Safety & Environmental Assurance Centre

Towards the Development of Animal Product-Free *in vitro* Systems for NGRA of Consumer Goods

Paul Russell, **Sarah Hatherell**, Ouarda Saib & John Kilgallon

Background

There is an increasing acceptance of the role *in vitro* assays can play in assuring consumer safety, particularly as part of Next Generation Risk Assessment (NGRA) (Baltazar *et al*, 2020). NGRA is an exposure-led, hypothesis-driven approach integrating new approach methodologies (NAMs) to ensure chemical safety without the use of animal testing. There is also a growing desire to remove animal products from these *in vitro* assays to make them more scientifically robust and human-relevant. For example, the use of foetal bovine serum (FBS) and animal-derived antibodies can introduce a lot of batch-to-batch variability potentially resulting in experimental quality (e.g. contamination of FBS; specificity of antibodies) and reproducibility issues (Baker *et al*, 2016; van de Valk *et al*, 2018). Additionally, it is more frequently becoming recognised that knowledge of all the constituents of the cell culture medium used and their influence on cellular processes are important for improved reproducibility (Hirsch & Schildknecht, 2019). Therefore, ideally chemically-defined media would be used to culture human cells for *in vitro* assays to eliminate any remaining scientific quality issues resulting from use of animal- or human-derived components (van der Valk *et al*, 2010) although this is technically very challenging. Here we will describe some of the challenges, opportunities and potential options for replacing animal-derived products in *in vitro* systems.

Key Focus Areas for Replacement of animal-derived products

Cell Culture Media/Reagents

In 2008, the ECVAM Scientific Advisory Committee (ESAC) advocated for the use of non-animal derived supplements for *in vitro* studies wherever possible and stated that *"for methods forwarded to ECVAM for validation/pre-validation where [the use of non-animal alternatives to serum] is not fulfilled a justification for future use must be provided, including measures taken to seek non-animal alternatives to [FBS]".*

The technical disadvantages of using serum (both animal & human-derived) include its undefined nature, batch-to-batch variability in composition, and the risk of contamination. Several OECD test guidelines* already use serum-free cell culture methods demonstrating that this is an area of growth with plenty of opportunities for further development. In particular it is hoped that rather than just removing or replacing (i.e. animal with human) serum in cell culture medium a fully chemically defined medium can be used as there are also reproducibility concerns due to the non-defined nature of human serum.

Chemically defined media
Standard *in vitro* cell culture media commonly consist of a basal medium supplemented with serum (animal or human-derived) as a source of nutrients and other ill-defined factors. In contrast, chemically defined media require that all of the components must be identified and have their exact concentrations known.

Current challenges include commercial availability of specialised reagents; technical challenges when it comes to cell adaptation (each cell type requires its own specific media) which requires expertise and can be a lengthy process; ensuring that the whole protocol is animal product free (i.e. from the plastic ware to the freezing media); lack of consensus around what constitutes an animal-free reagent.

Opportunities include the growing market for those who want more reproducible, reliable, ethical and human-relevant assays; faster research; improved assays.

*including OECD TGs no. 431, 439 and 492 (OECD, 2015a; OECD, 2015b; OECD, 2016) *in vitro* test methods for skin corrosion, skin irritation & eye irritation testing.

Antibodies

The recent EURL ECVAM paper on animal derived antibodies (Viegas Barosso *et al*, 2020) sets out a clear position: *"EURL ECVAM recommends that animals should no longer be used for the development and production of antibodies for research, regulatory, diagnostic and therapeutic applications. In the EU, the provisions of Directive 2010/63/EU should be respected, and EU countries should no longer authorise the development and production of antibodies through animal immunisation, where robust, legitimate scientific justification is lacking."*

A clear scientific **advantage** of using non-animal derived antibodies is again the removal of batch-to-batch variability. The main **challenge** here is that animal-derived antibodies are currently much more readily available as "off the shelf" and ready to use than those that don't involve animals.

We recognise that transition to routine use of non-animal-derived antibodies will be greatly helped by the availability of mature technologies and specialised laboratories/manufacturers. The hope is to work with those developers and other external collaborators to increase the uptake and use of these types of antibody, driving further development.

Existing collaborations

Unilever is sponsoring (with AstraZeneca) and co-funding NC3Rs CRACK IT challenge 36 (https://nc3rs.org.uk/crackit/animal-free-vitro) to adapt two established OECD Test Guideline *in vitro* systems to animal-product-free conditions.

The two *in vitro* tests are OECD TG 487 and OECD TG 455; they are important tests as they provide data towards establishing whether a chemical will cause cancer or endocrine disrupting effects in humans.

The aim of this work is to deliver a robust, human-relevant (and preferably chemically-defined) version of the assays that demonstrates improved data quality and reproducibility.

CRACK IT

NC3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research

XCellR8 have previously successfully adapted two skin sensitisation *in vitro* tests KeratinoSens™ (OECD TG442D) and h-CLAT (OECD TG442E) to xeno-free conditions by replacing FBS with human serum (Belot *et al*, 2017; Edwards *et al*, 2018)

We are currently collaborating with XCellR8 to take this a step further so that the tests are performed under fully chemically-defined conditions.

XCellR8 Redefining testing

Conclusion: Increased development, publication and commercial availability of animal-free reagents will increase the reproducibility and human relevance of the chosen assays and help drive the use of animal product-free *in vitro* systems for NGRA.

References

- Baker, M. (2016). Reproducibility: Respect your cells! Nature 537, 433-435. doi:10.1038/537433a
- Baltazar MT CS., Carmichael PL., Cubberley R. et al. (2020). A Next-Generation Risk Assessment Case Study for Coumarin in Cosmetic Products. Toxicol Sci 176(1):236-252. doi: 10.1093/toxsci/kfaa048.
- Edwards A., Roscoe, L., Longmore, C. et al. (2018). Adaptation of the human Cell Line Activation Test (h-CLAT) to Animal-Product-Free Conditions. ALTEX 35(4):477-488. doi: 10.14573/altex.1710051.
- Belot, N., Sim, B., Longmore, C. et al. (2017). Adaptation of the KeratinoSens™ skin sensitization test to animal-product-free cell culture. ALTEX 34(4):560-564. doi: 10.14573/altex.1701311.
- ESAC (2008). ESAC statement on the use of FCS and other animal-derived supplements. Ispra, Italy: European Centre for the Validation of Alternative Methods. Available at: https://eurl-ecvam.jrc.ec.europa.eu/about-ecvam/archive-publications/publication/ESAC28_statement_FCS_20080508.pdf
- Hirsch, C. & Schildknecht, S. (2019) In Vitro Research Reproducibility: Keeping Up High Standards. Front Pharmacol. 10:1484. doi:10.3389/fphar.2019.01484
- van der Valk, J., Bieback, K., Buta, C. et al. (2018). Fetal Bovine Serum (FBS): Past-Present-Future. ALTEX 35 (1), 99-118. doi:10.14573/altex.1705101.
- van der Valk, J., Brunner, D., De Smet, K. et al. (2010) Optimization of chemically defined cell culture media – Replacing fetal bovine serum in mammalian in vitro methods. Toxicol In Vitro 24, 1053-1063. doi:10.1016/j.tiv.2010.03.016.
- Viegas Barroso, J.F., Halder, M.E. and Whelan, M., EURL ECVAM Recommendation on Non-Animal-Derived Antibodies, EUR 30185 EN, Publications Office of the European Union, Luxembourg, 2020, ISBN 978-92-76-18347-1, doi:10.2760/091625, JRC120199.