July, 2013, Seoul

Our practical examples of International validation studies for establishing OECD test guidelines

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JaCVAM, NIHS

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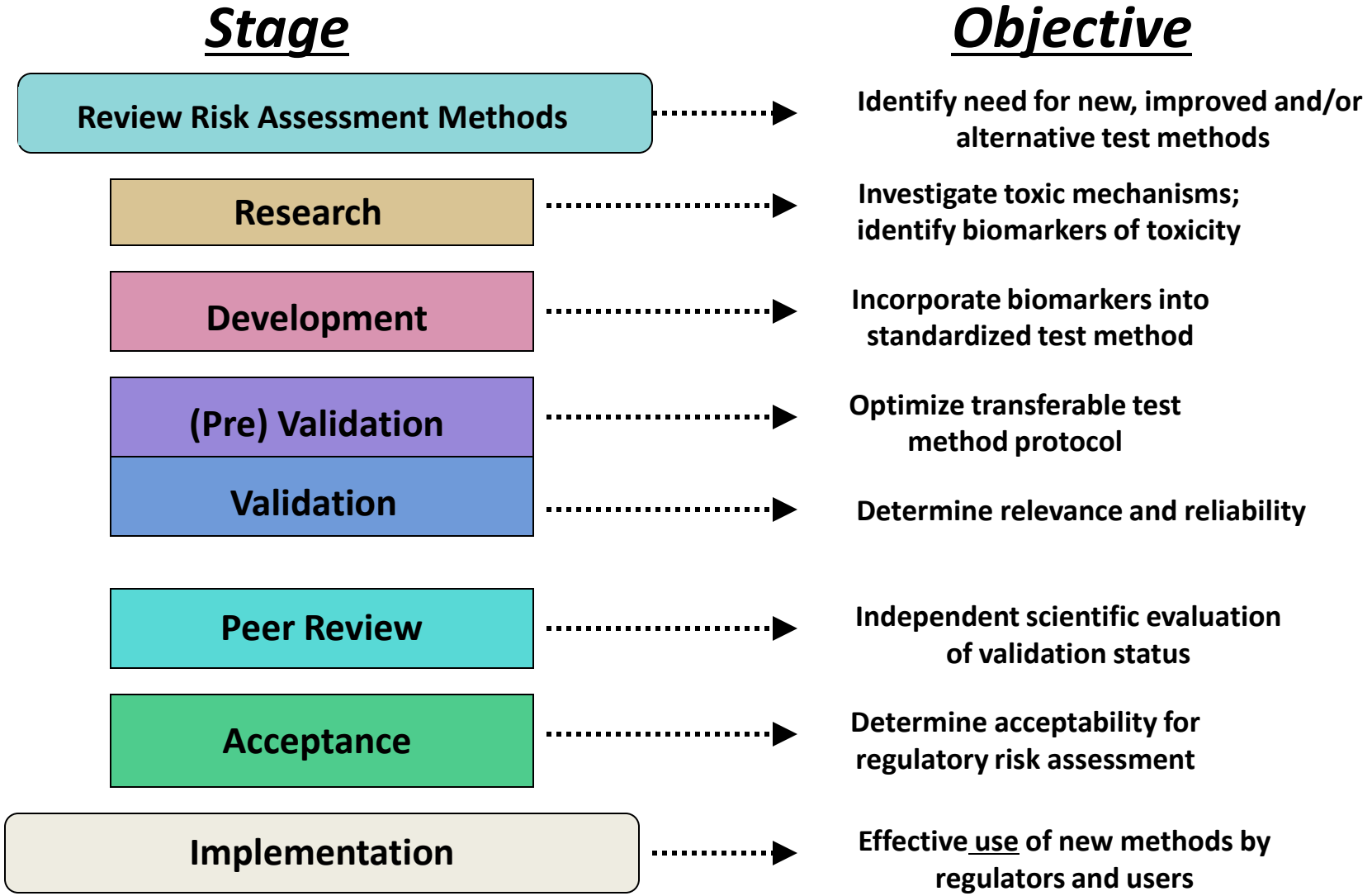
Test Method Evolution and Translation Process: Concept to Implementation



A general connectional framework

Module 1: Test Definition

Module 2: Within-laboratory repeatability and reproducibility

Module 3: Between-laboratory transferability

Module 4: Between-laboratory reproducibility

Module 5: Predictive capacity

Module 6: Applicability domain

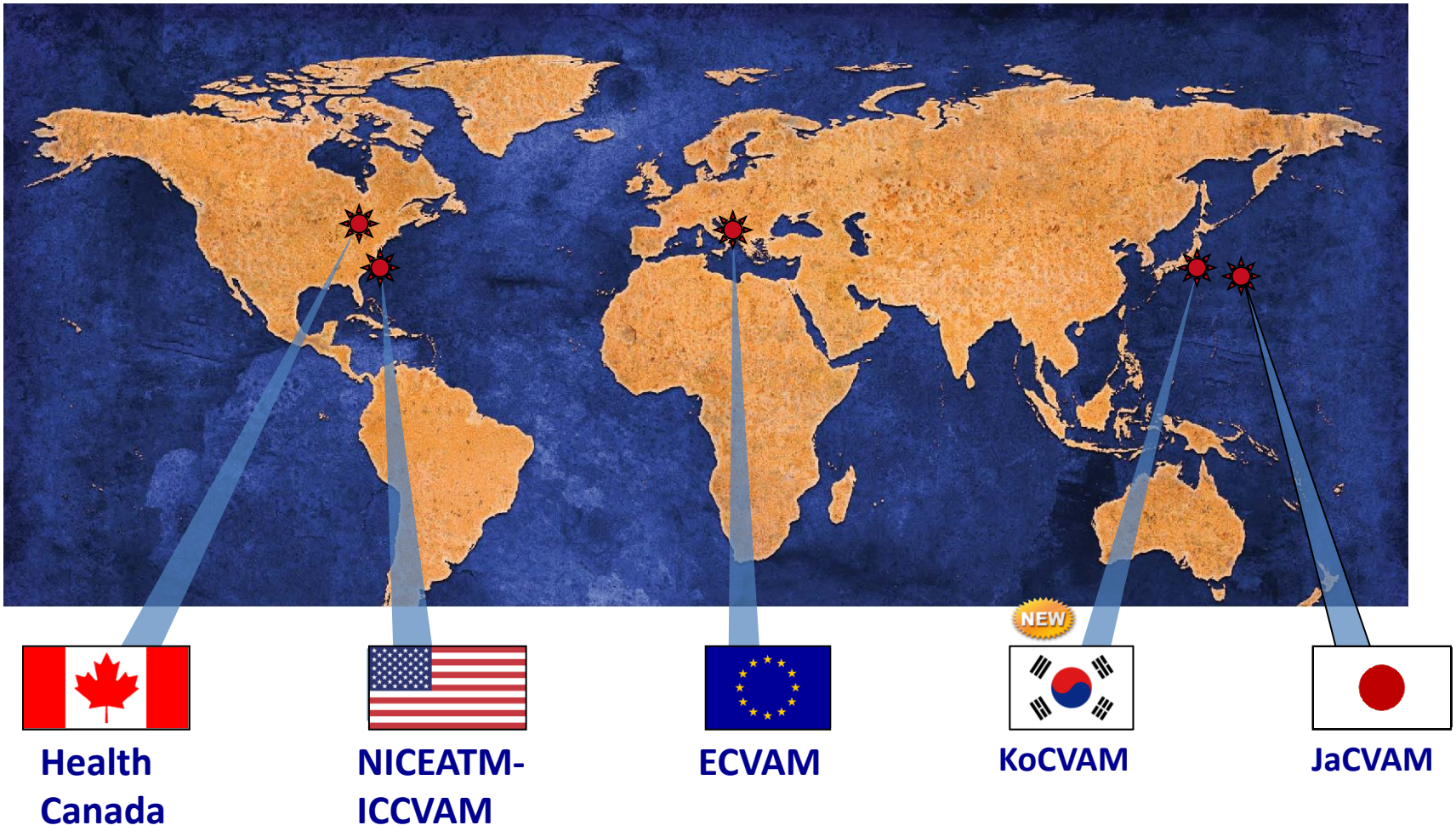
Module 7: Performance standards

JaCVAM roles

- JaCVAM assesses the utility, limitations, and suitability for use in regulatory studies of test methods for determining the safety of chemicals and other materials and also performs validation studies when necessary. In addition, JaCVAM cooperates and collaborates with similar organizations in related fields, both in Japan and internationally.
- JaCVAM activities are also beneficial to application and approval for the manufacture and sale of pharmaceutical and other products as well as to revisions to standards for cosmetic products.

ICATM Framework

ICATM is a **voluntary** international cooperation of national organizations: Canada, the European Union, Japan, South Korea, and the United States.



OECD Test Guidelines developed by Japanese

- Performance-Based Test Guideline for Stably Transfected Transactivation In Vitro Assays to Detect Estrogen Receptor Agonists No.455
- Skin sensitization assay, LLNA：DA No.442A
- Skin sensitization assay, LLNA：BrdU-ELISA No.442B
- Skin irritation assay with LabCyte EPI-MODEL 24

Preparing Draft Test Guideline

- Bhas 42 cell transformation assay
- Short Time Exposure (STE) assay for eye irritation testing
- *in vivo* comet assay for genotoxicity testing

During the OECD WNT commenting round

Japanese developed methods undergoing International peer review

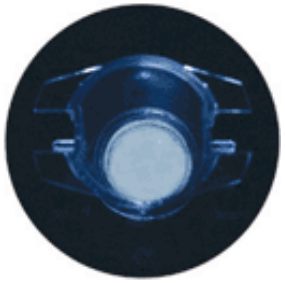
- h-CLAT assay for skin sensitization testing
  (In preparation with EURL ECVAM)
- Short Time Exposure (STE) assay for eye irritation testing
  (On-going by ICCVAM)
- *in vivo* comet assay for genotoxicity testing
  (On-going by OECD expert)
- Reactive Oxygen Species (ROS) assay for photoxicity testing
  (On-going by JaCVAM)

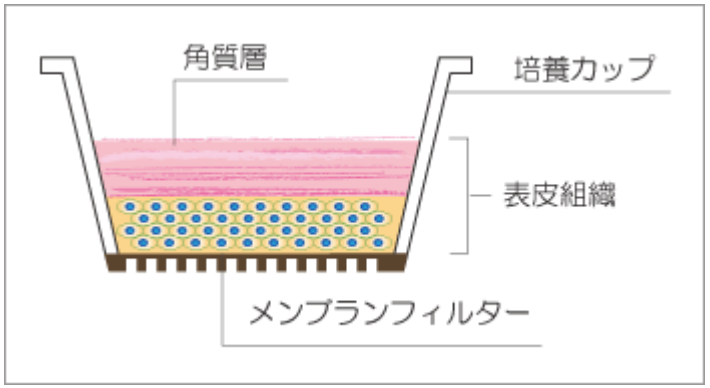
JaCVAM on-going International validation studies

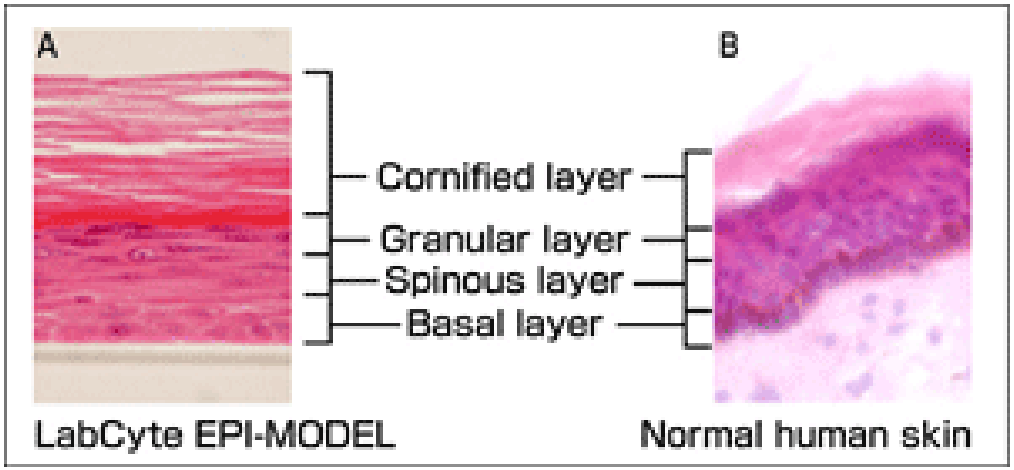
1. IL-8 reporter gene assay for skin sensitization testing
2. SIRC-CVS assay for eye irritation tesitng
3. Stable transfected transcriptional activation (STTA) antagonist assay for endocrine disruptor screening
   Experimental part ended in March 2013
4. Hand-1 Luc assay for reproductive testing

Example 1:

LabCyte







The LabCyte EPI-MODEL is produced by culturing human epidermal cells on a culture plate. After human epidermal cells have been cultured and proliferated, exposing their surface to the air causes it to keratinize*, creating a cultured epidermis model similar to the human epidermis (Figures A and B).

*QC batch release criteria IC50=1.4-4.0mg/mL(mean 2.57mg/mL),
18 hr treatment with SLS.

OECD/OCDE

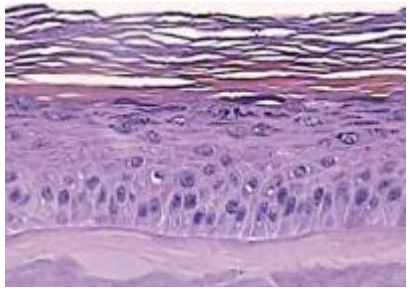
439

Adopted:
22 July 2010

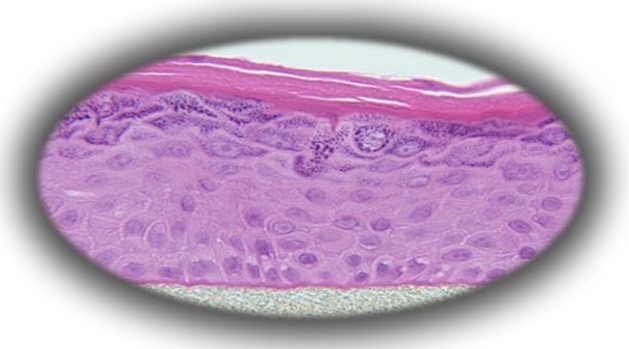
OECD GUIDELINE FOR THE TESTING OF CHEMICALS

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

4. There are three validated test methods that adhere to this Test Guideline. Prevalidation, optimisation and validation studies have been completed for an *in vitro* test method (10) (11) (12) (13) (14) (15) (16) (17) (18) (19) (20), using a RhE model, commercially available as EpiSkin™ (designated the Validated Reference Method – VRM). Two other commercially available *in vitro* skin irritation RhE test methods have shown similar results to the VRM according to PS-based validation (21), and these are the EpiDerm™ SIT (EPI-200) and the SkinEthic™ RHE test methods (22).



EpiSkin



EpiDerm Tissue Model

Table 1: Minimum List of Reference Chemicals for Determination of Accuracy and Reliability Values for Similar or Modified RhE Skin Irritation Test Methods[1]

Chemical	CAS Number	Physical state	*In vivo* score	VRM* Cat. based on *in vitro*	UN GHS Cat. based on *in vivo* results
NON-CLASSIFIED CHEMICALS					
1-bromo-4-chlorobutane	6940-78-9	Liquid	0	Cat. 2	No Cat.
diethyl phthalate	84-66-2	Liquid	0	No Cat.	No Cat.
naphthalene acetic acid	86-87-3	Solid	0	No Cat.	No Cat.
allyl phenoxy-acetate	7493-74-5	Liquid	0.3	No Cat.	No Cat.
isopropanol	67-63-0	Liquid	0.3	No Cat.	No Cat.
4-methyl-thio-benzaldehyde	3446-89-7	Liquid	1	Cat. 2	No Cat.
methyl stearate	112-61-8	Solid	1	No Cat.	No Cat.
heptyl butyrate	5870-93-9	Liquid	1.7	No Cat.	No Cat. (*Optional Cat. 3*)
hexyl salicylate	6259-76-3	Liquid	2	No Cat.	No Cat. (*Optional Cat. 3*)
cinnamaldehyde	104-55-2	Liquid	2	Cat. 2	No Cat. (*Optional Cat. 3*)
CLASSIFIED CHEMICALS					
1-decanol[2]	*112-30-1*	*Liquid*	*2.3*	*Cat. 2*	*Cat. 2*
cyclamen aldehyde	103-95-7	Liquid	2.3	Cat. 2	Cat. 2
1-bromohexane	111-25-1	Liquid	2.7	Cat. 2	Cat. 2
2-chloromethyl-3,5-dimethyl-4-methoxypyridine HCl	86604-75-3	Solid	2.7	Cat. 2	Cat. 2
di-n-propyl disulphide[2]	*629-19-6*	*Liquid*	*3*	*No Cat.*	*Cat. 2*
potassium hydroxide (5% aq.)	1310-58-3	Liquid	3	Cat. 2	Cat. 2
benzenethiol, 5-(1,1-dimethylethyl)-2-methyl	7340-90-1	Liquid	3.3	Cat. 2	Cat. 2
1-methyl-3-phenyl-1-piperazine	5271-27-2	Solid	3.3	Cat. 2	Cat. 2
heptanal	111-71-7	Liquid	3.4	Cat. 2	Cat. 2
tetrachloroethylene	127-18-4	Liquid	4	Cat. 2	Cat. 2

Within-laboratory reproducibility

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

Between-laboratory reproducibility

11. An assessment of between-laboratory reproducibility is not essential if the proposed test method is to be used in a single laboratory only. For methods to be transferred between laboratories, the concordance of classifications (UN GHS Category 2 and No Category) obtained in different, independent test runs of the 20 Reference Chemicals between preferentially a minimum of three laboratories should be equal or higher (≥) than 80%.

Table 2: Required predictive values for sensitivity, specificity and accuracy for any similar or modified test method to be considered valid

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

Process of validation study

- Phase I transferability using 3 chemicals
- Phase II me-too study using 20 chemicals based on the ECVAM original performance standard
- Phase III me-too study using 6 chemicals based on the ECVAM revised performance standard

Validation report No.155 and a paper accepted by ATLA

- Peer review –

- Phase IV me-too study using 20 chemicals based on the draft OECD performance standard

Validation report No.159

- Phase V An additional study of phase IV study using 6 chemicals based on the OECD performance standard

Re-analyzed results (median) in LabCyte phase II & III validation studies

NO.	Code	GHS label	a	B	c	d	f	g
1	01	no	11.6	16.1	12.4	9.6	11.2	10.6
2	02	no	76.5	66.9	88.1	89.8	75.3	96.0
3	04	no	96.5	98.6	97.8	100.9	92.8	104.8
4	05	no	78.5	71.9	91.4	70.5	55.1	89.9
5	06	no	82.4	80.5	81.0	91.3	90.7	81.2
6	07	no	17.8	12.6	16.2	19.8	21.3	22.5
7	08	no	95.3	100.6	77.2	107.5	100.9	101.1
8	10	no	104.1	111.3	103.7	108.2	101.2	108.4
9	11	no	112.6	105.0	94.6	102.7	98.0	102.8
10	A	no	14.0	11.1	13.2	13.2	11.4	13.7
11	14	Category 2	6.8	8.8	9.5	10.7	16.7	12.0
12	15	Category 2	8.2	9.9	13.1	8.6	7.1	9.2
13	16	Category 2	59.8	92.0	81.7	37.7	59.6	79.6
14	B	Category 2	1.5	2.2	2.9	3.9	2.6	3.9
15	C	Category 2	0.7	0.8	1.0	2.0	1.0	0.4
16	18	Category 2	78.3	50.6	87.5	69.9	71.9	92.4
17	D	Category 2	14.5	16.0	12.6	18.3	13.8	15.2
18	E	Category 2	3.9	3.4	3.4	3.9	4.2	4.1
19	20	Category 2	23.3	14.0	8.6	19.2	8.0	8.1
20	F	Category 2	5.6	6.1	6.5	5.4	5.2	7.2

1-bromohexane

SUMMARY REPORT OF THE PEER REVIEW PANEL ON LABCYTE EPI-MODEL 24 IN VITRO TEST METHOD FOR THE ASSESSMENT OF SKIN IRRITATION POTENTIAL OF CHEMICALS

Future work should focus especially on the following aspects. Most importantly, the issue of ①misclassifying 1-bromohexane should be resolved.

Furthermore, an ②extensive analysis of the within- and between reproducibility referring to the performance standards of the draft OECD Test Guideline should be carried out and appropriately documented. It is also recommended to assess variability between replicate tissues and to define a respective acceptance criterion. In order to comply better with the performance standards, analyses using the ③mean instead of the median for deriving a final classification for a complete run sequence of a given laboratory should be carried out. Finally, ④ appropriate documentation describing and demonstrating the adherence to GLP principles should be provided.

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Outline of phase IV & V validation studies

Organization: JaCVAM Validation Management Team
Participated Lab.: Lab 1-3 : Three of four lab. Participated at phase I-II validation studies
Duration: September to November, 2010
Chemicals : Twenty chemicals based on the draft OECD performance standard(Coded samples distributed by JaCVAM)
Objects: To resolve misclassifying 1-bromohexane , the protocol has been revised by Japan tissue Engineering (J-TEC). To confirm general versatility on the revised protocol, we performed phase IV validation study.

Table. Modifications to rinsing operation in SOP versions 8.1, 8.2, and 8.3

Modification points	SOP ver. 8.1	SOP ver. 8.2	SOP ver. 8.3
1. Handling of PBS stream from washing bottle	Not described	Specifies that PBS stream is to avoid direct contact with tissue surface.	
2. Removal of PBS by swishing water off	Not described	Described briefly.	
3. Correct use of cotton pad	Not described	Specifies that cotton pad is to avoid direct contact with tissue surface.	
4. Removal of chemicals		Not described	Remove chemicals prior to washing by swishing water off
5. Washing fluid volume		Not described	Wash with large volume of PBS
6. No. of wash cycles		More than 10	More than 15
7. Swishing water off .after washing		Not described	Only once
8. Swishing water off .after final washing		Not described	Not done

Table . Classification using three independent cell viabilities based on merged results of validation and supplementary studies

No.	UN GHS *in vivo* Cat.	Lab A				Lab B				Lab C			
		1	2	3	F	1	2	3	F	1	2	3	F
1	No Cat.	P	P	P	P	P	P	P	P	P	P	P	P
2		N	N	N	N	P	N	N	N	N	N	N	N
3		N	N	N	N	N	N	N	N	N	N	N	N
4		N	N	N	N	P	N	N	N	N	N	N	N
5		N	N	N	N	N	N	N	N	N	N	N	N
6		P	P	P	P	P	P	P	P	P	P	P	P
7		N	N	N	N	N	N	N	N	N	N	N	N
8		N	N	N	N	N	N	N	N	N	N	N	N
9		N	N	N	N	N	N	N	N	N	N	N	N
10		P	P	P	P	P	P	P	P	P	P	P	P
11	Cat.2	P	P	P	P	P	P	P	P	P	P	P	P
12		P	P	P	P	P	P	P	P	P	P	P	P
13		P	P	P	P	P	P	P	P	P	P	P	P
14		P	P	P	P	P	P	P	P	P	P	P	P
15		N	N	N	N	P	P	P	P	P	N	N	N
16		P	P	P	P	P	P	P	P	P	P	P	P
17		P	P	P	P	P	P	P	P	P	P	P	P
18		P	P	P	P	P	P	P	P	P	P	P	P
19		P	P	P	P	P	P	P	P	P	P	P	P
21		P	P	P	P	P	P	P	P	P	P	P	P

P: Positive,
N: Negative,
F: Final determination by median,
ND: Not detected for invalid

← 1-bromohexane

Table. 2x2 tables with merged results of validation studies

		Lab A			Lab B			Lab C		
		UN GHS *in vivo* Cat.								
		Cat. 2	No	total	Cat. 2	No	total	Cat. 2	No	total
in vitro	Irritant	9	3	12	10	3	13	9	3	12
	Non-irritant	1	7	8	0	7	7	1	7	8
	Total	10	10	20	10	10	20	10	10	20
Sensitivity		90% (9/10)			100% (10/10)			90% (9/10)		
Specificity		70% (7/10)			70% (7/10)			70% (7/10)		
Accuracy		80% (16/20)			85% (17/20)			80% (16/20)		

April, 2013

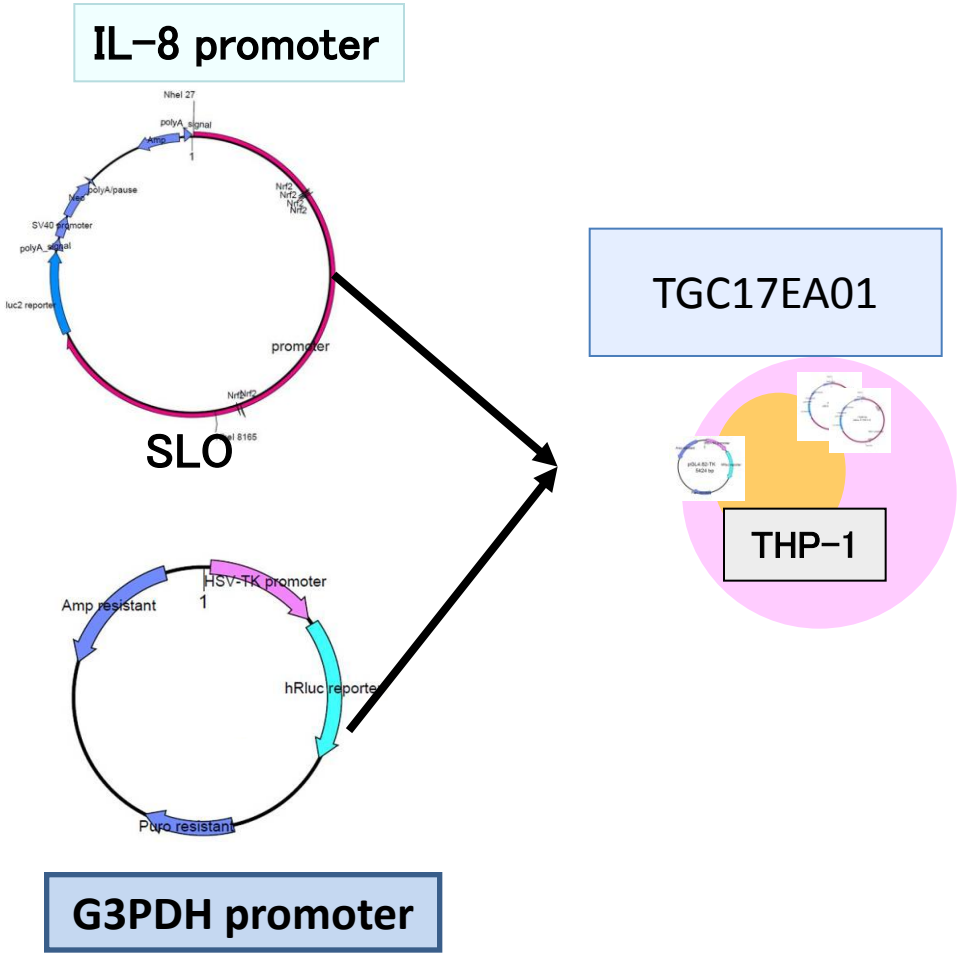
DRAFT UPDATED GUIDELINE 439 FOR THE TESTING OF CHEMICALS

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 substance 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 replacement test for *in vivo* skin irritation testing or as a partial replacement test within a tiered testing strategy (4).

Example 2: IL-8 Luc assay

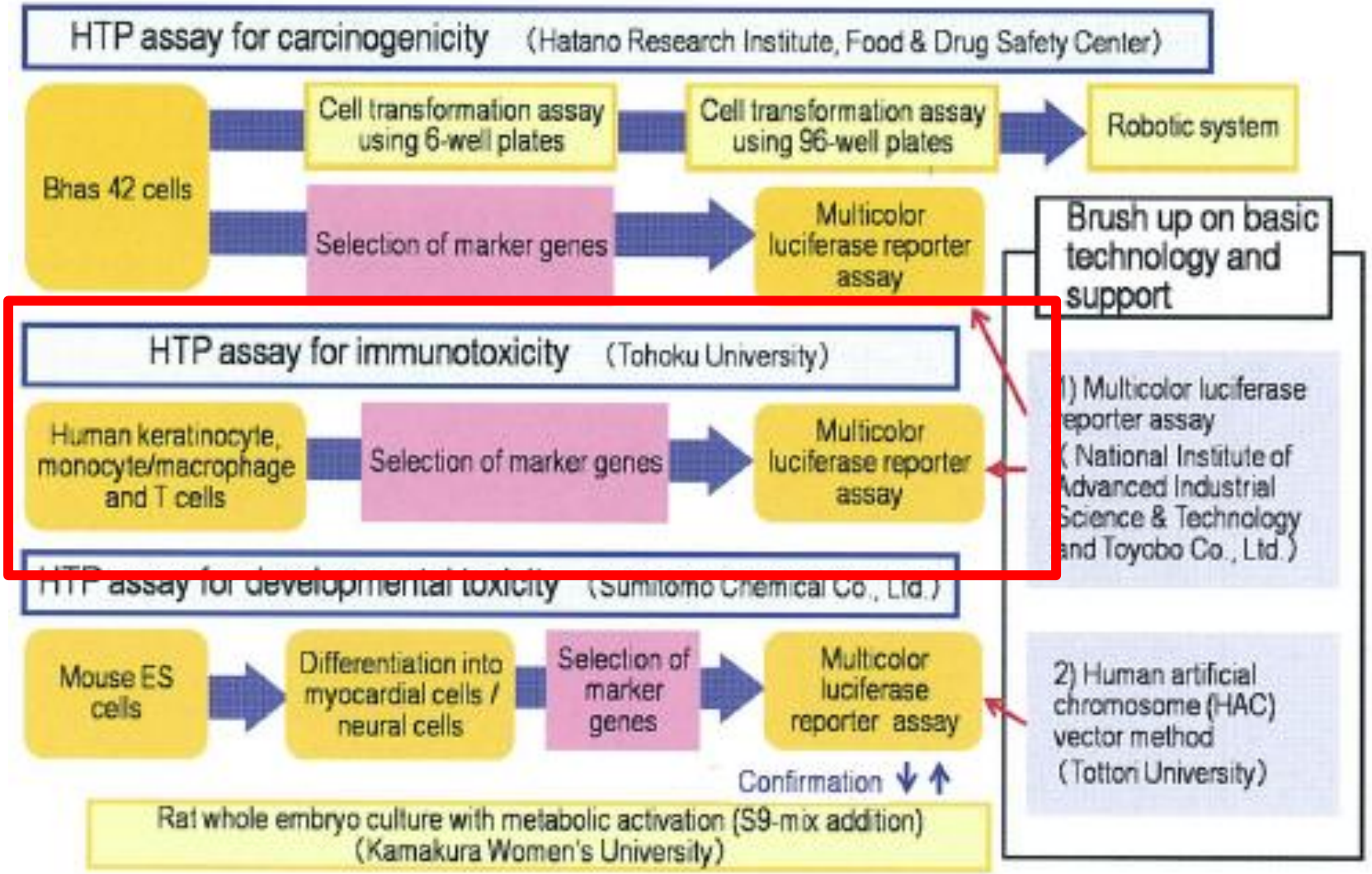


Validation activities: ECVAM

- Myeloid U937 Skin Sensitization Test (MUSST) - 1999
- Human Cell Line Activation Test (h-CLAT) - 2000
- Direct Peptide Reactivity Assay (DPRA) - 2003

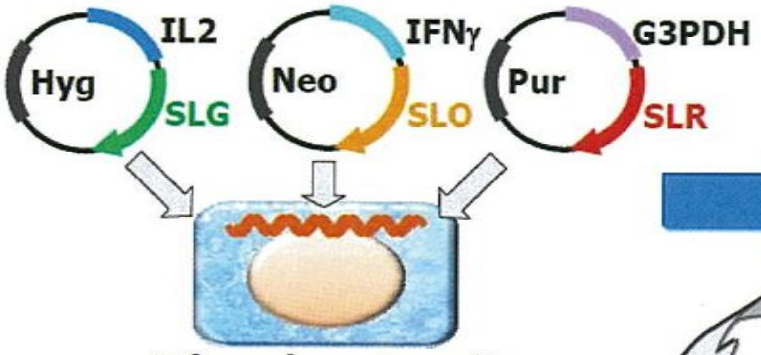
- Keratinosens – a HaCaT based system with a reactive cysteine linked to luciferase - 2007

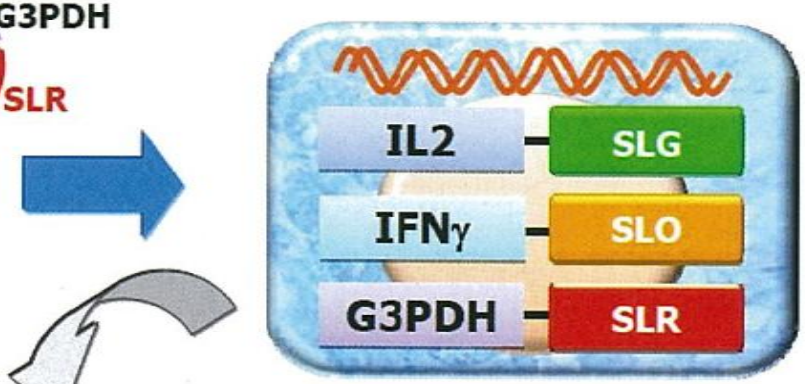
Each of these has been submitted to ECVAM for a formal independent view on their suitability, stage of validation and gap analysis

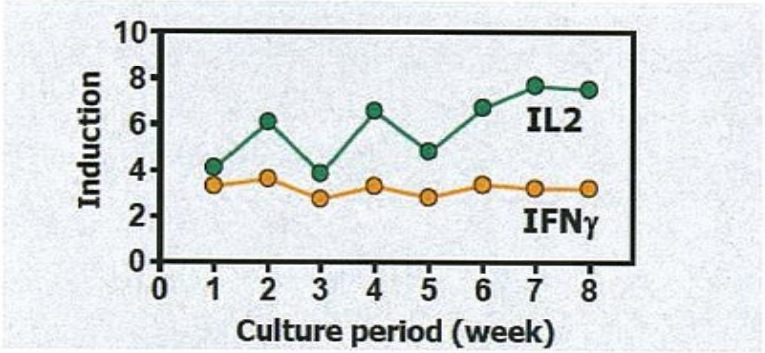

HTP assay for carcinogenicity (Hatano Research Institute, Food & Drug Safety Center)
Bhas 42 cells
Cell transformation assay using 6-well plates
Cell transformation assay using 96-well plates
Robotic system
Selection of marker genes
Multicolor luciferase reporter assay
Brush up on basic technology and support
HTP assay for immunotoxicity (Tohoku University)
Human keratinocyte, monocyte/macrophage and T cells
Selection of marker genes
Multicolor luciferase reporter assay
1) Multicolor luciferase reporter assay
(National Institute of Advanced Industrial Science & Technology and Toyobo Co., Ltd.)
HTP assay for developmental toxicity (Sumitomo Chemical Co., Ltd.)
Mouse ES cells
Differentiation into myocardial cells / neural cells
Selection of marker genes
Multicolor luciferase reporter assay
2) Human artificial chromosome (HAC) vector method
(Tottori University)
Confirmation
Rat whole embryo culture with metabolic activation (S9-mix addition)
(Kamakura Women's University)

Example of toxicity test for immunology using a multireporter assay

Generation of T cells stably express SLG, SLO and SLR enzymes under two marker gene promoters and internal control gene promoter.



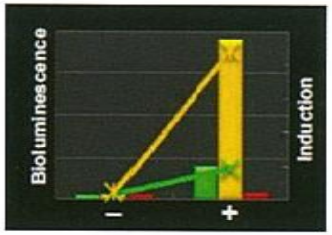




Cells display stable luciferase expressions and respond to chemicals during prolonged culture.

Chemical risk analysis in a 96well plate format HTP assay

Reaction with Tripluc® assay Reagents



Main members for IL-8 Luc assay Validation Management Team

Name	Role and expertise	Affiliation
Trial Coordinator Noriho Tanaka	VMT Chairperson,	**HRI and OTIP, Japan**
Lead Lab Yutaka Kimura* Setsuya Aiba**	*VMT Co-chair **Developer of this assay Test method, expertise underlying science	**Tohoku Univ., Japan**
Hajime Kojima	Management of quality control	**JaCVAM, NIHS, Japan (JaCVAM representative)**
Takashi Omori	Data analysis, biostatistics dossier	**Doshisha Univ., Japan**
Liaison members		
ECVAM liaison Emanuela Corcini	Test system expertise, multi-study validation expertise, immunotoxicity expertise	**Mila Univ., Italy**
ICCVAM liaison Warren Casey	Test system expertise, multi-study validation expertise	**NICEATM, USA**
KoCVAM liaison Ai-Young Lee	Test system expertise, multi-study validation expertise	**KoCVAM, Korea**

Stages of IL-8 Luc assay pre-validation study under Modular approach

Module 2: Within-lab Reproducibility (5 coded)

Module 3: Transferability

Phase 1 (*finished*) 10 non-coded

Module 4: Between-Lab Reproducibility

Phase 2 20 coded

Present time

Module 5: Predictive capacity

Phase 3 ?? coded

History of IL-8 Luc assay pre-validation studies

2011 Phase 1 Transferability

Revised protocol

2012 Phase 2-a Within-& Between lab reproducibility

Revised protocol

2013 Phase 2-b Within-& Between lab reproducibility

Revised protocol

Phase 2-c Within-& Between lab reproducibility?

Main revised points: change of positive control, dilution procedure of chemicals, acceptance criteria, etc.

Summary

It is difficult with make the optimize transferable test method protocol in the pre-validation study.

In order to conduct easy and simple validation study, the protocol and study plan of new test method should be examined strictly by the funding agency and validation center.

Japanese Center for the Validation of Alternative Methods

Office : New Testing Method Assessment, Division of Pharmacology,
National Biological Safety Research Center (NBSRC),
National Institute of Health Sciences (NIHS)

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About JaCVAM | Update on JaCVAM | Academic activities | Submission of Alternative Methods | International Cooperation

Thank you for your attention

Policy and Mission: JaCVAM's policy and mission is to promote the 3Rs in animal experiments for the evaluation of chemical substance safety in Japan and establish guidelines for new alternative experimental methods through international collaboration.

the 3Rs in animal experiments---Reduction (of animal use)
Refinement (to lessen pain or distress and to enhance animal well-being)
Replacement (of an animal test with one that uses non-animal systems or phylo-genetically lower species)
(OECD GD34)

News

- 【NEW】news texts dummy texts news texts dummy texts news texts dummy texts(2009.7.16)
- news texts dummy texts news texts (2009.7.3)
- news texts dummy texts news texts dummy texts news texts dummy texts (2009.7.3)

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- JaCVAM Activities

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インターネット | 保護モード: 有効