MTT Assay with EpiDerm™ Cultures : *IN VITRO* SKIN CORROSION ASSAY

Theory: The purpose of this study is to evaluate the potential skin corrosivity of the test article by measuring the conversion of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) to a formazan product by EpiDerm™ after exposure to the test article(s). The method involves a 3 minute exposure for corrosive and a 1 hour confirmation exposure for a non-corrosive classification that is consistent with the ECVAM prevalidation EpiDerm™ Skin Corrosivity Test protocol[1].

Applications and Use

- The EpiDerm™ model is composed of human keratinocytes stratified into a 3-dimensional dermal structure consisting of basal, spinous, and granular layers, including a functioning stratum corneum with characteristic lipid lamellae.
- EpiDerm™ is suited to address the corrosive potential of test materials.
- Test materials are applied topically at formulation strength.
- Suited for both water soluble and insoluble formulations.
- Suitable for testing creams, pastes, highly viscous materials, and powders otherwise precluded from testing in other models.
- Cells are of human origin.

Experimental Procedure

Receipt and Preparation of Cultures

- Each culture is removed with sterile forceps from the agarose gel, inspected, and transferred to a pre-labeled 6-well plate containing 0.9 ml of assay medium per well. The EpiDerm™ cultures will be incubated at 37±1°C in a humidified atmosphere of 5±1% CO_2 in air for at least one hour prior to dosing.

Assay Procedure

- The test materials are tested NEAT by topical application. Pastes and highly viscous materials may be "creamed" to effect application.
- The positive control is 8N KOH and exposed for 3 and 60minutes.
- The negative control is sterile, deionized water generally exposed concurrently with the longest exposure time of the test or positive control articles.
- 50 µl (liquids) or 25 mg (solids) of the test or control article are applied topically onto the tissue surface.
- The cultures are returned to the incubator for the appropriate exposure times of 3 to 60 minutes.
- After the appropriate exposure time, the test articles are rinsed from the cultures using DPBS without Ca^{++} and Mg^{++}.
- The cultures are transferred to wells containing 0.3 ml of MTT reagent (1 mg/ml) and incubated for 3 hours.
- After incubation, the cultures are blotted on absorbent paper and extracted in 2 ml of isopropanol for 2 hours, while shaking.
- 200 µl of each extraction solution are transferred to a 96-well plate and the absorbance at 550nm (OD_{550}) recorded.

Data Evaluation

- The relative survival is determined by comparing the mean corrected OD_{550} of the test article-treated wells to the mean corrected OD_{550} of the negative control-treated wells.
- **Interpretation**: Test materials that reduce tissue viability to <50% after a 3 minute exposure are classified corrosive. In addition, test materials which result in tissue viability =50% after a 3 minute exposure **and** <15% after a 60 minute exposure are also classified corrosive. Tissue viabilities of =50% after a 3 minute exposure and =15% after a 60 minute exposure are classified non-corrosive.
- Occasionally, a test article may directly reduce the MTT giving erroneous results. A direct MTT reduction test is performed as a pre-screen, and "killed tissue" controls may be assayed concurrently.

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[1] Liebsch. M., Traue, D., Barrabas, C., Spielmann, H., Uphill, P., Wilkins, S., McPherson, J.P., Wiemann, C., Kaufmann, T., Remmele, M., and Holzhütter, H.G. (2000) The ECVAM prevalidation study on the use of EpiDerm for skin corrosivity testing. ATLA **28**:371-401.