

August, 2013, Tokyo

皮膚基礎研究クラスターフォーラム

代替法の国内外動向



小島 肇

JaCVAM, NIHS

内容

1. 2012年に成立したTGおよび2013年に成立する予定の試験法
2. 2012年度にJaCVAMが認めた試験法
3. 化粧品・医薬部外品ガイダンス2012-2013
4. 眼刺激性試験代替法の最新情報
5. 皮膚感作性試験代替法の最新情報
6. AOP



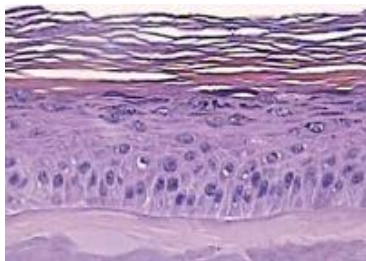
2012年に成立したTGおよび2013年に成立する予定の試験法

Method	Lead Country	International acceptance
BG1Luc Estrogen Receptor Transactivation Test Method for Identifying Estrogen Receptor Agonists and Antagonists	USA	OECD TG 457 (2012)
Performance-Based Test Guideline for Stably Transfected Transactivation In Vitro Assays to Detect Estrogen Receptor Agonists Test	USA & Japan	OECD updated TG 455 (2012)
Fluorescein Leakage (FL) test method	EU	OECD TG 460 (2012)
Use of anesthetics, analgesics, and humane endpoints for routine use in the TG 405	USA	OECD updated TG 405 (2012)
<i>In vitro</i> skin irritation testing including LabCyte EPI-Model	Japan	OECD TG updated 439 (2013)
Bovine Corneal Opacity and Permeability (BCOP) Test Method	USA & EU	OECD TG updated 437 (2013)
Isolated Chicken Eye (ICE) Test Method	Netherlands	OECD TG updated 438 (2013)

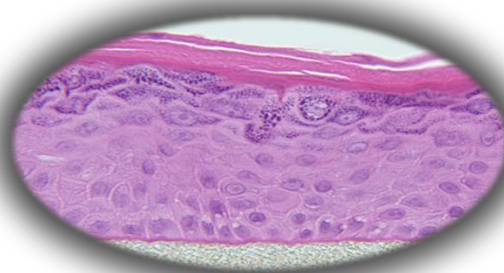
OECD GUIDELINE FOR THE TESTING OF CHEMICALS

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

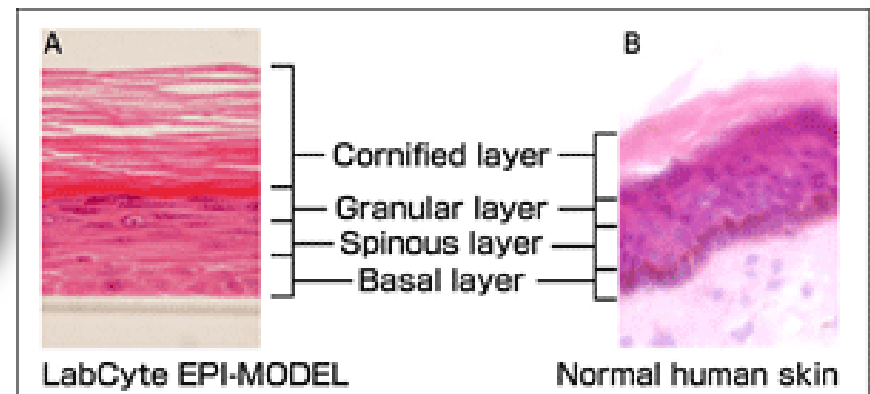
4. There are three validated test methods that adhere to this Test Guideline. Prevalidation, optimisation and validation studies have been completed for an *in vitro* test method (10) (11) (12) (13) (14) (15) (16) (17) (18) (19) (20), using a RhE model, commercially available as EpiSkin™ (designated 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



新規試験法提案書

平成 25 年 1 月 20 日

No. 2012-04

皮膚感作性試験代替法 Local Lymph Node Assay (LLNA): BrdU-ELISA の判定基準の変更に関する提案

平成 24 年 10 月 1 日に東京、国立医薬品食品衛生研究所にて開催された新規試験法評価会議（通称：JaCVAM 評価会議）において以下の提案がなされた。

提案内容：皮膚外用剤として用いる医薬品、医療機器、化粧品、皮膚適用の医薬部外品、農薬等に含まれる物質又はそれらの製品の皮膚感作性を予測する皮膚感作性試験代替法 Local Lymph Node Assay (LLNA): BrdU-ELISA は、RI を使用せずとも従来試験法と同等の結果が得られることから、行政上利用することは可能である。

この提案書は、米国 Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) LLNA: BrdU-ELISA Evaluation Report (2010)、LLNA: BrdU-ELISA の JaCVAM 評価報告 (2011) および OECD Test Guideline (TG) 442B をもとに、皮膚感作性試験代替法評価委員会によりまとめられた文書を用いて JaCVAM 評価会議が評価および検討した結果、その有用性が確認されたことから作成された。

以上の理由により、行政当局の安全性評価方法として「皮膚感作性試験代替法 LLNA: BrdU-ELISA の判定基準の変更」に関する提案をするものである。

吉田武美 

JaCVAM 評価会議 議長

西川秋佳 

JaCVAM 運営委員会 委員長

JaCVAM statement on the Local Lymph Node Assay (LLNA): BrdU-ELISA for skin sensitization assay

At the meeting concerning the above method, held on 1 October 2012 at the National Institute of Health Sciences (NIHS), Tokyo, Japan, the members of the Japanese Center for the Validation of Alternative Methods (JaCVAM) Regulatory Acceptance Board unanimously endorsed the following statement:

The LLNA: BrdU-ELISA can be used to identify substances as potential skin sensitizers or nonsensitizers as well as LLNA for regulatory use, without Radio-isotope.

Following the review of the results of the ICCVAM (Interagency Coordinating Committee on the Validation of Alternative Methods, USA) Evaluation Report, JaCVAM peer review panel reports, and OECD (Organisation for Economic Co-operation and Development) Test Guideline revised No. 442B, it is concluded that the LLNA: BrdU-ELISA for skin sensitization assay is clearly beneficial.

The JaCVAM Regulatory Acceptance Board has been regularly kept informed of the progress of the study, and this endorsement is based on an assessment of various documents, including, in particular, the evaluation report prepared by the JaCVAM ad hoc peer review panel for skin sensitization assay.



Takemi Yoshida
Chairperson
JaCVAM Regulatory Acceptance Board



Akiyoshi Nishikawa
Chairperson
JaCVAM Steering Committee

20 January, 2013

2012年度にJaCVAM評価会議が認証した試験法

No.	Test Method
1	Skin sensitization assay, LLNA : DA
2	Skin sensitization assay, LLNA : BrdU-ELISA
3	Skin sensitization assay, rLLNA
4	<i>In vitro</i> skin irritation testing: EpiDerm, SKINEthics
5	Fluorescein leakage (FL) Test Methods for Identifying Ocular Corrosives and Severe Irritants

事務連絡
平成24年4月26日

事務連絡
平成25年5月30日

各都道府県衛生主管部（局）
薬務主管課 御中

各都道府県衛生主管部（局）
薬務主管課 御中

厚生労働省医薬食品局審査管理課

厚生労働省医薬食品局審査管理課

皮膚感作性試験代替法及び光毒性試験代替法を化粧品・医薬部外品の
安全性評価に活用するためのガイドランスについて

皮膚感作性試験代替法（LLNA：DA、LLNA：BrdU-ELISA）を化粧品・医薬部外品の
安全性評価に活用するためのガイドランスについて

今般、皮膚感作性試験代替法及び光毒性試験代替法について、その利用促進を図るため、平成23年度レギュラトリーサイエンス総合研究事業（研究代表者 小島肇）において、それぞれ化粧品・医薬部外品の安全性評価に活用するためのガイドランスを作成したので、貴管下関係業者に対して周知願います。

今般、皮膚感作性試験代替法（LLNA：DA、LLNA：BrdU-ELISA）について、その利用促進を図るため、平成24年度レギュラトリーサイエンス総合研究事業（研究代表者 小島肇）において、それぞれ化粧品・医薬部外品の安全性評価に活用するためのガイドランスを作成したので、貴管下関係業者に対して周知願います。

なお、その他の代替法に関するガイドランスについては、順次、作成する予定です。

なお、その他の代替法に関するガイドランスについては、順次、作成する予定です。

（添付資料）

- ① 皮膚感作性試験代替法としての LLNA を化粧品・医薬部外品の安全性評価に活用するためのガイドランス
- ② 光毒性試験代替法としての *in vitro* 3T3 NRU 光毒性試験を化粧品・医薬部外品の安全性評価に活用するためのガイドランス

（添付資料）

- ① 皮膚感作性試験代替法としての LLNA：DA を化粧品・医薬部外品の安全性評価に活用するためのガイドランス
- ② 皮膚感作性試験代替法としての LLNA：BrdU-ELISA を化粧品・医薬部外品の安全性評価に活用するためのガイドランス

眼刺激性試験代替法の国際動向1

Method	Current status	Lead Organization	International acceptance
Bovine Corneal Opacity and Permeability (BCOP) Test Method	Completed		OECD TG 437 (2009)
Isolated Chicken Eye (ICE) Test Method	Completed		OECD TG 438 (2009)
Bovine Corneal Opacity and Permeability (BCOP) Test Method and the Isolated Chicken Eye (ICE) Test Method	Completed	NICEATM-ICCVAM, EURL ECVAM and the Netherlands	Adopted by WNT 25 in April 2013.
Use of Histopathology as an additional endpoint in Ocular Safety Testing	Completed		OECD GD 160 (2011)
Cytotoxicity test: SIRC CVS	JaCVAM-sponsored validation study ongoing	JaCVAM; EURL ECVAM, NICEATM-ICCVAM, and Health Canada VMT	
Cytotoxicity test: three-dimensional dermal model (MATREX)	JaCVAM-sponsored validation study in the planning stage	JaCVAM; EURL ECVAM, NICEATM-ICCVAM, and Health Canada VMT	

眼刺激性試験代替法の国際動向2

Method	Current status	Lead Organization	International acceptance
Cytotoxicity test: Short Time Exposure (STE) test	JaCVAM-sponsored validation study completed –peer review completed by NICEATM-ICCVAM	JaCVAM; EURL ECVAM, NICEATM-ICCVAM, and Health Canada VMT liaisons	SPSF submitted and approved in 2012.
Use of anesthetics, analgesics, and humane endpoints for routine use in the TG 405	Completed		OECD updated TG 405 (2012)
Cytosensor Microphysiometer® (CM) Test method	The draft TG submitted to OECD.	EURL ECVAM; NICEATM-ICCVAM	Adoption of draft TG expected by WNT 26 in 2014.
Fluorescein Leakage (FL) test method	Completed		OECD TG 460 (2012)
Human reconstructed tissue models for eye irritation - EpiOcular EIT™ - SkinEthic HCE	EURL ECVAM validation study completed. Peer review anticipated by 2013.	EURL ECVAM; JaCVAM, NICEATM-ICCVAM, and Health Canada VMT liaisons	

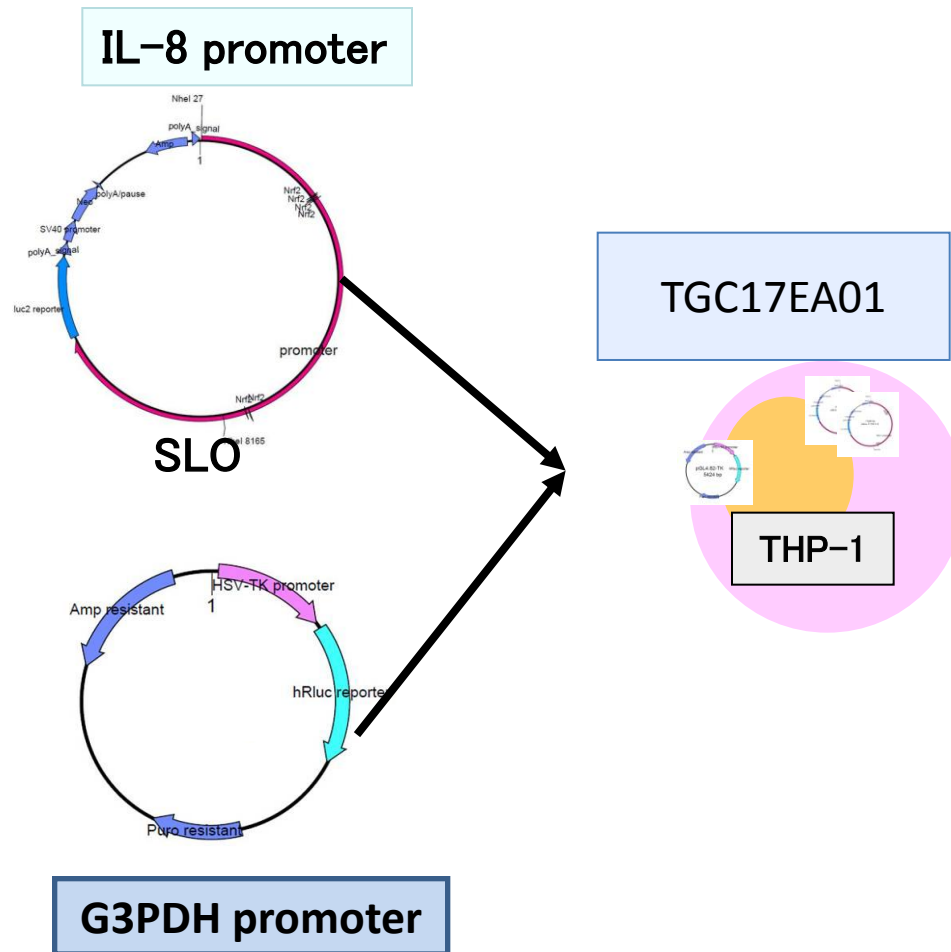
皮膚感作性試験代替法の国際動向1

Method	Current status	Lead Organization	International acceptance
Murine Local lymph Node Assay (LLNA)	Completed		OECD TG 429 (2002), ISO (2002)
Updated LLNA	Completed		Updated OECD TG 429 (2010), ISO (2010)
Reduced LLNA (rLLNA)	Completed		Updated OECD TG 429 (2010)
LLNA:DA	Completed		OECD TG442A (2010)
LLNA:BrdU-ELISA	Completed		OECD TG 442B (2010)
Nonradioactive LLNA protocol (LLNA: BrdU-Flow Cytometry)	<ul style="list-style-type: none"> - ICCVAM international peer review, 2009 ➤ KoCVAM validation study on-going 	NICEATM-ICCVAM, KoCVAM	

皮膚感作性試験代替法の国際動向2

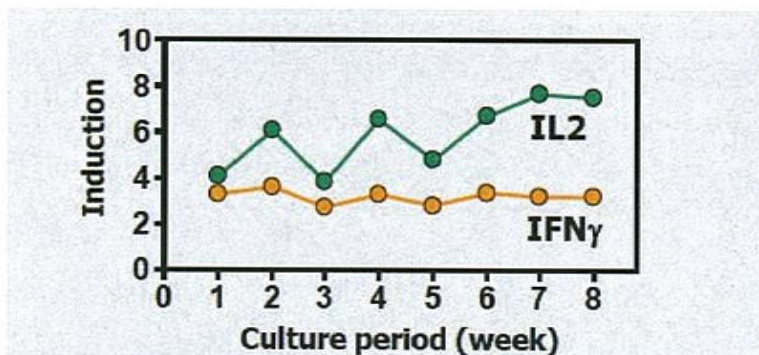
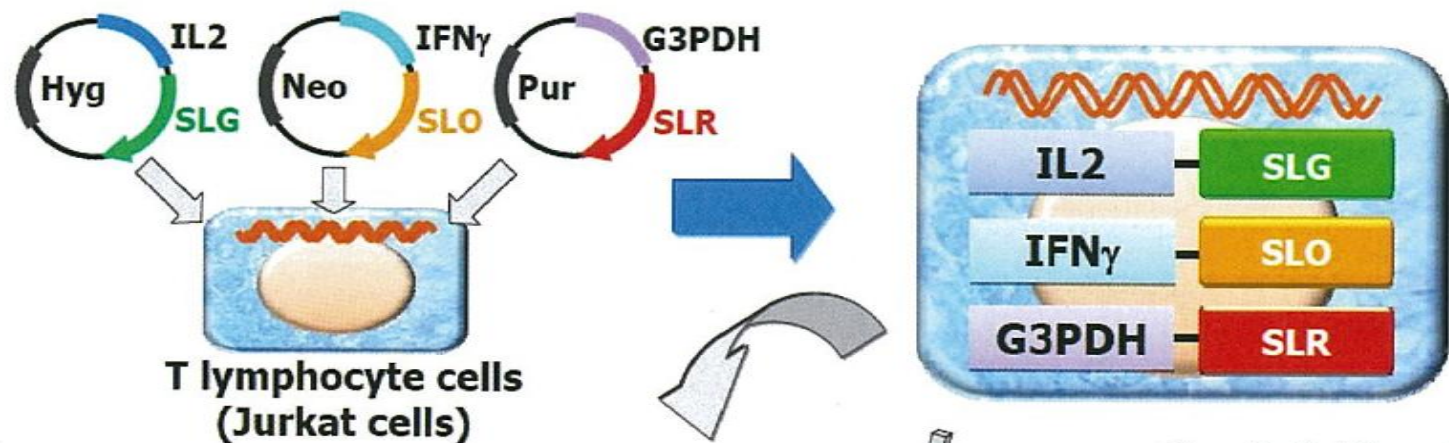
Method	Current status	Lead Organization	International acceptance
<i>In vitro</i> skin sensitization assays (h-CLAT; DPRA; MUSST)	Multi-laboratory validation ends in August 2012 (h-CLAT and MUSST). DPRA peer review is over.	EURL ECVAM; JaCVAM and NICEATM-ICCVAM VMT liaison members	Proposal for an Adverse Outcome Pathway for skin sensitization has been approved by the OECD. SPSFs approved for the DPRA, and h-CLAT.
<i>In vitro</i> skin sensitization assay KeratinoSens	External Validation Study, peer review is over.	EURL ECVAM	SPSF approved
<i>In vitro</i> skin sensitization assay IL-8 Luc assay	METI-sponsored validation study ongoing	JaCVAM; EURL ECVAM, NICEATM-ICCVAM, KoCVAM and Health Canada VMT liaisons	

IL-8 Luc assay



Example of toxicity test for immunology using a multireporter assay

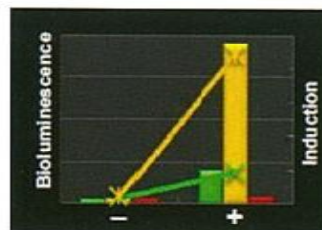
Generation of T cells stably express SLG, SLO and SLR enzymes under two marker gene promoters and internal control gene promoter.



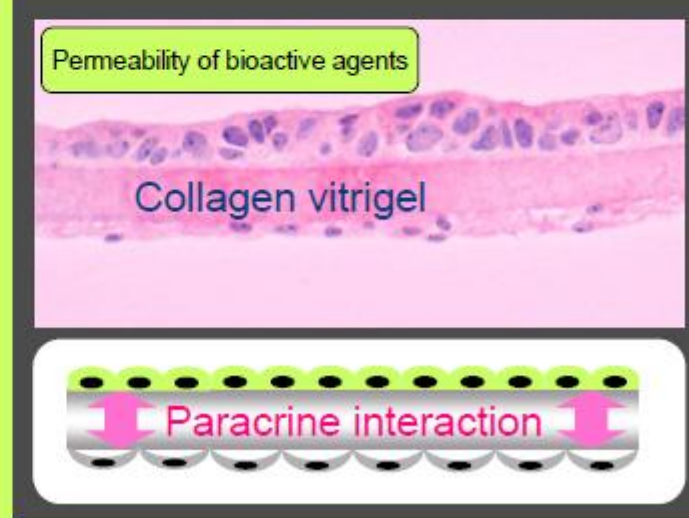
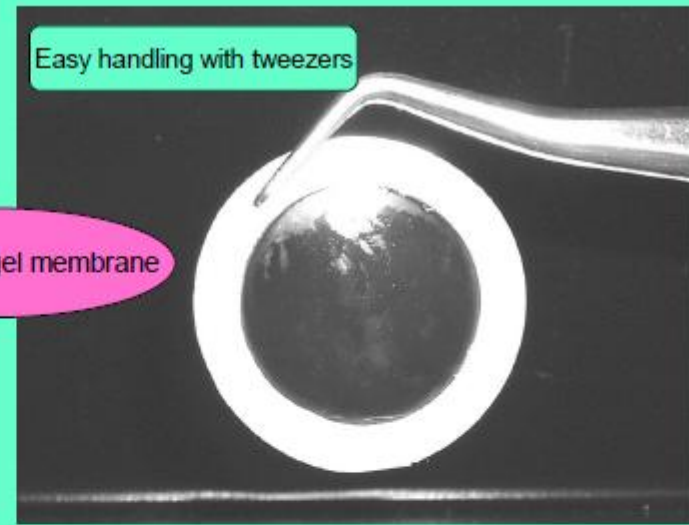
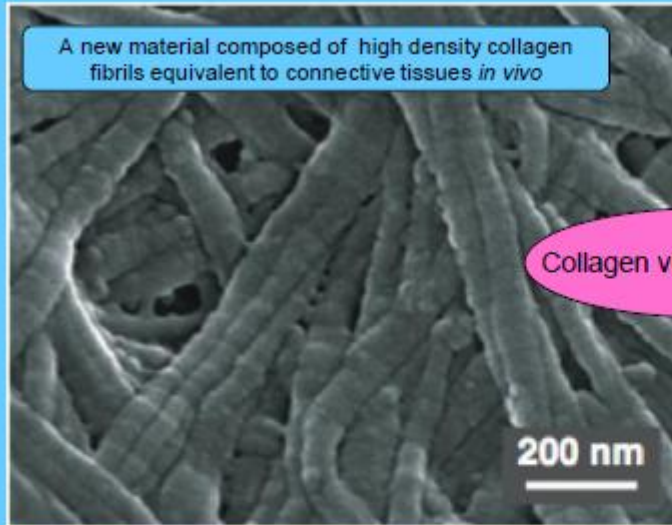
Cells display stable luciferase expressions and respond to chemicals during prolonged culture.

Chemical risk analysis
in a 96well plate
format HTP assay

Reaction with Tripluc[®] assay Reagents



Background- 1 : Collagen vitrigel membrane (CVM)



Takezawa T, *et al.*, Cell Transplantation, 13: 463-473, 2004
Takezawa T, *et al.*, Tissue Engineering, 13: 1357-1366, 2007

Takezawa T, *et al.*, Cell Tissues Organs, 185: 237-4241, 2007
Takezawa T, *et al.*, Yakugaku Zasshi, 130: 565-574, 2010

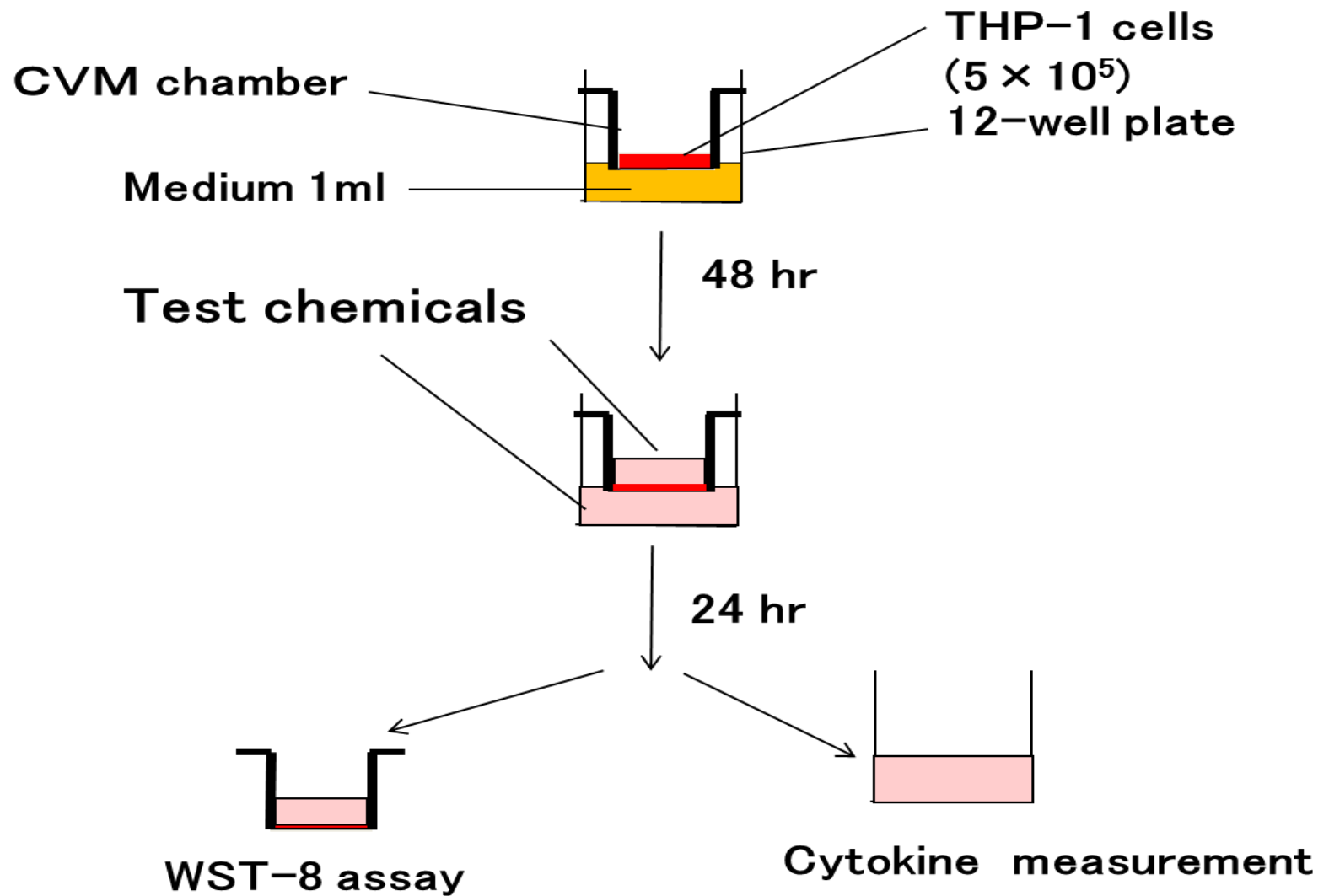
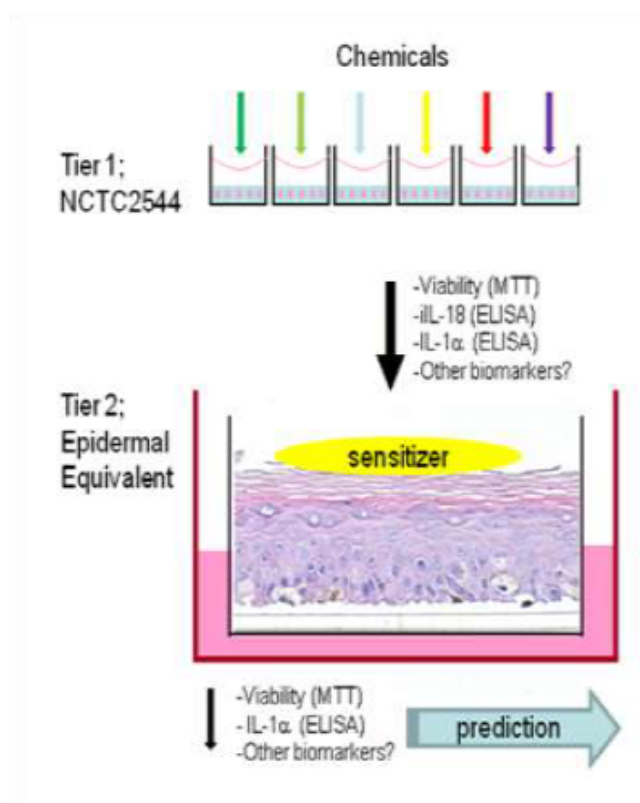


Fig.1 Vitrigel-SST model

A 2-tiered approach for identification and classification of skin sensitizers

3RsMC

3Rs Management
and Consultancy
www.3RsMC.eu



• Identification of contact sensitizers in Tier 1

-IL-18 expression

- Identification of potency of sensitizers in Tier 2

- EC50, MTT

- IL-1 α expression

NCTC2544/IL-18:

- 30 chemicals tested
- WLR: >95%
- Transferable
- BLR: >95%
- Accuracy: 97% (labeling)

RHE potency test:

- 16 chemicals tested
- WLR: >95%
- Transferable
- BLR: >95%
- Concordancy: 92% (classification)

Assessed by Cosmetics Europe

- 10 coded compounds

まとめ: 各試験法の限界と特徴 (EURL-ECVAM)

Test	Concordance (%)	Potency class dependent	Pro-haptens	Irritant	Respiratory sensitizations	Dye interference	HTS	Solubility issue	Potency information
DPRA*	85	No(?)	No	Yes	Yes	No	No	Yes	(Yes)
Keratino-sens*	87	Yes	Yes	+/-	?	Yes	Yes	Yes	No
IL-18 detection	97	Yes	Yes	No	No	No	Yes	No	(Yes)
Skin GARD (Genomic Allergen Rapid Detection)	>95	No	Yes	No	Res GARD	No	Yes	Yes	(Yes)
VitroSense	89	No(?)	Yes	No	?	No	Yes	Yes	(Yes)
h-CLAT**	78	Yes	?	Yes	?	?	No	Yes	(Yes)
MUSST**	78	Yes	?	Yes	?	?	No	Yes	No

*: ESAC opinion, **: ECVAM validation performed

皮膚感作性試験の有害性転帰事象 (AOP)

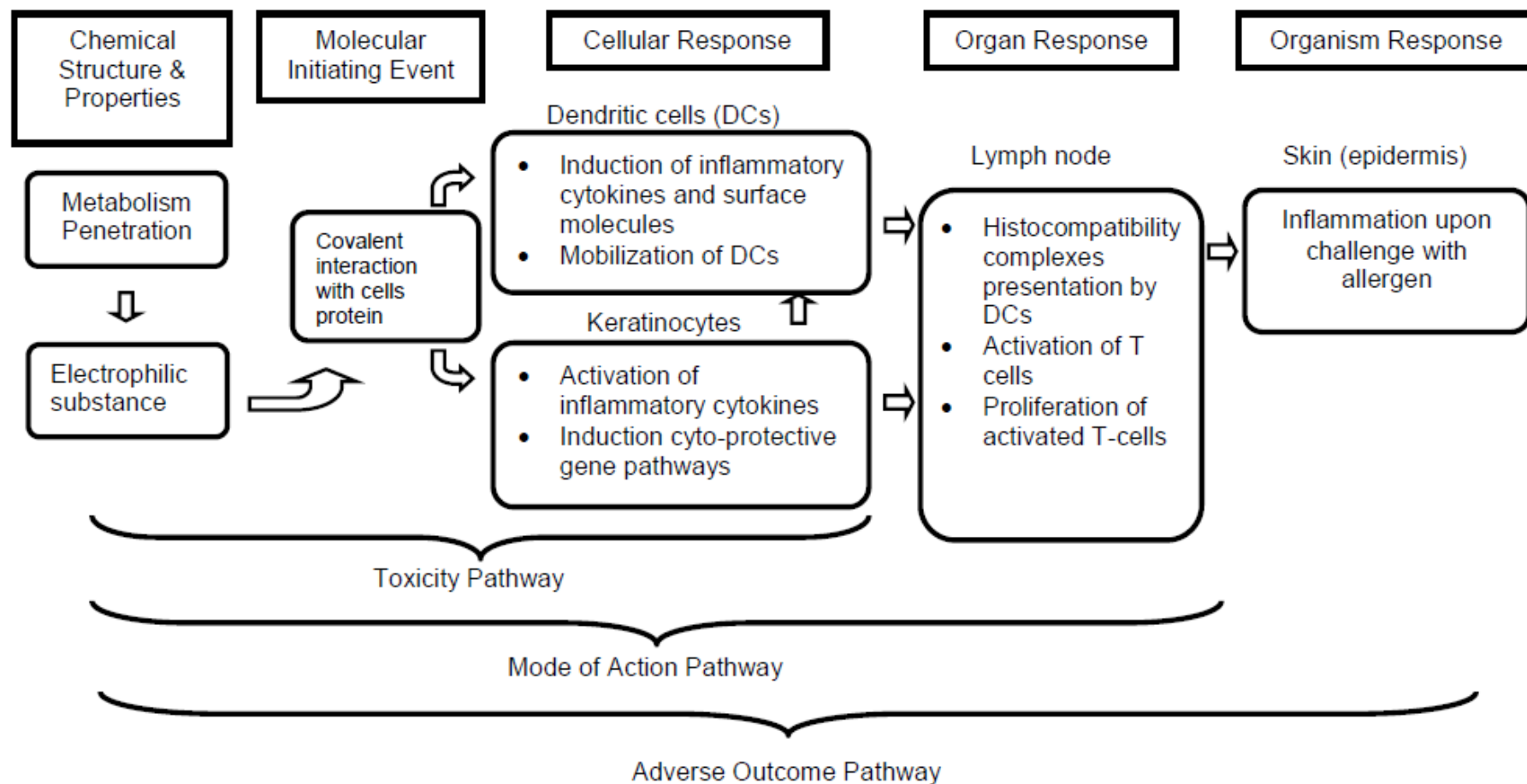
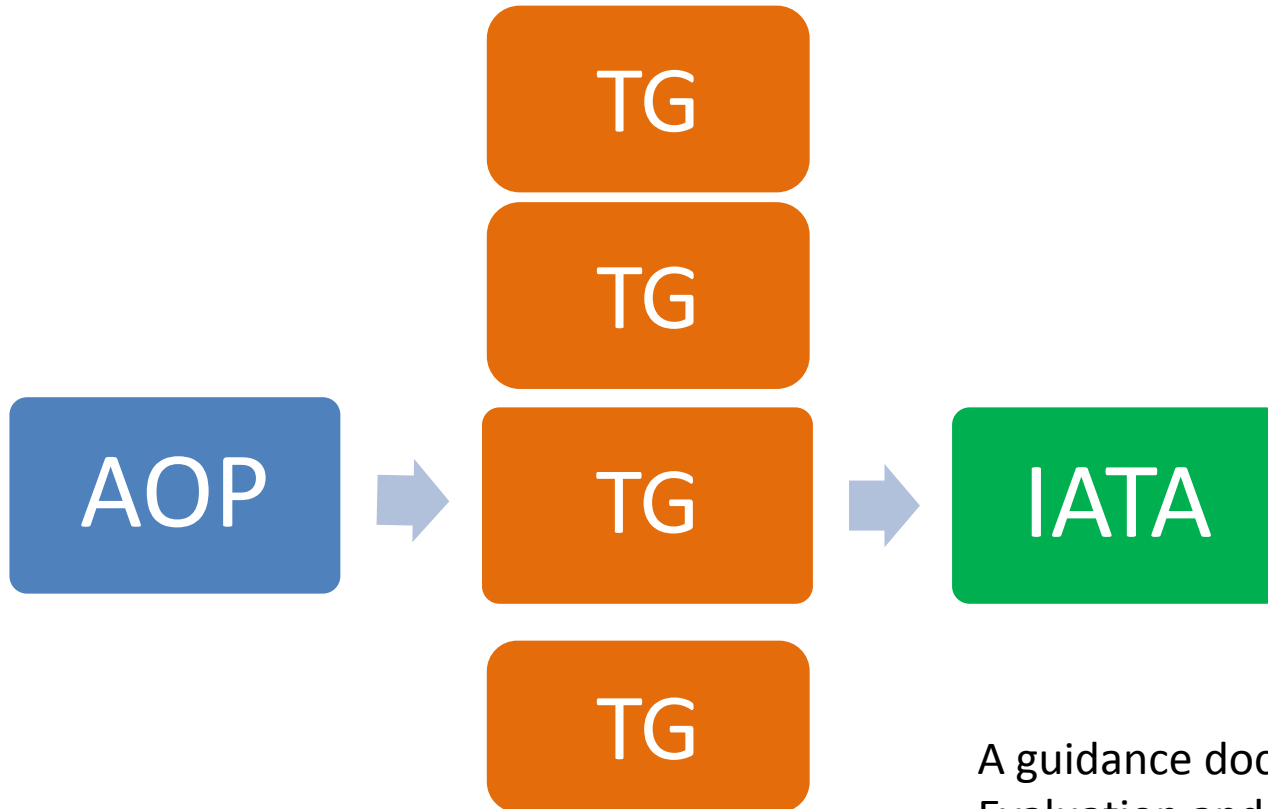


Figure 3. Flow diagram of the pathways associated with skin sensitisation.

行政的な受入れに関するOECDの戦略

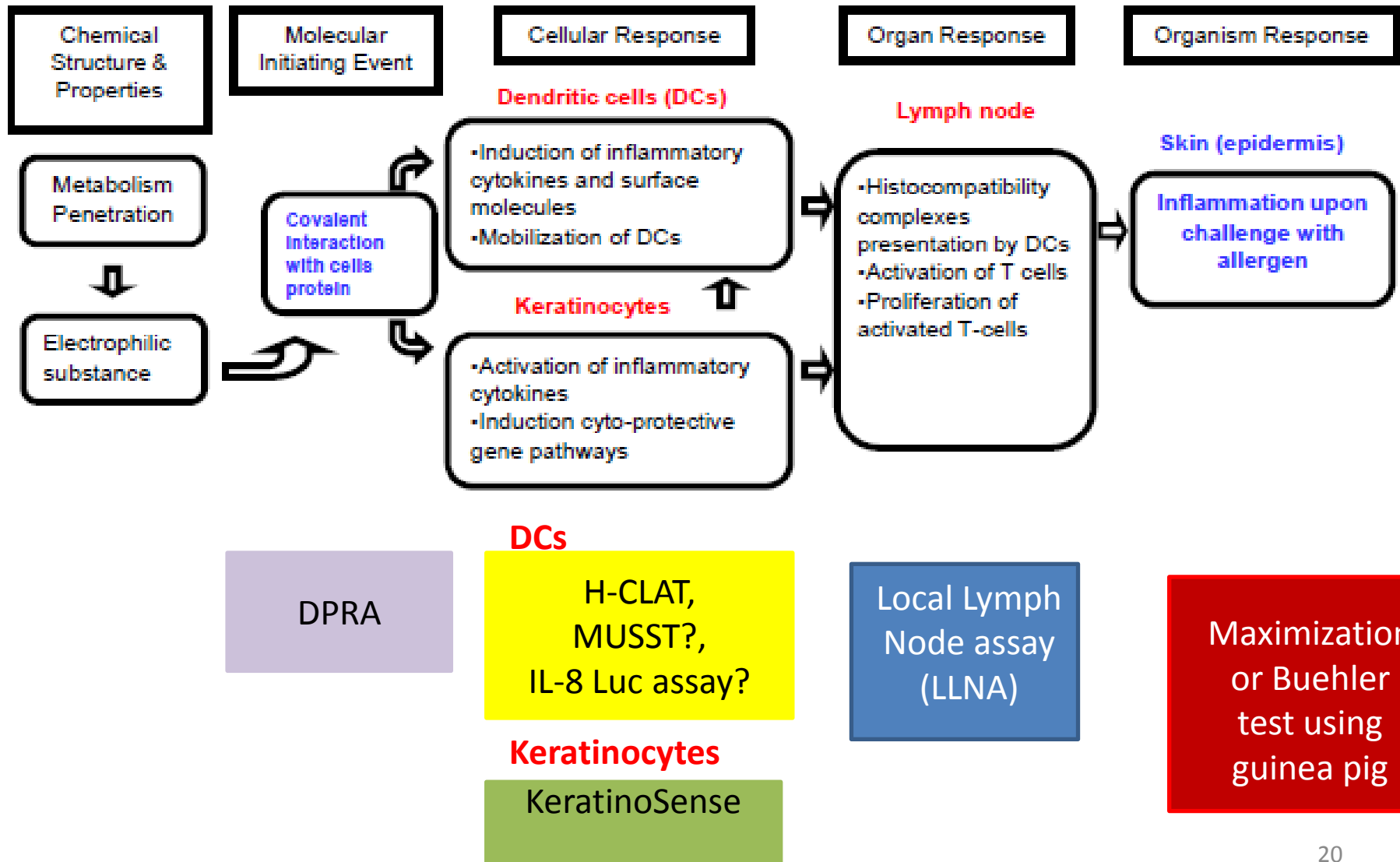


AOP: Adverse
Outcome Pathway

TG: Test Guideline

A guidance document on the
Evaluation and Application of
Integrated Approaches to Testing
and Assessment (IATA)

皮膚感作性試験の有害性転帰事象 (AOP) と試験法





About JaCVAM



Update on JaCVAM



Academic activities



Submission of Alternative
Methods to JaCVAM



International Cooperation

御静聴ありがとうございました

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

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news texts dummy texts (2009.7.16)

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texts dummy texts (2009.7.3)

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