Application of the Reconstructed Human Epidermis (RhE) Model as an *In Vitro* Skin Irritation Test for Detection of Irritant Activity in Medical Device Extracts

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Conflict of Interest Statement

Neither myself nor members of my family, have any financial interest or affiliation with a commercial organization that has a direct or indirect interest in the subject matter of my presentation.

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Objectives/Outline

- Introduction to the subject
- Discussion of problem
- Safety testing of medical devices (ISO 10993-1)
- How to do irritant testing of medical devices (ISO 10993-10)
- Irritant testing of chemicals (OECD 439)
- Human reconstructed epidermis models
- Preparation of positive and negative test samples
- Preliminary studies with medical device extracts
- Organization of international round robin study

Abbreviations

- ISO: International Organization for Standardization
- OECD: Organisation for Economic Co-operation and Development
- MTT: Methylthiazolyldiphenyl-tetrazolium bromide, 3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide
- NC: Negative control
- PC: Positive control
- PVC: Polyvinyl chloride
- RhE: Reconstructed human epidermis
- SDS: Sodium dodecyl sulfate

Medical Device Safety Testing

- Medical devices are a specific category for which safety evaluation is essential before the device can be marketed and applied in patients.
- For the safety evaluation the whole device needs to be investigated.
- The nature of the medical device does not allow simple toxicity testing.
- Common extracts are used that are considered to reflect possible leakage of toxic components from the device.

ISO 10993-1

- Biological evaluation of medical devices Part-1. Evaluation and testing within a risk management process
- This is Part 1 of a series of standards (10993 series) that describe various aspects of medical device toxicity testing
- Other parts address other aspects of toxicity testing
  - Genotoxicity, carcinogenicity, and reprotoxcity
  - Cytotoxicity

10993 Series Dealing with Toxicity Testing

- Part 2. Animal welfare
- Part 3. Genotoxicity, carcinogenicity, reproduction toxicity
- Part 4. Blood compatibility
- Part 5. Cytotoxicity
- Part 6. Implantation
- Part 10. Irritation and sensitization testing
- Part 11. Systemic toxicity
- Part 16. Toxicokinetic study design
- Part 20. Immunotoxicity

10993 Series Other Standards

- Part 7. Ethylene sterilization
- Part 9. Identification of degradation products
- Part 12. Sample preparation and reference materials
- Part 13. Degradation of polymers
- Part 14. Degradation of ceramics
- Part 15. Degradation products of metals and alloys
- Part 17. Allowable limits of leachables
- Part 18. Chemical characterization
- Part 19. Physicochemical, morphological, and topological characterization
- Part 22. Guidance on use of nanomaterials in medical devices

Choice of Tests

- Medical devices compose a wide range of products varying from hospital beds, walking aids to cardiac pacemakers, and hip implants.
- Depending on their use more or less information can be needed on the safety evaluation of the device.
- Use of device determines what kind of testing needs to be done.

Summary of the Systematic Approach to a Biological Evaluation of Medical Devices as Part of a Risk Management Process ISO 10993-1:2009

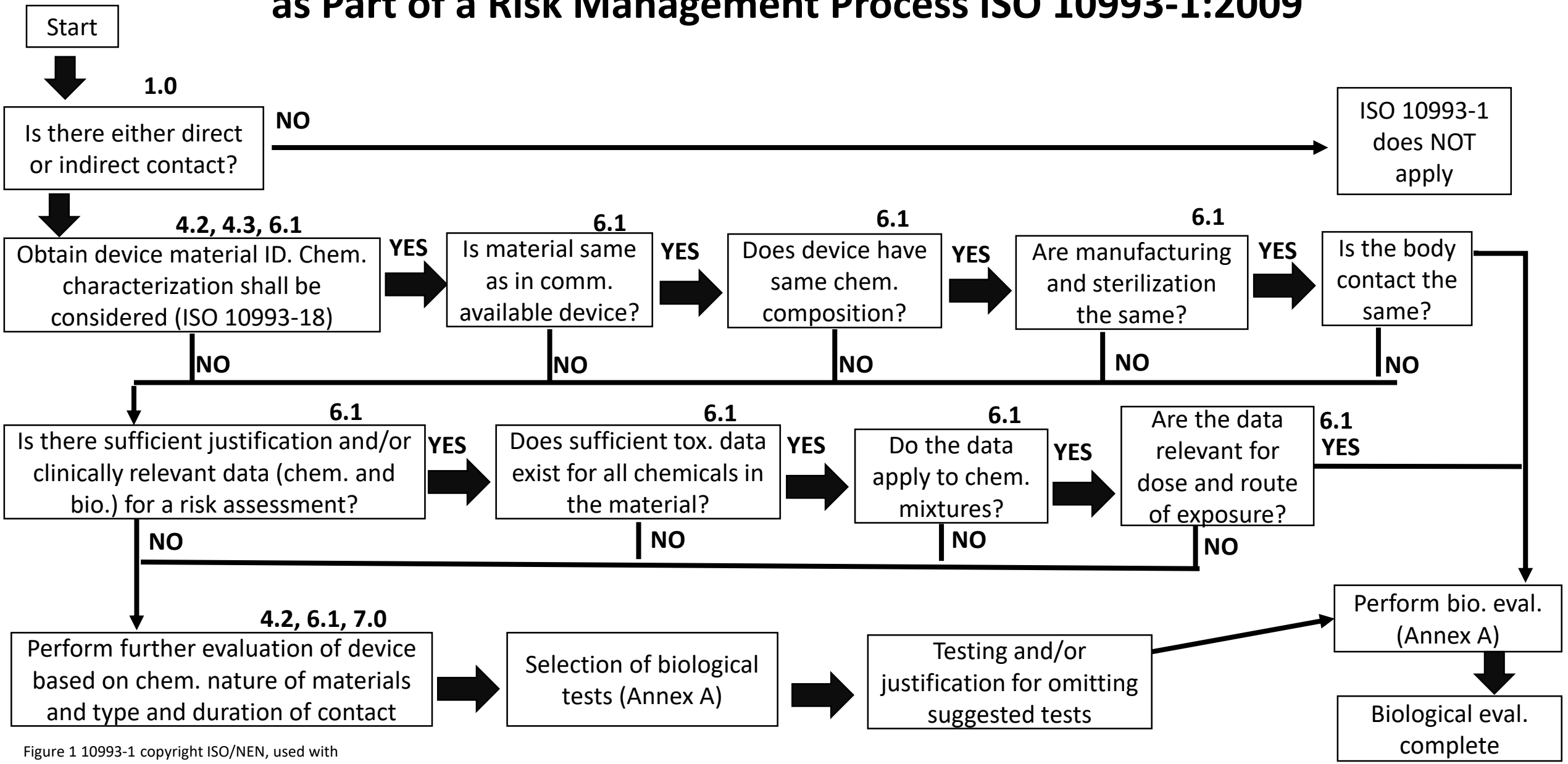


Figure 1 10993-1 copyright ISO/NEN, used with permission of NEN, Delft, the Netherlands.

Safety Testing Medical Devices ISO 10993 -1

- Category of device
  - Surface device
  - External communicating device
  - Implant device
- Contact
  - Skin/Mucosal membrane/Breached or compromised surface
  - Blood path/Tissue/Bone/Dentin/Circulating blood
- Contact duration
  - Limited (≤ 24 h)
  - Prolonged (> 24 h to 30 days)
  - Permanent (> 30 days)

Table A.1 — Evaluation tests for consideration

Medical device categorization by			Biological effect							
Category (nature of body contact, see 5.2)	**Contact**	**contact duration** (see 5.3) **A – limited** (⩽ 24 h) **B – prolonged** (> 24 h to 30 d) **C – permanent** (> 30 d)	Cytotoxicity	Sensitization	Irritation or intracutaneous reactivity	Systemic toxicity (acute)	Subchronic toxicity (subacute toxicity)	Genotoxicity	Implantation	Haemocompatibility
Surface device	Skin	A	X [a]	X	X					
		B	X	X	X					
		C	X	X	X					
	Mucosal membrane	A	X	X	X					
		B	X	X	X					
		C	X	X	X		X	X		
	Breached or compromised surface	A	X	X	X					
		B	X	X	X					
		C	X	X	X		X	X		
External communicating device	Blood path, indirect	A	X	X	X	X				X
		B	X	X	X	X				X
		C	X	X		X	X	X		X
	Tissue/bone/dentin	A	X	X	X					
		B	X	X	X	X	X	X	X	
		C	X	X	X	X	X	X	X	
	Circulating blood	A	X	X	X	X				X
		B	X	X	X	X	X	X	X	X
		C	X	X	X	X	X	X	X	X
Implant device	Tissue/bone	A	X	X	X					
		B	X	X	X	X	X	X	X	
		C	X	X	X	X	X	X	X	
	Blood	A	X	X	X	X	X		X	X
		B	X	X	X	X	X	X	X	X
		C	X	X	X	X	X	X	X	X

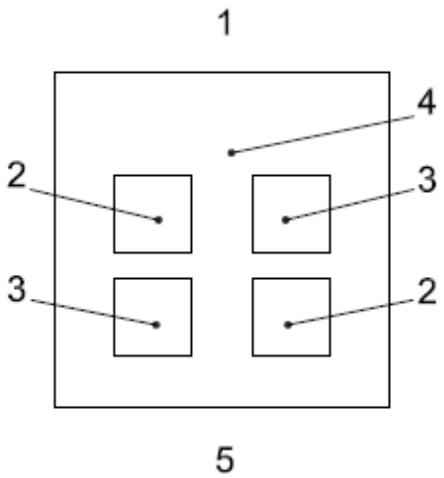
[a] The crosses indicate data endpoints that can be necessary for a biological safety evaluation, based on a risk analysis. Where existing data are adequate, additional testing is not required.

ISO 10993-10 Irritation and Sensitization

For almost all medical devices irritation testing needs to be considered and performed.

10993-10 Irritation Testing

- *In vivo* patch test
  - 3 healthy young adult albino rabbits (pretesting possible n=1)
  - 0.5 g or 0.5 ml applied directly to skin
  - 2.5x2.5 non-occlusive dressings
  - Observation periods
    - (1 ± 0.1 h, 24 ± 2 h, 48 ± 2 h, 72 ± 2 h)



Key

1 cranial end
2 test site
3 control site
4 clipped dorsal region
5 caudal end

Figure 1 — Location of skin application sites

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Irritation Score ISO 10993-10

Reaction	Irritation score
Erythema and eschar formation	0 – 4
Oedema formation	0 – 4
Maximum possible score for irritation	8
Other adverse changes at the skin sites shall be recorded and reported	

Table 1 — Scoring system for skin reaction

Reaction	Primary Irritation Score
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate erythema	3
Severe erythema (beet-redness) to eschar formation preventing grading of erythema	4
Oedema formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Well-defined oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond exposure area)	4
Total possible score for irritation	8
Other adverse changes at the skin sites shall be recorded and reported.	

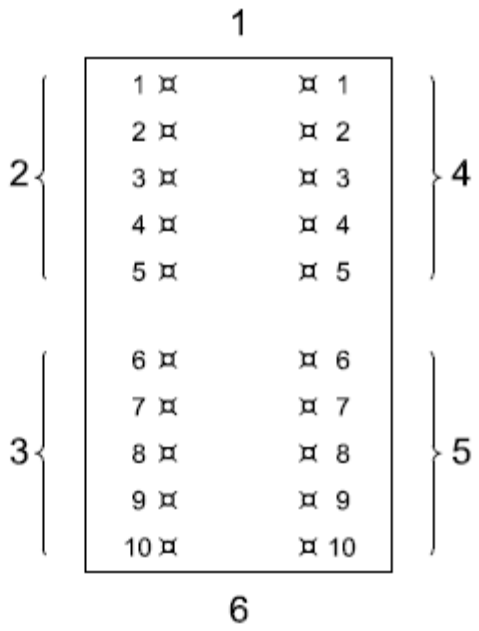
Response Category

Primary or cumulative irritation index categories in a rabbit

Mean score	Response category
0 to 0.4	Negligible
0.5 to 1.9	Slight
2 to 4.9	Moderate
5 to 8	Severe

Intracutaneous Irritation Test

- *In vivo* intracutaneous administration
  - 3 healthy young adult albino rabbits (pretesting possible n=1)
  - Intracutaneous injection 0.2 ml of extracts with polar or non-polar solvent.
  - Scoring (24 ± 2 h, 48 ± 2 h, 72 ± 2 h)
  - At 72 ± 2 h all score are totalled



Key

1 cranial end
2 0,2 ml injections of polar extract
3 0,2 ml injections of non-polar extract
4 0,2 ml injections of polar solvent control
5 0,2 ml injections of non-polar solvent control
6 caudal end

Figure 2 — Arrangement of injection sites

Erythema Score

Reaction	Numerical grading
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptable)	1
Well defined erythema	2
Moderate erythema	3
Sever erythema (beet-redness) to eschar formation preventing grading of erythema	4

Oedema Score

Reaction	Numerical grading
Oedema formation	
No oedema	0
Very slight oedema (barely perceptable)	1
Well-defined oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond exposure area)	4

Reaction	
Maximal score for irritation (combined score for erythema and oedema)	8
Other adverse changes at the injection site shall be recorded and reported	

OECD 439 Reconstructed Human Epidermis (RhE)

- OECD 439 adopted by OECD 28 July 2015
  - *In vitro* skin irritation: reconstructed human epidermis test method
  - Sensitivity 80%, specificity 70%, accuracy 75%
- Four models accepted
  - EpiSkin™
  - EpiDerm™ SIT (EPI-200)
  - SkinEthic™RHE
  - LabCyte EPIMODEL24 SIT
- Based on short exposures (15 minutes up to 60 minutes depending on model)
- Viability determined by MTT method after post treatment period to allow recovery from weak effects and for appearance of clear toxic effects

Round-Robin Study on Use of RhE for Irritant Testing of Medical Device Extracts

- Organized within ISO TC 194 WG8 on irritation and sensitization
- Trigger to really start RR initiative by publication of Casas et al., TIV 2013.

Toxicology in Vitro 27 (2013) 2175–2183

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

In vitro human skin irritation test for evaluation of medical device extracts

J.W. Casas [a], G.M. Lewerenz [a], E.A. Rankin [a], J.A. Willoughby Sr. [b,1], L.C. Blakeman [b,1], J.M. McKim Jr. [b,1], K.P. Coleman [a,*]

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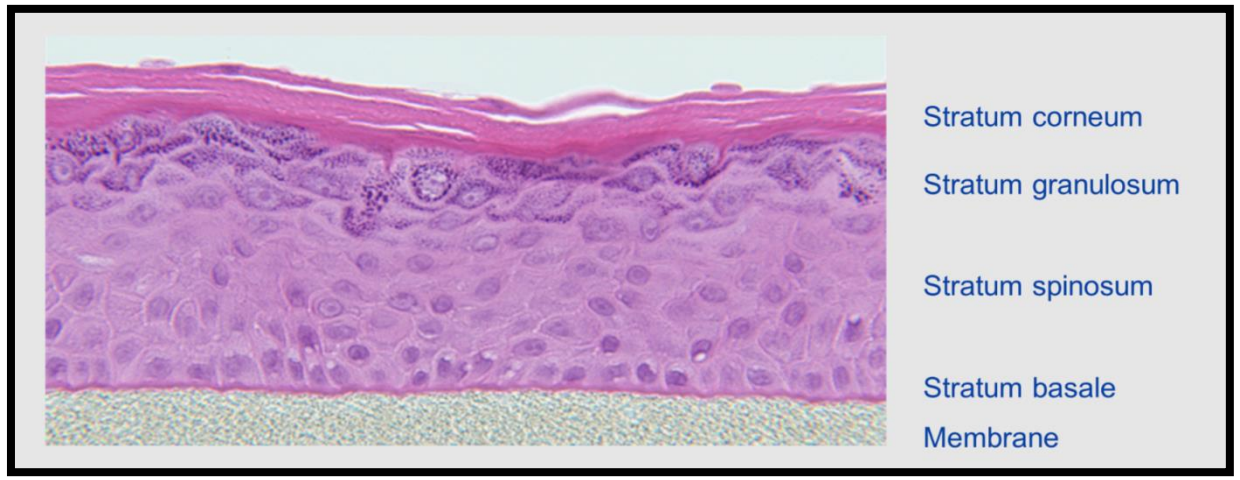
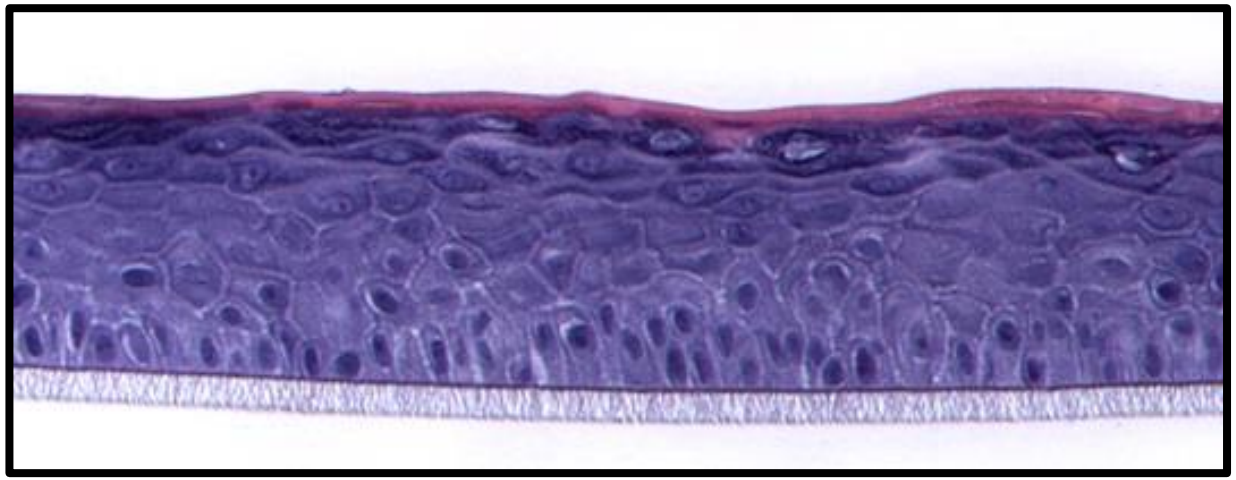
RhE Models Used in Round-Robin Study



MatTek EpiDerm™ (EPI-200) RhE model



EPISKIN SkinEthic™ RHE model

Preparation of RR started with 27 laboratories/organizations

Several Laboratories Were Lost during RR Preparation and Testing Phase

- Reasons for dropping out
  - Building/refurbishing activities
  - Custom/import problems with RhE tissues
  - Staff changes
  - Insufficient training
  - Time restrictions

Organization of RR study
Participants and Stakeholder Involvement

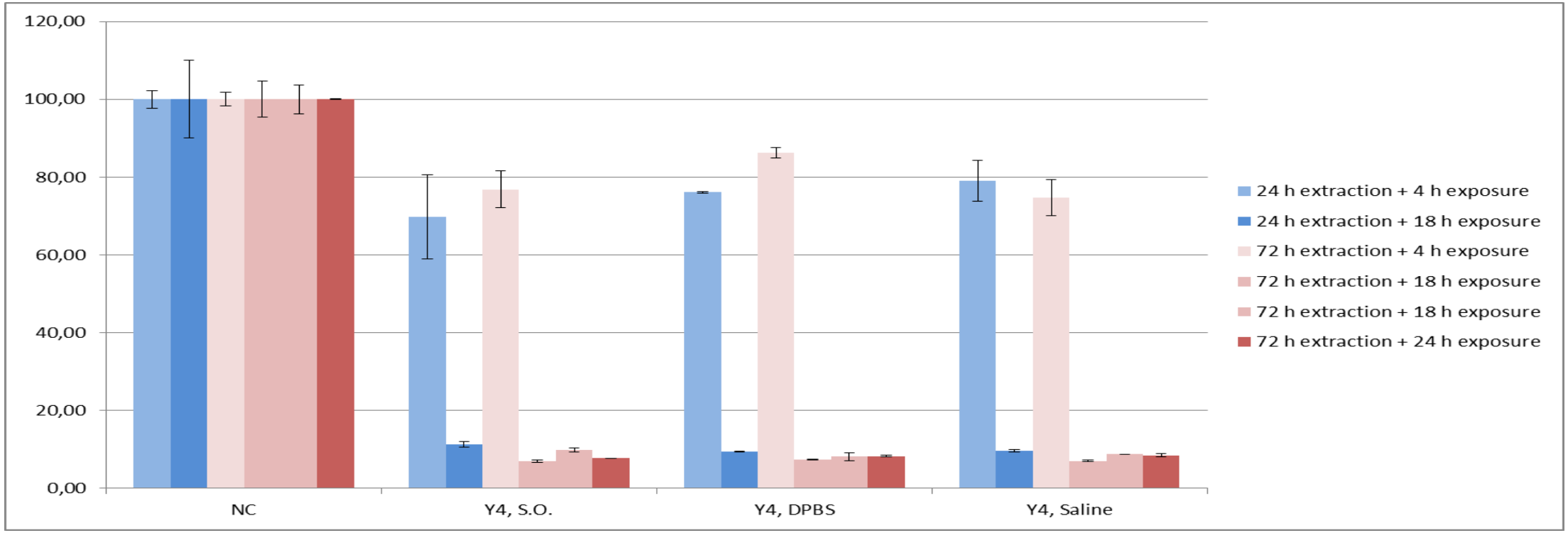
- **Governmental Laboratories**
  - US FDA (USA), NIHS (Japan), RIVM (Netherlands)
- **University Laboratories**
  - SP-TRI (Sweden), Yonsei University (South Korea)
- **Contract Research Organizations**
  - APS (USA), Cyprotex (USA), Envigo (Germany), Eurofins Biolab (Italy), Eurofins BioPharma (Germany), NAMSA (USA, France), Nelson Laboratories (USA), VitroScreen (Italy), WuXi Apptec (USA)
- **Medical Device Companies**
  - Beckton Dickinson (USA), Arthrex Inc. (USA), Medtronic lpc (USA)
- **RhE Model Manufacturers**
  - EPISKIN (France), MatTek (Slovakia, USA)
- **Independent Consultancy** for the statistical evaluations
  - seh consulting+services (Germany)

Preliminary Studies

- Adaptation of exposure protocol
  - Medical device extracts can contain low levels of chemicals
  - Extension of exposure time (OECD 439, 15 minutes up to 60 minutes depending on model used)
- Evaluation of exposure times 4–24 hours (Casas et al., 2013, 24 hours)
- Extraction of polymers for 72 ± 2 h
- No recovery period
- Viability determined directly after exposure and washing procedure
- Protocols provided by RhE manufacturers almost similar

Preliminary Studies

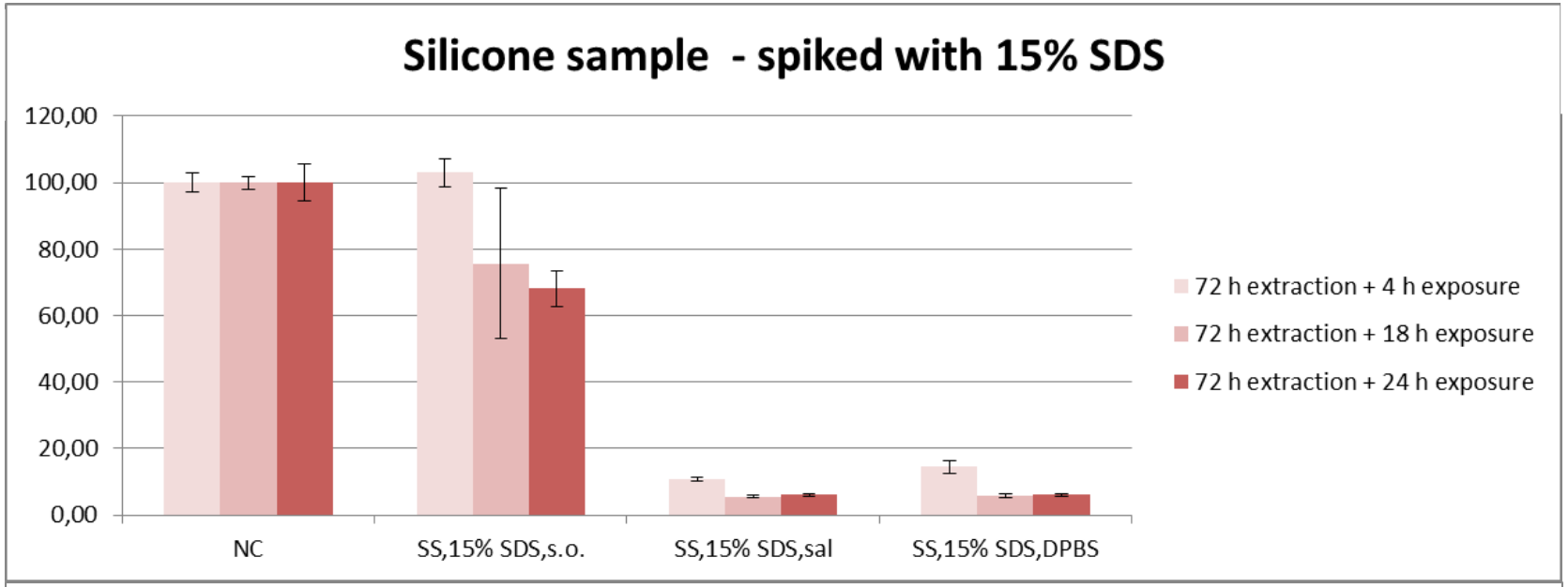
Conditions of extraction: 24hrs (blue) and 72 hrs (red) in Sesame Oil (S.O.), DPBS and Saline
Exposure time: 4, 18 and 24 hours

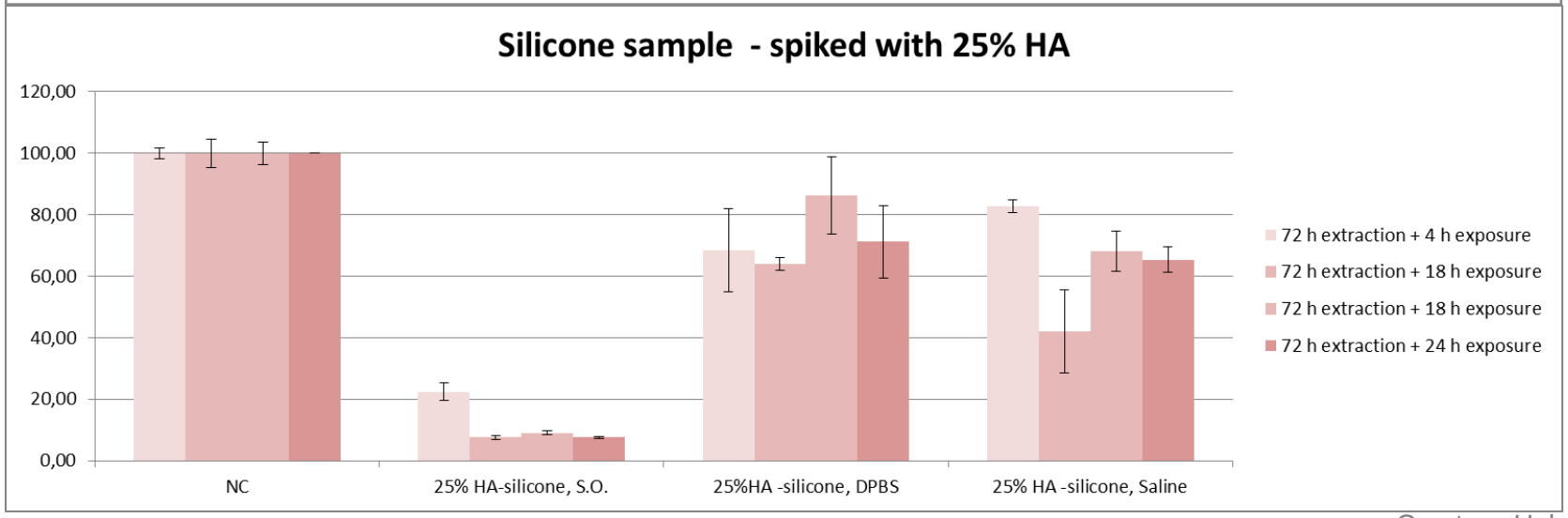


1. No difference between 24 and 72 hrs extraction conditions, and between 18 and 24 hrs exposure
2. No significant difference between extractions in DPBS and Saline (watery solutions)

Courtesy Helena Kandarova & Silvia Letasiova

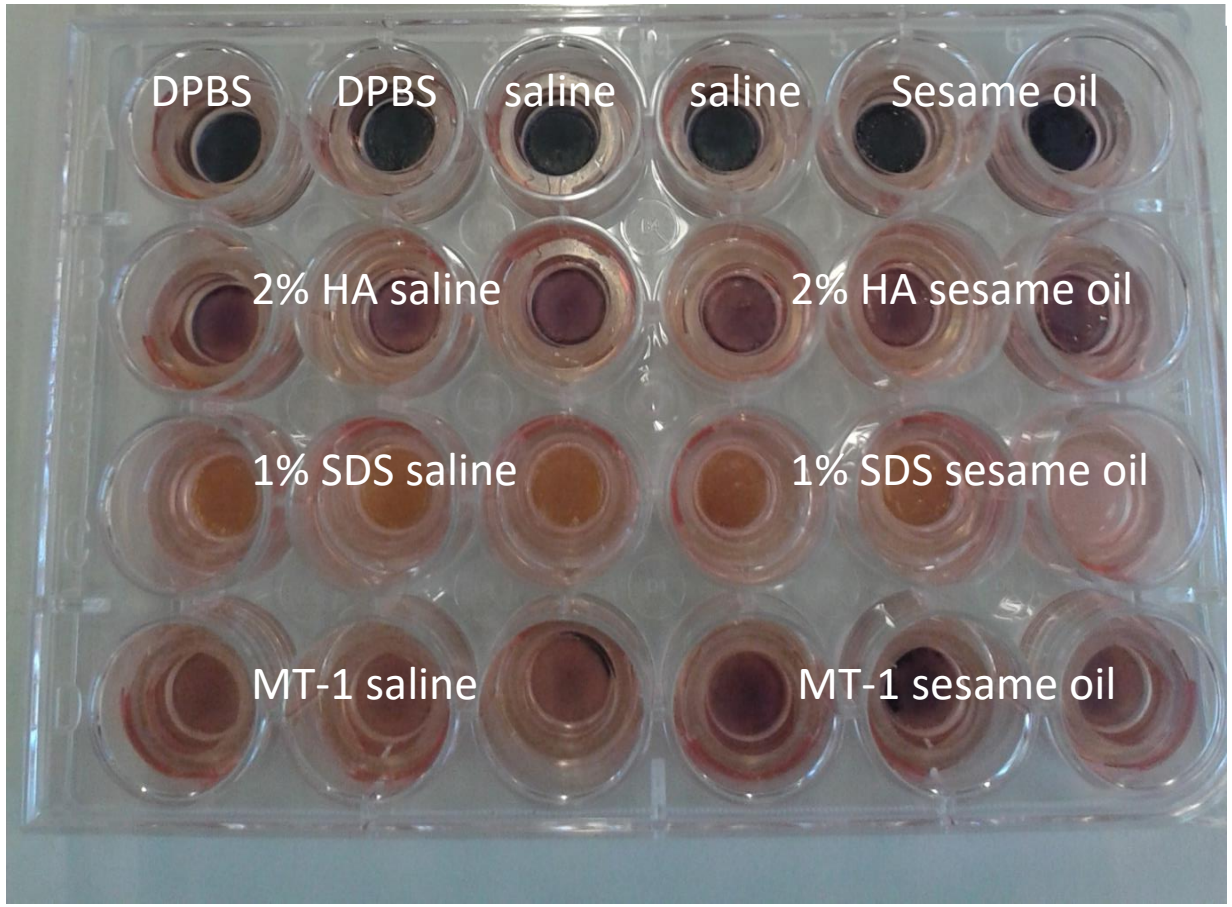
Preliminary Studies Show Dependence on Extraction Vehicle

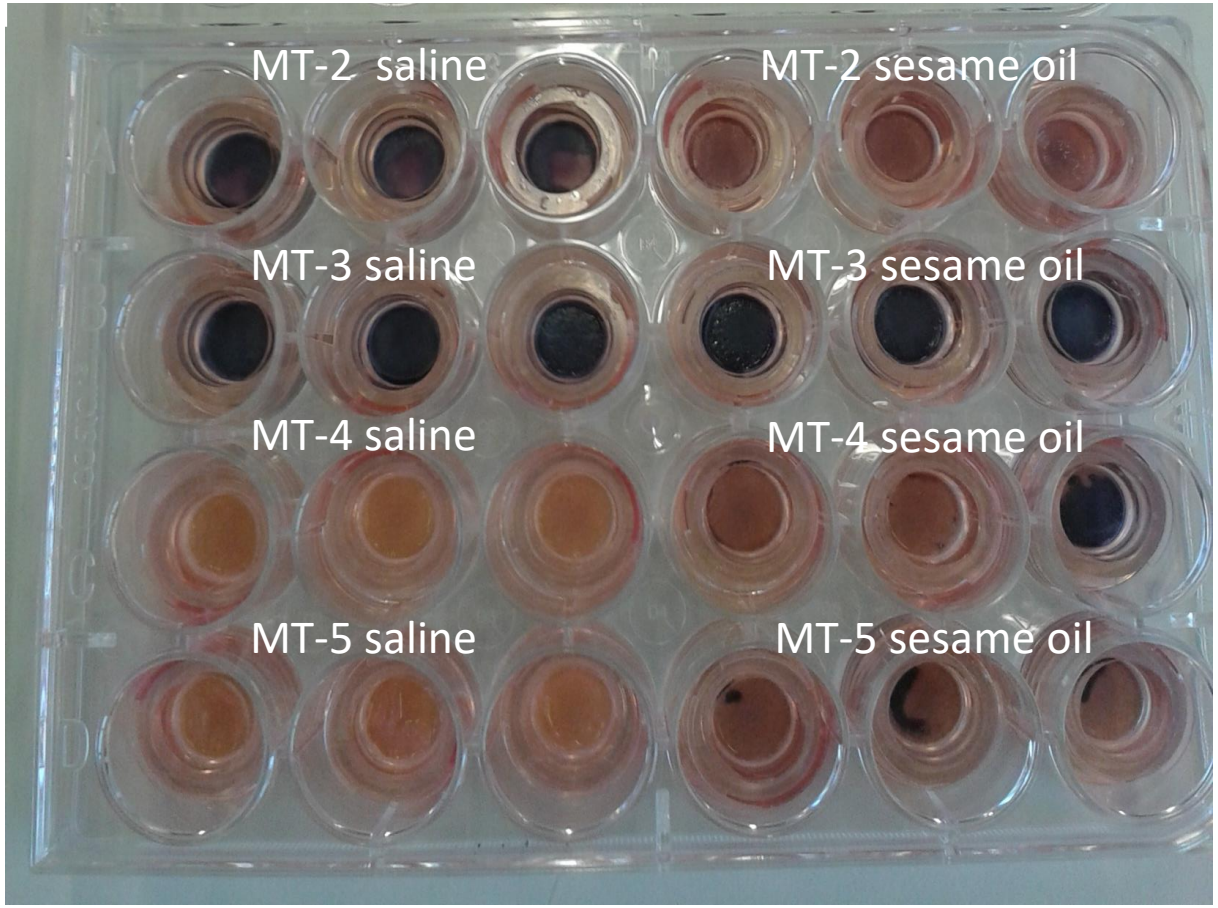




Courtesy Helena Kandarova & Silvia Letasiova

Example of RhE Method (EpiDerm™ Tissues)





MT-1 Y4-Polymeer
MT-2 25% HA-Silicone
MT-3 Silicone Control, A
MT-4 15% SDS- Silicone, D
MT-5 17% SDS- Silicone, G

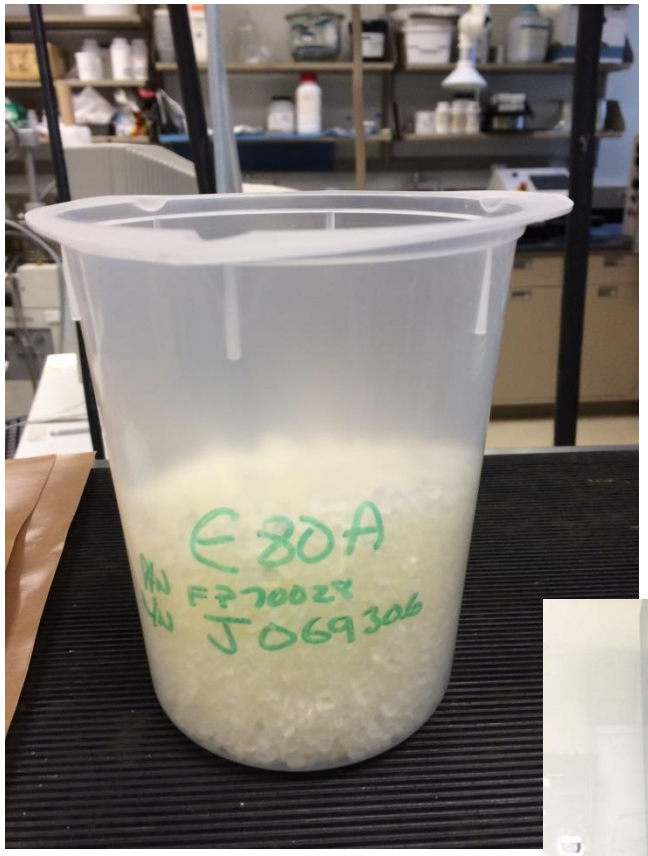
Positive Irritant Polymers

- Difficult to find irritant polymers
- Therefore we made most on our own
- Lots of trial and error
- By mixing, casting, and hot-melt
- Extractions, LC-MS, and tissue testing

Preparation Test Samples

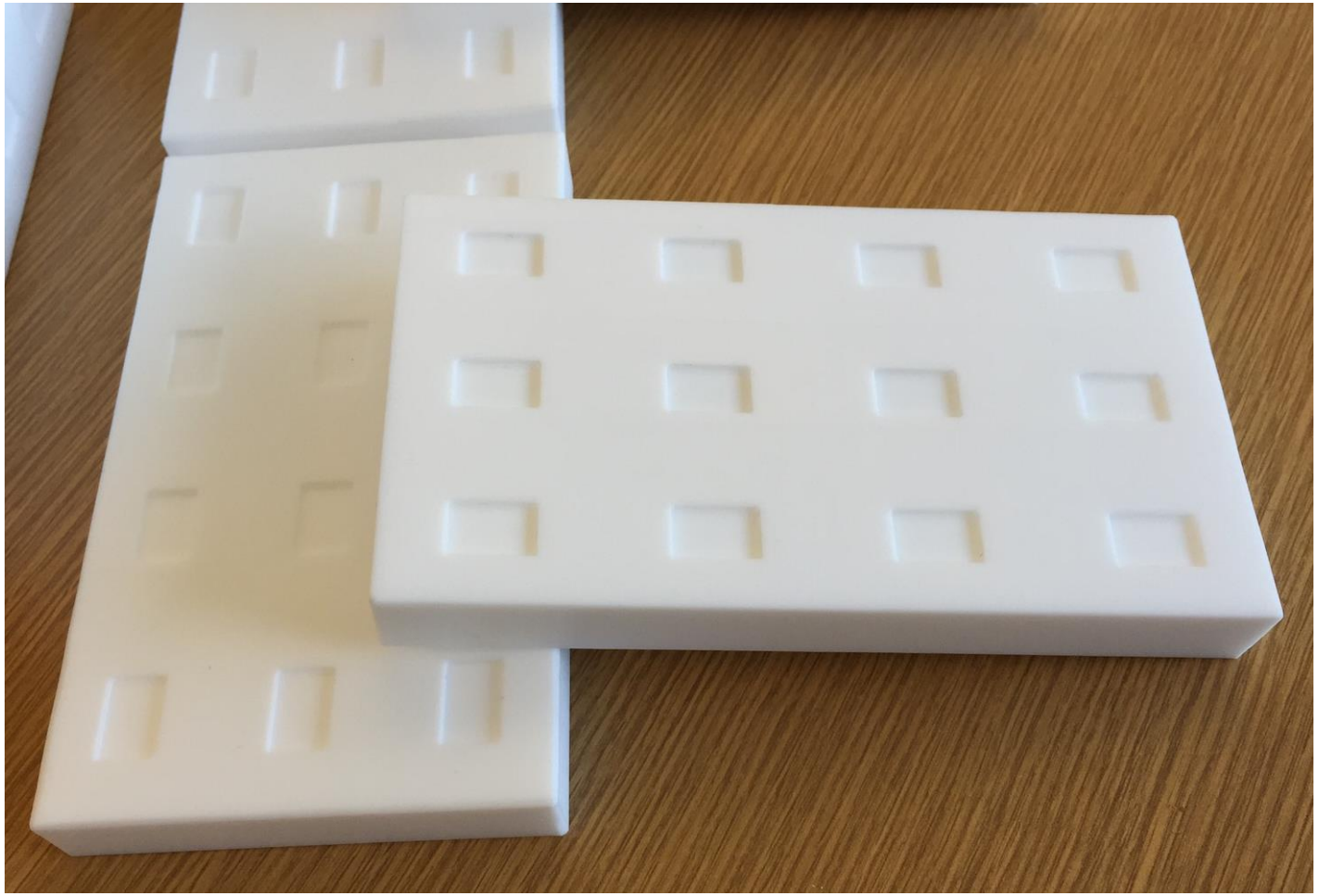
Hi-speed Mixing

Courtesy Kelly Coleman, Medtronic plc.


E80A
J069306


COPLEY

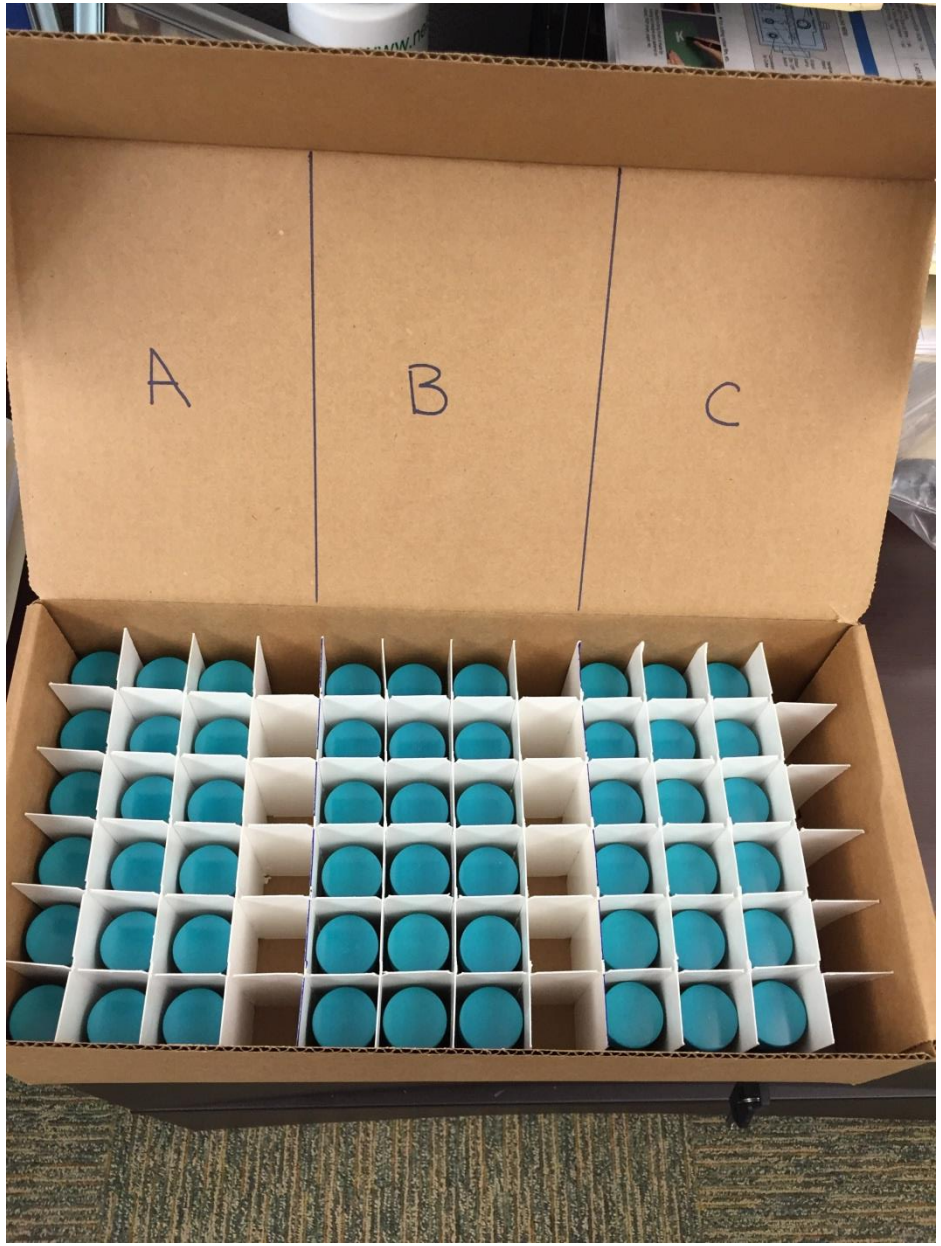
Preparation Test Samples
Teflon® Casting Blocks

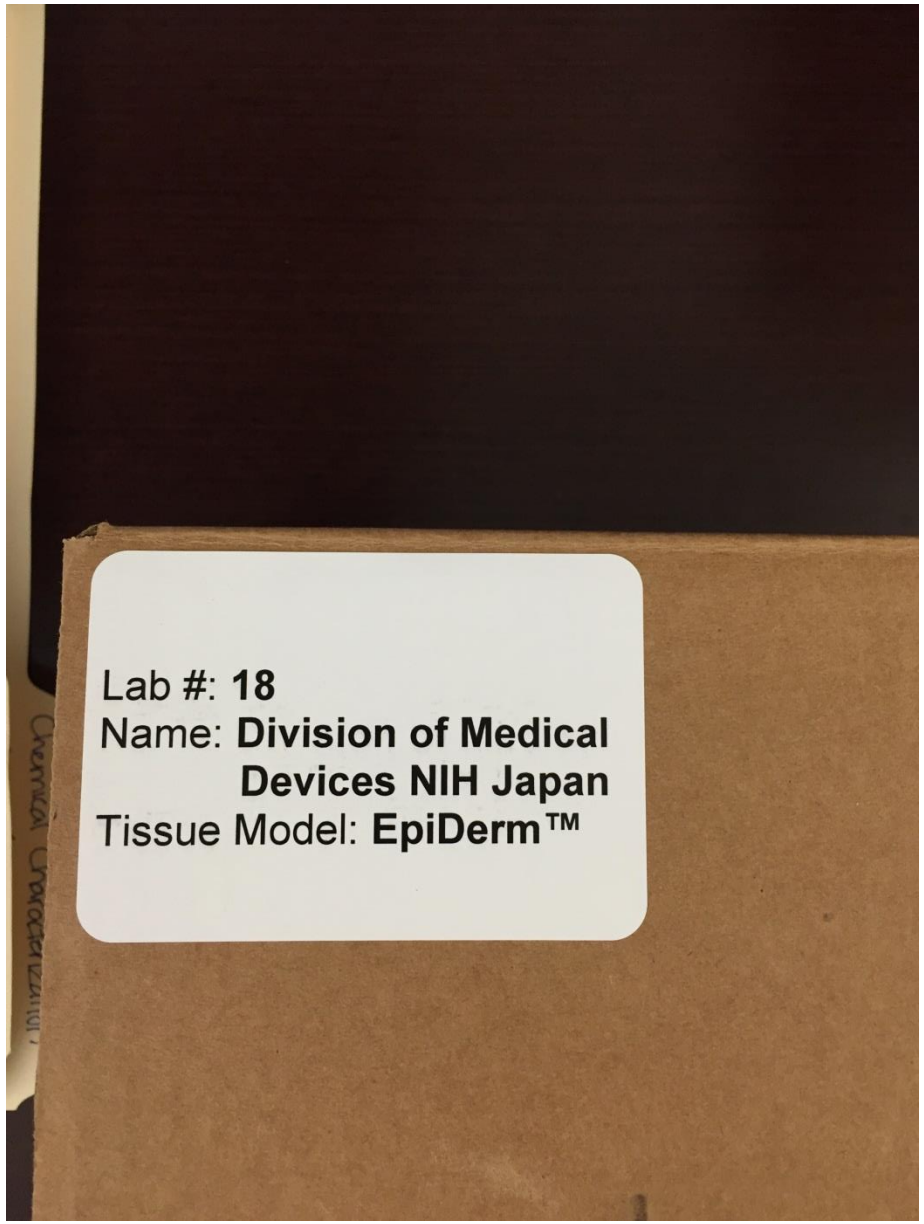


Courtesy Kelly Coleman, Medtronic plc.

Test Samples Used for Extraction

Material	Expected classification
100% Silicone	Non – irritant
100% Polyurethane E80A	Non – irritant
100% PVC (Y-1)	Non – irritant
PVC + 5.8% Genapol X-80 (Y-4)	Irritant
PVC + 4% Genapol X-100	Irritant
Silicone + 15% SDS	Irritant
Silicone + 25% Heptanoic Acid	Irritant


A
B
C


Lab #: 18
Name: Division of Medical Devices NIH Japan
Tissue Model: EpiDerm™

Overall Protocol(s)

- Polymer extraction 72 ± 2 hours, 37°C under agitation/shaking
  - 3 cm^2 (approximately 1 cm x 1.5 cm sample, x2 upper/under site) or 0.2 g
  - 1 ml saline or sesame oil
- RhE tissue exposure for:
  - 18 hours (EpiDerm™ method)
  - 24 hours (SkinEthic™ RHE method)
- Viability measurment with MTT formazan production
  - 3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide
- Each assay performed in triplicate (3 tissues)
- Each assay performed three times on three independent days
- All samples coded and unknown to test operator
- Evaluation centrally by independent statistician

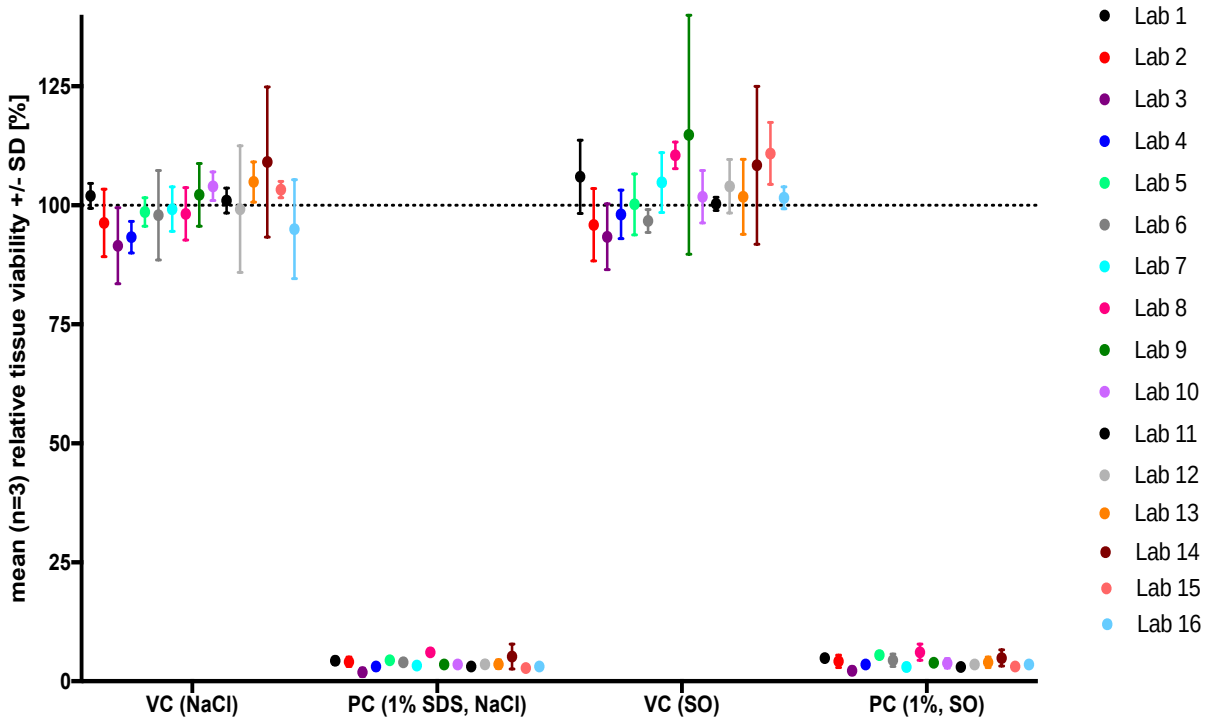
Example of Test Outcomes

EpiDerm™ test method: Laboratory 12

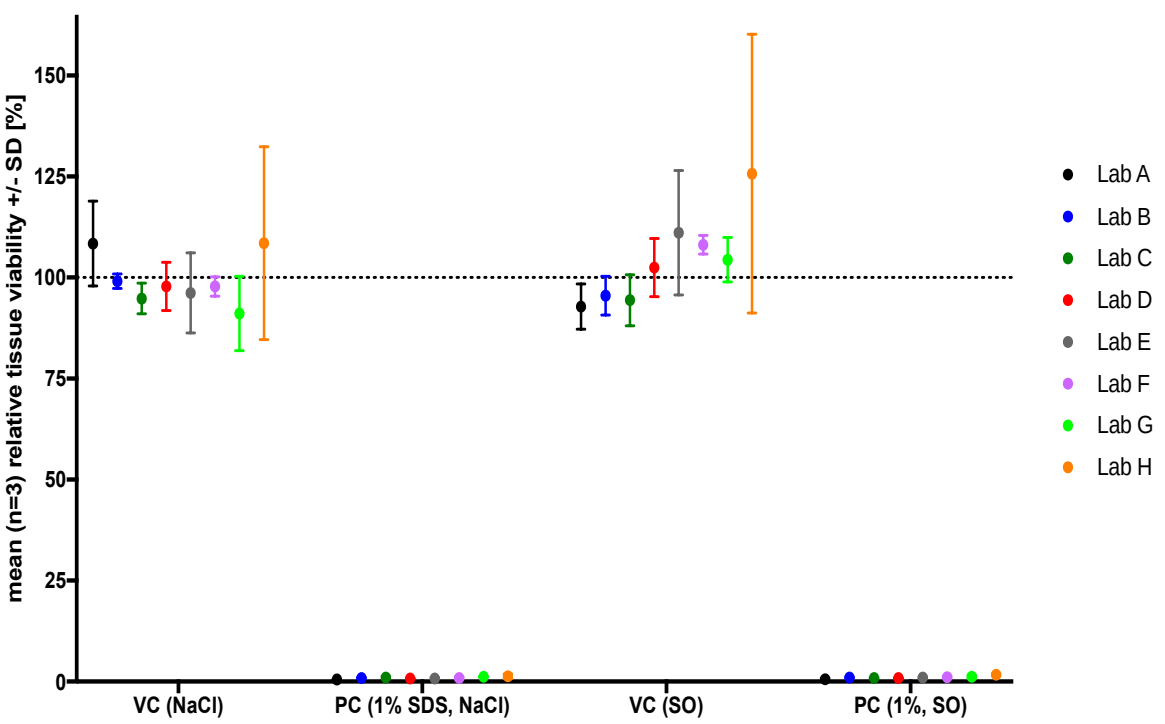
	Experiment B			Experiment C			Experiment R#		
test material	code	% cell viability	SD	code	% cell viab.	SD	code	% cell viab.	SD
DPBS	-	100.0	5.45	-	100.0	11.90	-	100.0	6.07
Saline	-	102.6	9.37	-	110.5	8.41	-	84.5	10.04
1% SDS in saline	-	3.7	0.31	-	4.2	0.30	-	2.9	0.29
100% silicone	B286-1	102.5	8.55	C539-1	121.7	5.17	A30R-1	107.7	3.46
Polyurethane E80A	B283-1	102.8	11.30	C537-1	115.2	12.02	A25R-1	102.7	10.62
PVC + 4% Genapol	B282-1	20.9	2.43	C543-1	12.5	6.40	A26R-1	24.4	7.04
Silicone + 15% SDS	B285-1	4.6	0.57	C544-1	4.4	0.38	A27R-1	3.5	0.21
Silicone + 25% Heptanoic Acid	B288-1	100.6	2.20	C542-1*	103.9	5.97	A31R-1*	78.0	10.33
Y-1	B284-1	97.0	6.98	C538-1	127.3	6.17	A29R-1	106.8	1.59
Y-4 Lot 1, 2, & 3	B281-1	6.8	1.59	C540-1	6.1	0.14	A28R-1	5.5	0.64
1% SDS in sesame oil	-	3.6	0.28	-	3.7	0.20	-	3.6	0.23
Sesame oil	-	102.5	0.87	-	119.7	2.56	-	105.5	1.13
DPBS	-	100.0	8.04	-	100.0	2.09	-	100.0	6.07
Sesame oil	-	97.8	0.92	-	108.7	7.76	-	105.5	1.13
1% SDS in sesame oil	-	3.4	0.18	-	3.5	0.19	-	3.6	0.23
100% silicone	B286-2	97.7	4.15	C539-2	120.3	9.53	A30R-2	110.7	7.25
Polyurethane E80A	B283-2	111.0	9.00	C537-2	124.7	2.19	A25R-2	110.8	7.36
PVC + 4% Genapol	B282-2	11.7	2.48	C543-2	7.2	2.15	A26R-2	12.5	2.66
Silicone + 15% SDS	B285-2	113.7	7.05	C544-2	113.3	3.92	A27R-2*	101.0	2.98
Silicone + 25% Heptanoic Acid	B288-2*	113.2	6.38	C542-2*	116.5	4.97	A31R-2*	7.0	1.90
Y-1	B284-2	104.7	0.22	C538-2	123.0	8.22	A29R-2	110.9	3.78
Y-4 Lot 1, 2, & 3	B281-2	30.9	1.59	C540-2	40.5	4.20	A28R-2	40.9	1.80
1% SDS in saline	-	3.7	0.34	-	4.0	0.25	-	2.9	0.29
Saline	-	99.0	9.52	-	106.2	5.42	-	84.5	10.04

#For Experiment A the data were lost due to an ICT error. *test material was attached to the bottom of the container

Cell Viability Tesults for Vehicle (VC) and Positive (PC) Controls

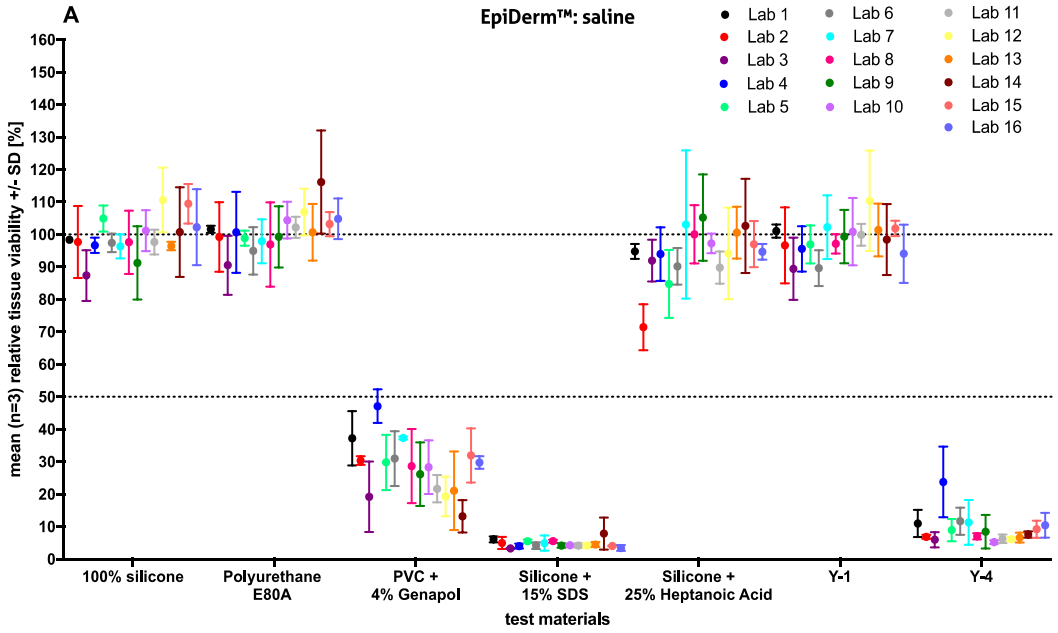


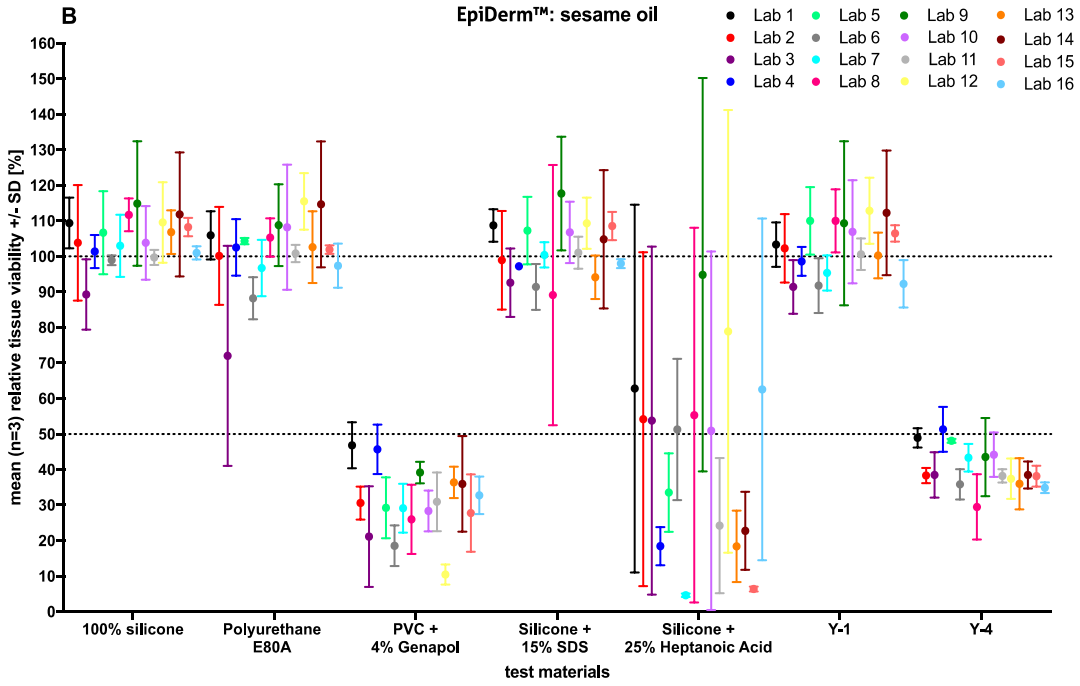
EpiDerm™ test method

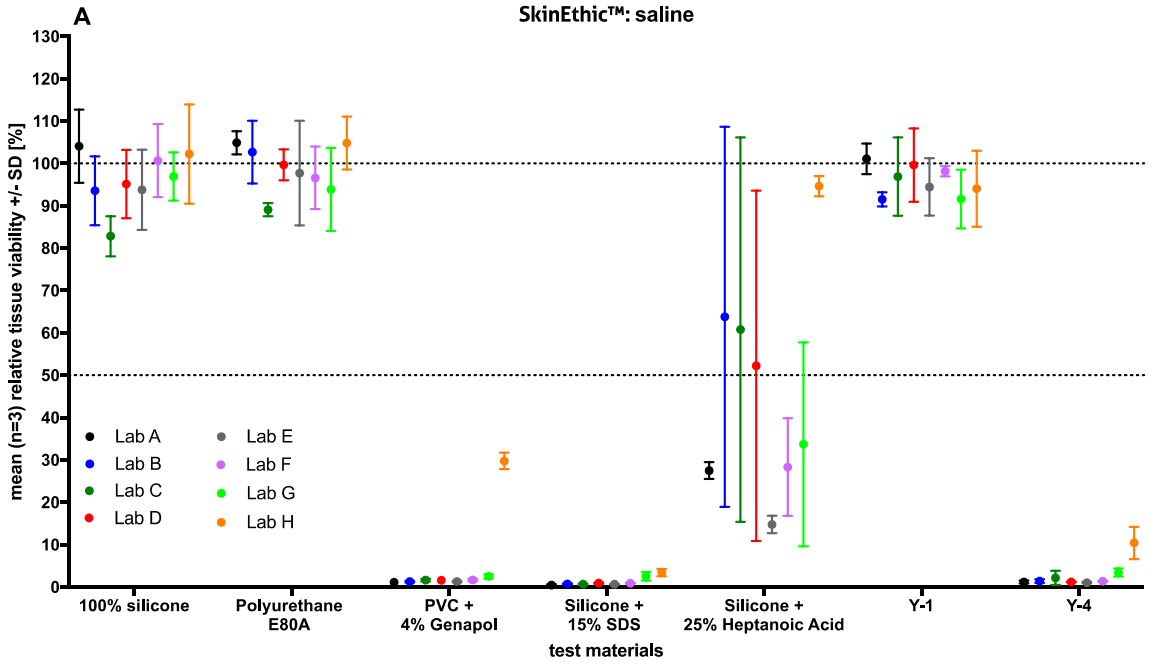


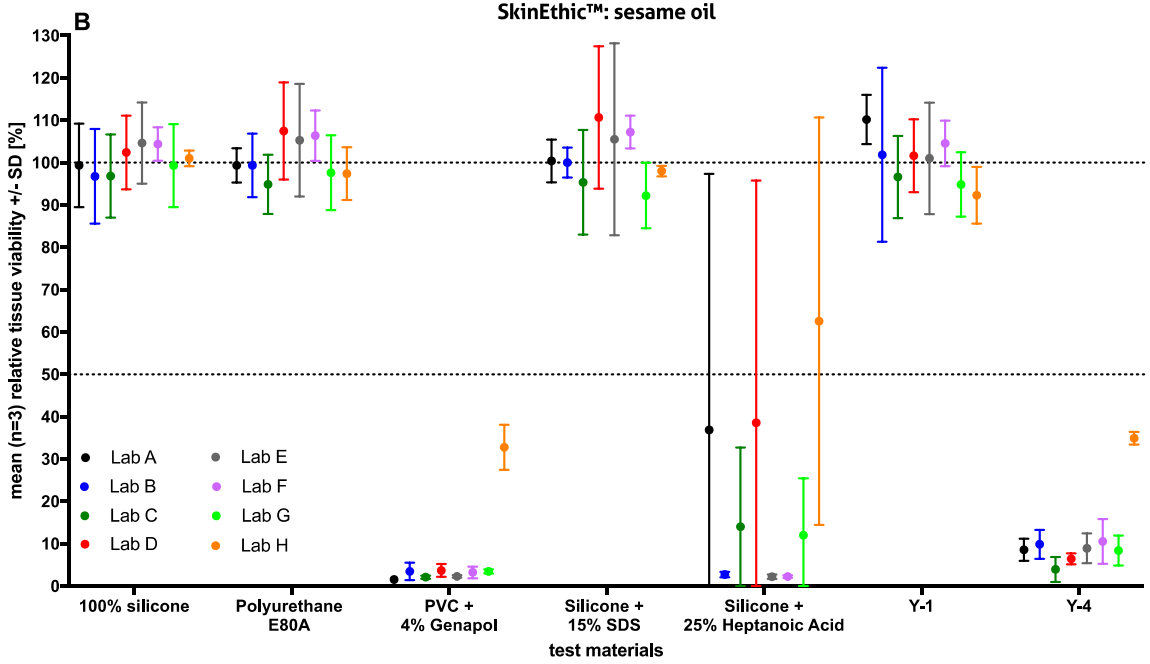
SkinEthic™RhE test method

Mean cell viability and standard deviation (SD) of three individual experiments per laboratory are displayed. The dotted line at 100% represents the respective negative (DPBS) control, which is used for normalization.


A
EpiDerm™: saline
Lab 1
Lab 2
Lab 3
Lab 4
Lab 5
Lab 6
Lab 7
Lab 8
Lab 9
Lab 10
Lab 11
Lab 12
Lab 13
Lab 14
Lab 15
Lab 16
mean (n=3) relative tissue viability +/- SD [%]
100% silicone
Polyurethane E80A
PVC + 4% Genapol
Silicone + 15% SDS
Silicone + 25% Heptanoic Acid
Y-1
Y-4
test materials


B
EpiDerm™: sesame oil
Lab 1
Lab 2
Lab 3
Lab 4
Lab 5
Lab 6
Lab 7
Lab 8
Lab 9
Lab 10
Lab 11
Lab 12
Lab 13
Lab 14
Lab 15
Lab 16
mean (n=3) relative tissue viability +/- SD [%]
100% silicone
Polyurethane E80A
PVC + 4% Genapol
Silicone + 15% SDS
Silicone + 25% Heptanoic Acid
Y-1
Y-4
test materials


A
SkinEthic™: saline
Lab A
Lab B
Lab C
Lab D
Lab E
Lab F
Lab G
Lab H
mean (n=3) relative tissue viability +/- SD [%]
100% silicone
Polyurethane E80A
PVC + 4% Genapol
Silicone + 15% SDS
Silicone + 25% Heptanoic Acid
Y-1
Y-4
test materials


B
SkinEthic™: sesame oil
Lab A
Lab B
Lab C
Lab D
Lab E
Lab F
Lab G
Lab H
mean (n=3) relative tissue viability +/- SD [%]
100% silicone
Polyurethane E80A
PVC + 4% Genapol
Silicone + 15% SDS
Silicone + 25% Heptanoic Acid
Y-1
Y-4
test materials

Overall Results

EpiDerm™ EPI-200 predictivity

	Mode		Mean		Classifications of individual experiments		
16 labs	Saline	Sesame oil	Saline	Sesame oil	Saline	Sesame oil	Overall
Average predictivity	100% (111/111)	96.4% (106/110)	100% (111/111)	91.8% (101/110)	99.7% (334/335)	94.0% (314/334)	95.5% (319/334)

SkinEthic ™ RHE predictivity

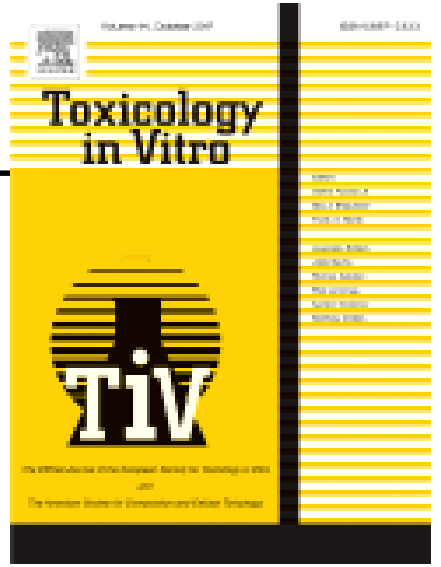
	Mode		Mean		Classifications of individual experiments		
8 labs	Saline	Sesame oil	Saline	Sesame oil	Saline	Sesame oil	Overall
Average predictivity	89.3% (50/56)	100% (56/56)	92.9% (52/56)	100% (56/56)	90.5% (152/ 168)	98.2% (165/168)	100% (168/168)

Conclusions

- RhE tissues are robust models for detecting low irritant levels in medical device abstracts

- Consequently, these *in vitro* assays are an acceptable replacement for the rabbit skin irritation test.

Next Steps

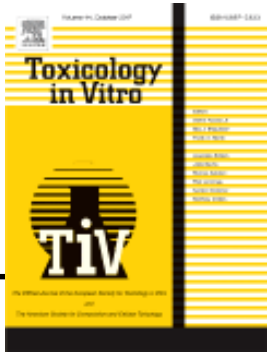


- Results to be published in a special edition of *Toxicology In Vitro 2018*.

- Drafting of ISO 10993-23 standard for irritation testing of medical devices including the *in vitro* RhE as primary test (ISO TC 194).

- An application to US FDA's Medical Device Development Tools (MDDT) program is under discussion.

Special Issue of *Toxicology In Vitro* 2018



- Coleman KP, Grailer TP, McNamara LR, Rollins BL, Cristiano NJ. Preparation of Irritant Polymer Samples for an In Vitro Round Robin Study.
- Kandárová H, Willoughby JA, De Jong WH, Letasiova S et al. Pre-validation of an In Vitro Skin Irritation Test for Medical Devices Using the Reconstructed Human Tissue Model EpiDerm.
- Pellevoisin C, Videau C, Briotet D, Grégoire C et al. Use of SkinEthicTM RHE for In Vitro Evaluation of Skin Irritation of Medical Device Extracts.
- Olsen D, Lee M, Turley AP. Assessment of Test Method Variables for In Vitro Skin Irritation Testing of Medical Device Extracts.
- Kandárová H, Bendova H, Letasiova S, Coleman KP et al. Evaluation of Medical Device Round Robin Irritants using Human Patch Testing and an RhE In Vitro Assay.
- De Jong WH, Hoffmann S, Lee M, Kandárová H, Pellevoisin C et al. 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.

Development ISO standards

NWIP approved 16-04-2018: Revision of ISO 10993-10 now only on sensitization testing including extended informative annex on *in vitro* alternatives for sensitization testing.

NWIP approved 18-01-2018: Drafting of new standard ISO 10993-23 on irritation testing including *in vitro* Reconstructed Human Epidermis (RhE) model and *in vivo* assays for irritation testing of medical device extracts.

Medical Device Development Tools (MDDT)

- U.S. FDA Center for Devices and Radiological Health (CDRH) is offering the MDDT program, to reduce the regulatory burden faced by medical device sponsors by promoting the development of tools that streamline the review process.
- Importantly, this program provides a clear pathway for companies to receive agency approval to use **nonanimal methods** in place of animal tests that are routinely required or recommended.
- MDDT is a process to provide early qualification of tools used by medical device sponsors in the development and evaluation of medical devices. Notably, the process can be used as a collaborative platform for industry, academia, and research organizations to discuss early concepts and build partnerships in sponsoring the development of a tool for potential qualification by US FDA.
- One example of an application currently under discussion is the *in vitro* irritation test using reconstructed human epidermis (RhE) models as recently evaluated in a global round robin study within ISO/TC 194 “Biological and Clinical Evaluation of Medical Devices”, Working Group 8 on “Irritation and Sensitization”.

- FDA Webinar Link
- MDDT Final Guidance Document

The People

Wim H. De Jong, Sebastian Hoffmann, Michelle Lee , Helena Kandárová, Christian Pellevoisin, Haishima Yuji, Beau Rollins, Austin Zdawzcyk, Jamin Willoughby, Michael Bachelor, Timothy Schatz, Shelby Skoog, Sherry Parker, Anita Sawyer, Paolo Pescio, Kristina Fant, Kwang-Mahn Kim, Jae Sung Kwon, Helge Gehrke, Hana Hofman- Hüther, Marisa Meloni, Conrad Julius, Damien Briotet, Sylvia Letasiova, Reiko Kato, Atsuko Miyajima, Liset De La Fonteyne, Christelle Videau, Carine Tornier, Audrey Turley, Nicholas Christiano, Thor Rollins, Kelly P. Coleman.