

Animal alternative evaluation of skin irritants and contact sensitizers

Any new product introduced to the market is required to be evaluated for its irritant and/or sensitizing potential. Skin irritants and sensitizers must first penetrate through the stratum corneum into the epidermis where they may induce a direct toxic effect (irritants) and/or an immune reaction (sensitizers). The migration of epidermal Langerhans cells (LCs) to draining lymph nodes is thought to be necessary for development of an immune response to a chemical. However, while both irritants and sensitizers can induce LC migration at toxic concentrations only sensitizers cause LC migration at non-toxic concentrations. Derphartox has developed a routine test system, using organotypic skin explant cultures (hOSEC), for determining the skin irritant and contact sensitizing potential of chemicals based on their abilities to stimulate LC migration at non-toxic concentrations.

Basic protocol

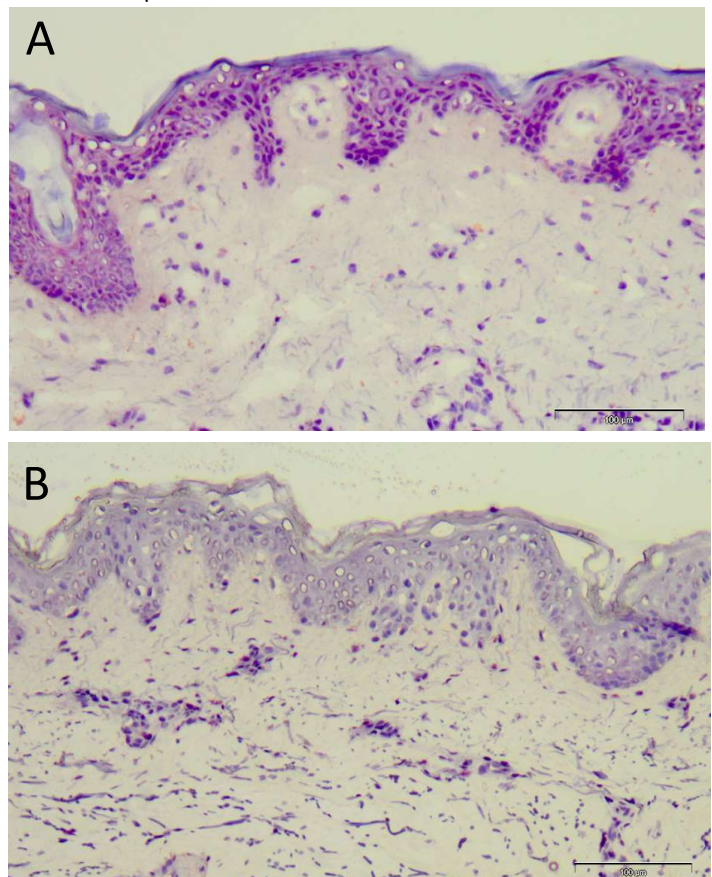
Skin is obtained as waste product after cosmetic surgery according to the internal procedures from the hospital with the informed consent of the patient.

- Skin explants are incubated with various doses of the test compound in order to determine the epidermal cytotoxicity
- Incubations are performed in triplicate using human skin from 3 different donors
- Skin explants (5-8 mm diameter) are cultured in medium supplemented with heat-inactivated 10% normal human serum
- The skin explants are incubated for 4, 24 and 48 hours at 37°C in a 5% CO₂ humidified atmosphere and then fixed in 4% formalin and/or snap-frozen in liquid nitrogen

End points

- Epidermal cytotoxicity. This is assessed using methyl-green pyronine (MGP) staining. Absence of pyronine (RNA) staining in the epidermis is regarded as toxic effect of the chemical (Figure 1).
- Dermal distribution of LCs. LCs are stained using a CD1a/anti-Langerin antibody and their epidermal distribution is measured (Figure 2).

Figure 1. MGP staining of cryosections (4 hour incubation). **A.** Vehicle control. Viable epidermis with RNA (violet-purple) and DNA (blue). **B.** Irritant. Non-viable epidermis with RNA depleted.



Classification of chemicals ^{Table 1}

- A chemical is classified as “Corrosive” if it has a toxic effect during a 4hr exposure.
- A chemical is classified as an “Irritant” if it is toxic during a 48 hr exposure.
- A chemical is classified as a “Sensitizer” if it induces LC migration during a 24 hr exposure and is non-toxic during a 48 hr exposure.

Table 1. Validation of the hOSEC system.

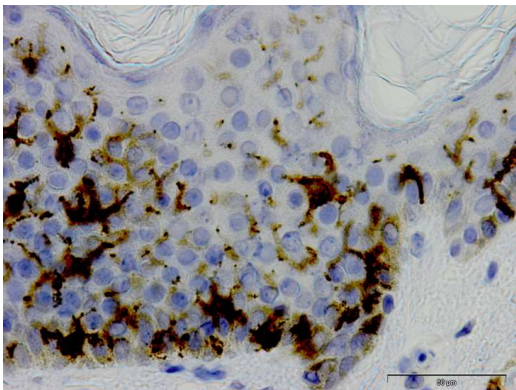
Chemical	Classification as sensitizer			
	hOSEC	Human	Guinea pig	Mouse
Croton oil	-	-	n.d.	+
Nonanoic acid	-	-	n.d.	+
SDS	-	-	-	+
CoCl ₂	+	+	+	+
DNCB	+	+	+	+
Eugenol	+	+	+	+
K ₂ Cr ₂ O ₇	+	+	+	+
Neomycine	+	+	-	-
NiSO ₄	+	+	+	-

Various chemicals from the ECVAM panel have been tested and the results compared with LLNA, GPMT and human data. Classifications of chemicals obtained using hOSEC (irritant or sensitizer) agree well with classifications using volunteers and are at least as good as the GPMT, if not better than the LLNA classifications based on animal models.

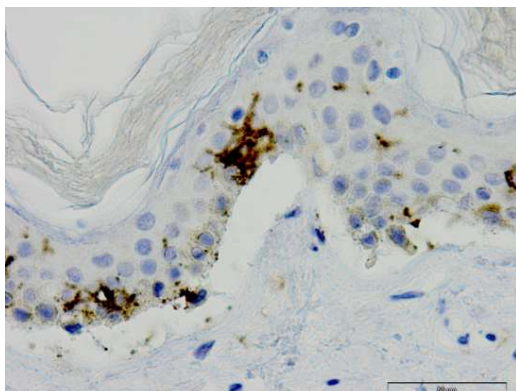
Human data; human maximization test (HMT) or human patch test (HPT). Guinea pig; guinea pig maximization test (GPMT) or Buehler Test (BT). Mouse; local lymph node assay (LLNA).

Figure 2. Effect of a sensitizer at non-toxic concentration on Langerhans cells numbers, morphology and distribution (48hr incubation).

A. Control



B. Sensitizer



Note: Additional services e.g. RNA isolation followed by gene expression analysis, phenotyping of skin émigrés using FACS, analysis f.e. from the incubation medium, or other properties and mechanism of action of the compound, can also be performed upon the client's request.

Time lines

These will vary depending on the client's wishes.

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References

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- Rustemeyer, T., et al., Exp Dermatol, 2003. 12(5): p. 682-91.
- Lehe, C.L., et al., Altern Lab Anim, 2003. 31(6): p. 553-61.
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Advantages of the model

- Closely resembles the in vivo human situation
- Correctly identifies potential human contact allergens, even those that cannot be detected using LLNA