IL-8 Luciferase (IL-8 Luc) Assay

Report of the Peer Review Panel

on

a JaCVAM co-ordinated study programme addressing the validation status of the IL-8 Luc assay for the prospective identification of skin sensitising substances

Report completed by the Peer Review Panel on April 21st , 2016.

Table of Contents

Executive Summary

The IL-8 Luc assay has been proposed as an in vitro alternative, providing information on key event 3 (KE3) in the adverse outcome pathway for skin sensitisation. The assay has some advantages in terms of technical performance and time required compared to existing methods concerning KE3. The peer review panel (PRP) found the Validation Management Team's report presented the necessary information for an independent review. Consequently, the PRP were able to conclude that the IL-8 Luc assay was well defined, with a clear protocol and criteria for data interpretation. Both within and between laboratory reproducibility information were satisfactory, and along with the predictive capacity, very similar to other recently validated methods. Adequate performance standards were also detailed. Accordingly, the PRP concluded that the IL-8 Luc assay validation has demonstrated that the method should be acceptable as part of an integrated testing strategy for the predictive identification of skin sensitisation hazard.

Peer Review Panel Composition

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Background

For many years, chemicals with the capacity to induce contact allergy in humans, termed skin sensitisers, have been identified by a variety of *in vivo* methods (reviewed in 1). However, for animal welfare, ethical and scientific reasons there has been a desire to replace *in vivo* methods with non-animal alternatives (2, 3). To this end, a number of *in silico*, *in chemico* and *in vitro* approaches have been developed (reviewed in 4 - 6). Three of these methods have achieved formal validation approval and been translated into OECD Test Guidelines (reviewed in 7 - 9). Nevertheless, any individual alternative method possesses a spectrum of advantages and disadvantages, some characterised by the applicability domain, whilst others reflect aspects such as time, cost and "user friendliness". Against this background, several Japanese academic and industrial partners came together to develop, refine and evaluate an *in vitro* predictive test based on the release of interleukin-8 (IL-8) from the dendritic cells surrogates, THP-1 (a human monocytic leukemia cell line) using a luciferase read out marker (the IL-8 Luc assay) (10, 11). This assay is directed towards the detection of key event 3 (KE3), dendritic cell activation, in the adverse outcome pathway (AOP) for skin sensitisation (12, 13).

The PRP was assembled at the end of 2014 and met in March 2015 to review a progress report on the IL-8 Luc assay prepared by the Validation Management Team (VMT). Following the commentary on this work by the PRP, the VMT refined the protocol, adopted a single prediction model and conducted additional work, notably on interlaboratory reproducibility. The PRP met for a final time in October 2015 and engaged in follow-up telephone conferences in January and February 2016. With the provision of all of the amended, updated and additional material, including the final VMT report and final IL-8 Luc protocol, this PRP Validation Report was prepared.

IL-8 Luc Test Method Definition

The PRP concluded that the IL-8 Luc test method has been fully described in the report of the Validation Management Team (VMT) and in the associated detailed test protocol. The validation report covers well the need for the assay in the current regulatory context. Furthermore, a clear rationale for the assay has been given and helpfully includes reference to existing *in vitro* methods and Adverse Outcome Pathway (AOP) for skin sensitization that have been validated and adopted into OECD guidelines. The PRP agreed that the mechanistic basis of the method and how it related to the skin-sensitization endpoint also was well described in the report.

The PRP agreed that, when used on its own, the regulatory application of this assay could contribute to hazard identification (sensitizer/non-sensitizer). However, follow-up work suggests the assay could contribute also to potency categorization when used in integrated

approaches such as IATA.

The PRP agreed that this method is intended to contribute to the replacement of animal usage for skin sensitization assessment and that when compared to other *in vitro* cell-based methods which address the same key event in the AOP, it is likely to offer time, throughput, and cost benefits. Furthermore, the potential issues surrounding restriction of use of the cell line could be overcome by a distribution and costing plan similar to that put in place for Keratinosens™.

Within Laboratory Reproducibility

Although subject to modifications associated with the development of the IL-8 Luc protocol (i.e. change of dilution procedure from DMSO to X-VIVO), the PRP agreed that the results which emerged have demonstrated a sufficient degree of intra-laboratory reproducibility. For achieving such conclusion, the PRP focused on results obtained with the final protocol and prediction model (Criterion 3). Furthermore, the VMT provided the PRP with a rationale for combining the two datasets obtained with different dilution procedures (9 chemicals using the DMSO dilution procedure and 5 chemicals using the X-VIVO dilution procedure). Based on such assumptions, the success criterion of >85% within laboratory reproducibility was achieved in each of the three participating laboratories (Lab. A: 85.7% (12/14), Lab. B: 91.7% (11/12), Lab. C: 85.7% (12/14)).

The PRP notes that extensive documentation of within laboratory reproducibility data for the final and all the development phases of the IL-8 Luc has been displayed in the VMT report and its appendices. Furthermore, justification for combining the two dilution procedures was given to the PRP as an answer to its comments. Taken into account, the data lends support to the view that the assay has a sufficient level of reproducibility within laboratories.

Transferability

The PRP noted that the technical transfer of the IL-8 Luc assay involved training and successful assessment of 3 test substances (not blinded) on two separate occasions by each of the participating laboratories prior to their approval to participate in the subsequent validation work.

Between Laboratory Reproducibility

As with within laboratory reproducibility, the PRP discussions focused on results obtained with the final protocol and prediction model (i.e., criterion 3), ignoring a number of incomplete experiment sets incorporated into the review material. The PRP concluded that the data on between laboratory reproducibility (87.5%, 28/32) exceeded the success criterion of >80% between laboratory reproducibility.

Again, the PRP notes that extensive and transparent documentation of between laboratory reproducibility data for the final and all the development phases of the IL-8 Luc has been displayed in the VMT report and its appendices. The data lends support to the view that the assay has a sufficient level of between laboratory reproducibility.

Predictive Capacity

Demonstration of a test method's performance should be based on the testing of representative, preferably coded, reference chemicals. The PRP concluded that the validation study used an appropriate level of test chemical coding to ensure fully blinded evaluation. With respect to predictive capacity, the PRP confirms that a suitable balance of known stronger, weaker, and non-classified test chemicals was selected, with classifications generally reflecting what is understood of the human health effects of those substances (where such information was available). In addition, it is noted that these test chemicals largely comprise test chemicals from the ECVAM validation study, with the majority differing from those used in initial method development.

The ultimate protocol for the IL-8 Luc assay involves use of X-VIVO 15 (a chemically defined serum-free medium) as a medium for the dissolution of test substances. When combined with the existing dataset and using the final prediction model (Criterion 3), the validation dataset showed an accuracy of 82.4% (28/34), a sensitivity of 79.2% (19/24) and a specificity of 90.0% (9/10). In addition, two extra datasets have been tested using either the DMSO or the X-VIVO dilution procedures with a larger amount of test chemicals (122 and 143 respectively). The VMT report the following predictive capacity for the DMSO and X-VIVO dilution procedures respectively: accuracy of 73% and 80% (n=122 and 143 respectively), sensitivity of 72% and 86% (n=88 and 107 respectively) and specificity of 74% and 64% (n= 34 and 36 respectively). Taken altogether these values are in line to other assays currently adopted (or under consideration) as *in vitro* alternatives for skin sensitisation. However, if certain test substances were omitted such as detergents (see the section below on the applicability domain) and considerations on human sensitizing potential were taken into account, the predictive accuracy increased to 73% and 83% (n=117 and 138 respectively for the DMSO and X-VIVO dilution procedures), with a sensitivity of 69% and 84% (n=94 and 113 respectively) and a specificity of 87% and 80% (n=23 and 25 respectively). Finally, the PRP recognizes that it is not the intention that the assay is used in standalone mode ', but any consideration of how results

from the IL-8 Luc method might be combined with other *in vitro* methods for skin sensitisation was outside the scope of the present review.

Applicability Domain

Inevitably, the IL-8 Luc assay shares limitations common to many suspension cell based techniques, not least in dealing with highly hydrophobic but poorly cytotoxic substances. The PRP considered that these issues were appropriately and comprehensibly documented in the VMT report, but special mention is made of the need to exclude detergent/surfactant substances as well as materials known to interfere with luciferase measurement. Potential concerns with the identification of *respiratory* sensitising anhydrides also are mentioned, but the PRP did not conclude that this aspect presented an issue of significance for adoption of the IL-8 Luc assay for identification of skin sensitisers.

Performance Standards

Whilst recognising that these would be discussed in more detail post-validation, nevertheless, the PRP was of the view that the list of performance standard substances placed in an appendix to the VMT report was satisfactory. This is supplemented by a short list of proficiency chemicals to be used as a routine check on performance of the assay.

Additional Comments

The PRP concluded that the validation study management and conduct met the criteria set out in OECD GD 34 (2005). The PRP concluded also that the study was conducted in the spirit of GLP.

The PRP appreciated the transparency with which all the IL-8 Luc assay material was presented, but noted also that modifications to the protocol and the prediction model during the validation process added a degree of complexity to some aspects of the evaluation. A diagram depicting the protocol modifications during the various phases of the assay refinement and validation included in the report has helped to clarify the process and timeline of event. Nevertheless, a clear separation between assay research/development and subsequent validation is desirable, an approach the PRP recommends for any future work deriving from JaCVAM or elsewhere.

The PRP notes that during the conduct of the review, it did not have access to the full raw data files associated with the IL-8 Luc assay development/validation work.

Conclusions and Recommendations

The PRP concluded that the IL-8 Luc assay validation has demonstrated that the method should be acceptable as part of an integrated testing strategy for the predictive identification of skin sensitisation hazard.

Acknowledgements

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