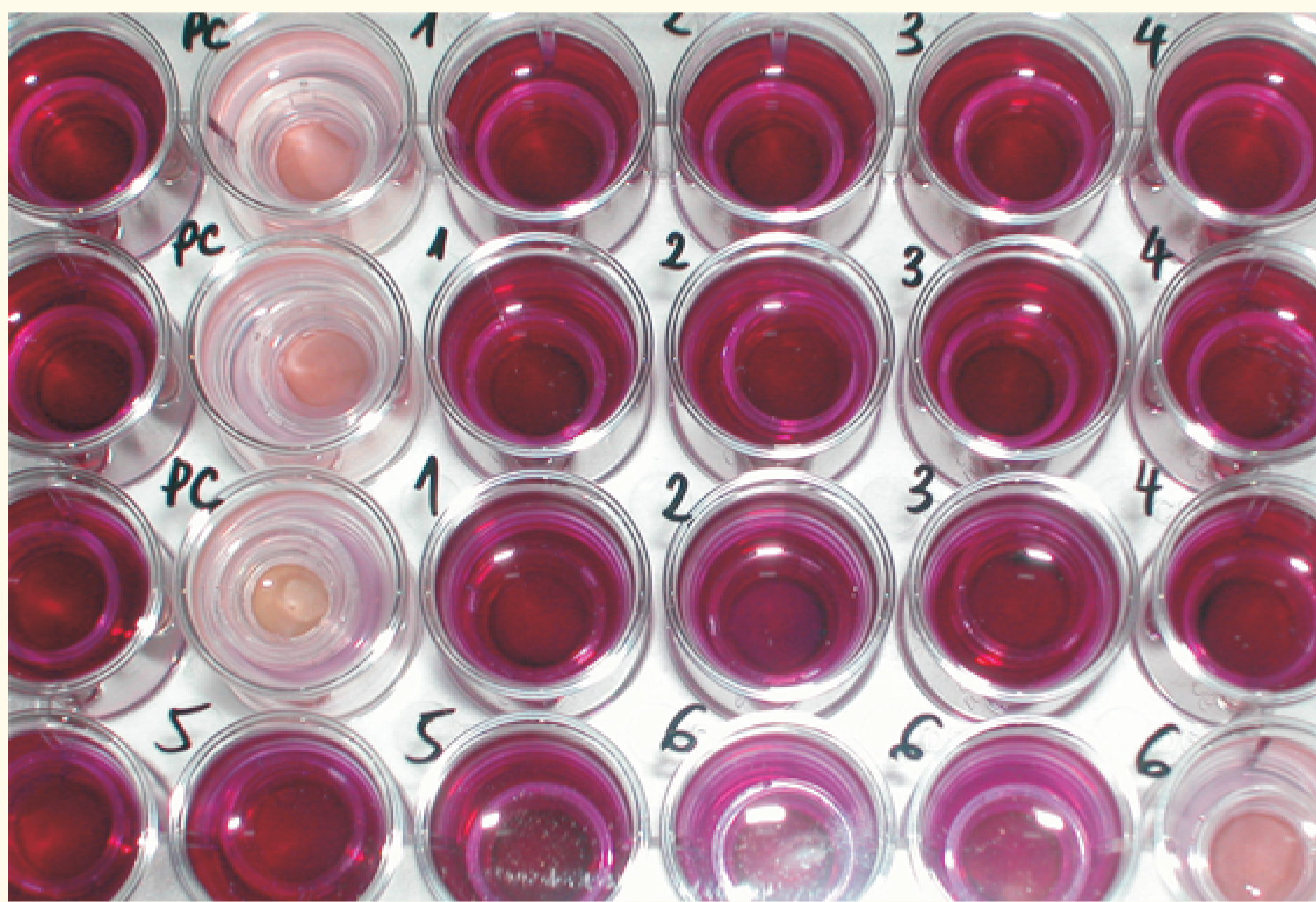
Aim of the study

The aim of this study was to evaluate the dermal irritation potential of different cosmetic products using the *in vitro* skin irritation test.

Methods

EpiDerm™ skin irritation test

In vitro skin irritation test was performed according to Standard Operating Procedure evaluated in the European Centre of the Validation of Alternative Methods (ECVAM) Skin Irritation Validation Study or the MTT Effective Time -50 (ET-50) protocol developed at MatTek Corporation. This test allows for assessing irritating properties of both cosmetic ingredients and ready made products. It consists in putting a sufficient amount of cosmetic preparation on the surface of the epidermis model. After a certain incubation period, cell viability is assessed with the use of MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide) colorimetric test. The sensitivity of the test may be increased by measuring the level of 1α interleukin concentration (IL- 1α) as one of the mediators of inflammatory reaction. *In vitro* results were compared to the dermatological test data.



Skin irritation test on EpiDerm™ model

MTT Effective Time-50 (ET-50) protocol for use with EpiDerm™ (EPI-200) MTT kit (MTT-100), MK-24-007-0001 09/06/05 developed at MatTek Corporation.



Correlation of *in vitro* and *in vivo* results:
tissue viability = 50% - irritant (R38)
tissue viability = 50% - non irritant
% viability = $100 \times [OD(\text{sample})/OD(\text{negative control})]$

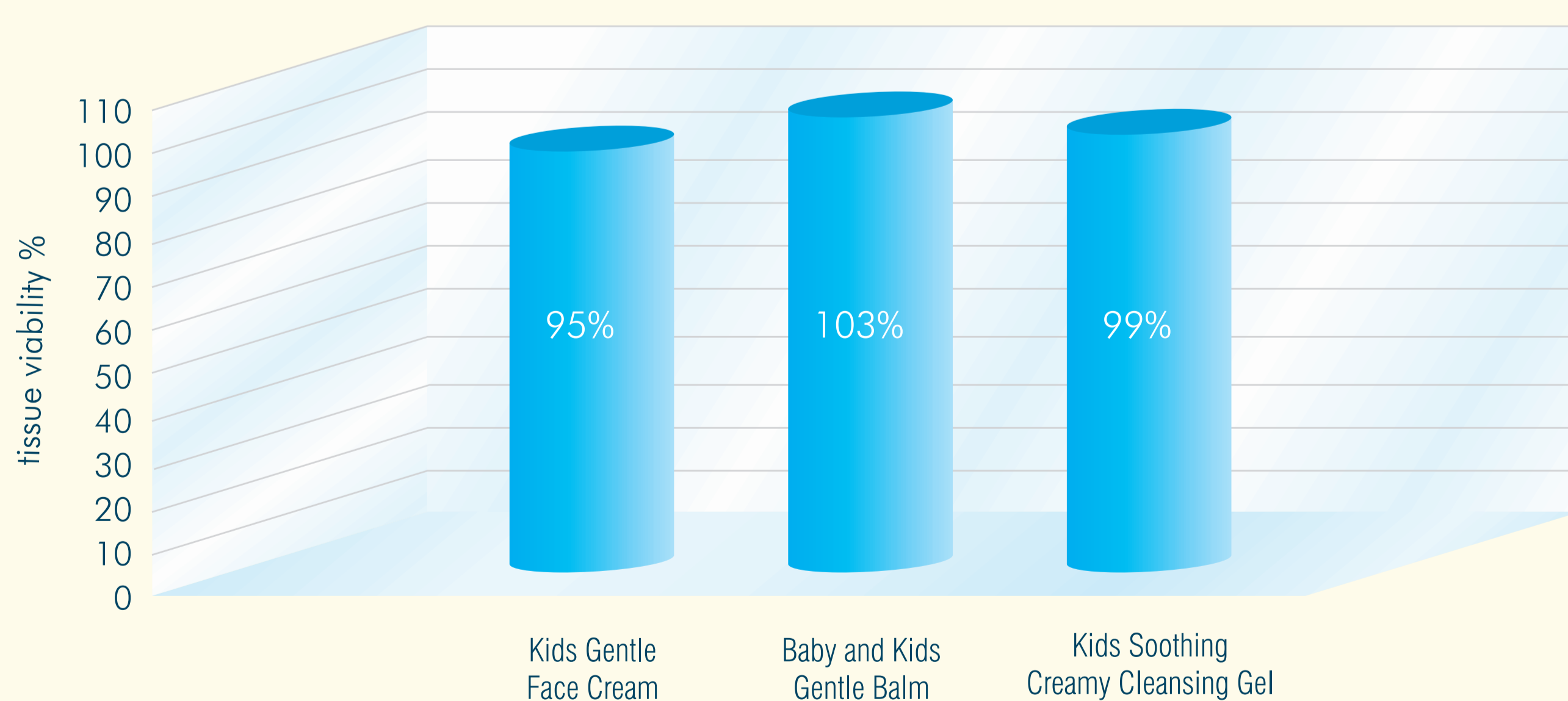
Patch test

In order to determine dermal irritation or sensitization potential of tested cosmetic formulations, patch tests were performed. For every cosmetic tested, the tests were conducted on the skin of 30 adult subjects, who had allergy of different organs. The patches were applied on the subjects' backs for 48 hours. Skin responses were evaluated after 48 and 72 hours after the patches application.

Results

EpiDerm™ skin irritation test

Evaluation of the irritation potential of dermocosmetics



Patch test

Product	Patch test
Kids Gentle Face Cream	no dermal reaction
Baby and Kids Gentle Balm	no dermal reaction
Kids Soothing Creamy Cleansing Gel	no dermal reaction

Tested dermocosmetics:

Kids Gentle Face Cream – tissue viability = 95% - non irritating

Baby and Kids Gentle Balm – tissue viability = 103% - non irritating

Kids Soothing Creamy Cleansing Gel – tissue viability = 99% - non irritating

Conclusion

The *in vitro* skin irritation test results could be used as a preliminary assessment of dermal irritation after the application of skin care products. Nevertheless, further studies are required to adapt the human epidermis model EpiDerm™ to predict *in vivo* skin irritation caused by cosmetics.