Amino acid Derivative Reactivity Assay (ADRA)

Report of the Peer Review Panel

on

a JaCVAM co-ordinated study programme addressing the validation status of the Amino acid Derivative Reactivity Assay for the prospective identification of skin sensitising substances

Report completed by the Peer Review Panel on July 6th, 2018.

Table of Contents

Executive Summary

The Amino acid Derivative Reactivity Assay (ADRA) has been proposed as an in vitro alternative, providing information on key event 1 (KE1) in the adverse outcome pathway for skin sensitisation. The assay has several advantages in terms of technical performance and time required compared to existing methods concerning KE1. 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 ADRA was well defined, with a clear protocol and criteria for data interpretation. Both within and between laboratory reproducibility information exceeded the pre-defined success criteria, and along with the predictive capacity, were similar to, often better than, other recently validated methods. Adequate performance standards were also detailed. Accordingly, the PRP concluded that the ADRA 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

David Basketter (Chair)	DABMEB Consultancy Ltd, Sharnbrook, UK
Takao Ashikaga	JaCVAM, Kawasaki, Japan
Steven Enoch	Liverpool John Moores University, Liverpool, UK
Hiroshi Itagaki	Yokohama National University, Yokohama, Japan
Joanna Matheson	Consumer Product Safety Commission, Bethesda, USA
Kyung-Min Lim	EWHA Womens University, Seoul, Korea
Stanislav Vukmanovic	Food and Drug Administration, College Park, USA

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). To this end, a number of in silico, in chemico and in vitro approaches have been developed (reviewed in 3 - 6). Several of these methods have achieved formal validation approval and been translated into OECD Test Guidelines (detailed 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. In addition, each method must be judged, independently, in terms of its relevance and reliability via the process of validation

Against this background, the Japanese company Fujifilm undertook to address certain limitations of one of the new in vitro methods, the Direct Peptide Reactivity Assay (DPRA) (OECD, 2015). Fujifilm Corporation synthesized a pair of amino acid derivatives, N-(2-(1-naphthyl)acetyl)-L-cysteine (NAC) and α-N-(2-(1-naphthyl)acetyl)-L-lysine (NAL), for use as nucleophilic reagents in the Amino acid Derivative Reactivity Assay (ADRA) (10, 11). The use of these reagents was described as offering, amongst other things, cost and accuracy benefits (see below).

The PRP was assembled at the end of 2017 and met in March 2018 to review the detailed report on the ADRA prepared by the Validation Management Team (VMT). Following the commentary on this work by the PRP, the VMT clarified some minor details in the protocol and made some small adjustments to their validation report. The PRP engaged in follow-up telephone conference in June 2018. With the provision of all of the amended and updated materials, including the final VMT report and final ADRA protocol (12), this PRP Validation Report was prepared.

ADRA Test Method Definition

The PRP concluded that the ADRA 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 methods which address the same key event in the AOP, it offers several potential advantages. These include the application of more sensitive nucleophilic reagents that permit use of lower test concentrations (and therefore reduce the chance of difficulties with hydrophobic materials), limit precipitation and turbidity, reduce the risk of co-elution and enhance overall accuracy. These advantages are more fully detailed in the VMT report.

Within Laboratory Reproducibility

The PRP noted that the studies which comprised both the within and the between laboratory reproducibility were completed with the involvement of four participating laboratories, rather than the usual three, which had the effect of increasing the quality and reliability of the information.

With regard to within-laboratory reproducibility, the PRP recognized that the test results of greater than a 97% average concordance and the reasons for the non-concordant results are well described. The lowest concordance value was 90%, which still substantially exceeded the target of 80% set for within-laboratory reproducibility. The PRP was surprised, however, that Sumitomo was not instructed by the VMT to fill the gaps in its Phase I data set, where only 7 of the 10 test chemicals were tested. However, it was accepted that the sole reason was time constraints associated with the wish to ensure timely completion and progress of the validation work package.

The PRP concluded that the ADRA demonstrated successful within laboratory reproducibility.

Transferability

The PRP noted that the technical transfer of the ADRA involved training of four laboratories and assessment of 5 test substances (not blinded), adaptation/learning, and then an evaluation of a further 10 substances in each of the participating laboratories prior to their approval to participate in the subsequent validation work. The successful training and transfer is fully documented in the VMT report.

The PRP concluded that the transferability of the ADRA was properly documented and successfully achieved.

Between Laboratory Reproducibility

With regard to between-laboratory reproducibility, the PRP recognized that the test results gave 91.9% average concordance (based on an analysis of any 3 of the four participating laboratories) and also that the reasons for the non-concordant results were well and satisfactorily described. The lowest concordance value was 90%, which substantially exceeded the target of 80% set for within-laboratory reproducibility.

The PRP requested that the VMT also calculate between laboratory reproducibility based on four laboratories and add this information to the report as reference and to review all tables to ensure accuracy. This has been done and shows that even with 4 laboratories, the performance exceeded the target by a good margin.

The VMT report repeatedly compares ADRA results with those of DPRA, and the PRP recognized that the ADRA results do exceed those of DPRA, which is an accepted OECD assay.

The PRP concluded that the ADRA demonstrated successful between laboratory reproducibility.

Predictive Capacity

The PRP noted that an independent chemical selection committee selected and encoded all chemicals used in the validation study, guided by those recommended by EURL/ECVAM and the reference chemicals from OECD TG 429 (13). The chemicals selected covered an appropriate range of potency and physicochemical characteristics.

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.

However the PRP considered that, when judged on the basis of LLNA results, ADRA has a level of predictive performance for the 40 test chemicals tested for within and between laboratory reproducibility that is comparable to that of DPRA.

Applicability Domain

The PRP noted that the VMT report clearly delineated potential applicability limitations of ADRA. However, metal allergens are already well defined, such that assay functionality in this area is deemed not to be of any practical importance. Similarly, although the VMT noted potential limitations with regard to pre- and pro-haptens, the PRP agreed with the conclusions of Patlewicz and colleagues (14), that virtually all such materials appear to be correctly identified by in vitro assays. The results presented in the VMT report with several pre-/pro-haptens are consistent with this conclusion, the exception being resorcinol. However, the PRP is aware that when tested in the absence of EDTA (and therefore in the presence of copper ions), resorcinol yields positive results (10,11).

Although the VMT noted in the report the potential for co-elution problems, the PRP is aware that the assay has been designed so that these are likely to occur with much lower frequency compared to the DPRA.

Although the VMT has suggested in the report that the use of lysine and cysteine alone may miss some sensitizers, the PRP noted that the nucleophilic sites in these amino acids are identical to those of DPRA and is of the opinion that reactivity beyond these two amino acids is very unlikely.

Finally, although the solubility of test substances can be a limitation for any test, the PRP agreed that the requirement for much lower test concentrations in the ADRA is anticipated to give this test a significant advantage.

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 used in the transferability training could be considered suitable. Note should be taken also of the performance standard chemicals used for the DPRA. It was suggested also to the PRP that performance standards could be developed as part of a performance-based test guideline (PBTG) and that it would therefore be unnecessary to establish a set of substances specifically for ADRA. However, the PRP concluded that it might be helpful for the VMT to elaborate a set of performance standards which then may be used to inform the OECD process.

The PRP suggested that the ten substances used as proficiency chemicals for DPRA could also be adopted for ADRA.

Additional Comments

The PRP concluded that the validation study management and conduct met the criteria set out in OECD GD 34 (15). The PRP concluded also that the study was conducted in the spirit of good laboratory practice.

The PRP notes that during the conduct of the review, it was detailed how to gain access to the full raw data files associated with the assay development/validation work. However, no need to do this was identified during the review.

The PRP encouraged Fujifilm to resolve any doubts related to patent issues concerning the assay as soon as possible.

Although the PRP understood why EDTA was incorporated for the validation study, there remained a concern that reducing the low level of dimerization that occurs within 24 hours may at the same time result in discrepancies with other test results from either DPRA or the "ADRA without EDTA." The data set of 82 chemicals presented in Appendix 11 indicated that a small percentage of weak sensitizers become false negatives in the presence of EDTA. Consequently, the PRP was grateful for the clarification from the VMT regarding the need for the use of EDTA which is detailed in their report. The PRP also encouraged Fujifilm to publish a paper describing an extended dataset based on the protocol in the final SOP (adding EDTA) (12) as soon as possible.

The PRP is aware of the potential for use of differential reactivity between lysine and cysteine as a means for the positive identification of potential respiratory sensitizers. Further research and evaluation on this topic is encouraged.

Conclusions and Recommendations

The PRP concluded that the Amino acid Derivative Reactivity Assay validation has demonstrated that this method should be acceptable as part of an integrated testing strategy for the predictive identification of skin sensitisation hazard.

Acknowledgements

The PRP is grateful to the members of the VMT for their hard work and patience. Thank are due also to JaCVAM for their support in setting up and hosting meetings in Japan, as well as for the setting up of telephone conferences.

References

1. Thyssen J P, Giménez-Arnau E, Lepoittevin J-P, Menné T, Boman A, Schnuch A. (2012) The critical review of methodologies and approaches to assess the inherent skin sensitization potential (skin allergies) of chemicals. Parts 1 - 3. Contact Dermatitis, 66: Suppl 1: 11 - 70.

2. Adler S, Basketter D A, Creton S, Pelkonen O, van Benthem J, Zuang V et al. (2011) Alternative (non-animal) methods for cosmetics testing: current status and future prospects—2010. Arch. Toxicol. 85: 367 - 485.

3. Basketter D A, Ashikaga T, Casati S, Hubesch B, Jaworska J, de Knecht J, Landseidel R, Manou I, Mehling A, Petersohn D, Rorije E, Rossi L, Steiling W, Teissier S and Worth A. (2015) Alternatives for Skin sensitisation testing and assessment. Regul. Toxicol. Pharmacol., 73: 660 - 666.

4. Reisinger K, Hoffmann S, Alépée N, Ashikaga T, Barroso J, Elcombe C, Gellatly N, Galbiati V, Gibbs S, Groux H, Hibatallah J, Keller D, Kern P, Klaric M, Kolle S, Kuehnl J, Lambrechts N, Lindstedt M, Millet M, Martinozzi-Teissier S, Natsch A, Petersohn D, Pike I, Sakaguchi H, Schepky A, Tailhardat M, Templier M, van Vliet E, Maxwell G. (2015) Systematic evaluation of non-animal test methods for skin sensitisation safety assessment. Toxicol. in Vitro, 29: 259 - 270.

5. Urbisch D, Mehling A, Guth K, Ramirez T, Honarvar N, Kolle S, Landsiedel R, Jaworska J, Kern P S, Gerberick F, Natsch A, Emter R, Ashikaga T, Miyazawa M, Sakaguchi H. (2015) Assessing skin sensitization hazard in mice and men using non-animal test methods. Regul. Toxicol. Pharmacol., 71: 337 - 351.

6. Kleinstreuer NC, Hoffmann S, Alépée N, Allen D, Ashikaga T, Casey W, Clouet E, Cluzel M, Desprez B, Gellatly N, Göbel C, Kern PS, Klaric M, Kühnl J, Martinozzi-Teissier S, Mewes K, Miyazawa M, Strickland J, van Vliet E, Zang Q, Petersohn D. Non-animal methods to predict skin sensitization (II): an assessment of defined approaches. Crit Rev Toxicol. 2018 48: 359-374.

7. OECD (2015). OECD Guideline for the Testing of Chemicals No. 442C: In Chemico Skin Sensitisation: Direct Peptide Reactivity Assay (DPRA). Paris, France: Organisation for Economic Cooperation and Development. Available at: http://www.oecd.org/env/testguidelines.

8. OECD (2015). OECD Guideline for the Testing of Chemicals No. 442D: In Vitro Skin Sensitisation: ARE-Nrf2 Luciferase Test Method. Paris, France: Organisation for Economic Cooperation and Development. Available at: http://www.oecd.org/env/testguidelines.

9. OECD (2018). OECD Guideline for the Testing of Chemicals No. 442E. In Vitro Skin Sensitisation assays addressing the Key Event on activation of dendritic cells on the Adverse Outcome Pathway for Skin Sensitisation. http://www.oecd.org/env/test-no-442e-in-vitro-skin-sensitisation-9789264264359-en.htm

10. Fujita M, Yamamoto Y, Tahara H, Kasahara T, Jimbo Y, Hioki T. (2014). Development of a prediction method for skin sensitization using novel cysteine and lysine derivatives. Journal of pharmacological and toxicological methods. 70:94-105

11. Yamamoto Y, Tahara H, Usami R, Kasahara T, Jimbo Y, Hioki T, Fujita M. (2015). A novel in chemico method to detect skin sensitizers in highly diluted reaction conditions, Journal of Applied Toxicology, 35:1348-1360.

12. Amino acid Derivative Reactivity Assay (ADRA) Standard Operating Procedure Version 1.2. Available at: http://www.jacvam.jp/en_effort/effort02.html

13. OECD (2010). OECD Guideline for the Testing of Chemicals No. 429. Local lymph node assay. www.oecd-ilibrary.org/environment/test-no-429-skin-sensitisation_9789264071100-en

14. Patlewicz G, Casati S, Basketter DA, Asturiol D, Roberts DW, Lepoittevin J-P, Worth A and Aschberger K (2016) Can currently available non-animal methods detect pre and pro haptens relevant for skin sensitisation? Regul Toxicol Pharmacol, 82, 147-155.

15. OECD (2012). Guidance document on the validation and international acceptance of new or updated test methods for hazard assessment. Paris, France. www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2005)14&doclanguage=en