INTERLABORATORY COMPARISON OF OECD TG 439 IN BRAZIL

Pan-American Conference for Alternative Methods
August 23-24, 2018

Luciene Bottentuit Lopez Balottin, D.Sc.

INMETRO
Instituto Nacional de Metrologia, Qualidade e Tecnologia

Ministry of Industry, Foreign Trade and Services

The National Institute of Metrology, Quality and Technology (Inmetro) was created by law in December, 1973, ***to support Brazilian enterprises*** *, to increase their productivity and the quality of goods and services.*

PROVIDE CONFIDENCE TO THE SOCIETY CONCERNING MEASUREMENTS AND PRODUCTS

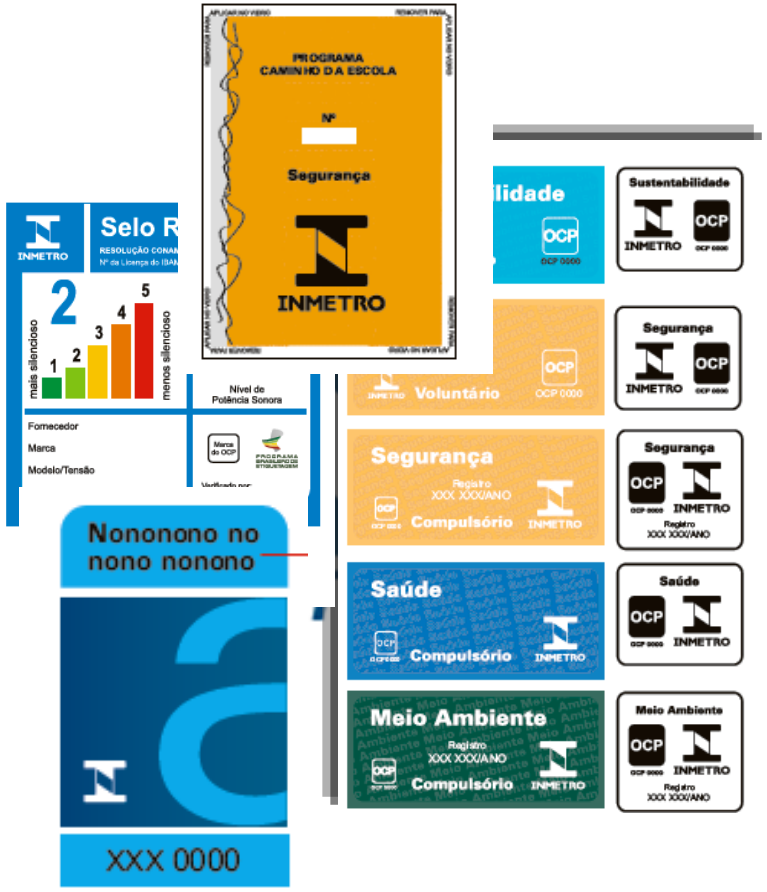
PROMOTING HARMONIZATION IN CONSUMPTION RELATIONS, INOVATION AND COMPETITIVENESS

through:

METROLOGY and CONFORMITY ASSESSMENT

Inmetro's Main Activities

- Scientific and Industrial Metrology;
- Legal Metrology;
- Conformity Assessment Regulations;
- Accreditation of Laboratories and Certification Bodies;
- GLP Monitoring Authority;
- WTO/TBT Enquiry Point.
- Market Surveillance



RENAMA

Rede Nacional de Métodos Alternativos

The National Network of Alternative Methods - RENAMA was created under the Ministry of Science, Technology, Innovation and Communication - MCTIC, which will be supervised by a Steering Committee.

Created 2012

BRACVAM

Associated Labs

I - promote the implementation, development and validation of alternative methods to the use of animals;

II - to promote the adoption of alternative methods to the use of animals in teaching and research activities;

III - to stimulate the implantation of alternative methods to the use of animals through technical training and implementation of validated methodologies;

IV - periodically monitor the performance of the associated laboratories through interlaboratory comparisons;

V - to promote the quality of the tests using the development of certified chemical and biological reference materials, where applicable;

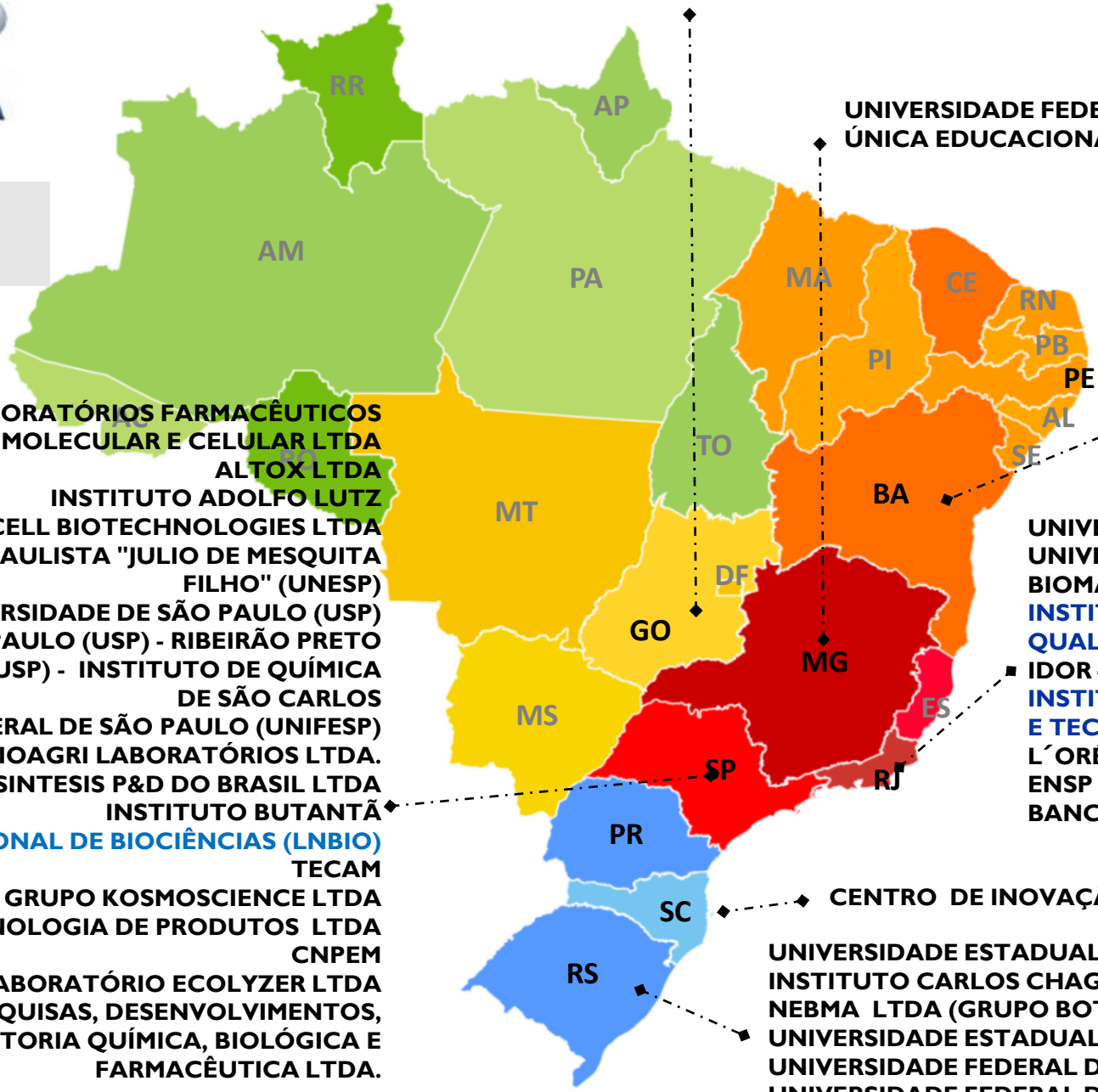
VI - to encourage the implementation of the laboratory quality system and the principles of good laboratory practice (GLP);

VII - to disseminate knowledge in the theme of alternative methods to the use of animals;

VIII - offer, within the Network's laboratories, services for toxicological tests using alternative methodologies to the use of animals.

RENAMA

ASSOCIATED LABS: 39
CENTRAL LABs: 3



UNIVERSIDADE FEDERAL DE GOIÁS (UFG)

UNIVERSIDADE FEDERAL DE MINAS GERAIS (UFMG)
ÚNICA EDUCACIONAL - FACULDADE INFORIUM DE TECNOLOGIA

UNIVERSIDADE FEDERAL DE PERNAMBUCO (UFPE)

UNIVERSIDADE FEDERAL DA BAHIA (UFBA)

ACHE LABORATÓRIOS FARMACÊUTICOS
INVITROCELL AVALIAÇÃO MOLECULAR E CELULAR LTDA
ALTOX LTDA
INSTITUTO ADOLFO LUTZ
PLURICELL BIOTECHNOLOGIES LTDA
UNIVERSIDADE ESTADUAL PAULISTA "JULIO DE MESQUITA FILHO" (UNESP)
UNIVERSIDADE DE SÃO PAULO (USP)
UNIVERSIDADE DE SÃO PAULO (USP) - RIBEIRÃO PRETO
UNIVERSIDADE DE SÃO PAULO (USP) - INSTITUTO DE QUÍMICA DE SÃO CARLOS
UNIVERSIDADE FEDERAL DE SÃO PAULO (UNIFESP)
BIOAGRI LABORATÓRIOS LTDA.
LABORATÓRIOS BIOSINTESIS P&D DO BRASIL LTDA
INSTITUTO BUTANTÃ
LABORATÓRIO NACIONAL DE BIOCIÊNCIAS (LNBIO)
TECAM
GRUPO KOSMOSCIENCE LTDA
NATURA INOVAÇÃO E TECNOLOGIA DE PRODUTOS LTDA
CNPEM
LABORATÓRIO ECOLYZER LTDA
T&E ANALÍTICA - CENTRO DE PESQUISAS, DESENVOLVIMENTOS, ANÁLISES E CONSULTORIA QUÍMICA, BIOLÓGICA E FARMACÊUTICA LTDA.

UNIVERSIDADE FEDERAL DO RIO DE JANEIRO (UFRJ)
UNIVERSIDADE FEDERAL FLUMINENSE (UFF)
BIOMANGUINHOS - FIOCRUZ
INSTITUTO NACIONAL DE CONTROLE DA QUALIDADE EM SAÚDE (INCQS)- FIOCRUZ
IDOR – PESQUISA E ENSINO
INSTITUTO NACIONAL DE METROLOGIA, QUALIDADE E TECNOLOGIA (INMETRO)
L´ORÉAL PESQUISA E INOVAÇÃO BRASIL
ENSP – FIOCRUZ
BANCO DE CÉLULAS DO RIO DE JANEIRO (BCRJ)

CENTRO DE INOVAÇÃO E ENSAIOS PRÉ-CLÍNICOS (CIENP)

UNIVERSIDADE ESTADUAL DE MARINGÁ (UEM)
INSTITUTO CARLOS CHAGAS - FIOCRUZ
NEBMA LTDA (GRUPO BOTICÁRIO)
UNIVERSIDADE ESTADUAL DE LONDRINA (UEL)
UNIVERSIDADE FEDERAL DO PARANÁ (UFPR)
UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE (UFCSPA)

CONCEA. 2014. Normative Resolution N° 18/2014 on alternative methods of 24 September 2014. Diário Oficial da União. N° 185, Seção 1. p.9. http://pesquisa.in.gov.br/imprensa/jsp/visualiza/index.jsp?data=25/09/2014&jornal=1&pagina=9&totalArquivos=120 (accessed 31/5/17)

➢ **CONCEA** – National Council for the Control of Animal Experimentation

- *Normative Resolution n.* ***18/2014***

❖ *recognizes the following validated alternative methods:*

✓ **Skin Corrosion/Irritation: OECD TG** 430, 431**,** 435, and **439;**
✓ Eye Corrosion: OECD TG 437, 438, and 460;
✓ Phototoxicity: OECD TG 432;
✓ Skin Absorption: OECD TG 428;
✓ Skin Sensitisation: OECD TG 429, 442A, and 442B;
✓ Acute Toxicity: OECD TG 420, 423, 425, and 129*;
✓ Genotoxicity: OECD TG 487

* Note: The CONCEA's publication *"OECD TG 129"* refers to the Guidance Document n. 129 (*Guidance document on using cytotoxicity tests to estimate starting doses for acute oral systematic toxicity tests).*

SEPTEMBER 2019 !!

"Organization of interlaboratory comparisons as a tool for increasing laboratory quality, harmonizing methods and integration/strengthening of the National Network of Alternative Methods (ReNaMA)".

CNPq/MCTIC Nº 19/2016

INMETRO

Consortium Formation

natura bem estar bem

L'ORÉAL EPISKIN ACADEMY

biosintesis

TECAM Laboratórios

INVITROCELL

Ministério da Saúde
FIOCRUZ - PARANÁ
Instituto Carlos Chagas

UNIVERSIDADE FEDERAL DE PERNAMBUCO
VIRTUS IMPAVIDA

Pharmaceutical and Cosmetics Development Center (NUDFAC)

INSTITUTO ADOLFO LUTZ

USP Ribeirão Preto

Laboratory of cosmetic technology applied to photoprotection

CIEnP

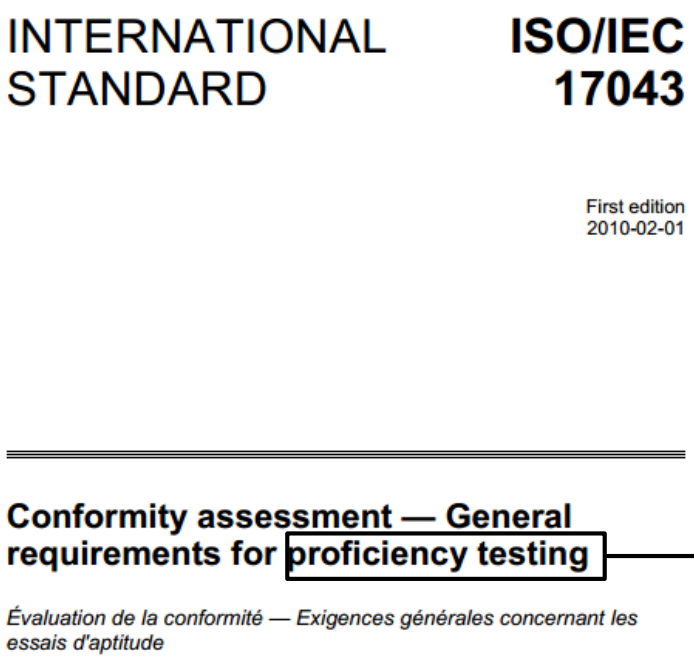
unesp

Grupo kosmo science

UFMG

International Organization for Standardization

independent, non-governmental *international organization* with a membership of 163 national standards bodies



INTERNATIONAL STANDARD

ISO/IEC 17043

First edition
2010-02-01

Conformity assessment — General requirements for proficiency testing

Évaluation de la conformité — Exigences générales concernant les essais d'aptitude

Evaluation of participant performance against pre-established criteria by means of interlaboratory comparisons

Interlaboratory comparison

Organization, performance and evaluation of measurements or tests on the same or similar items by two or more laboratories in accordance with predetermined conditions.

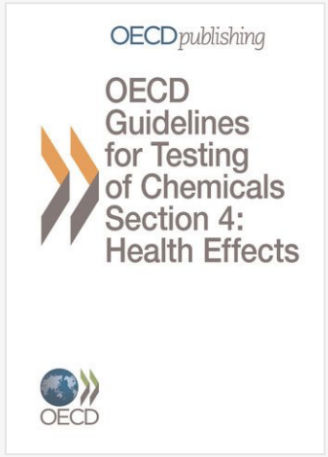
What is the purposes for interlaboratory comparisons?

- **Evaluation of the performance of laboratories for specific tests or measurements and monitoring laboratories' continuing performance;**
- **Identification of problems in laboratories and initiation of actions for improvement which, for example, may be related to inadequate test or measurement procedures, effectiveness of staff training and supervision, or calibration of equipment;**
- **Education of participating laboratories based on the outcomes of such comparisons;**

ISO/IEC 17043:2010

INTERLABORATORY COMPARISON OECD TG 439

Organization, performance and evaluation of measurements or TESTS on the same or similar items by two or more laboratories in accordance with predetermined conditions.

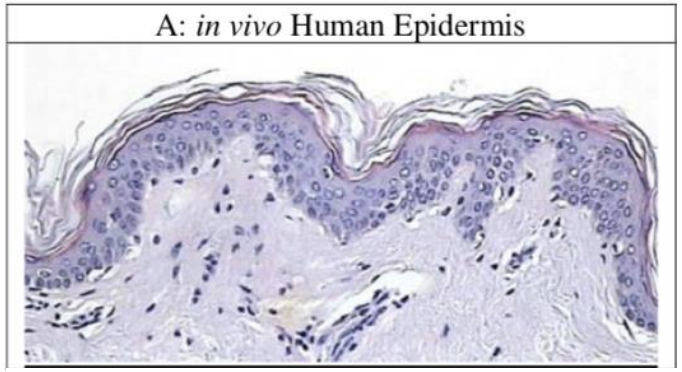


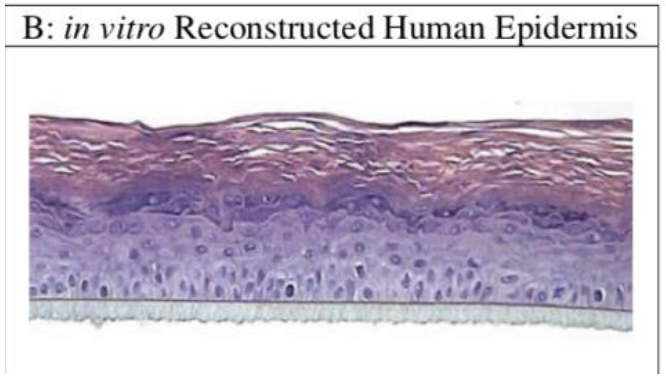
Test No. 439: In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method

Replaces : Test No. 439: In Vitro Skin Irritation - Reconstructed Human Epidermis Test Method

This Test Guideline describes an in vitro procedure that may be used for the hazard identification of irritant chemicals (substances and mixtures) in accordance with the UN Globally Harmonized System of Classification and Labelling (GHS) Category 2. It is based on reconstructed human epidermis (RhE), which in its overall design closely mimics the biochemical and physiological properties of the upper parts of the human skin. Cell viability is measured by enzymatic conversion of the vital dye MTT into a blue formazan salt that is quantitatively measured after extraction from tissues. Irritant test substances are identified by their ability to decrease cell viability below defined threshold levels (below or equal to 50% for UN GHS Category 2). Coloured chemicals can also be tested by used of an HPLC procedure. There are three validated test methods that adhere to this Test Guideline. Depending on the regulatory framework and the classification system in use, this procedure may be used to determine the skin irritancy of test substances as a stand-alone replacement test for in vivo skin irritation testing, or as a partial replacement test, within a tiered testing strategy.

Test No. 439: In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method





N. Alépée et al. / Toxicology in Vitro 24 (2010) 257–266

Model: SkinEthic™ RHE

DESCRIPTION

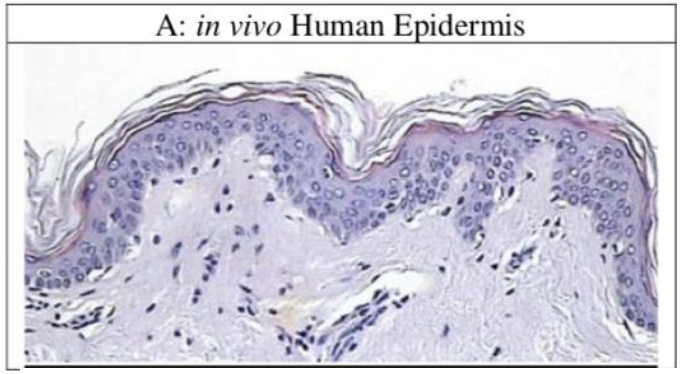
Culture inserts equilibration period: Incubate at least 2 h (37°C)
↓
Transfer tissues to fresh medium
↓

PREDICTION MODEL

In vitro Result	Classification (*In vivo* Prediction)
Mean tissue viability ≤ 50%	Category 2 (previously R38), Irritant (I)
Mean tissue viability > 50%	No Category (previously No label), Non Irritant (NI)

Extraction: Transfer the inserts in 750 µL isopropanol, add 750 µL on the top of each tissue
↓
Extract formazan at least 2 h
↓
Perforate the insert and homogenize formazan extract
↓
Read OD with microplate spectrophotometer at 570 nm

Test No. 439: In Vitro Skin Irritation: Reconstructed Human Epidermis Test Met



N. Alépée et al. / Toxicology in Vitro 24 (2010) 257–266

ANNEX 2

TEST METHODS INCLUDED IN THIS TG

Nr.	Test method name	Validation study type	References
1	**EpiSkin™**	Full prospective validation study (2003-2007). The test method components of this method were used to define the essential test method components of the original and updated ECVAM PS (39) (40) (21)*. Moreover, the method's data relating to identification of non-classified vs classified substances formed the main basis for defining the specificity and sensitivity values of the original PS*.	(2) (10) (11) (14) (15) (16) (17) (18) (19) (20) (21) (23) (32) (39) (40)
2	**EpiDerm™ SIT (EPI-200)**	EpiDerm™ (*original*): Initially the test method underwent full prospective validation together with Nr. 1. from 2003-2007. The test method components of this method were used to define the essential test methods components of the original and updated ECVAM PS (39) (40) (21)*. **EpiDerm™ SIT (EPI-200):** A modification of the original EpiDerm™ was validated using the original ECVAM PS (21) in 2008*	(2) ((10) (12) (13) (15) (16) (17) (18) (20) (21) (23) (33) (39) (40) (2) (21) (22) (23) (33)
3	**SkinEthic™ RHE**	Validation study based on the original ECVAM Performance Standards (21) in 2008*.	(2) (21) (22) (23) (31)
4	**LabCyte EPI-MODEL24 SIT**	Validation study (2011-2012) based on the Performance Standards (PS) of OECD TG 439 (8) which are based on the updated ECVAM PS* (39) (40).	(24) (25) (26) (27) (28) (35) (39) (40) and PS of this TG (8)*

Unclassified

Organisation de Coopération et de Dév
Organisation for Economic Co-operati

PERFORMANCE STANDARD
IN VITRO RECONSTRUCTEI
IRRITATION TESTING AS DI

(Intended for the developers of
Series on Testing and Assessme
No. 220

ENV/JM/MONO(2015)27
Unclassified

OECD/OCDE

439
Adopted:
28 July 2015

OECD GUIDELINES FOR THE TESTING OF CHEMICALS

In Vitro Skin Irritation: Reconstructed Human *Epidermis* Test Method

INTRODUCTION

1. Skin irritation refers to the production of reversible damage to the skin following the application of a test chemical for up to 4 hours [as defined by the United Nations (UN) Globally Harmonized System of Classification and Labelling of Chemicals (GHS)](1). This Test Guideline (TG) provides an *in vitro* procedure that may be used for the hazard identification of irritant chemicals (substances and mixtures) in accordance with UN GHS Category 2 (1) (2). In member countries or regions that do not adopt the optional UN GHS Category 3 (mild irritants), this Test Guideline can also be used to identify non-classified chemicals. Therefore, depending on the regulatory framework and the classification system in use, this Test Guideline may be used to determine the skin irritancy of chemicals either as a stand-alone

INTERLABORATORY COMPARISON OECD TG 439

Organization, performance and evaluation of measurements or tests on the same or similar items by two or more laboratories in accordance with **PREDETERMINED CONDITIONS.**

OECD TG 439 + PERFORMANCE STANDARD BASED RELATED TO TG 439

- ✓ **ESSENTIAL TEST METHOD COMPONENTS;**
- ✓ **REFERENCE CHEMICALS - WELL-KNOWN UN GHS CLASSIFICATION;**
- ✓ **DEFINED RELIABILITY AND PREDICTIVE CAPACITY VALUES.**

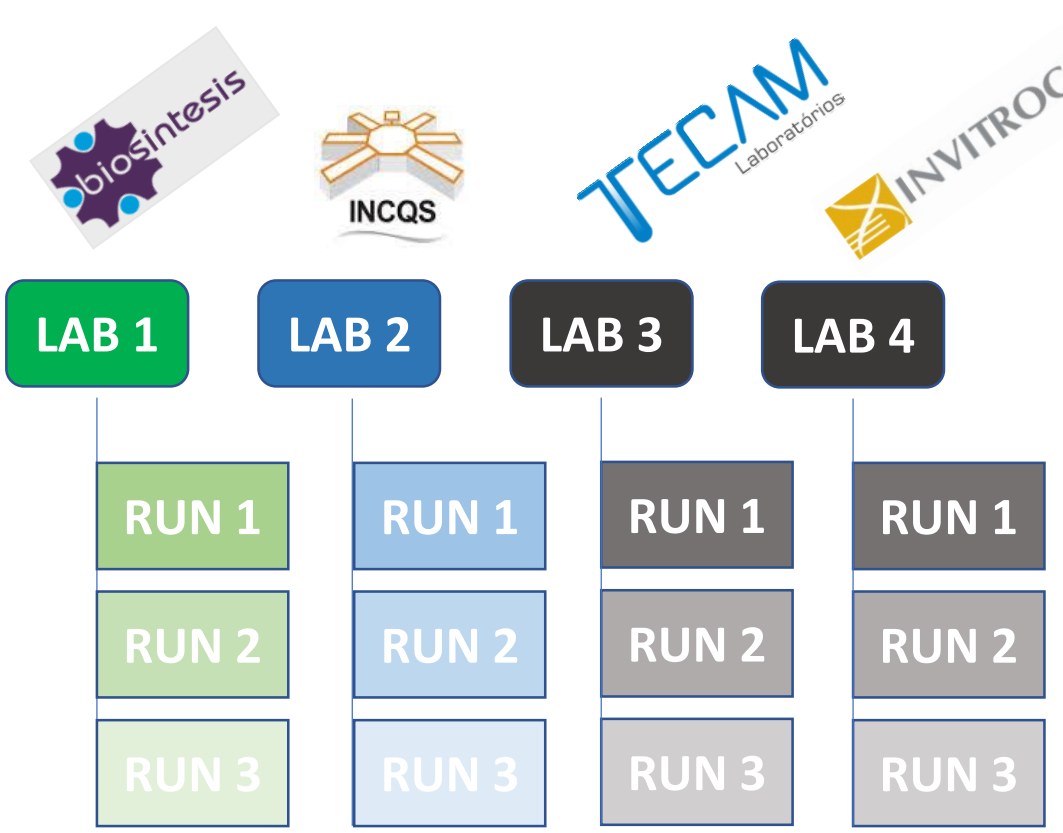
INTERLABORATORY COMPARISON OECD TG 439

Organization, performance and evaluation of measurements or tests **ON THE SAME OR SIMILAR ITEMS BY TWO OR MORE LABORATORIES** in accordance with predetermined conditions.

L'ORÉAL

SkinEthics RHE™ supplier

- ✓ **ESSENTIAL TEST METHOD COMPONENTS;**
- ✓ **20 REFERENCE CHEMICALS - WELL-KNOWN UN GHS CLASSIFICATION;**
- ✓ **DEFINED RELIABILITY AND PREDICTIVE CAPACITY VALUES.**
- ✓ **4 LABS;**



INTERLABORATORY COMPARISON OECD TG 439

ORGANIZATION, performance and evaluation of measurements or tests on the same or similar items by two or more laboratories in accordance with predetermined conditions.

- **ISO/IEC 17043:2011 BASED (INMETRO) + INTERNATIONAL VALIDATION EXPERIENCE (L'Oréal);**
- **CLEARLY DEFINED RESPONSABILITIES**

We are in the begining of the learning process!

Study Coordination Management

L'Oreal/ INMETRO

- Study coordination
- Study goal and project plan
- Test chemicals selection
- Final reports and publications

Test chemicals sourcing

INMETRO

- Liaison with test chemicals suppliers
- Liaison with the Study Coordinator
- Chemical acquisition, coding and distribution
- Point of contact for chemicals and follow-up during the experimental phase
- Decoding, reception and check of sealed envelopes

Lead Laboratory

L'Oreal

- SOP
- Training
- Testing

Participating Laboratories

- Transfer
- Testing

Study data Coordination

INMETRO

- Data storage, reporting and archiving
- Supervision of the laboratories and follow-up of the experimental phase
- Clarification of any data related issues once testing is completed
- Statistical analysis of the study data
- Reporting of the study results obtained

INTERLABORATORY COMPARISON OF OECD TG 439 IN BRAZIL

RESULTS

ESSENTIAL TEST METHOD COMPONENTS

- **Tissue morphology,**
- **Cell viability, and the assessment of the barrier function through the measure of ET50.**

De Vecchi et al ,2018

Implementation, availability and regulatory status of an OECD accepted Reconstructed Human Epidermis model in Brazil

Implementação, disponibilidade e contexto regulatório de um modelo de Epiderme Humana Reconstruída no Brasil aceito pela OECD

ESSENTIAL TEST METHOD COMPONENTS

- **Tissue morphology,**
- **Cell viability, and the assessment of the barrier function through the measure of ET50.**

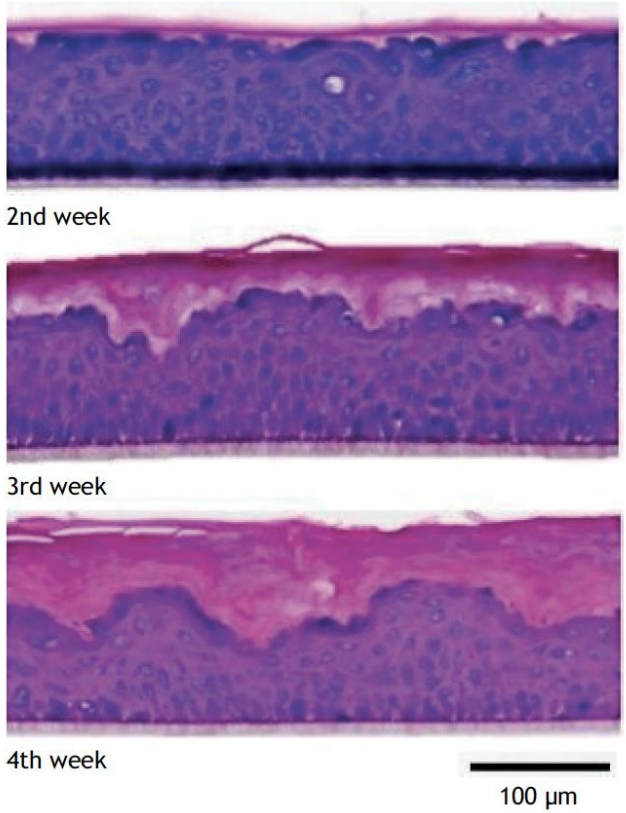
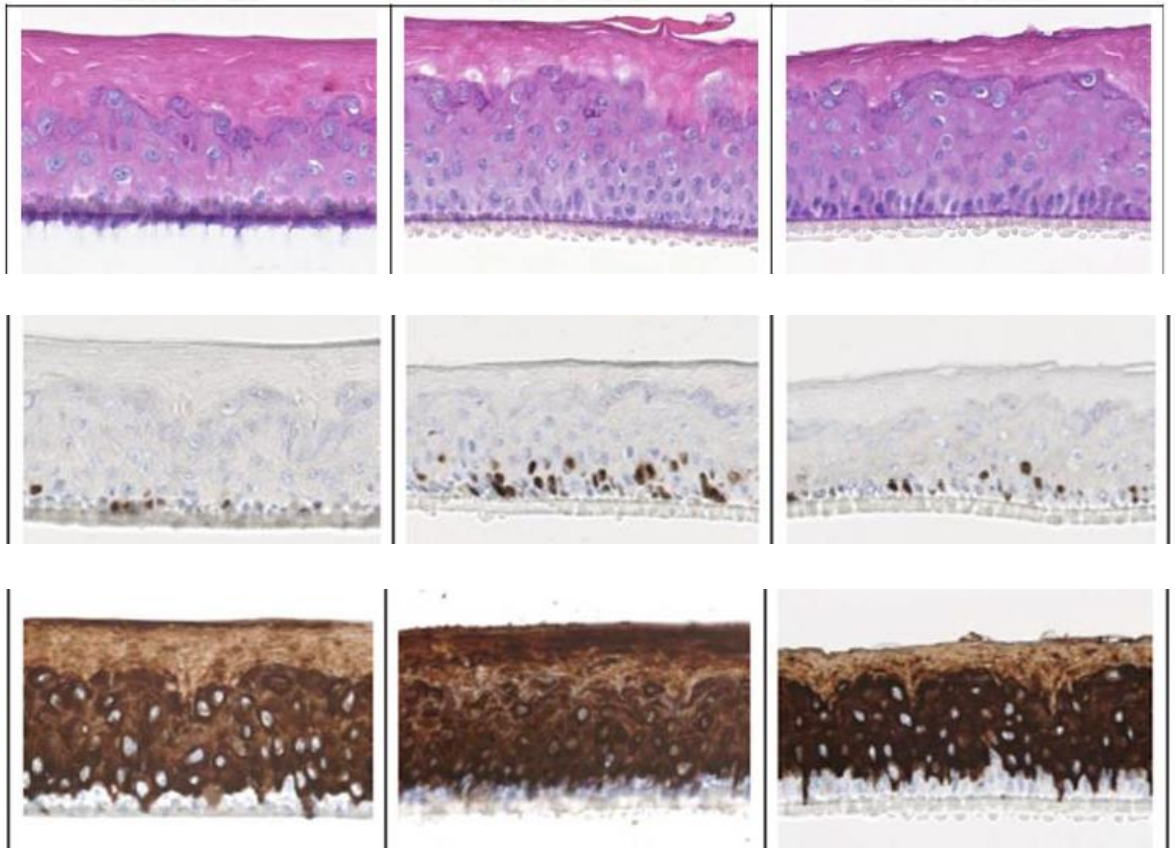


Figure 2. Histology of SkinEthic™ RHE using Hematoxylin/Eosin staining of one representative batch during 2nd, 3rd and 4th week of differentiation *in vitro*. Scale bar: 100 µm.

HE

Ki67
Proliferation marker

CK10
Suprabasal marker



SkinEthic RHE tissues in Brazil

ESSENTIAL TEST METHOD COMPONENTS

- **Tissue morphology,**
- **Cell viability, and the assessment of the barrier function through the measure of ET50.**

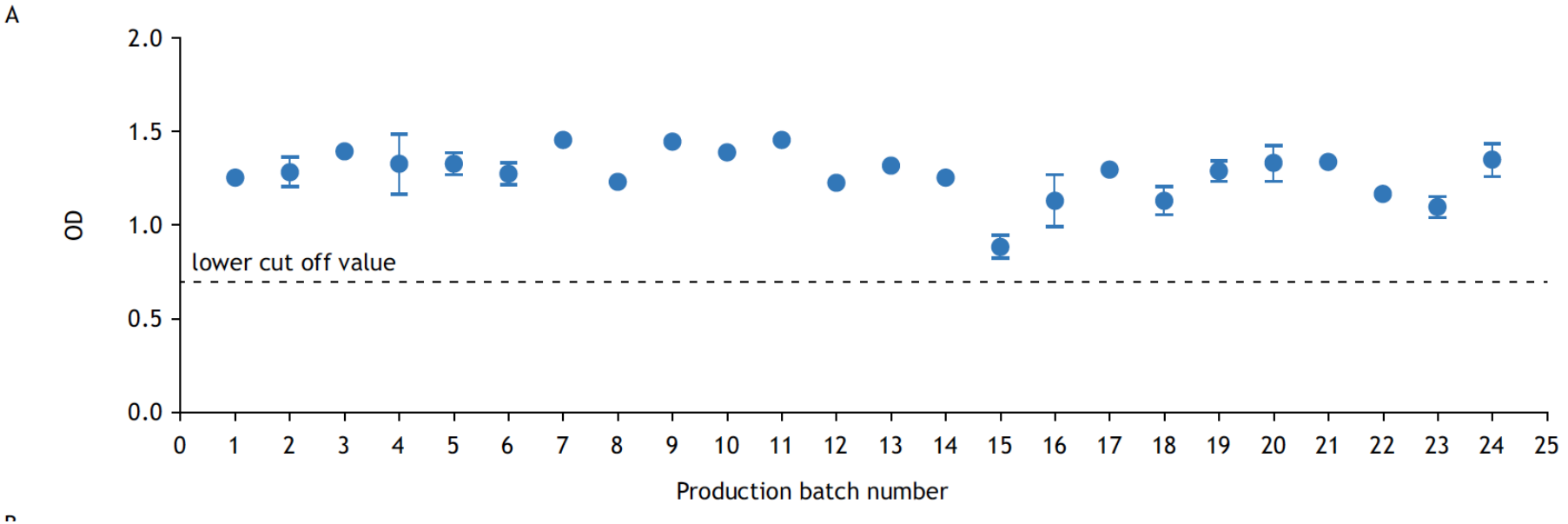


Table 2: Acceptability ranges for negative control OD values of the test methods included in this TG

	Lower acceptance limit	Upper acceptance limit
EpiSkin™ (SM)	≥ 0.6	≤ 1.5
EpiDerm™ SIT (EPI-200)	≥ 0.8	≤ 2.8
SkinEthic™ RHE	≥ 0.8	≤ 3.0
LabCyte EPI-MODEL24 SIT	≥ 0.7	≤ 2.5

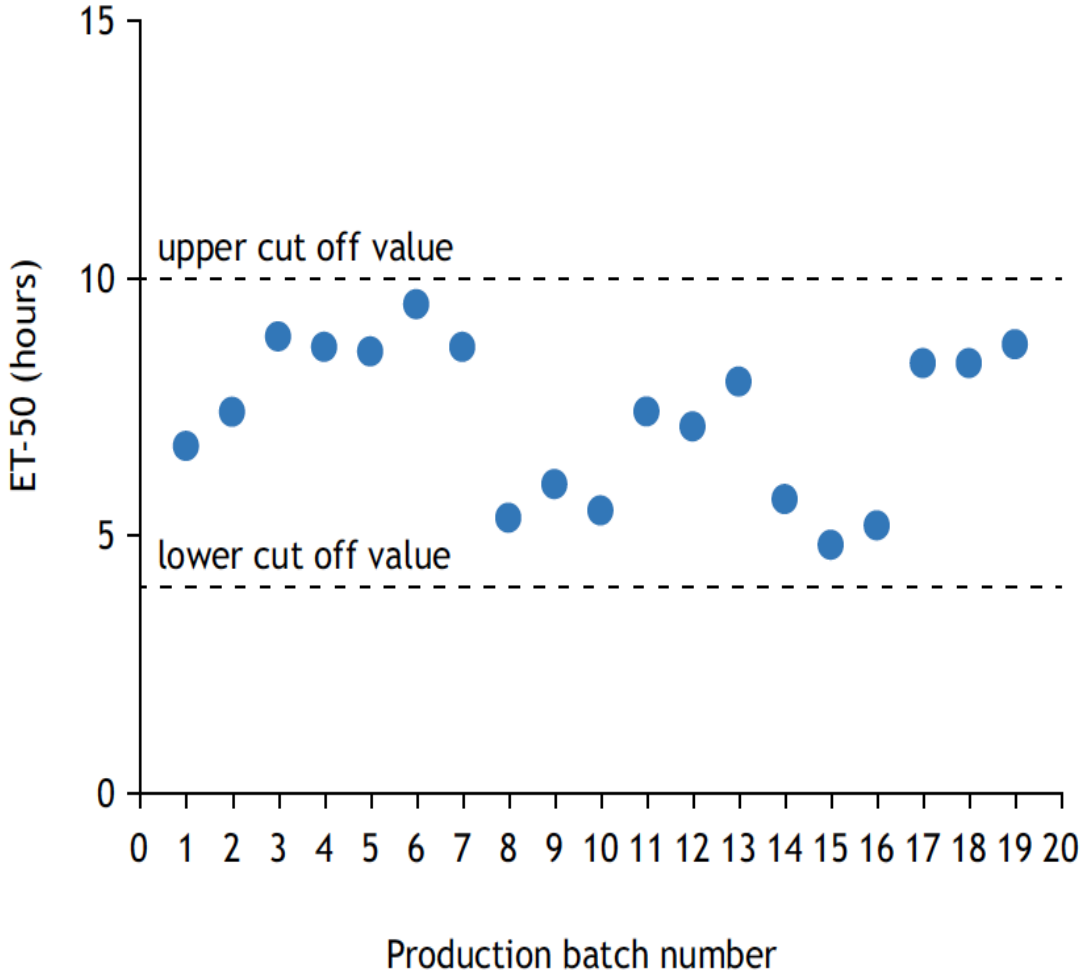


Figure 4. ET-50 values for 19 batches generated in Brazil, showing a high repeatability and reproducibility.

ESSENTIAL TEST METHOD COMPONENTS

- **Tissue morphology,**
- **Cell viability, and the assessment of the barrier function through the measure of ET50.**

Table 3: QC batch release criteria of the test methods included in this TG

	Lower acceptance limit	Upper acceptance limit
EpiSkin™ (SM) (18 hours treatment with SDS) (32)	IC_{50} = 1.0 mg/ml	IC_{50} = 3.0 mg/ml
EpiDerm™ SIT (EPI-200) (1% Triton X-100) (33)	ET_{50} = 4.0 hr	ET_{50} = 8.7 hr
SkinEthic™ RHE (1% Triton X-100) (34)	ET_{50} = 4.0 hr	ET_{50} = 10.0 hr
LabCyte EPI-MODEL24 SIT (18 hours treatment with SDS) (35)	IC_{50} = 1.4 mg/ml	IC_{50} = 4.0 mg/ml

REFERENCE CHEMICALS

❖ 20 CHEMICALS SELECTED ELIGIBLE TO FULFILL THE FOLLOWING CRITERIA:

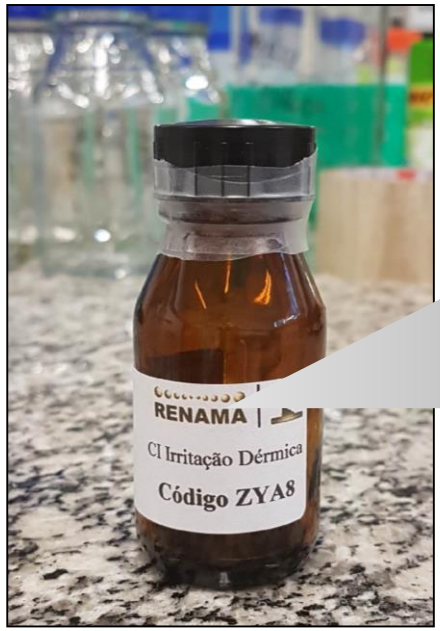
- **Availability of high quality *in vivo* Draize reference data**
- **50±2.5% split for classified (UN GHS Cat 2) vs. not-classified (GHS No Cat)**
- **Good representation of all skin irritation effects driving classification**
- **50% split for physical form (solids vs. liquids)**
- **All chemicals available from commercial sources**
- **Diverse structural and chemical classes**

…. MTT reducers, no colour interfering chemicals.

• **Independent coding and distribution of chemicals** (Random coding of the chemicals; different codes for each chemical for each laboratory).

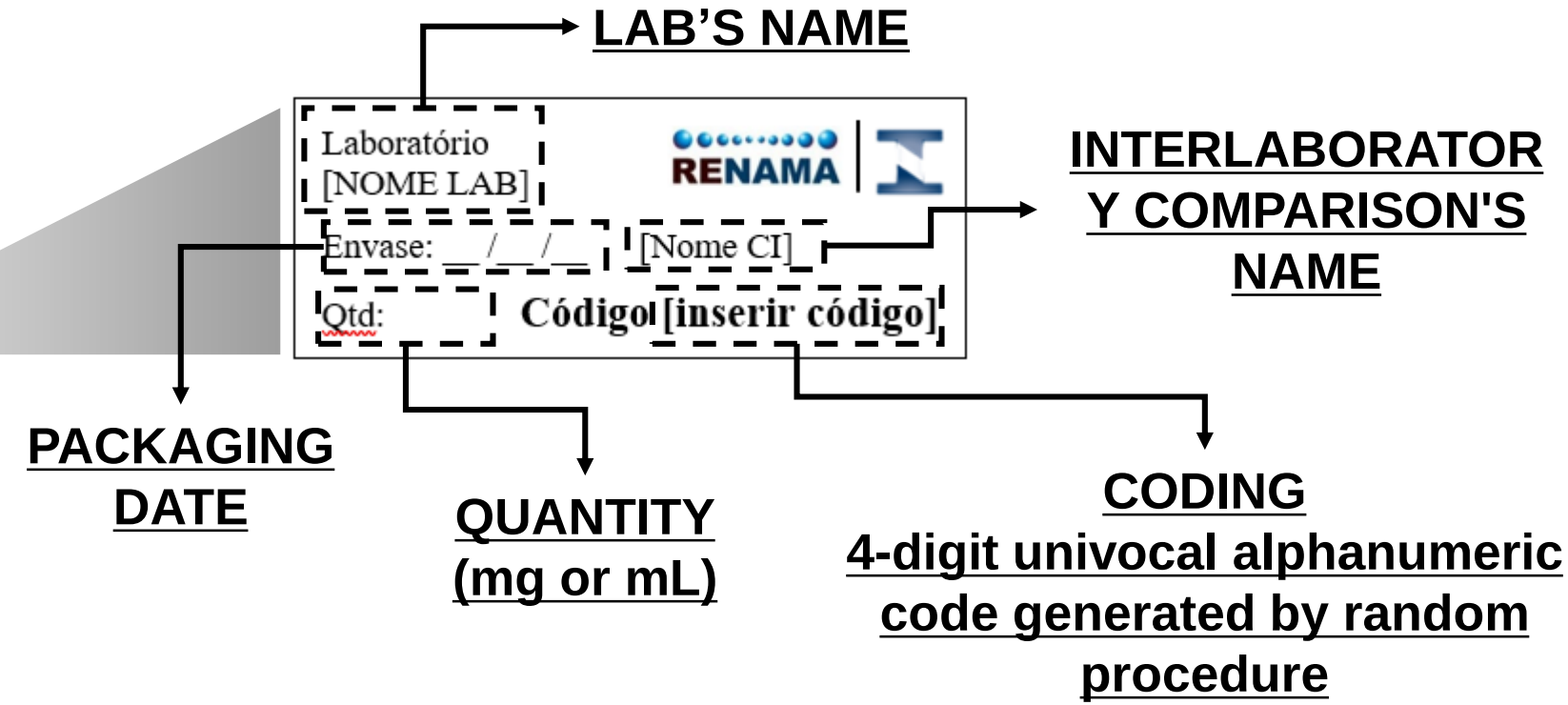
TEST ITENS

20 TEST ITENS



Clean borosilicate ambar bottle with airtight seal

Packaging and identification



TEST ITENS

SECONDARY PACKAGING

Light, moisture, and mechanical shocks protection

INTERLABORATORY COMPARISON'S NAME

Ministério da Ciência, Tecnologia, Inovações e Comunicações

RENAMA Rede Nacional de Métodos Alternativos

INMETRO

[NOME E COD CI]

LABORATÓRIO: [código do laboratório]

LAB'S NAME

CÓDIGO DA SUBSTÂNCIA

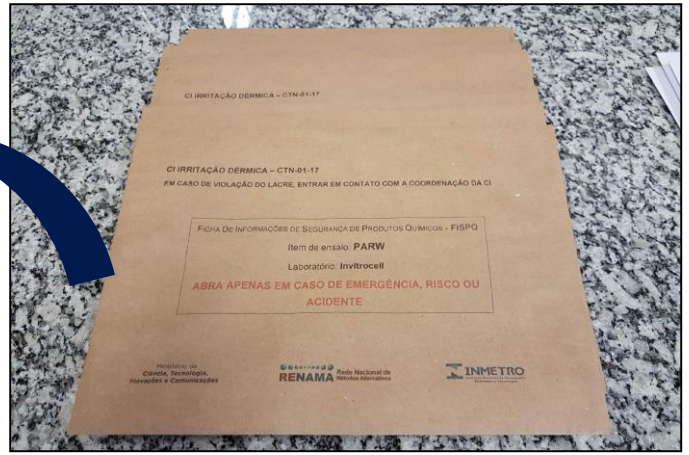
[INSERIR CODIFICAÇÃO]

CODING

4-digit univocal alphanumeric code generated by random procedure

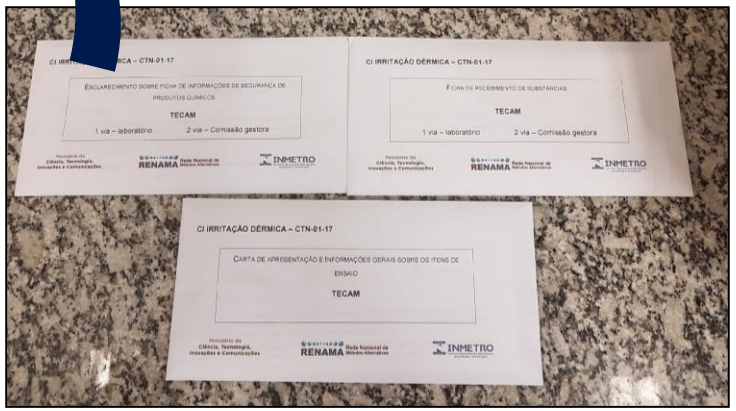
TEST ITENS

DOUBLE INDEPENDENT CHECK IN ALL STEPS



MATERIAL SAFETY DATA SHEET

Sent in sealed tamper-proof envelope and can only be opened in case of emergency. Integrity will be checked at the end of the study



SIGNED BY THE LABORATORY OFFICER

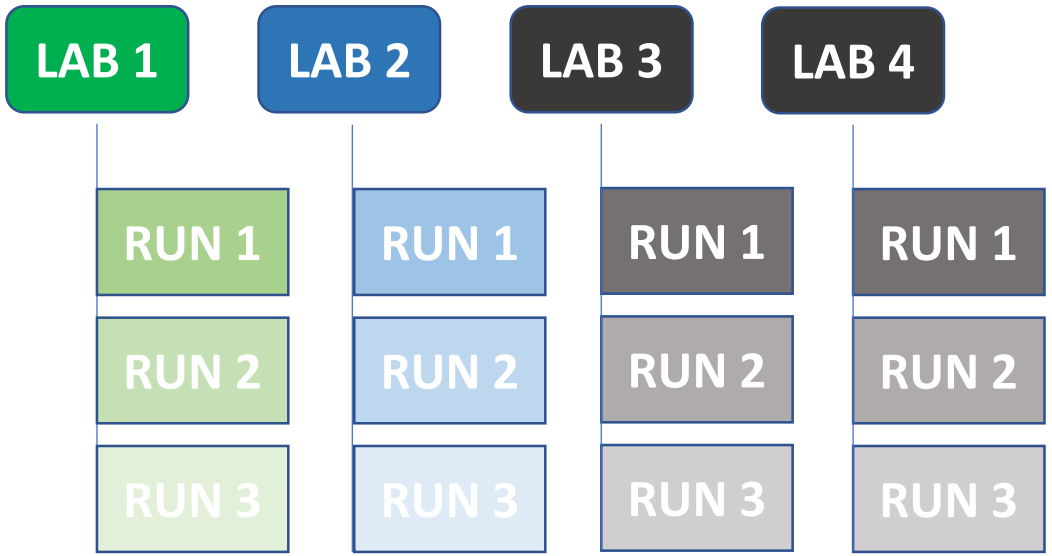
Cover letter and instructions

Receipt form – integrity check of test items

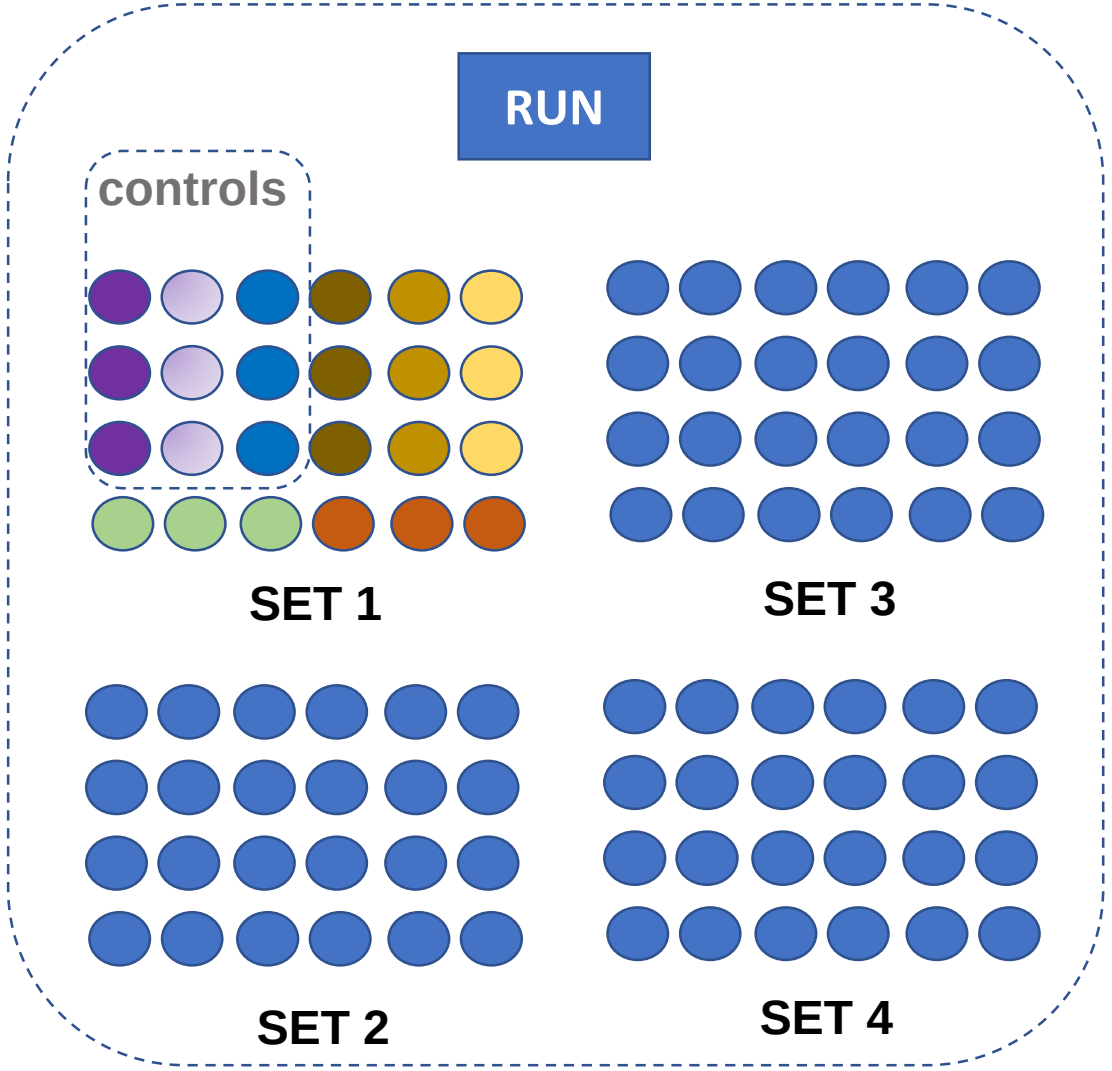
Maintenance of MSDS confidentiality

DEFINED RELIABILITY AND PREDICTIVE CAPACITY VALUES

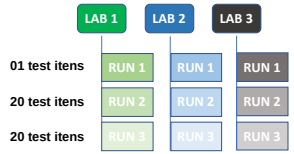
20 Chemicals



Raw data: optical density (O.D).

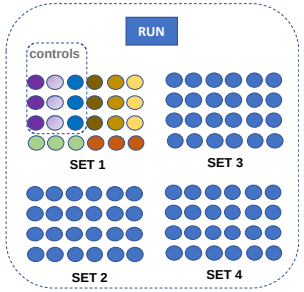


DEFINED RELIABILITY AND PREDICTIVE CAPACITY VALUES



Raw data: optical density (O.D).
Viability (%control)- Average (O.D)
Standard Deviation (O.D)

Viability – UN GHS Classifications



DB ALM

DB-ALM Protocol

***IN VITRO* SKIN IRRITATION TEST: SkinEthic™ Reconstructed Human Epidermis MODEL (RHE)**

Last Update: May 2017 (v.9.5_Stand-alone document)

Part B. Procedure Details, Latest Version: May 22nd 2017.

Protocol Name: SKINETHIC™ RHE SKIN IRRITATION TEST

B.1 Contact person

Contact Person: Dr ALEPEE Nathalie
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17
BU

Application volumes/quantities

Liquid and viscous test chemicals:

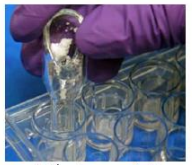
1) Dispense 16±2 µL (i.e. 32 µL/cm²) of the undiluted test chemical on the top of each epidermis tissue (3 per test chemical: replicate 1, replicate 2, and replicate 3), using positive displacement pipette. Use the tip to spread the test chemical gently on the epidermis topical surface. See Picture 5.

Annexes

Annex 1: Evaluation of test chemicals - MTT direct interaction
Annex 2: Evaluation of test chemicals - Color interaction (at least 15 min)
Annex 3: Incubation timings
Annex 4: Validation of an analytical method on a HPLC/UPLC-spectrophotometry endpoint
Annex 5: Illustrative flowchart providing guidance on how to identify and handle direct MTT-reducers and/or colour interfering chemicals

3) Use special glass weigh boats (or similar tools avoiding electrostatic electricity and allowing a targeted application directly in the insert with no risk of test chemical scattering in the medium subnatant) to apply 16±2 mg (i.e. 32 mg/cm²) of the powder to the epidermis surface. If necessary, spread it on the epidermal surface. See Pictures 9, 10 and 11.


Picture 9

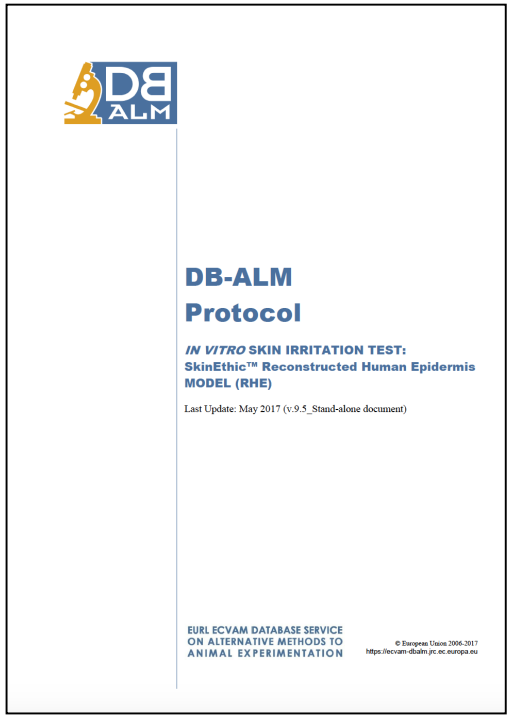

Picture 10


Picture 11

DEFINED RELIABILITY AND PREDICTIVE CAPACITY VALUES

Training in Skin Irritation/Corrosion – OECD TG 439

DB-ALM PROTOCOL



INMETRO

ReNaMA Central Lab

Laboratory infrastructure and Training supplies

L'ORÉAL EPISKIN ACADEMY

Task Leader Conducting the theoretical-practical course with Reconstructed Human Epidermis (RHE)

TECAM Laboratórios

biosintesis

INVITROCELL

INCQS

Grupo kosmo science

CIEnP

USP Ribeirão Preto

Laboratory of cosmetic technology applied to photoprotection

DEFINED RELIABILITY AND PREDICTIVE CAPACITY VALUES

Training in Skin Irritation/Corrosion – OECD TG 439

DB-ALM PROTOCOL

EPISKIN ACADEMY

TRAINING SESSIONS

2 DAYS WORKSHOP ON ALTERNATIVES AND SKIN IRRITATION/CORROSION ACCORDING TO OECD TG439

The program alternates during 2 days lectures (5 hours) in classroom and hands-on manipulation into laboratory (10 hours) with real tissues models. The handling part of the workshop is a real added value for participants and is limited to 8 trainees per session due to laboratoryies's constraints. The training is based on the OECD TG439 for in vitro skin irritation of chemical with SkinEthic reconstructed human epidermis (RHE). This method has been selected in accordance to its status of regulatory method based on RHE model, robust and widely used, easy to handle and easy to implement. Each participant has to assess the skin irritation potential of 2 unknown products, a solid and a paste, compared to a negative (PBS) and a positive control (SDS).

PROGRAM

Day 1	
9h-11h	*Presentation* – Regulation, Alternative methods, Tissue Engineering
11h-12h30	*Lab* – Preincubation
12h30-13h30	Lunch
13h30-18h30	*Lab* – Exposition, Rinsing- Post-incubation
Day 2	
9h-11h	*Lab* – MTT, manipulation of other 3D models
11h-12h	*Presentation* – Applications of 3D models to efficiency testing, to drug development and to medical devices assessment
12h-12h30	*Lab* – Isopropanol extraction
12h30-13h30	Lunch
13h30-15h30	*Lab* OD reading
15h30-17h30	Data interpretations
17h30-18h00	Discussion on alternative methods – Conclusion

Contacts
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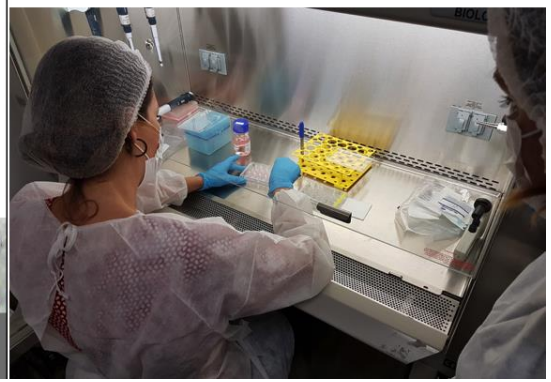
http://www.episkin.com



TIME	SCHEDULE
	WEDNESDAY, August 9, 2017
09:00 am	Arrival at INMETRO, welcome participants Meeting and facilities visit
10:00 am	Quick flow chart presentation by trainer **Practice 1** for trainees (application and rinsing step)
12:00 am	Lunch
13:30 pm -> 16:30 pm	Tissue treatment : **Application, rinsing, post treatment incubation start -** for trainer and trainees
16:30 pm	Practice QA - Training Documents – Schedule
17:30 pm	Conclusion day 1
	THURSDAY, August 10, 2017
9:30 am	Arrival at INMETRO, welcome participants SOP Questions & Answers session Pre-check MTT & color interaction
12:00 am	Lunch
13:30 pm	Killed tissue preparation by trainer and trainees **Practice 2** on particular cases MTT & color interaction - application for trainees
16:30 pm	Conclusion day 2
	FRIDAY, August 11, 2017
8:30 am	Arrival at INMETRO, welcome participants Presentation of the training day Part II - Training documents
9:00 am -> 15:30 am	**MTT incubation, Formazan extraction, Data acquisition -** for trainer and trainees
16:00 am -> 18:00 pm	**Data calculation steps** SOP and Calculations Question & Answers session Conclusion day 3

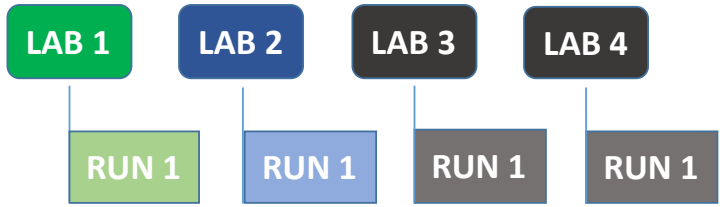
DEFINED RELIABILITY AND PREDICTIVE CAPACITY VALUES

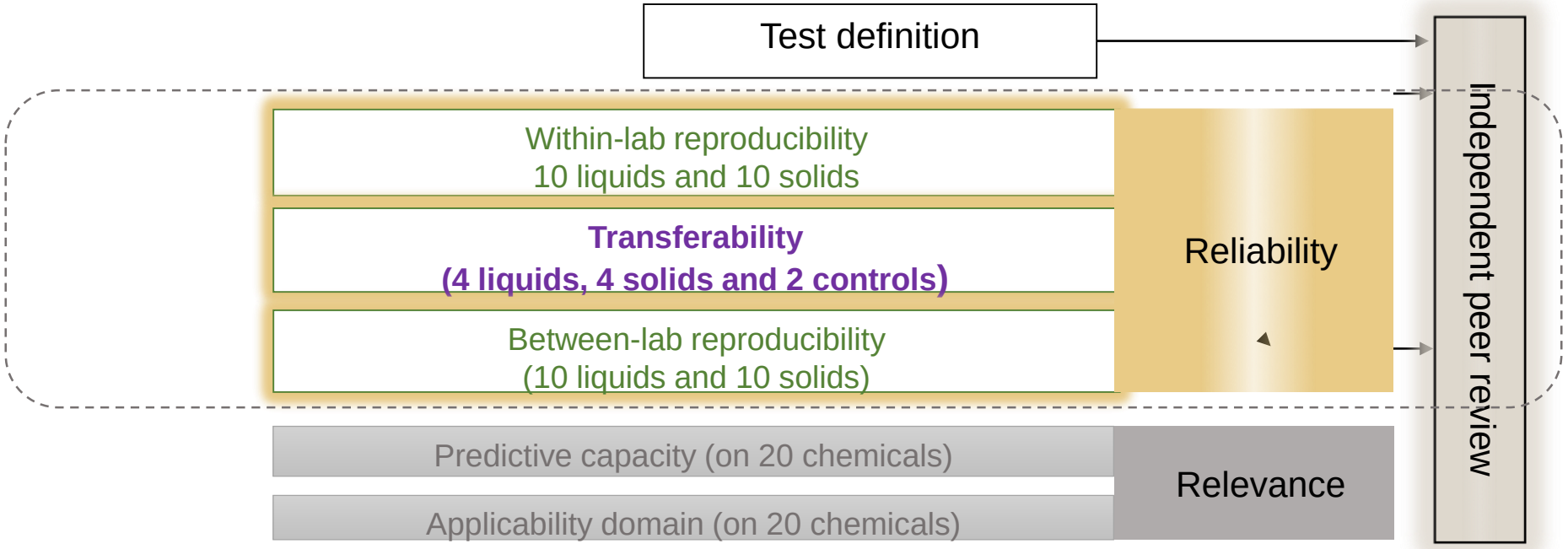
Training in Skin Irritation/Corrosion – OECD TG 439

DB-ALM PROTOCOL

Protocol implementation : TRANSFERABILITY

08 Test itens – NOT CODED - 4 liquids, 4 solids and 2 controls)





DEFINED RELIABILITY AND PREDICTIVE CAPACITY VALUES

Protocol implementation : TRANSFERABILITY

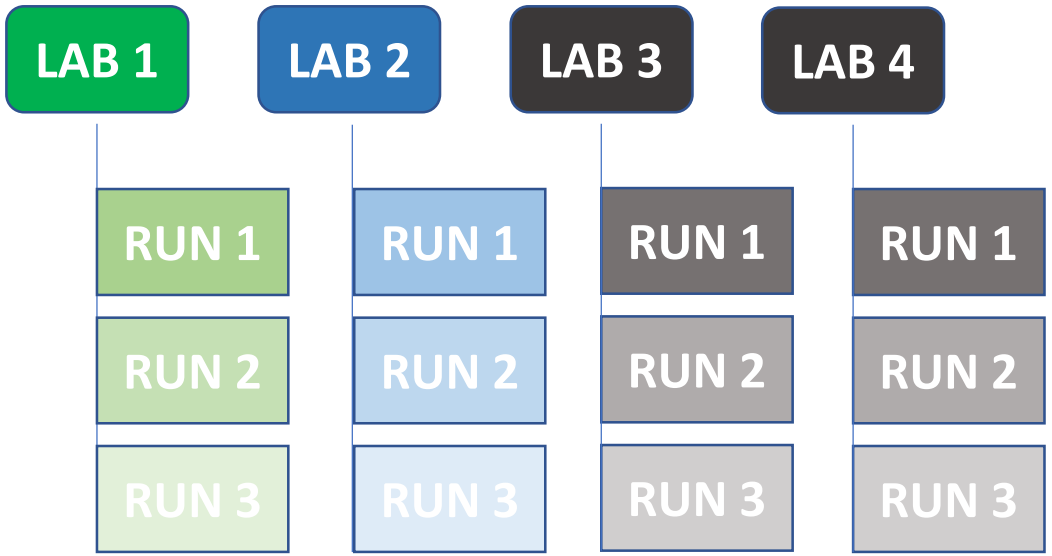
08 Test itens – NOT CODED - 4 liquids, 4 solids and 2 controls)

Interlaboratorial comparison skin irritaion (OECD TG 439) on SkinEthic RHE - TRANSFER PHASE														
Chemical	CAS	CLASS	LAB 1			LAB 2			LAB 3			LAB 4		
			N1	SD	Class N1	N1	SD	Class N1	N1	SD	Class N1	N1	SD	Class N1
Naphthalene acetic acid	86-87-3	NC	85,4	16,1	NC	111,3	12,2	NC	93,9	7,7	NC	/		
Methyl palmitate	112-39-0	NC	94,6	11,6	NC	113,7	6,47	NC	115,1	10,2	NC	/		
3,3'-Dithiodipropionic acid	1119-62-6	NC	99,9	4,9	NC	120	13,87	NC	95,4	3,8	NC	112,1	12,3	NC
2-Chloromethyl-3.5-dimethyl-4-methoxypyridine HCl	86604-75-3	C	0,4	0,1	C	1,1	0,03	C	1,0	0,3	C	1,5	0,2	C
N-Butyl methacrylate	97-88-1	C	3,1	3,1	C	1,1	0,38	C	1,1	0,1	C	3,7	3,4	C
Dipropylene glycol (mixture of isomers)	25265-71-8	NC	99,6	9,8	NC	126,1	11,18	NC	SD>18%	---	---	125,1	12,8	NC
Allyl heptanoate	142-19-8	NC	78,4	2,9	NC	73,6	4,01	NC	SD>18%	---	---		>18	
Heptylamine	111-68-2	C	0,2	0,2	C	2,1	0,83	C	1,0	0,2	C	3,2	1,6	C
Heptyl butyrate	5870-93-9	NC	/			/			/			118,4	7,36	NC
Cyclamen aldehyde	103-95-7	C	/			/			/			0,7	0,6	C

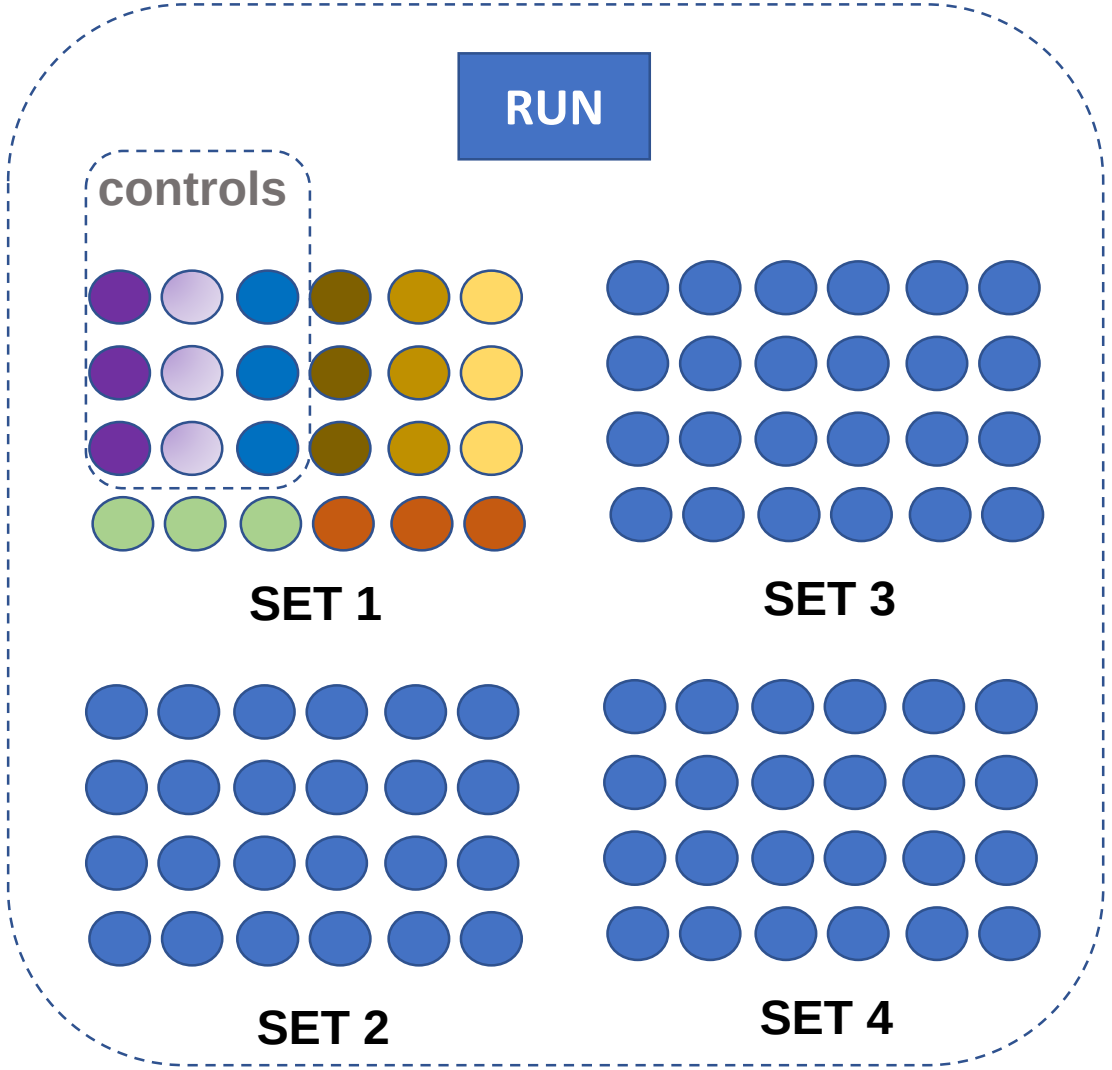
INTERLABORATORY COMPARISON OF OECD TG 439 IN BRAZIL

DEFINED RELIABILITY AND PREDICTIVE CAPACITY VALUES

20 Chemicals



Waiting for the last results!



DEFINED RELIABILITY AND PREDICTIVE CAPACITY VALUES

RELIABILITY - concordance of predictions

Within-laboratory reproducibility

An assessment of within-laboratory reproducibility should show **in one single** laboratory, a **concordance of predictions (UN GHS Category 2 and No Category)** obtained in different, independent test runs **of the 20 Reference Chemicals** equal or higher (≥) than **90%.**

Between-laboratory reproducibility

For methods to be transferred between laboratories, the **concordance of predictions (UN GHS Category 2 and No Category)** obtained in different, independent test runs **of the 20 Reference Chemicals** between a minimum of three laboratories should be **equal or higher (≥) than 80%.**

DEFINED RELIABILITY AND PREDICTIVE CAPACITY VALUES

RELEVANCE – PREDICTIVE CAPACITY -concordance of predictions

Table 4: Required sensitivity, specificity and accuracy values for similar or modified RhE test method to be considered valid to discriminate skin irritants (UN GHS Category 2) from non-classified (UN GHS No Category)

Sensitivity	Specificity	Accuracy
≥ 80%	≥ 70%	≥ 75%

Positives (Cat 2)

Negatives (no Cat)

Concordance of classification

INTERLABORATORY COMPARISON OF OECD TG 439 IN BRAZIL

NEXT STEPS

- ❑ DATA ANALYSIS (AUG_SET/2018)
- ❑ IDENTIFICATION OF POSSIBLE ACTIONS FOR IMPROVEMENT
- ❑ FIRST STUDY REPORT
- ❑ FINAL STUDY REPORT
- ❑ PUBLICATION (DEC/2018)

OECD TG 439
SkinEthics™ in Brazil

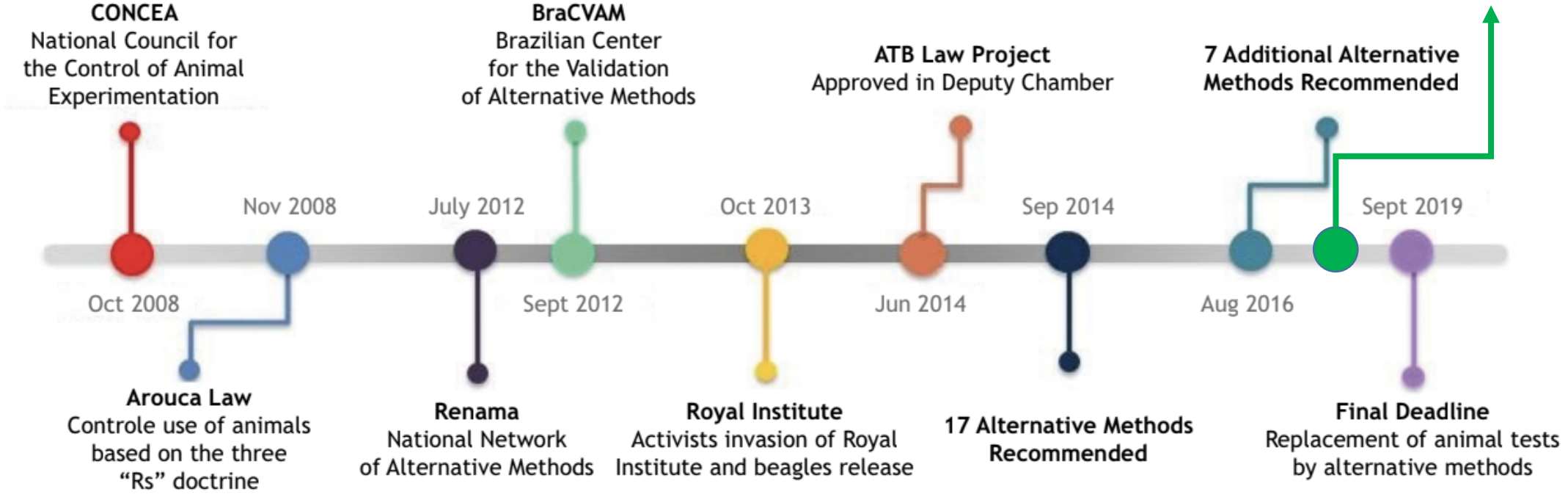


Figure 1. Regulatory evolution related to the control of animal experimentation and alternative methods recognition in Brazil. Since the creation of Concea in 2008, Brazil had significant regulatory advances and its counting down to ban animal testing for several endpoints on September 2019.

De Vecchi et al ,2018

Thank you!

45 anos INMETRO

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