

Version 1.0 September, 2018

Study plan for the validation trial on multicolor reporter assay using THP-G1b (TGCHAC-A4) (IL-1 Luc assay) as a test evaluating the immunotoxic potential of chemicals

Conducted by:

IL-1 Luc assay Validation Management Team

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1. Background

The use of multicolor reporter assay using THP-G1b (TGCHAC-A4), IL-1 Luc assay is an important for evaluating the immunotoxic potential of chemicals as a part of Multi-ImmunoTox assay (MITA), because of its technical simplicity, short-term test period and accuracy of test result based on a mechanism of immunotoxicity.

The aim of this trial is to (pre)validate the IL-1 Luc assay method to assess transferability and inter-laboratory variability, in order to incorporate this test for screening the immunotoxic chemicals. The IL-1 Luc 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 [Cooper-Hannan et al., 1999] where the whole concept of the validation trials is described in the context of GLP, iv) and in line with the ISO procedure JRC.I.03.GP.01v.01 (<http://ihcpnet.jrc.it/quality-safety/quality-documents/unit-03-ivm/doc/JRC.I.03.GP.01v.01.pdf>).

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 IL-1 Luc assay. The IL-1 Luc 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 IL-1 Luc 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 IL-1 Luc assay Validation Management Team

Name	Role and expertise	Affiliation
<u>Trial Coordinator</u> Hajime Kojima	VMT trial coordinator , Management of quality control	JaCVAM, NIHS, Japan (JaCVAM representative)
<u>Lead Lab</u> Yutaka Kimura* Setsuya Aiba*	*Developer of this assay Test method, expertise underlying science	Tohoku Univ., Japan
Takao Ashikaga	Chemical supplier	JaCVAM, NIHS, Japan (JaCVAM representative)
Takashi Omori	Data analysis, biostatistics dossier	Kobe Univ., Japan
International expert members		
<u>EU liaison</u> Emanuela Corcini	Test system expertise, validation expertise, immunotoxicity expertise	Milan Univ., Italy
<u>EU liaison</u> Erwin L. Roggen,	Test system expertise, validation expertise, immunotoxicity expertise	3Rs Management and Consulting ApS, Denmark
<u>ICCVAM liaison</u> Dori Germolec	Immunotoxicity expertise	NTP/NIEHS, USA
<u>JSIT liaison</u> Tomoaki Inoue	Immunotoxicity expertise	Chugai Pharmaceutical Co., Ltd.

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) : Kohji Yamakage

Test Facility 2: AIST, Tsukuba

SD : Rie Yasuno

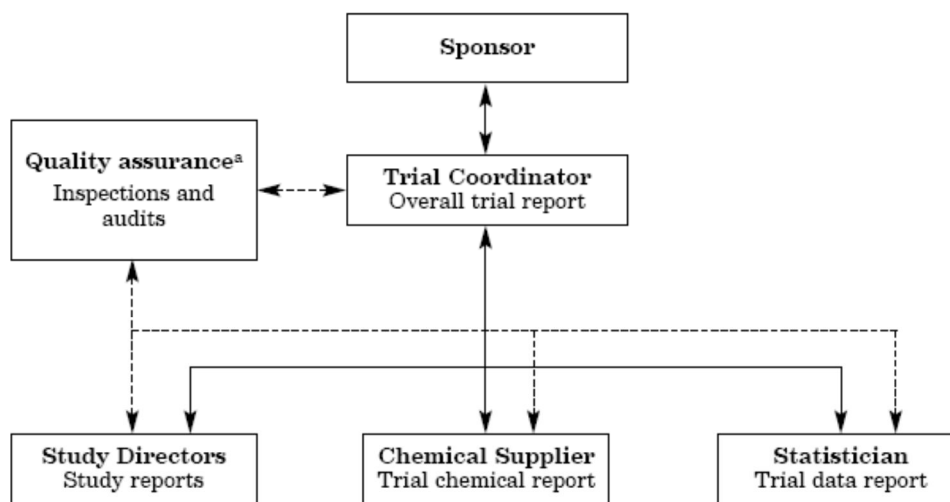
Test Facility 3: AIST, Takamatsu

SD : Yoshihiro Nakajima

Information relevant for Modules 1, 2, 3 performed by all laboratories. Data obtained by these laboratories have demonstrated that the IL-1B Luc 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 IL-1B Luc assay method under non-GLP conditions (GLP principle).

3.1 Trial management structure

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



^aSeveral Quality Assurance units might be involved in a multi-study trial.

Dashed lines indicate assurance staff involvement.

Figure 1: Management Structure of the IL-1B Luc assay validation trial

1) Chemical management group

The members of chemical management group are elected by recommendation of

the IL-1 Luc 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 IL-1 Luc 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 IL-1 Luc 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.

3.2 Sponsor

The validation trial for assessing the validity of IL-1 Luc assay will be financed by the Ministry of Health, Labour and Welfare (MHLW), Japan.

The lead laboratory will support the IL-1 Luc 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

3.3 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 IL-1 Luc 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.

3.4 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 IL-1 Luc assay protocols. In case any critical observations are made a new version of the IL-1 Luc assay protocols might necessary be issued to the other test facilities before initiating the between-laboratory transferability.

3.5 [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 IL-1 Luc assay to the all test facility, the Phase 0 study using non-coded three chemicals was performed. A few concentrations of each test item will be tested in triplicate in 2 independent runs according to the IL-1 Luc assay protocol describing the details of the experimental design. 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.

3.6 [Module 2] Within-laboratory reproducibility

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.

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.

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).

3.7 [Module 4] Between-laboratory reproducibility

Ten coded test items have been selected to confirm the between-laboratory reproducibility in the phase I study. A few concentrations of each test item will be tested

in triplicate according to the IL-1 Luc assay method 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,

3.8 [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 automatization 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.

A measurement of bioluminescence intensity induced with chemical treatment will be measured by luminometer (Phelios: ATTO, Cat #:AB-2350) calibrated using stabilized SLG, SLO and SLR enzymes in this validation trial.

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 IL-1 Luc 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	2	Between-lab transferability
Phase I	5 coded	3	Within & between-lab reproducibility
Phase II (planning)	20 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 IL-1 Luc 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 IL-1 Luc 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.

7. Study timeline

An approximate schedule for IL-1 Luc assay validation trial is shown in Table 3.

Duration of this validation trial is around twenty -month from August 2018 to 2020.

Table 3. Schedule of IL-1 Luc assay validation trial

Month	Activity
August, 2018	Establish the VMT
	Selection of participating research laboratories
	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
October, 2018	Technical transfer using five known chemicals (non-coded) Start of technical transfer to know between laboratory transferability
	Data collection of technical transfer (Phase 0 study)
	Phase I study
October, 2018	Coding and distribution of five coded test chemicals
November, 2018	Start of Phase I study
March, 2019	End of Phase I study
May, 2019	<u>2nd VMT Meeting</u> / Phase I results and planning of Phase II study

<u>Phase II study to know between- and within-laboratory reproducibility</u>	
2019	Coding and distribution of coded test chemicals and positive chemicals
2019	Start of Phase II study using 20 coded test chemicals
2019	End of Phase II study
2020	<u>3rd VMT Meeting</u> /reviewing of Phase II study results
2020	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