Human Skin Model Test with Medical Devices: *In Vitro* Skin Irritation

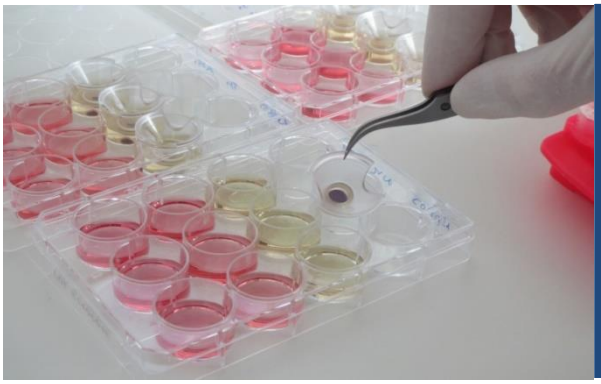
(EpiDerm™)

The prediction and classification of medical devices by the human skin model test of skin irritation potential can be done by the assessment of the effect on EpiDerm™ model.

The modified human skin model test for medical devices is a reliable *in vitro* test method and is performed in accordance with the draft ISO guideline 10993-10 Part 10 at Eurofins BioPharma Product Testing Munich GmbH.[1]

Assessment of Skin Irritation Potential of Medical Devices

- Acute irritation characterizes a local, reversible inflammatory response of normal living skin to direct injury after application of irritant substances.[2]
- The EpiDerm™ represents a reconstituted three-dimensional human epidermis (RhE) model which consists of human epidermal keratinocytes. This *in vitro* model mimics biochemical and physiological properties of the upper human skin.
- To determine skin irritation potential the test item is extracted and applied directly on the skin tissue surface. The cell viability is measured by the MTT assay.
- The EpiDerm™ human skin model test can be used as a reliable *in vitro* test method to identify extracts from medical devices as "irritant" or "non-irritant".[3]



Acute irritation characterizes a local, reversible inflammatory response of normal living skin to direct injury after application of irritant substances [2]. After an incubation period of 18 ± 0.5 h the viability of the cells is measured by the MTT assay.

Procedure

Principles of the Human Skin Model Test with Medical Devices

Protocol	
Model	Human skin model EpiDerm™ supplied from MatTek
Analysis	Skin irritation potential: cytotoxicity measurement with MTT (mean tissue viability compared to negative control tissues) Optional: Interleukin-1α release into the tissue culture medium
Test chemical concentrations	100 µL undiluted extract in 0.9% NaCl (polar) 100 µL undiluted extract in sesame oil (non-polar)
Extraction and absorption capacity	Extraction at 37 ± 1 °C for 72 ± 2 h in 0.9% NaCl or sesame oil with continuous agitation / shaking Determination of the absorption capacity of the test item in 0.9% NaCl (polar) or in sesame oil (non-polar)
Exposure time	18 ± 0.5 h in the incubator
Quality controls	Positive control: 1% SDS in NaCl and sesame oil Negative control: DPBS Vehicle controls: → 0.9% NaCl as solvent for polar extracts → sesame oil as solvent for non-polar extracts
Pre-tests	**To determine if additional controls are needed:** **NSMTT***: mixing of test item extract with MTT medium to determine if test item alone can reduce MTT → blue colouring: in main experiment two killed tissues treated with test item and two untreated killed tissues were added as controls **NSC_{living}**[#]: mixing of test item extract with H_2O or isopropanol to determine if strong own colour of test item can discolour at contact with this liquids → optical discolouring (measuring of spectrum): in main experiment two living tissues without incubation with MTT medium were added as controls **NSC_{killed}**[§]: if the two other controls were determined → in main experiment two killed tissues without incubation with MTT medium were added as controls
Application	Direct topical application of extracts on skin tissues Three tissue replicates per dose group
Data delivery	Optical density (OD) value with microplate spectrophotometer at 570 nm Tissue viability of each dose group
Prediction model	**Mean tissue viability (% negative control):** ≤ 50%: Irritant; (Interleukin-1α > 60 pg/mL) > 50%: Non-Irritant; (Interleukin-1α ≤ 60 pg/mL)

*NSMTT: non-specific MTT reduction
[#]NSC_{living}: non-specific colouring of living tissues
[§]NSC_{killed}: non-specific colouring of killed tissues

References

1) ISO 10993-10, 2010(E), “Biological evaluation of medical devices - Part 10: Tests for irritation and skin sensitization“. Annex D: *In vitro* tests for skin irritation
2) UN (2015). United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS), sixth revised edition, UN New York and Geneva
3) DeJong W., Hoffmann S., Lee M., Kandárova H., Pellevoisin, C., Haishima Y., Rollins B., Zdawczyk A., Willoughby J., Bachelor M., Schatz T., Skoog S., Parker S., Sawyer A., Pescio P., Fant K., Kim, KM., Kwon JS., Gehrke H., Hofman-Hüther H., Meloni, M., Julius C., Briotet D., Letasiova S., Kato R., Miyajima A., De La Fonteyne L., Videau C. Tornier C., Turley A.P., Christiano N., Rollins T.S. and Coleman K.P., (2018) Round robin study to evaluate the reconstructed human epidermis (RhE) model as an *in vitro* skin irritation test for detection of irritant activity in medical device extracts. Toxicology *in Vitro*.