Appendix 1
Study plan

Version 1.3 September, 2019

Study plan for the validation trial on the EpiSensA assay as a test evaluating the skin sensitization of chemicals

EpiSensA assay Validation Management Team

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1. Background

The EpiSensA assay for assessing skin sensitisation potential was developed by Kao Corporation as an alternative to in vivo testing.

The aim of this trial is to validate the EpiSensA assay method to assess transferability and inter-laboratory variability, in order to incorporate this test for screening the skin sensitisation chemicals. The EpiSensA assay for the validation trial will be undertaken i) in accordance with the principles and criteria documented in the OECD No. 34 Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment [OECD, 2005], ii) according to the Modular Approach to validation [Hartung et al., 2004] ,iii) according to the concept discussed on the validation trials with participation of GLP Test Facilities [OECD, 2000] where the whole concept of the validation trials is described in the context of GLP.

The studies part of a validation trial should ideally be performed in accordance with GLP [OECD, 1998-2007; FDA, 1999; EPA, 1998a&b; JSQA, 2010; SCC, 2010]. As a minimum, but not necessary limited, use of standard operating procedures (SOP), adequate data recording, reporting and record keeping are essential.

A general conceptional framework [Hartung et al., 2004; OECD, 2005] will be used for documenting all the study to assess the validation status of a test method, called "modular approach" to validation. In this approach, the information needed to support the validity of the method is organized into modules that provide the following information:

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

The Modular approach as introduced by Hartung et al., allows using datasets from various data sources and studies. This advantage is used in the following proposal to assess the scientific validity of the EpiSensA assay. This assay for the validation trial has performed under the GLP principle.

2. Objective of the trial

The validation trial will assess the reliability (reproducibility within and between laboratories) and relevance (predictive capacity) of the EpiSensA assay with a challenging set of test substances (test items) for which high quality *in vitro and in vivo*

data are available.

3. Validation Management Team (VMT)

The VMT encompasses collective expertise with the test, in the underlying science and the scientific design, management and evaluation of a validation trial.

The VMT, which plays a central role overseeing the conduct of the validation trial, includes:

Table 1. Members for the EpiSensA assay Validation Management Team

Name	Role and expertise	Affiliation
Trial Coordinator Hajime Kojima	VMT trial coordinator, Management of quality control, Chemical supplier	JaCVAM, NIHS, Japan (JaCVAM representative)
Lead Lab *Masaki Miyazawa *Hideyuki Mizumachi	*Developer of this assay Test method, expertise underlying science	Kao Cooperation, Japan
Takashi Sozu	Data analysis, biostatistics dossier	Tokyo Univ. of Science, Japan
International expert members		
EU liaison David Basketter	Test system expertise, validation expertise	DABMEB Consultancy Ltd, UK
EU liaison Chantra Eskes	Test system expertise, validation expertise	Services & Consultation on Alternative Methods (SeCAM), Switzerland
EU liaison Sebastian Hoffmann	Test system expertise, validation expertise	seh consulting + services, Germany
ICCVAM liaison David Lehman	Test system expertise	U.S. Environmental Protection Agency
Korean liaison Tae Sung Kim	Test system expertise	KoCVAM, Korea
Japanese liason Masahiro Takeyoshi	Test system expertise	CERI, Japan
Japanese liason Takao Ashikaga	Test system expertise	JaCVAM, NIHS, Japan

3.1 Participating Test Facilities

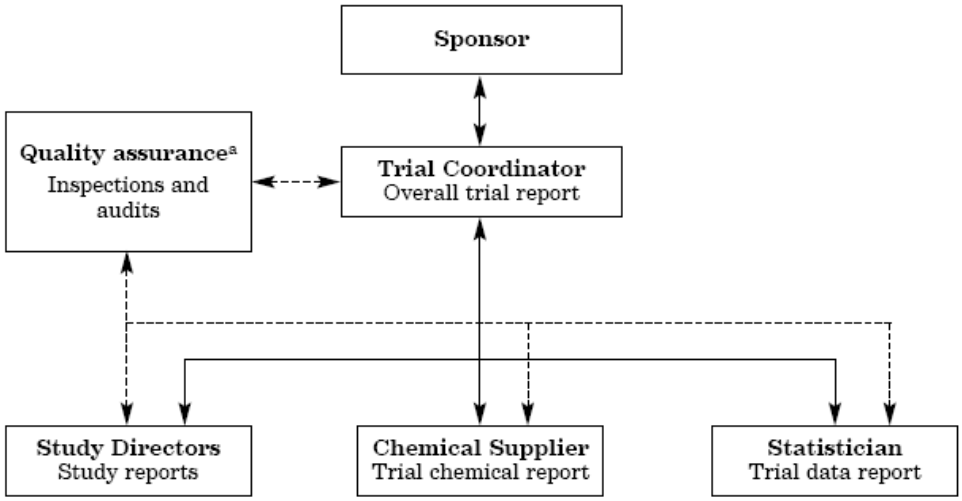
The laboratories participating in the trial are defined as follow:

Test facility 1: Hatano Res. Inst., FDSC. Study Director (SD) : Mika Wartanabe
Test facility 2: KOSE Cooperation SD : Noriyasu Imai
Test facility 3: LION Cooperation SD : Shinichi Watanabe

Information relevant for Modules 2, 3, 4 performed by all laboratories. Data obtained by these laboratories have demonstrated that the EpiSensA assay is transferable and reproducible between experienced laboratories. The all facility will be the laboratory participating in this validation trial acting as unexperienced laboratory to assess between laboratory transferability, reliability and relevance of the EpiSensA assay method under non-GLP conditions (GLP principle).

3.2 Trial management structure

The management structure of the validation trial is shown in **Figure 1**



[a]Several Quality Assurance units might be involved in a multi-study trial.
Dashed lines indicate assurance staff involvement.

Figure 1: Management Structure of the EpiSensA assay validation trial

1) Chemical management group

The members of chemical management group are elected by recommendation of the EpiSensA assay VMT. They prepare a tentative list of test chemicals and works with the VMT to make a final decision on the test chemicals to be used in the validation trial. The coded test chemicals listed are distributed by JaCVAM.

2) Data analysis group

The members of data analysis group are elected by recommendation of the EpiSensA assay VMT, and check and analyze the data obtained in this validation trial from a third-party standpoint. They also take charge of statistical processing in this validation trial.

3) Quality assurance group

The members of record management group are elected by recommendation of the EpiSensA assay VMT. They prepare protocol, test chemical preparation record forms, blank data sheets, etc. and distributes them to the research laboratories participating in this validation trial. They also collect filled out forms and data sheets after completion of experiments, pointing out omissions or flaws in recording, if any, and requesting correction of such errors.

4) Lead laboratory

The lead laboratory representing the test method is responsible for providing the test method protocol and the eventually necessary data recording or calculation templates. The Trial Coordinator has to ensure that such data recording or calculation templates have been validated before distribution to the test facilities involved in the validation trial. The lead laboratory is also responsible for providing, if necessary, new versions of the protocols during the entire validation trial. The lead lab and the other participating test facilities might be contacted by the VMT for technical issues.

3.3 Sponsor

This validation study is supported by research grants provided by the Japan Chemical Industry Association (JCIA) and the Japanese Society for Alternative to Animal Experiments (JSAAE).

Japanese Tissue Engineering Co., Ltd also provides LabCyte EPI-MODEL24 to test facilities in accordance with the discount system.

The lead laboratory will support the EpiSensA assay validation trial by assuring that reliability is assessed. At the same time, preliminary results of the test method can be evaluated. For this purpose, Lead laboratory will support:

- the financial aspects related to the coordination of a validation trial (e.g. organization of VMT meetings where also the involved test facilities can be invited for technical clarifications to the VMT, the publication of the validation trial results)

- the test, reference and control item purchase, coding and distribution to the test facility
- the availability of the test systems to the participating laboratories by supporting the Lead laboratory with the logistics for delivering the test system to the facility
- the independent data analysis and statistical support (biostatistician) based on the study reports generated
- the other costs for participating laboratories

3.4 Trial coordination

Dr. Hajime Kojima was appointed as the Trial Coordinator with well-defined roles and responsibilities to coordinate the trial and to establishment of a VMT by supporting of JaCVAM.

The name and location of the Trial Coordinator should be identified in each individual study plan. For the EpiSensA assay validation trial, the Trial Coordinator has direct access to the test item coding.

The Trial Coordinator's responsibilities include:

a) Establishment of/support to lead laboratory, including meeting organization
b) Trial communication and coordination with test facilities
c) Recording of document and data flow between test facilities
d) Assessing and documenting the impact of any amendments and/or deviations from the trial plan and study plans on the quality and integrity of the validation trial
e) Ensuring that the individual study reports are forwarded, in a timely manner, for data and statistical analysis
f) Preparing the trial plan and report, which can be based on the study reports from the lead laboratories and other test facilities involved in the validation trial, and should reflect the overall trial
g) Approval with date and signature of all protocols, Study Plans and Study Reports
h) The communication of the results of the trial into the public domain

The role of Trial Coordinator (as the formal representative of the VMT and the single contact point with the SDs) is of fundamental importance. The Trial Coordinator is the single critical point of trial control and must ensure clear lines of communication between the involved test facilities in the trial. The communication line of the Trial Coordinator is with the SDs of the different test facilities. The SDs are the single point of contact with the Trial coordinator (unless otherwise communicated by the participating Test

Facilities) to assure a transparent and recorded documentation flow during the trial. The Trial Coordinator should also ensure that appropriate arrangements have been made for the supply of the test systems, and test, control and reference items, which meet the requirements of the trial, and that there are appropriate test method protocols (dated signature by the trial coordinator and the Lead Laboratories) and, if appropriate, validated data recording, data analysis, data reporting sheets for the test method.

It is the responsibility of the Trial Coordinator to approve the study plans send for approval by the test facilities, and any amendments to the study plan, by dated signature.

3.5 Training

The lead laboratory will be responsible for issuing a training agenda to the Trial Coordinator for further distribution to the all test facility giving details what training aspects will be covered during the training of the other SDs and Study Personnel at the lead laboratory. Furthermore, after the training, the lead laboratory will issue to the Trial Coordinator a training report and indicating if critical observations are made by the other test facilities regarding the EpiSensA assay protocols. In case any critical observations are made a new version of the EpiSensA assay protocols might necessary be issued to the other test facilities before initiating the between-laboratory transferability.

3.6 [Module 2] Within-laboratory reproducibility

Fifteen coded test items have been selected to confirm the between-laboratory reproducibility in the phase I study. The within-laboratory reproducibility of the all test facility has been done by an independent biostatistical analysis, under the VMT. The proportion of concordance should be equal or more than 80% as tentative acceptance criteria for phase I validation.

3.7 [Module 3] Between-laboratory transferability

This between-laboratory transferability (Module 3, identical to ICCVAM proficiency testing phase) is performed in order to assess the successful transfer of the assay to a test facility unexperienced with that particular test method but having knowledge of similar test systems and endpoint detection methods.

For the transfer of EpiSensA assay to the all test facility, the Phase 0 study using non-coded five chemicals was performed. A few concentrations of each test item will be tested in triplicate in 3 independent runs according to the EpiSensA assay protocol describing

the details of the experimental design.

The five test items selected for the phase I study are coded as follows: A, B, C, D, and E. The all facility will prepare a study according to internal GLP principle. This plan will be submitted to the Trial Coordinator and lead laboratory for approval.

The results of the between-laboratory transferability will be reviewed before progressing with module 4 on the between laboratory reproducibility. If the transferability data do not meet test acceptance criteria, the Trial Coordinator representing the VMT will try to identify the problems and make corrections where needed. At the end of the testing, the test facilities will submit a QC certified copy of whole study dossier to the Trial Coordinator (study plan in GLP principle, raw data, records and data analysis, study report in GLP principle).

3.8 [Module 4] Between-laboratory reproducibility

Thirty coded test items have been selected to confirm the between-laboratory reproducibility in the phase I and II study. A few concentrations of each test item will be tested in triplicate according to the EpiSensA assay protocol describing the details of the experimental design.

At the end of the testing, the test facilities will submit a QC certified copy of whole study dossier to the trial coordinator (study plan in GLP principle, raw data, records and data analysis, study report in GLP principle). The proportion of concordance between-laboratory reproducibility should be equal or more than 80% as acceptance criteria,

3.9 [Module 5] Predictive capacity

The necessity for further chemical analysis will be subject to a VMT decision once the data of the between laboratory reproducibility has been assessed. Depending on the statistical analysis the lean design for validation as well as the automatisation of the test leading to an increased dataset will be considered.

4. Protocol

In this validation trial, the protocol (ver. 1E) will be used (attached Document #2). This protocol will make up a draft by the lead laboratory and be finalized by VMT.

5. Chemicals

5.1 Chemicals Selection

Test chemicals have been selected by chemical repository based on published papers on in vivo immunotoxicity

The applied selection criteria were:

- information on mode/site of action
- coverage of range of relevant chemical classes and product classes quality and quantity of reference data (*in vivo* and *in vitro*)
- high quality data derived from animals and (if available) also humans
- knowledge on interspecies variations (for example: variability with regard to the uptake of chemicals, metabolism, etc.)
- coverage of range of toxic effects/potencies
- chemicals that do not need metabolic activation
- appropriate negative and positive controls
- physical and chemical properties (feasibility of use in the experimental set-up as defined by the CAS No.)
- single chemical entities or formulations of known high purity
- availability
- costs

In the first phase of the selection procedure, the Chemical Selection Committee identified and collected several existing lists of potential chemical sensitizing in order to establish a primary database. These chemicals had originally been compiled by international experts for various purposes e.g. as reference compounds for validation studies. An extensive literature research was performed by the Chemical Selection Committee in order to insure that the preselected chemical fulfilled the selection criteria described above.

Emphasis was laid on the fact that different potencies (strong, weak and no activity) have been chosen. In addition, it was decided that at least 20% of the total substances to be tested should be negative in order to increase the statistical power of the data analysis.

In the first phase EpiSensA assay validation trial with data generation at the test facilities, five chemicals will be tested three times in each test chemical for between-laboratory reproducibility and to confirm transferability. After discussion of Phase I results, detailed test planning of the Phase II will be determined. At this moment, twenty chemicals will be planned in the phase II trial for predictive capacity (Table 2).

Table 2. Outline of test planning at each study in the validation trial.

Study	Chemicals	Test Number	Information obtained
Phase 0	3 non- coded	1	Between-lab transferability
Phase I	15 coded	3	Within & between-lab reproducibility To clarify the protocol carefully, phase I is separated 3 steps such as A, B and C.
Phase II	15 coded	1	Between-lab reproducibility & predictability

(Planning of Phase II will be determined after discussion of the results of Phase I)

5.2 Chemicals Acquisition, Coding and Distribution

The assessment of within-laboratory reproducibility (Module 2), between laboratory transferability (Module 3) in the all test facilities have been performed with coded chemicals. This EpiSensA validation trial plan describes the generation of the missing data sets under coded test item. If the results obtained are not very similar to the previous obtained sets, the VMT has to assess if coded chemicals need to be tested in the all test facilities.

The coding will be supervised by the Trial Coordinator, in collaboration with the chemical repository responsible of coding and distribution of test, reference and control items for the validation trial.

5.3 Handling

Each test facility shall receive through the Trial Coordinator essential information about the test chemicals (physical state, weight or volume of sample, specific density for liquid test chemicals, and storage instructions). Moreover, the SD should receive the safety information concerning the hazards identification and exposure controls/personal protection.

6. Records and archiving

At the end of the trial, the EpiSensA assay validation trial report is prepared by the Trial Coordinator or the VMT personnel who appointed by the Trial Coordinator. The trial report summarizes the trial goals, procedures, results and conclusions of the

validation trial. This represents the whole validation trial, including archiving and, as such, will cover several study reports, as well as reports for test item supply, data management and statistics. The Trial Coordinator oversees the preparation of the trial report. The Trial Coordinator will be representing the VMT discussions responsible for preparation of the scientific conclusions. Signatories to the trial report include the Trial Coordinator, the statistician, and the SDs of the involved test facilities. Although the SDs may not be involved with the preparation of the trial report, their signatures confirm that the trial report is an accurate reflection of the management and study events. The trial report should contain a statement, signed by the Trial Coordinator, commenting on the accuracy and completeness of the trial report and identifying any significant issues which could have affected the integrity of the trial, including matters of GLP compliance. A QC statement will be included in the trial report, in order to identify what QC monitoring was done and to confirm whether or not the trial report is an accurate reflection of the validation trial data.

6. Study timeline

An approximate schedule for EpiSensA assay validation trial is shown in Table 3. Duration of this validation trial is around 3 years from May 2018 to March 2021.

Table 3. Schedule of EpiSensA assay validation trial

Month	Activity
May 2018	Technical transfer using three known chemicals (non-coded) Start of technical transfer **to know between laboratory transferability**
	Data collection of technical transfer (**Phase 0 study**)
July 2018	Establish the VMT, **1st F to F VMT Meeting**
	Deliberation, decision and read-through of draft study plan
	Deliberation and decision of protocol
	Preparation of a tentative list of test chemicals
	Distribution of test chemicals, standard chemicals and positive control chemicals
Phase I study **to know between- and within-laboratory reproducibility**	

August 2018	Coding and distribution of ten coded test chemicals
September 2018	Start of Phase IA study
July 2019	End of Phase IA study
September 2019	Conference call for VMT
October 2019	Start of Phase IB study
February 2020	End of Phase IB study
March 2020	**2nd F to F VMT Meeting** / Phase IA & 1B results and planning of Phase 1C & II study
April 2020	Coding and distribution of coded test chemicals
May 2020	Start of Phase IC study
October 2020	End of Phase IC study
November 2020	Conference call for VMT
Phase II study to know between-laboratory reproducibility	
December 2020	Coding and distribution of coded test chemicals and positive chemicals
January 2021	Start of Phase II study using 20 coded test chemicals
June 2021	End of Phase II study
July 2021	**3rd F to F VMT Meeting** /reviewing of Phase II study results
March 2022	Completed validation report

Abbreviations

CAS: Chemical Abstracts Service
GLP: Good Laboratory Practice
HRI: Hatano Research Institute
FDSC: Food and Drug Safety Center
JaCVAM: Japanese Centre for the Validation of Alternative Methods
NIHS: National Institute of Health Sciences
OECD: Organization for Economic Co-operation and Development
QC: Quality Control
TG: Test Guideline
VMT: Validation Management Team

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