Written comments submitted to the
SACATM meeting March 10-11, 2004

Acute eye irritation assay using *in vitro* reconstituted human corneal epithelium (HCE model)

Current industrial use of reconstituted human corneal epithelium (HCE model) for the *in vitro* determination of the eye irritation potential of finished products and chemicals as alternative method to the Draize eye test.

Content:

February 26, 2004

Introduction

This report summarizes a part of the current industrial usage of the SkinEthic in *vitro* reconstituted human corneal epithelium (HCE model) for the prediction of eye irritation of finished products and chemicals as alternative method to the Draize eye irritation test.

The test method principle (based on Multiple Endpoint Analysis MEA) as well as a detailed assay procedure are provided. *In vitro-in vivo* correlation analysis (and development of the prediction model) is being performed internally by each laboratory.

The number of available *in vitro* data is presented separately for finished products (Table 1) and chemicals (Table 2). Some of the data provided are proprietory and confidential and not yet published in literature. However all laboratories cited are willing to provided internal *in vitro* vs. *in vivo* results if ICCVAM review is required. A contact name for each laboratory is listed at the end of the report.

1. *In vitro* reconstituted human corneal epithelium

When cultivated at the air-liquid interface in chemically defined medium, the transformed human corneal epithelial cells of the cell line HCE (LSU Eye Centre, New Orleans, USA) form a corneal epithelial tissue (mucosa), devoid of stratum corneum, resembling ultra-structurally (tissue morphology and thickness) the corneal mucosa of the human eye (Figure 1,2). [10, 17].

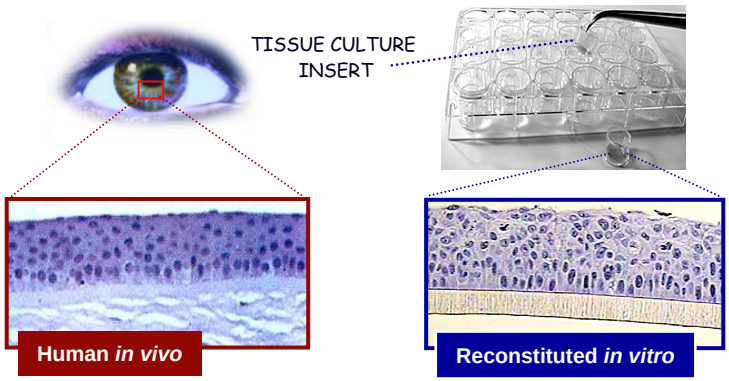


Figure. 1: Transversal section of human corneal epithelium *in vivo* (left), and reconstituted *in vitro* on a polycarbonate membrane in tissue culture inserts (right).

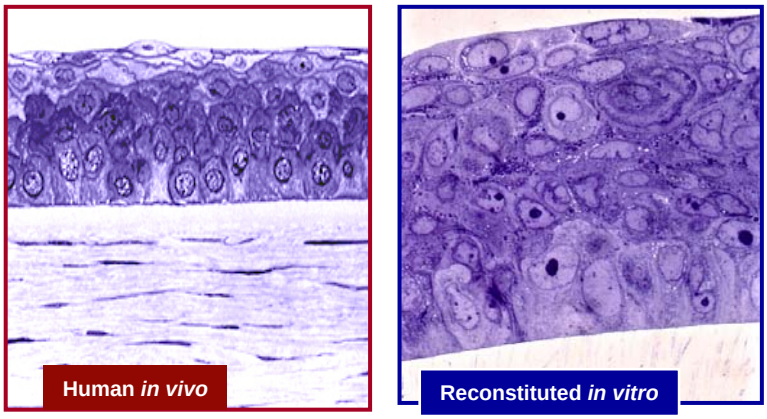


Figure 2. Ultra-structural results show that the *in vitro* 3D tissue (right) resembles normal *in vivo* corneal epithelium (left) featuring the typical presence of a columnar basal cell layer, 2-3 layers of transitional wing cells, and 2-3 layers of superficial squamous cells.

Recent results also indicate that the *in vitro* tissue constructs secrete the same mucins that are being found in the human cornea *in vivo* (Berry et al, personal communications, [15]).

2. Test method principle

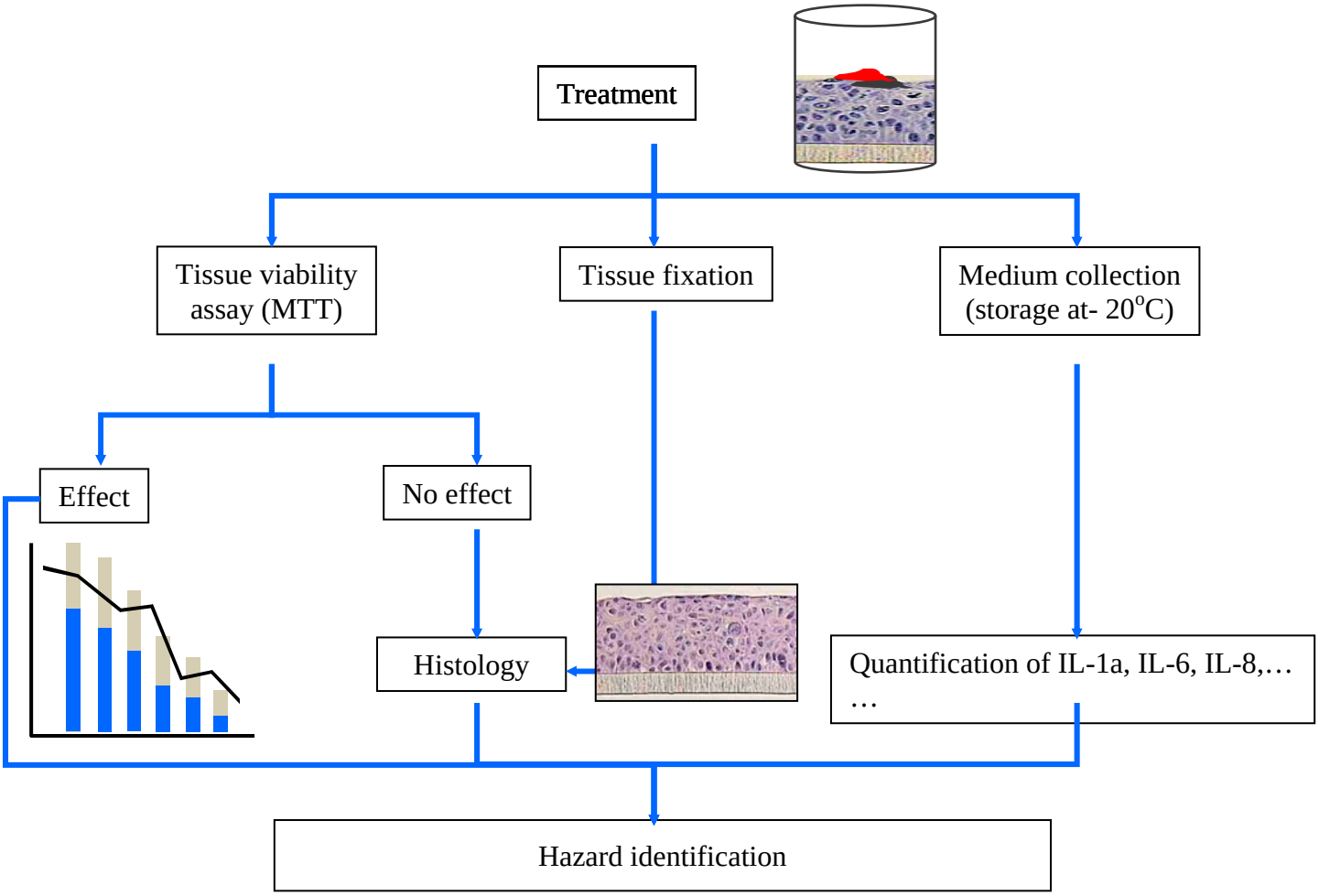
Triplicate in vitro reconstituted human corneal epithelial tissues (size 0.5 cm²) are dosed topically with a small amount of test agent for different time points:

- For finished products, tissues are dosed for 10 minutes, 1 hour, 3 hours and 24 hours.
- For chemicals, tissues are dosed for 5, 10, and 60 minutes.

Negative control (phosphate-buffered saline solution) as well as positive controls (SDS 0.5% and SDS 1%) are run in parallel.

At each time point, duplicate tissues are assessed for tissue viability (MTT assay), and one culture is fixed in a balanced 10% formalin solution for histological analysis, which is performed when the MTT assay data show no tissue toxicity. Additionally, the culture media underneath the tissues is being stored at –20°C for pro-inflammatory mediator analysis (IL-1a, Il-6, IL-8, amongst others).

Schematic representation of the MEA protocol principle:



3. Detailed assay procedure

Test method:
30 µl of each product (and controls) is deposited onto the surface of each of 12 equivalent cultures. The cultures are incubated at 37° C for 10 minutes, 1 hour, 3 hours and 24 hours for testing finished products, or 5, 10 and 60 minutes for testing chemicals.

Evaluation of cell viability:
For each of the tested products or controls, and for each time point, two treated cultures are rinsed with PBS and placed on 300 µl of 0,5 mg/ml MTT.

- Qualitative evaluation of cell viability: After a 30 minutes incubation at room temperature, the color of each culture is noted: Negative control cultures have to be of dark blue color, proof of the cell's viability. Positive control cultures have to be blue/white or white, evidence of cell death.

- Quantitative evaluation of cell viability: After 3 hours incubation on 300 µl of 0.5 mg/ml MTT at 37°C., 5% $C0_2$, cultures is placed in 1.5 ml of isopropanol. Extraction is performed at room temperature, for a minimum of 2 hours, by gentle shaking. Optical density is measured on 200 µl of extracts at 570 nm (reference filter: 690 nm). Results are expressed as percentage of viability compared to negative control (mean +/- SD of duplicate cultures):

$$\% \text{ Viability} = [OD_{(570nm - 690\ nm)} \text{ test product} / OD_{(570nm - 690\ nm)} \text{ negative control})] \times 100$$

Histology:
For each of the tested products or controls, at the end of each test period, one culture is fixed in a balanced 10% formalin solution and later embedded in paraffin. Four micron vertical sections are stained with hematoxylin/eosin, and photographed under a microscope.

- Histo-pathologic interpretation: Negative control cultures: The corneal epithelial tissues must have a constant thickness (corresponding to internal QC control sections), devoid of terminally differentiated cells, and a regular and compact shape. Cells are attached to the others via multiple desmosomes. Positive control cultures: Most of the upper cell-layers of the epithelial tissues must be disintegrated, and the remaining basal cells loosely attached to the polycarbonate substratum.

Release of inflammatory mediators:
After topical application of the test products, conditioned media underneath triplicate corneal tissues are collected and kept frozen at –20° C. for inflammatory mediator quantification (IL-1a, IL-6 and IL-8, using ELISA kits; IL-1a kit: R&D Systems, UK Cat # DLA50; IL-6 kit: R&D Systems, UK Cat # D6050; IL-8 kit: R&D Systems, UK Cat # 8050), amongst others.

4. Results for finished products

Table 1: Number of finished products tested in each laboratory.

Company	In vitro	Rabbit (Draize)	Human (Clinical)	References	Comments
Clarins, France	400	-	some	-	(1)
Lancaster-Coty, Monaco	187	187	-	[1,2,3,5,19]	(2)
AFSSAPS, France	149	149	-	[9]	(3)
LVMH, France	82	-	82	[4,14]	(4)
Avon Products, USA	20	-	10	[20]	(5)
VitroScreen, Italy	50	-	10	[6]	
Alberto Culver, USA	12	12			
Total	**900**	**348**	**102**		

(1): No animal data, some clinical data available.
(2): Data on 40 materials are published; the remaining will be published peer-reviewed later this year. Parts of the data is available for ICCVAM review on demand.
(3): AFSSAPS is a department of the French Ministry of Health.
(4): All 82 products were evaluated clinically in humans.
(5): Avon has a large internal data base (400 formulations) tested in other ocular tissue models.

5. Results for chemical raw materials

Table 2: Number of chemicals tested in each laboratory.

Company	In vitro	Rabbit (Draize)	Human (Clinical)	References	Comments
Lancaster-Coty, Monaco	48	48	-	[19]	(1)
Unilever, UK	8	8	-	[7]	(2)
Vitroscreen, Italy	20	20	-	-	
Novozymes, Denmark	18	18	-	[8]	(3)
SkinEthic Labs., France	22	22	-	[11,13]	(4)
Univ. of New Orleans, USA	1	1	1	[10,18]	(5)
Prevalidation multi-center: - J&J PRD, Belgium - Pfizer R&D, France - Novartis, Switzerland - SkinEthic Labs., France	25	25	-	[12,15]	(6)
Multi-center validation: -GSK/ SafePharm, UK - SkinEthic Labs., France	21	21	-	[21]	(7)
Total	**163**	**163**	**1**		

(1): Data is will be published peer-reviewed later this year. Parts of the data is available for ICCVAM review on demand.
(2): Evidence of *in vitro* corneal recovery was observed with slight to mild irritants.
(3): Test substances are enzymes.
(4): Data include *in vitro* corneal recovery study on 19 ECETOC chemical raw materials.
(5): Benzalkonium chloride was studied at different concentrations.
(6): 20 liquids and 5 solids were tested in 4 laboratories. The data is currently undergoing independent statistical analysis.
(7): 21 chemicals were tested in 2 labs. The data is currently undergoing independent statistical analysis.

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