Adaptation of Human Cell Based Safety Tests to Animal Product-Free Conditions

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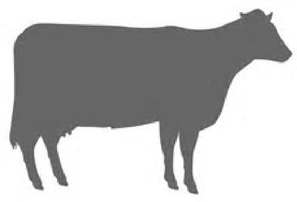
ABSTRACT

Much progress has been made in terms of the regulatory acceptance of human cell based methods for the assessment of human safety. However, most of these methods still use animal-derived components, such as serum, tissue extracts and antibodies, for which both scientific and ethical drawbacks have been well-documented. Such methods cannot be considered truly animal-free. XCellR8 has adapted 3 key human cell based safety tests to animal product free conditions: the regulatory KeratinoSens™ and h-CLAT tests for skin sensitisation, and the non-regulatory BlueScreen™ test for genotoxicity. In addition, we have developed a new pre-screen for acute toxicity using human cells in animal-free culture. Our in-house validation of these methods has shown equivalence and improvements to the fully validated published methods. Here we present an overview of this work so far and the steps taken to gain regulatory approval, demonstrating that truly animal-free regulatory safety testing is now achievable.

FOETAL BOVINE SERUM (FBS) – THE FACTS

- ! ~0.5 million litres FBS sold globally each year.
- ! ~1 million bovine foetuses harvested from cows at slaughter houses.
- ! Cutting the umbilical cord causes anoxia BUT this does not result in lowered brain function in the foetus as it would in adult animals.
- ! Calf has normal brain function at the time of cardiac puncture.
- ! "*The thought that cell culture techniques requiring FBS are a replacement for the use of animals is a misconception*" [1]

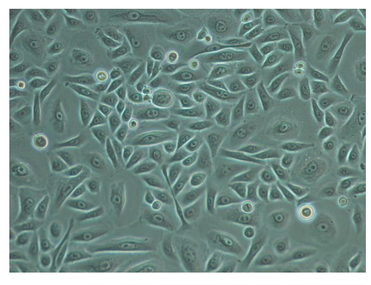


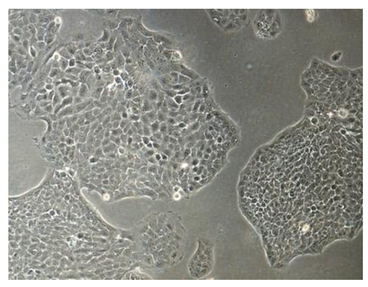
XCELLR8 ANIMAL PRODUCT FREE APPROACH

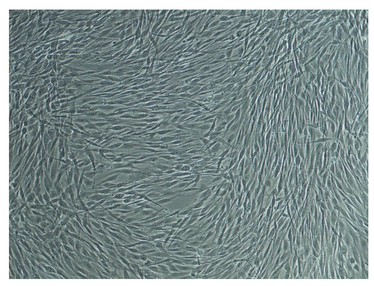
In
- ✓ Human Serum (from FDA-licensed facilities)
- ✓ Human Liver Extract (S9)
- ✓ Human Serum Albumin
- ✓ Trypzean (plant based enzyme)
- ✓ Human-based Antibodies (derived from human combinatorial libraries)

Out
- × Foetal Bovine/Calf Serum (FBS/FCS)
- × Horse Serum
- × Bovine Serum Albumin
- × Rat Liver Extract (S9)
- × Porcine Trypsin
- × Mouse Antibodies

Scientific advantage: enhanced human relevance of *in vitro* system
Ethical advantage: replace sacrifice of animals for *in vitro* testing with ethically derived human-based products







All cells in XCellR8's lab are cultured in animal product free conditions, including human epidermal keratinocytes (top) KeratinoSens™ (bottom left) and human dermal fibroblasts (bottom right)

ADAPTED REGULATORY & NON-REGULATORY TESTS

Endpoint	Method	Human Cell Type	Regulatory Status
Skin Sensitisation	KeratinoSens™ OECD TG 442d[2]	KeratinoSens™ cell line	ECHA approved for REACH; OECD approval for adoption into TG 442d 2017
Skin Sensitisation	h-CLAT OECD TG 442e	THP-1	ECHA approved for REACH; OECD submission for approval into TG 442e
Genotoxicity	BlueScreen™	TK6	Non-regulatory
Acute Toxicity	New screen developed at XCellR8	Human dermal fibroblasts	Non-regulatory – Regulatory development planned 2017-2020

DISCUSSION

In vitro testing is not truly animal-free unless it is free from animal-derived components. Foetal Bovine Serum (FBS) and other animal-derived cell culture products are still in common use for historical reasons, but they are no longer necessary due to the wide commercial availability of ethically sourced human equivalents. Uniquely, as a contract testing laboratory, XCellR8's GLP-accredited lab is completely animal product free. We have adapted existing skin sensitisation tests (KeratinoSens™ and h-CLAT) to animal product free conditions and demonstrated equivalence to the standard methods using the required proficiency testing and performance standards. Both methods have been accepted by the European Chemicals Agency (ECHA) for REACH purposes, and the adapted KeratinoSens™ method has recently gained OECD approval for incorporation into TG 442d by the end of 2017. In addition, we offer non-regulatory screens (validated in-house) for genotoxicity and acute toxicity in animal product free conditions. The validation of a new regulatory *in vitro* method requires significant time and financial resource: we urge laboratories developing new tests to adopt animal product free approaches from the outset.

References

[1]Jochems *et al* (2002). The use of fetal bovine serum: ethical or scientific problem? *Alter. Lab. Anim.* **30** (2): 219-227.
[2] Belot *et al* (2017). *ALTEX Online First*, 16 March 2017: 1-6.