

*National Toxicology Program
Interagency Center for the Evaluation of
Alternative Toxicological Methods*

*Interagency Coordinating Committee on
the Validation of Alternative Methods*

New Models in the Validation Pipeline for Allergic Contact Dermatitis Testing: DPRA, h-CLAT, and MUSST



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ICCVAM Workshop Series on Best Practices for Regulatory
Safety Testing: Assessing the Potential for Chemically Induced
Allergic Contact Dermatitis

January 20, 2011

William H. Natcher Conference Center
National Institutes of Health
Bethesda, MD



ICCVAM

NICEATM



ECVAM Phase III Pre-validation Study

- Three methods:
 - Direct Peptide Reactivity Assay (DPRA, Procter & Gamble)
 - Uses HPLC to monitor a chemical's potential to deplete a nucleophile-containing synthetic peptide
 - Myeloid U937 Skin Sensitization Test (MUSST, L'Oréal)
 - Uses flow cytometry to monitor induction of a protein marker on the surface of a human monocytic cell line following exposure to chemical
 - Human Cell Line Activation Test (h-CLAT, Kao and Shiseido)
 - Uses flow cytometry to monitor induction of two protein markers on the surface of a human monocytic leukemia cell line following exposure to chemical

ECVAM Study – Validation Management Team (VMT) Composition

- Validation Management Group
 - David Basketter – Chair
 - Silvia Casati – Co-chair
 - Alexandre Angers – ECVAM representative
 - Thomas Cole – Chair of Chemical Selection Group
 - André Kleensang – ECVAM biostatistician
 - Anna Compagnoni – alternate ECVAM biostatistician
 - Pierre Aeby – Industry representative
 - Sebastian Hoffmann – External expert
 - Jon Richmond – External expert
- Lead laboratory Representatives
 - G. Frank Gerberick – Procter & Gamble
 - Jean Marc Ovigne – L'Oréal
 - Takao Ashikaga – Shiseido
 - Hitoshi Sakaguchi – Kao Corporation
- Liaisons
 - JaCVAM (Hajime Kojima; alternate Yasuo Ohno)
 - NICEATM (William S. Stokes; alternate Eleni Salicru)
 - ICCVAM (Joanna M. Matheson; alternate Abigail Jacobs)

ECVAM Study – Objective and Goals

- Objective:
 - Evaluate the DPRA, MUSST, and h-CLAT in view of their future incorporation into a testing strategy for fully replacing current regulatory animal tests
- Primary Goal:
 - Assess the transferability and reliability (within- and between-laboratory reproducibility) of each of the three test methods when challenged with a set of coded chemicals
- Secondary Goals:
 - Preliminary assessment of the test methods' ability to discriminate between skin sensitizing and nonsensitizing chemicals
 - Preliminary assessment of the test methods' ability to categorize skin sensitizing chemicals into the GHS sub-categories 1A (strong sensitizer) and 1B (other than strong sensitizer)

ECVAM Study – Experimental Design

- Two sequential phases:
 - Phase A: Training of participating laboratories and test method transferability
 - Stage I: Lead laboratories issue SOP and training study plan for training personnel from other testing sites
 - Stage II: Trained personnel will transfer test method to their own laboratories
 - Phase B: Formal evaluation of test method reproducibility
 - 24 coded chemicals (15 tested three times at each site)
 - Stage I: Test methods evaluated with preliminary set of 9 coded chemicals tested once (1 experiment)
 - Stage II: Test methods evaluated with additional set of 15 coded chemicals tested 3 times (3 independent experiments)
 - After each phase, reports submitted to VMT for review

Overview of DPRA

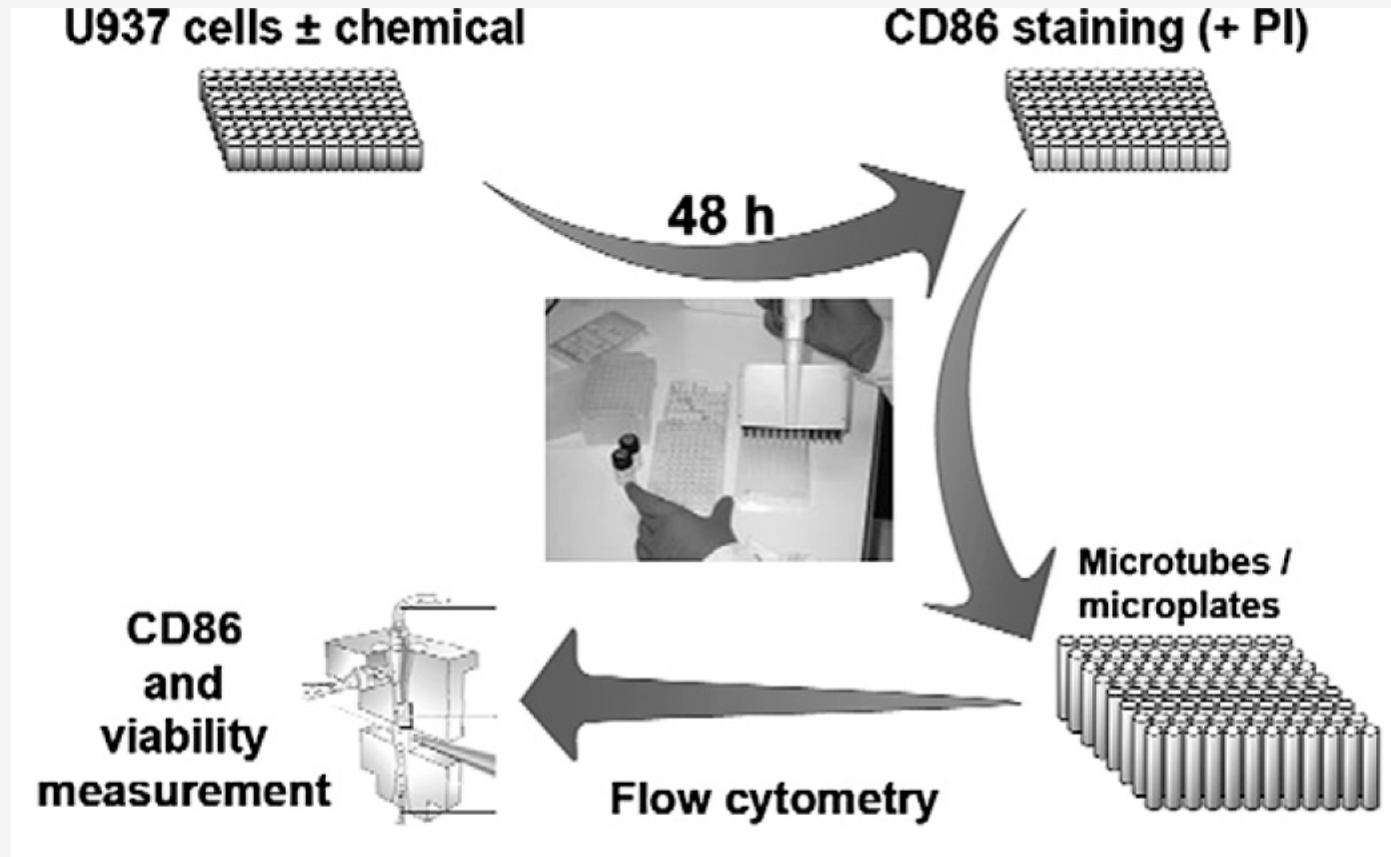
- Protein binding is a key step in induction of skin sensitization
- Cysteine and lysine containing peptides are mixed with test chemical at 1:10 or 1:50 ratio respectively
 - Reactivity is expressed as % peptide depletion measured by HPLC-UV after 24 hours incubation with test chemical
- Data analyzed using classification tree (recursive partitioning) methodology to rank reactivity as minimal, low, moderate or high
 - Minimal reactivity = nonsensitizers
 - Low, moderate or high reactivity = sensitizers
- Prediction model developed with 81 chemicals
 - Accuracy = 89%
 - Sensitivity = 88%
 - Specificity = 90%
- 157 chemicals tested to date
 - Accuracy = 85%

Overview of MUSST

- Dendritic cell activation is a key event in development of skin sensitization
- Based on flow cytometry assessment of CD86 (a co-stimulatory protein) upregulation in human monocytic U937 cells¹ after exposure to sensitizing chemicals
 - U937 are dendritic-like cells and serve as a surrogate for evaluation of CD86 dendritic cell expression
- A chemical is considered sensitizing if it induces a dose-dependant increase of CD86 expression at non-toxic doses
- The stimulation index calculated represents a ratio of the % of CD86+ cells in treated vs. control cells
 - The threshold for positive is set at SI = 150
- Data presented at the 6th World Congress showed comparable results from three independent laboratories with three sensitizers (MCI/MI, hydroquinone and ethylene diamine) and two irritants (SDS and lactic acid) indicating a transferable protocol

Sundstrom and Nilsson. 1976. Int J Cancer 17:565-577

Schematic View of the MUSST Protocol



Aeby P et al. 2010. Toxicol In Vitro 24(6):1465-1473.

Overview of h-CLAT

- Dendritic cell activation is a key event in development of skin sensitization
- Based on flow cytometry assessment of CD86 (co-stimulatory protein) and CD54 (adhesion protein) upregulation in human monocytic leukemia THP-1 cells after exposure to sensitizing chemicals
 - THP-1 are dendritic-like cells and serve as a surrogate for evaluation of CD86 and CD54 dendritic cell expression
- Experiments are conducted on three different days
 - THP-1 cells are cultured with test chemicals for 24 hr using 8 doses based on the dose that afforded 75% cell viability (CV75)
- A chemical is considered a potential skin sensitizer if ≥ 2 runs at any dose exceed the positive criteria
 - CD86 RFI $\geq 150\%$ and/or CD54 RFI $\geq 200\%$

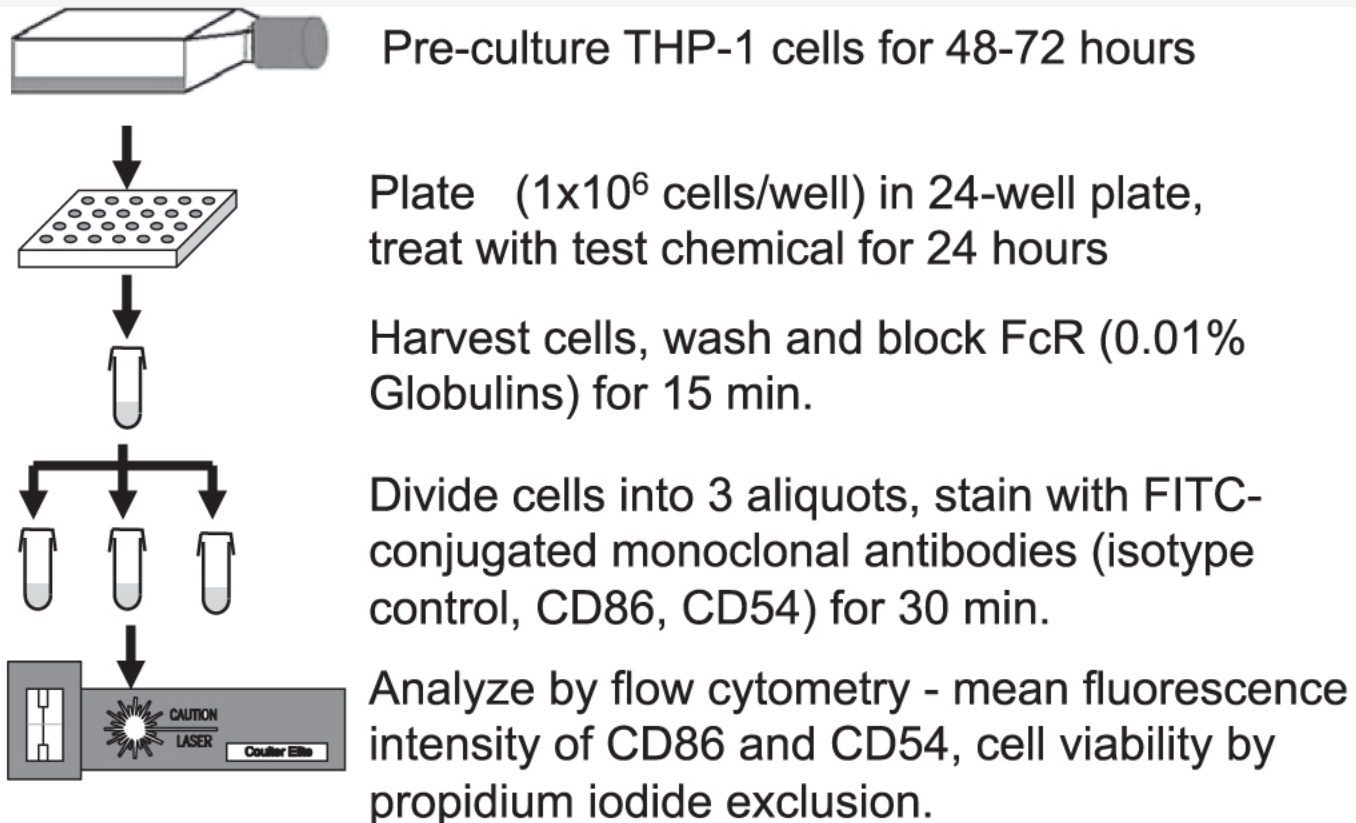
$$\text{RFI} = \frac{\text{MFI of chemical treated cells} - \text{MFI of chemical treated isotype control cells}}{\text{MFI of vehicle control cells} - \text{MFI of vehicle isotype control cells}} \times 100$$

Abbreviations :

RFI = relative fluorescence intensity

MFI = geometric mean fluorescence intensity

Schematic view of the h-CLAT protocol



From Aeby P et al. 2010. Toxicol In Vitro 24(6):1465-1473.

- More on h-CLAT from Dr. Hitoshi Sakaguchi, Ph.D., Kao Corporation

Other Nonanimal ACD Test Methods

Undergoing Evaluation/Prevalidation

■ KeratinoSens assay

- Undergoing ring trials in preparation for ECVAM review
- Test method submission and ECVAM assessment ongoing as of October 2010
- Based on the Nrf2-Keap1-ARE regulatory pathway
 - Innate cellular response
 - Induced by most sensitizers¹
- Uses a novel cell line based on the human HaCaT keratinocyte cell line² containing a reporter construct with a single copy of the ARE-element of the human AKR1C2 gene
- Have tested:
 - List of reference chemicals published by ECVAM
 - List of harmonized ICCVAM performance standards chemicals
 - 67 additional chemicals derived from the ICCVAM database
- Amenable to high-throughput format

¹Natsch et al. 2010. Toxicol Sci 113:284-292.

²Boukamp et al. 1988. J Cell Biol 106:761-771.

Summary

- DPRA, h-CLAT, and MUSST undergoing prevalidation at ECVAM in view of their future incorporation into a testing strategy for fully replacing current regulatory animal tests
- KeratinoSens assay to undergo evaluation by ECVAM
- Numerous assays from research and development designed to evaluate mechanisms of skin sensitization induction
 - SenCeeTox (epidermal Inflammation)
 - VITOSENS® (dendritic cell response)
 - Keratinocyte cultures
 - Keratinocyte and dendritic cell co-culture systems
 - T-cell proliferation assays

This presentation reflects the views of the author, has not been reviewed or approved by, and may not necessarily reflect the views of the U.S. Consumer Product Safety Commission