Adverse Outcome Pathways for skin sensitization assay

皮膚感作性試験代替法における最新動向

Update on the alternative to skin sensitization assay in Japan

Hajime Kojima,
JaCVAM, NIHS

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- International collaboration
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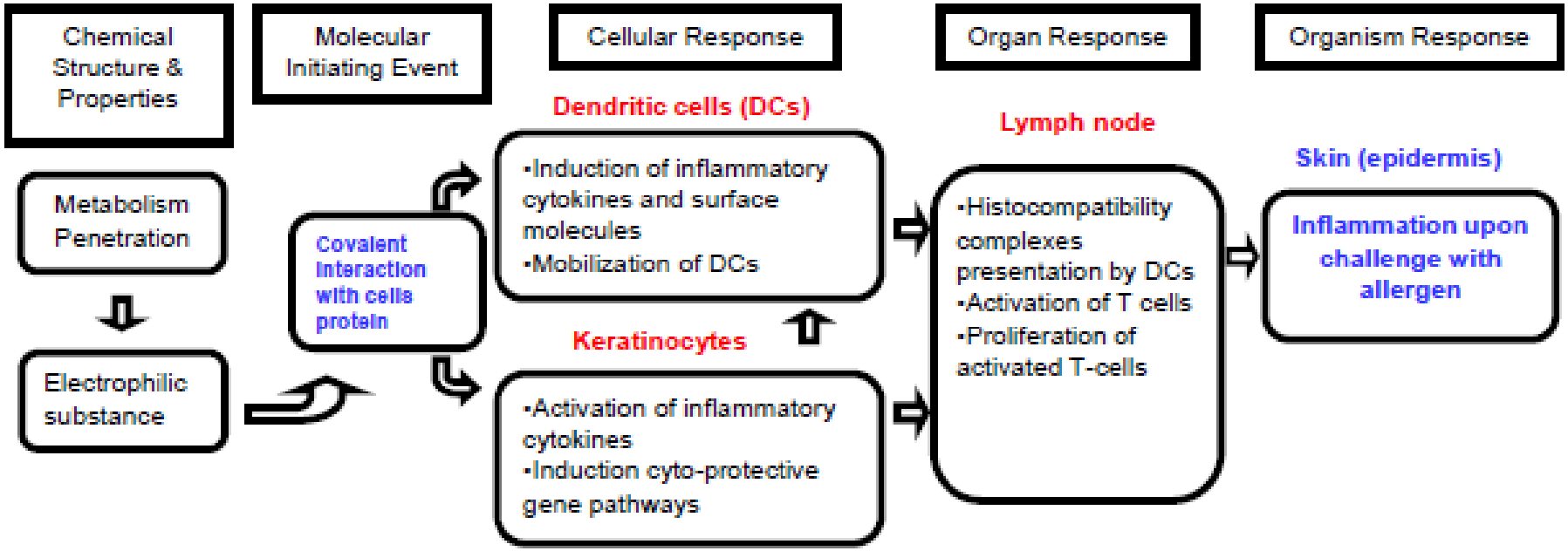
Adverse Outcome Pathways on skin sensitization assay for regulatory use



LLNA update

OECD/OCDE

429

Adopted:
22 July 2010

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Skin Sensitization: Local Lymph Node Assay

INTRODUCTION

1. OECD Guidelines for the Testing of Chemicals are periodically reviewed in light of scientific progress, changing regulatory needs, and animal welfare considerations. The original Test Guideline (TG) for the determination of skin sensitization in the mouse, the Local Lymph Node Assay (LLNA; TG 429) was adopted in 2002 (1). The details of the validation of the LLNA and a review of the associated work have been published (2) (3) (4) (5) (6) (7) (8) (9) (10) (11). The updated LLNA is based on the evaluation of experience and scientific data (12). This is the second TG to be designed for assessing skin sensitization potential of chemicals in animals. The other TG (*i.e.* TG 406) utilises guinea pig tests, notably the guinea pig maximisation test and the Buehler test (13). The LLNA provides advantages over TG 406 (13) with regard to animal welfare. This updated LLNA TG includes a set of Performance Standards (PS) (Annex 1) that can be used to evaluate the validation status of new and/or modified test methods that are functionally and mechanistically similar to the LLNA, in accordance with the principles of Guidance Document No. 34 (14).

Revised points on LLNA TG 429 from 2002 to 2010 versions

1. Reduced LLNA (rLLNA)
2. Limitation

   pesticide formulations
3. Pre-screen

   Erythema and ear-thickness
4. Performance standard

Adopted:
22 July 2010

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Skin Sensitization: Local Lymph Node Assay: DA

INTRODUCTION

1. OECD Guidelines for the Testing of Chemicals are periodically reviewed in light of scientific progress, changing regulatory needs, and animal welfare considerations. The first Test Guideline (TG) for the determination of skin sensitization in the mouse, the Local Lymph Node Assay (LLNA; TG 429) was adopted in 2002, and has since then been revised (1). The details of the validation of the LLNA and a review of the associated work have been published (2) (3) (4) (5) (6) (7) (8) (9). In the LLNA, radioisotopic thymidine or iodine is used to measure lymphocyte proliferation and therefore the assay has limited use in regions where the acquisition, use, or disposal of radioactivity is problematic. The LLNA: DA (developed by Daicel Chemical Industries, Ltd.) is a non-radioactive modification to the LLNA, which quantifies adenosine triphosphate (ATP) content via bio-luminescence as an indicator of lymphocyte proliferation. The LLNA: DA test method has been validated and reviewed and recommended by an international peer review panel as considered useful for identifying skin sensitizing and non-sensitizing substances, with certain limitations (10) (11) (12) (13). This Test Guideline is designed for assessing skin sensitization potential of chemicals in animals. TG 406 utilises guinea pig tests, notably the guinea pig maximisation test and the Buehler test (14). The LLNA (TG 429) and the two non-radioactive modifications, LLNA: DA (TG 442 A) and LLNA: BrdU-ELISA (TG 442 B), all provide an advantage over the guinea pig tests in TG 406 (14) in terms of reduction and refinement of animal use.

OECD/OCDE

442B

Adopted:
22 July 2010

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Skin Sensitization: Local Lymph Node Assay: BrdU-ELISA

INTRODUCTION

1. OECD Guidelines for the Testing of Chemicals are periodically reviewed in light of scientific progress, changing regulatory needs, and animal welfare considerations. The first Test Guideline (TG) for the determination of skin sensitization in the mouse, the Local Lymph Node Assay (LLNA; TG 429) was adopted in 2002, and has since then been revised (1). The details of the validation of the LLNA and a review of the associated work have been published (2) (3) (4) (5) (6) (7) (8) (9). In the LLNA, radioisotopic thymidine or iodine is used to measure lymphocyte proliferation and therefore the assay has limited use in regions where the acquisition, use, or disposal of radioactivity is problematic. The LLNA: BrdU-ELISA [Enzyme-Linked Immunosorbent Assay] is a non-radioactive modification to the LLNA test method, which utilises non-radiolabelled 5-bromo-2-deoxyuridine (BrdU) (Chemical Abstracts Service [CAS] No 59-14-3) in an ELISA-based test system to measure lymphocyte proliferation. The LLNA: BrdU-ELISA has been validated and reviewed and recommended by an international independent scientific peer review panel as considered useful for identifying skin sensitizing and non-sensitizing test substances, with certain limitations (10) (11) (12). This Test Guideline is designed for assessing skin sensitization potential of chemicals in animals. TG 406 utilises guinea pig tests, notably the guinea pig maximisation test and the Buehler test (13). The LLNA (TG 429) and the two non-radioactive modifications, LLNA: BrdU-ELISA (TG 442 B) and LLNA: DA (TG 442 A), all provide an advantage over the guinea pig tests in TG 406 (13) in terms of reduction and refinement of animal use.

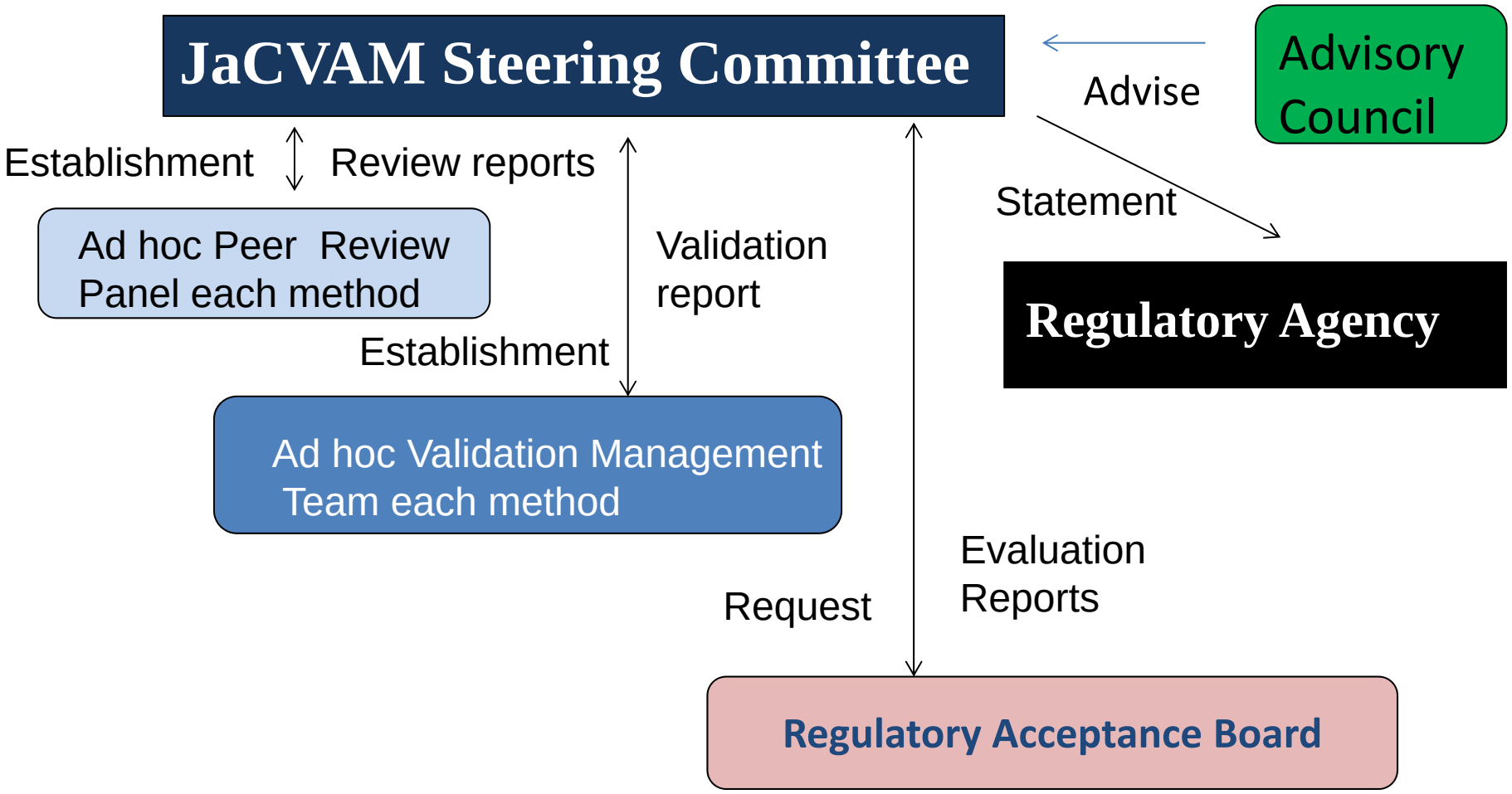
Comparison of LLNA methods in OECD Test Guideline *

	LLNA	LLNA：DA	LLNA:BrdU-ELISA
Species	CBA/Ca or CBA/J	CBA/J	CBA/JN
Number of application	3	4	3
Experiment periods	7	8	6
Period after final test substance application（day）	2	3（Between 3 and 4 application)	1
Determination of cellular proliferation	RI uptake	ATP contents	BrdU uptake
Application of endpoint volume and site	20μCi／250μL in tail vein	__	BrdU 5mg/0.5mL in i.p
Lymph node extraction after final test substance application	77	24-30	48
SI values	> 3	>1.8、1.8-2.5 : want to consider additional information	>1.6、1.6-2.9: want to consider additional information
rLLNA	Yes	No	No
Others		SLS1%solution pre-treatment for one hour	None

-：Not Described

＊：Different parts among test methods

Organization of JaCVAM



Accepted methods by the JaCVAM regulatory acceptance board

- The Bovine Corneal Opacity and Permeability (BCOP) Test Method for Identifying Ocular Corrosives and Severe Irritants
- The Isolated Chicken Eye (ICE) for Identifying Ocular Corrosives and Severe Irritants
- Skin sensitization assay, LLNA : DA
- Skin sensitization assay, LLNA : BrdU-ELISA
- *In vitro* skin irritation testing: EPISKIN
- *In vitro* skin corrosion testing: Vitrolife-Skin, EpiDerm
- *In vitro* cytotoxicity test methods for estimating starting doses for acute oral systemic toxicity tests
- rLLNA for skin sensitization assay
- Epiderm and SkinEthics for skin irritation assay

事　　務　　連　　絡
平成２３年２月４日

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

医薬部外品の承認申請資料作成等における動物実験代替法の利用と
JaCVAM の活用促進について

医薬部外品の承認申請資料の作成においては、下記に示す JaCVAM のホームページに掲載されている情報も参考の上、適切な資料を作成し、また化粧品のポジティブリスト改正要望等においても活用が図られるよう、貴管下関係業者に対し周知をお願いします。

記

JaCVAM ホームページ：http://jacvam.jp/

Administrative Notice for quasi-drug

RE: JaCVAM activities to promote the use of test results obtained from alternatives to animal testing in applications for approval of quasi drugs

We invite you to make reference to information available on the JaCVAM Website (http://jacvam.jp/) in ensuring proper preparation of applications for approval of manufacturing and sales of quasi drugs as well as to requests for revisions to positive lists for cosmetics. We also kindly request that you publicize this information to applicable businesses and other concerned parties operating under your jurisdiction.

The JaCVAM Website is available at http://jacvam.jp/.

事　務　連　絡
平成２４年４月２６日

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

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ダンスについて

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ダ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ダンスについては、順次、作成する予定です。

Administrative Notice for quasi-drug

Re: Guidance on the use of alternative methods to animal testing for skin-sensitization potency and phototoxicity in the safety assessment of quasi-drug and cosmetic products

- As part of its activities during 2011, a Regulatory Science Research program chaired by Dr. Hajime Kojima has prepared guidance on the use of alternative methods to animal testing for skin-sensitization potency and phototoxicity in the safety assessment of quasi-drug and cosmetic products and has made the guidelines available in order to promote the use of alternative methods. We kindly request that you publicize this information to concerned parties operating under your jurisdiction.
- The Evaluation and Licensing Division prepares and publishes guidance on the use of these and other alternative methods to animal testing as such information become available.

International collaboration

Acute Contact Dermatitis Test Methods 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)
Harminized performance standard for the LLNA	Completed		Updated OECD TG 429 (2010)

Acute Contact Dermatitis Test Methods 2

Method	Current status	Lead Organization	International acceptance
LLNA:BrdU-Flow Cytometry	ICCCAM international peer review, 2009, KoCVAM Validation study started	NICEATM-ICCVAM, KOCVAM	
In vitro skin sensitization assays (h-CLAT, MUSST)	Validation study ends in Fall, 2012	EURL ECVAM	
In vitro skin sensitization assay, DPRA	Peer review ongoing	EURL ECVAM	SPSF approved
In vitro skin sensitization assay, KeratinoSense	Peer review ongoing	EURL ECVAM	SPSF approved
In vitro skin sensitization assay, IL-8 Luc assay	METI-sponsored Validation study ongoing	JaCVAM	

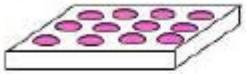
in vitro assay validation study update

JaCVAM correlated on-going International validation studies

1. h-CLAT assay for skin sensitization testing (with ECVAM)
2. IL-8 reporter gene assay for skin sensitization testing (with ICATM)
3. Stable transfected transcriptional activation (STTA) antagonist assay for endocrine disruptor screening (with OECD VMG-NA)
4. SIRC-CVS cytotoxicity assay for eye irritation tesitng (with ICATM)

Human Cell Line Activation Test (h-CLAT)*

Procedure



THP-1
1x10^6 cells /mL

Relative Fluorescence Intensity (RFI)

$$RFI = \frac{\text{MFI of chemical treated cells - MFI of chemical treated Isotype control cells}}{\text{MFI of vehicle control cells - MFI of vehicle Isotype control cells}} \times 100$$

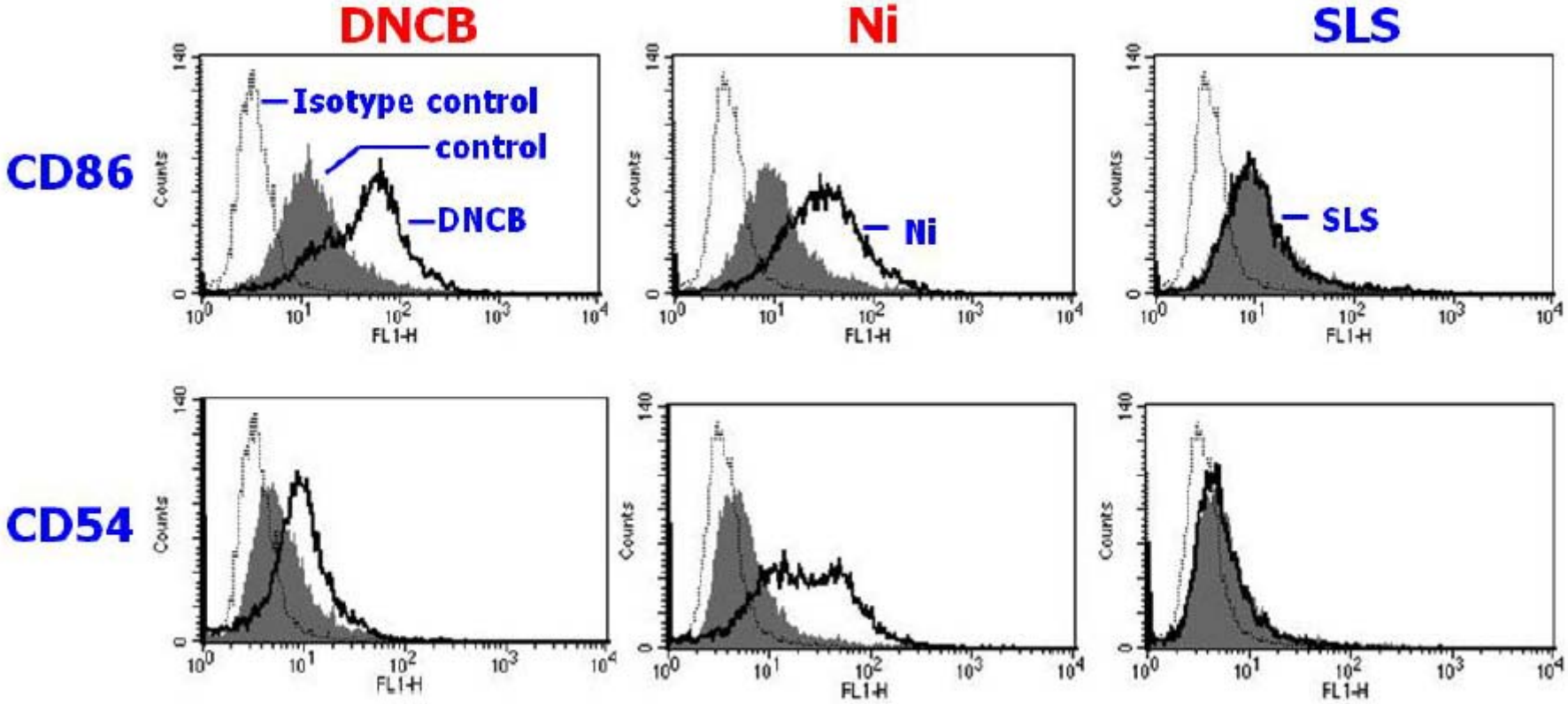
MFI = geometric mean fluorescence intensity

Prediction Model

- Viability ≥ 50% by Propidium Iodide
- Positive criteria: CD86 RFI ≥ 150% and/or CD54 RFI ≥ 200%
- Positive: 2 of 3 independent data at any dose should exceed the positive criteria

*: Ashikaga et al., 2006 Toxicol In Vitro 767-73., Sakaguchi et al., 2006 Toxicol In Vitro 774-84.

Histogram of CD86 / CD54 expression



DNCB and Ni (typical allergens) enhanced both CD86 and CD54 expressions but SLS (non-allergen) did not.

Miyazawa et al., Toxicology in Vitro 2007

Comparative evaluation with LLNA and human

h-CLAT vs LLNA

		h-CLAT	
		+(83)	-(34)
LLNA	+(85)	75	10
	-(32)	8	24

Sensitivity: 75/85 (88%)
Specificity: 24/32 (75%)
Positive predictivity: 75/83 (90%)
Negative predictivity: 24/34 (71%)
Accuracy: 99/117 (85%)

h-CLAT vs human

		h-CLAT	
		+(51)	-(20)
Human	+(55)	46	9
	-(16)	5	11

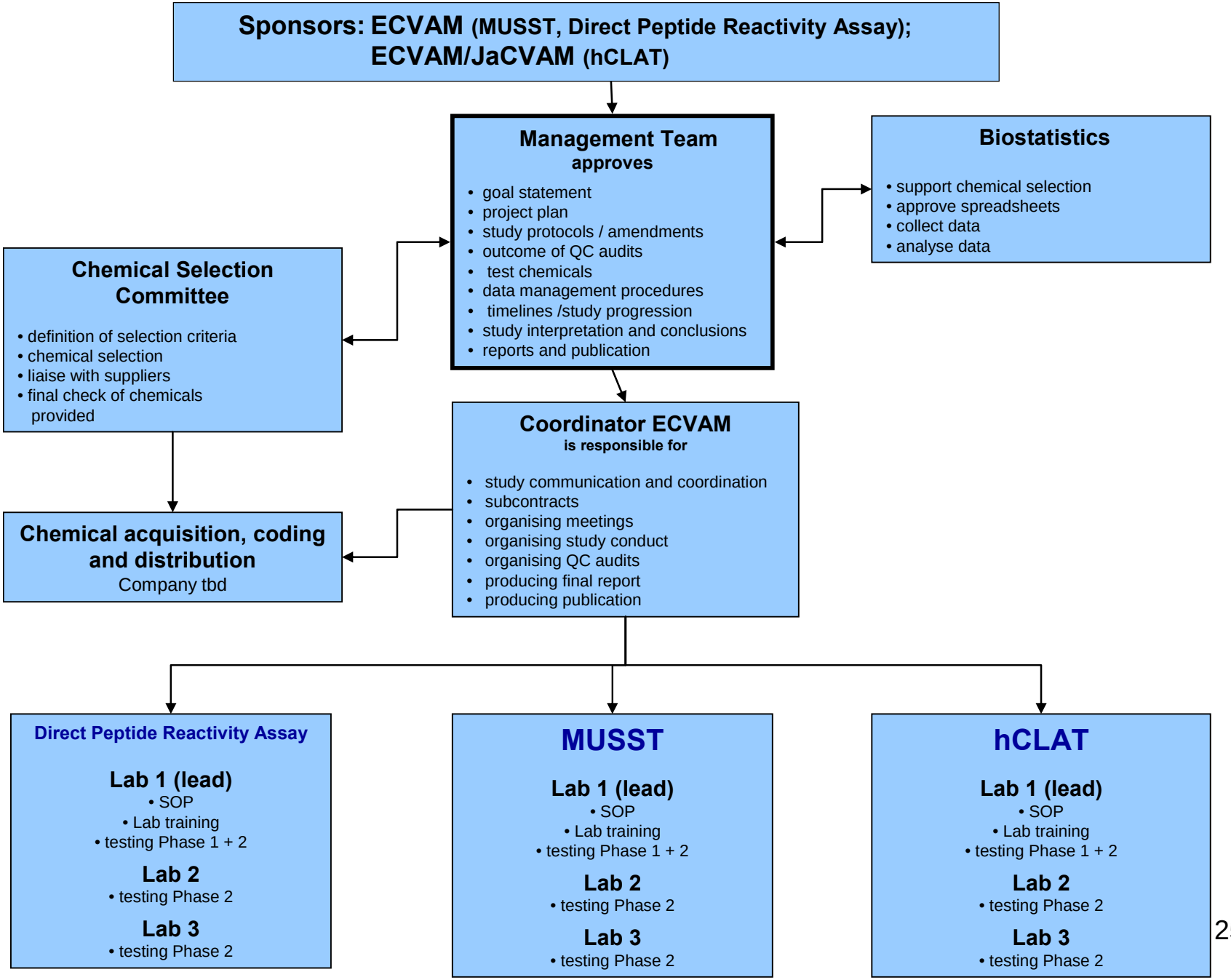
Sensitivity: 46/55 (84%)
Specificity: 11/16 (69%)
Positive predictivity: 44/51 (90%)
Negative predictivity: 11/20 (55%)
Accuracy: 57/71 (80%)

Good predictive capacity, but some false negative / positive

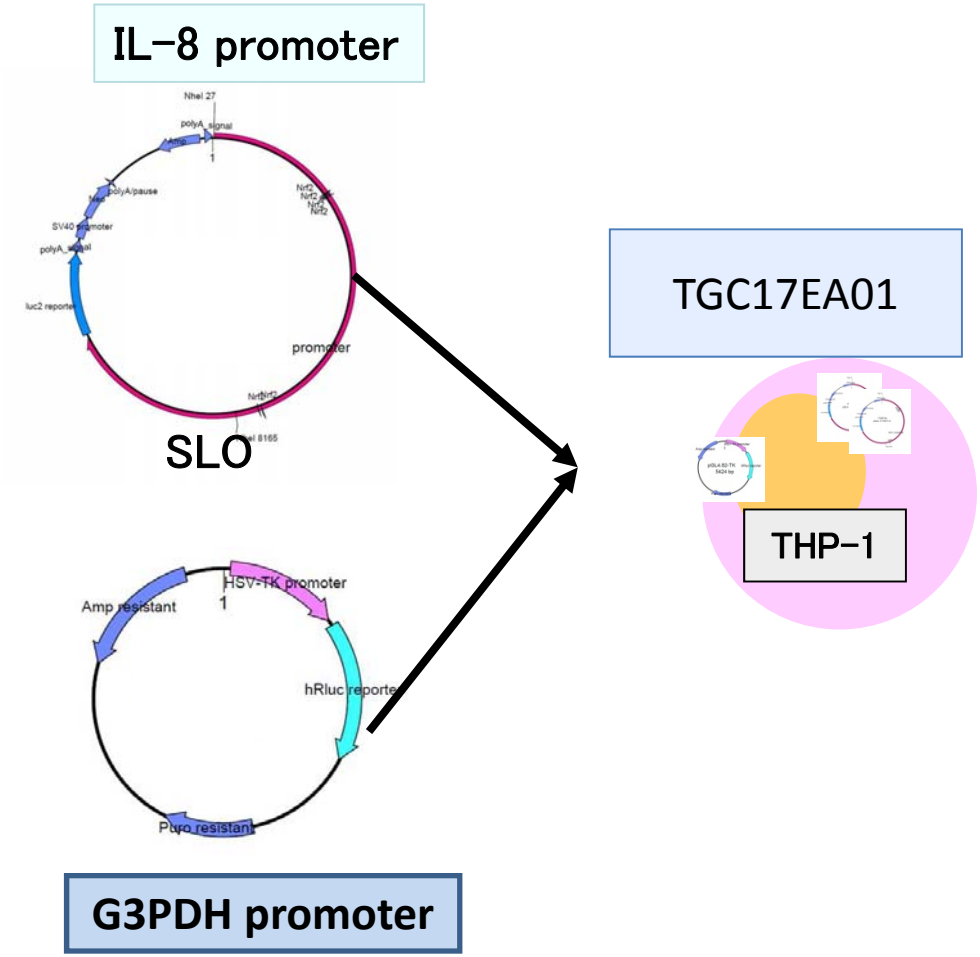
Nukada et al., WC7 2009, Ashikaga et al., ATLA 2010, Nukada et al., ESCD 2010

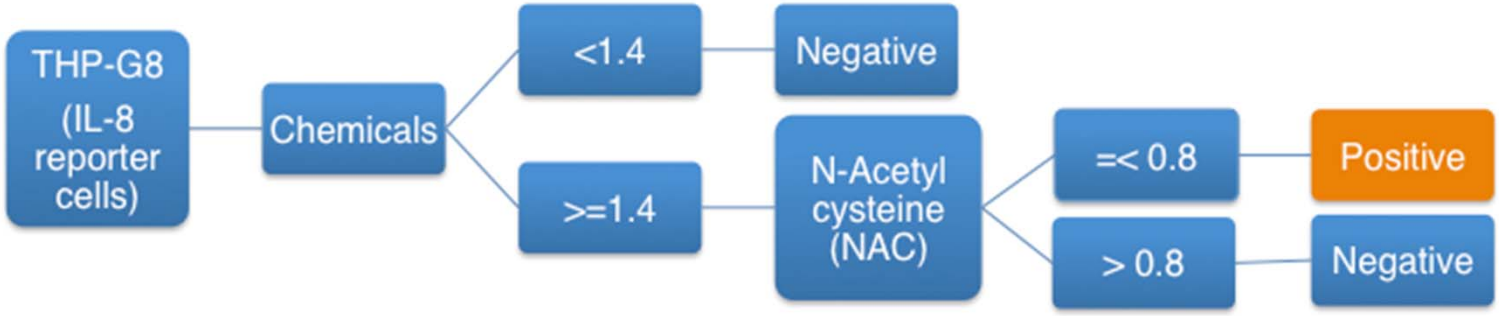
ECVAM prevalidation study

- **Liaison:**
  - JaCVAM and ICCVAM
- **Test methods:**
  - Direct Peptide Reactivity Assay (DPRA)
  - Myeloid U937 Skin Sensitization Test (MUSST)
  - **human Cell Line Activation Test (h-CLAT)**
- **Main purpose**
  - The assessment of the robustness and reliability
- **Experimental design**
- 24 coded chemicals in three (or four) laboratories each for the assessment of the within- and between-laboratory reproducibility


Sponsors: ECVAM (MUSST, Direct Peptide Reactivity Assay); ECVAM/JaCVAM (hCLAT)
Management Team
approves
• goal statement
• project plan
• study protocols / amendments
• outcome of QC audits
• test chemicals
• data management procedures
• timelines /study progression
• study interpretation and conclusions
• reports and publication
Biostatistics
• support chemical selection
• approve spreadsheets
• collect data
• analyse data
Chemical Selection Committee
• definition of selection criteria
• chemical selection
• liaise with suppliers
• final check of chemicals provided
Coordinator ECVAM
is responsible for
• study communication and coordination
• subcontracts
• organising meetings
• organising study conduct
• organising QC audits
• producing final report
• producing publication
Chemical acquisition, coding and distribution
Company tbd
Direct Peptide Reactivity Assay
Lab 1 (lead)
• SOP
• Lab training
• testing Phase 1 + 2
Lab 2
• testing Phase 2
Lab 3
• testing Phase 2
MUSST
Lab 1 (lead)
• SOP
• Lab training
• testing Phase 1 + 2
Lab 2
• testing Phase 2
Lab 3
• testing Phase 2
hCLAT
Lab 1 (lead)
• SOP
• Lab training
• testing Phase 1 + 2
Lab 2
• testing Phase 2
Lab 3
• testing Phase 2

IL-8 Luc assay





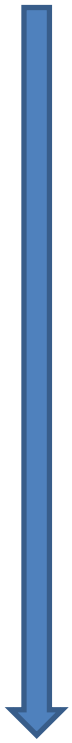
In at least two repeated experiments:

Criteria: chemicals that demonstrate FInSLO-LA ≥ 1.4 and I.I. ≤ 0.8 at the concentration of the chemical at which I.I.-SLR-LA was ≥ 0.2 are judged as positive. If chemicals make 2 or 3 positive results in 2 or 3 different experiments are determined as sensitizers.

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** **Joachim Kreysa**	Test system expertise, multi-study validation expertise, immunotoxicity expertise	**ECVAM, Italy**
ICCVAM liaison **William Stokes**	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 validation study under Modular approach



Module 2: Within-lab Reproducibility

Phase II 9 coded

Module 3: Transferability

Phase 0 (*finished*) non-coded

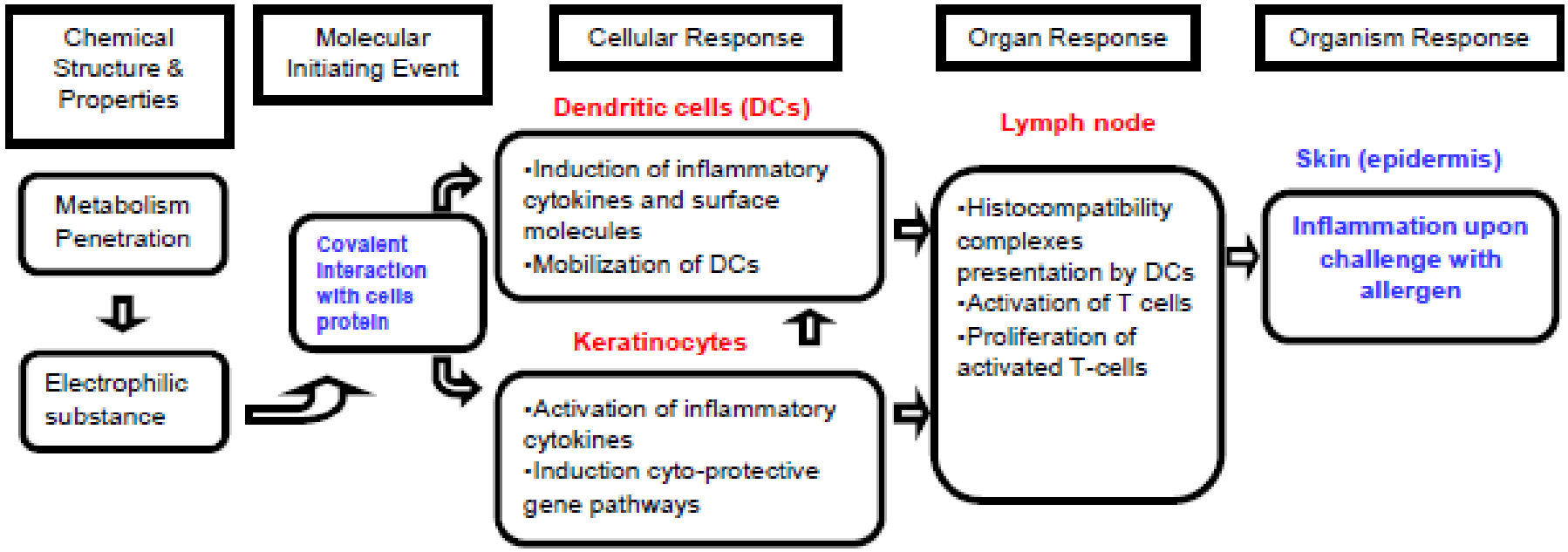
Module 4: Between-Lab Reproducibility

Phase II 9 coded

Module 5: Predictive capacity

Phase III 15 coded

Adverse Outcome Pathways on skin sensitization assay in near future



DPRA

DCs

H-CLAT,
MUSST,
IL-8 Luc assay

Keratinocytes

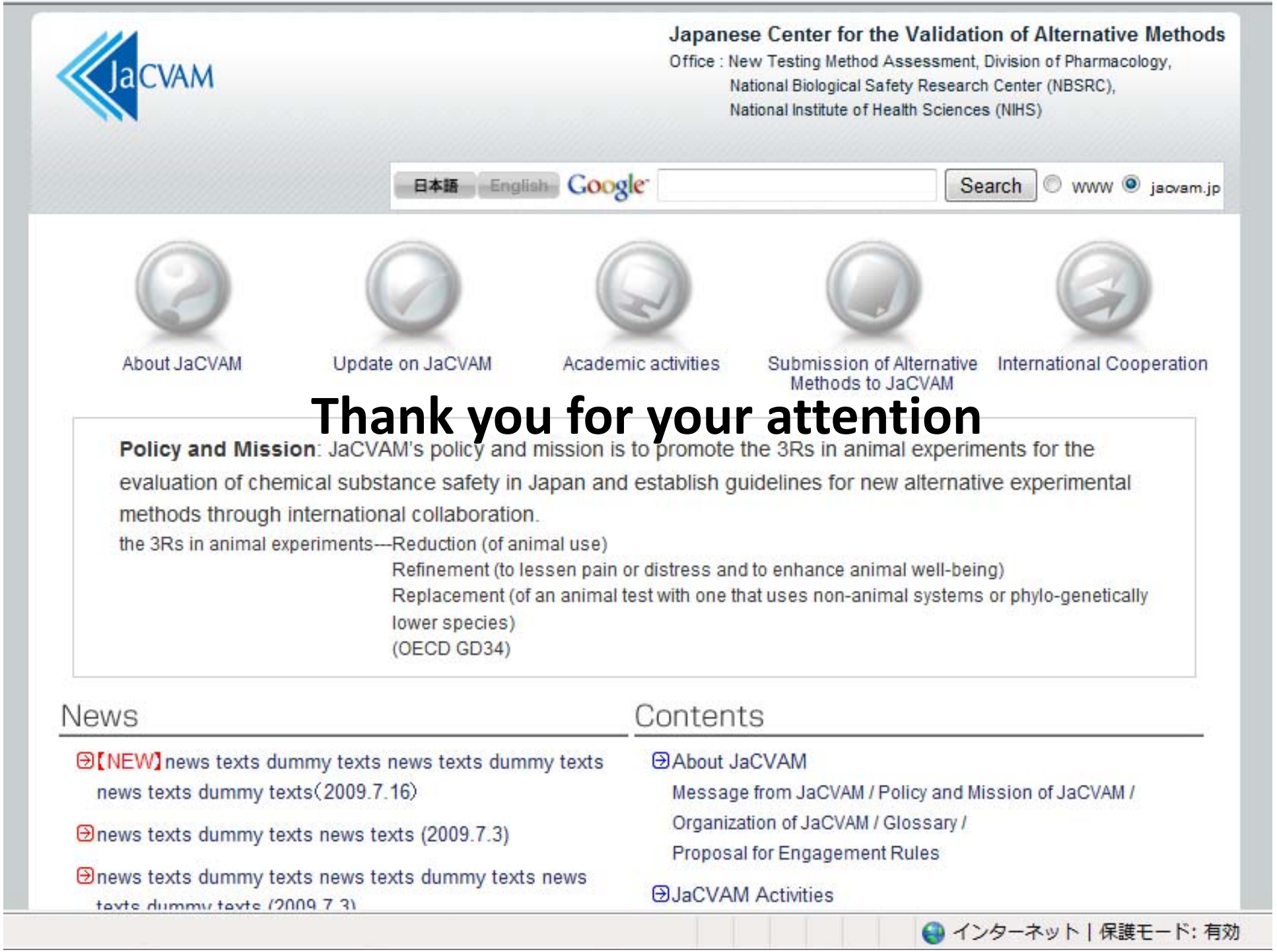
KeratinoSense

Local Lymph Node assay (LLNA)

Maximization or Buehler test using guinea pig

Alternative methods on skin sensitization assay for regulatory use

- Under the ICATM framework, JaCVAM expects to experience more efficient test validation and review, as well as more rapid international acceptance of scientifically valid methods.
- To ensure that new or revised tests are validated through comparison with domestically developed or internationally certified standard tests, peer reviewed, and officially accepted by the regulatory agencies.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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)
日本語 English Google Search www jacvam.jp
About JaCVAM
Update on JaCVAM
Academic activities
Submission of Alternative Methods to JaCVAM
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)
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