TOWARDS THE REPLACEMENT OF FOETAL BOVINE SERUM IN CELL CULTURE APPLICATIONS

PETA INTERNATIONAL SCIENCE CONSORTIUM LTD.

Gilly Stoddart, PhD, and Christopher Faßbender, PhD
PETA International Science Consortium Ltd., London, UK

PISC@piscltd.org.uk
PISCLTD.org.uk

ABSTRACT

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Foetal bovine serum (FBS), used as a supplement for cell culture media, presents significant scientific and animal-welfare concerns. The availability of supplements for cell culture media that are not animal-derived allows researchers to make a full transition away from the use of FBS, overcoming these concerns.

Advances in biotechnological protein production allow for the production of recombinant proteins. The use of application-specific non-animal cell culture media supplements decreases variability and biosafety issues and facilitates product purification. In basic research and R&D testing, FBS can be replaced relatively easily, and there is support for the use of serum-free media in regulatory testing.

This poster includes recommendations for the use of FBS alternatives in both regulatory and non-regulatory testing as well as information about companies that sell serum-free media or cell culture supplements.

FBS: SCIENTIFIC AND ETHICAL CONCERNS

FBS is a cell culture media supplement that provides an undefined mixture of macromolecules, including hormones, transport proteins, growth factors, lipids, minerals, elements, and detoxifying factors that maintain cell viability and facilitate cell metabolism, growth, proliferation, and spreading in culture.[1–3]

The use of FBS presents significant scientific and animal-welfare concerns.

Scientific Concerns

- **FBS batch variation and resulting reproducibility issues for *in vitro* studies:** Because serum is a complex mixture of biomolecules, significant batch-to-batch variation exists[4] and may explain the discrepancies in results from *in vitro* studies using FBS.[5,6]
- **Unexpected and undesired outcomes:** For example, FBS can suppress TGF-β1, thus preventing chondrogenesis in fibroblast-like type-B synoviocytes.[7]
- **Risk of contamination with animal proteins or pathogens:** Exogenous agents can contaminate cultured cells, and bovine proteins can contaminate biologics, which is especially problematic for human therapies.[8–10]

Ethical Concerns

- When pregnant cows are slaughtered, a large-gauge needle is used to draw blood from the beating heart of the foetus,[11,12] who is not anaesthetised and likely experiences pain.[13–15]
- In 1995, an estimated 500,000 litres of FBS were produced worldwide,[16] using more than 1 million foetuses.[17]

By Lilly_M (GFDL, http://www.gnu.org/copyleft/fdl.html)

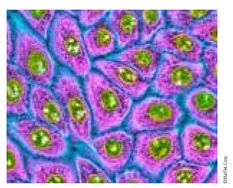
ALTERNATIVES TO FBS

There has been a push to develop and optimise serum-free culture systems.[18–20]

Laboratories conducting *in vitro* testing should make the transition to using supplements for cell culture media that are not animal-derived and publish their optimal concentrations.

Alternatives to FBS in Non-Regulatory Settings

- For some cell types, it may be possible to adapt the cells directly from growth in serum-containing to serum-free media, while maintaining cellular function.[21]
- Optimal concentrations of recombinant proteins have been determined for many cell types,[22–25] whereas for others, the concentration of each supplement will need to be optimised.[26]



Stained primary human epidermal keratinocytes cultured in serum-free NHEK-GM medium

Alternatives to FBS in Regulatory Settings

- The European Union Reference Laboratory for alternatives to animal testing Scientific Advisory Committee advocates for the use of supplements that are not animal-derived for *in vitro* studies.[27]
- Serum-free media should be incorporated into new validation efforts or new cell culture test guidelines.
- Several OECD test guidelines use serum-free media (e.g. TG 431 and TG 439), and many three-dimensional reconstructed human tissues (such as those from MatTek, Epithelix, and SkinEthic) are maintained in serum-free media.
- Currently, TG 442D on the ARE-Nrf2 luciferase test method (KeratinoSens™) is being updated to include FBS-free serum, and TG 442E on the Human Cell Line Activation Test (h-CLAT) is being validated using human serum and human antibodies.
- Testing with serum-free media can be conducted in parallel with OECD test protocols to evaluate the ability of a chemically defined medium to replace FBS.

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DATABASES OF NON-ANIMAL CELL CULTURE PRODUCTS

- Visit http://www.piscltd.org.uk/FBS-Alts-database for a list of examples of non-animal cell culture products and applications. The database includes 70 animal-free products for 12 groups of cell types and includes references, suppliers, and information on the products' applications.
- Animal Free Research UK provides a spreadsheet of commercially available FBS-free media: https://www.animalfreeresearchuk.org/serum-free-media.
- The 3Rs-Centre Utrecht Life Sciences and Animal Free Research UK plan to launch an interactive FBS-free database in August 2017.

Table: Extract From the Non-Animal Cell Culture Products and Applications Database

Medium Composition	Reference	Supplier	Product Link	Comments
CHO (Chinese hamster ovary)				
ProCHO – three different media systems. Protein-free. Very low levels of recombinant insulin.	Reinhart *et al*, 2013: Benchmarking of commercially available CHO cell culture media for antibody production[28]	www.lonza.com	http://www.lonza.com/products-services/bio-research/cell-culture-products/specialty-media/cho-expression-media/procho-protein-free-cho-medium.aspx	Medium developed specifically to facilitate the production and downstream processing of recombinant proteins expressed in CHO cells; supports high-density cultures. Animal-free.
HEK 293 cells (human embryonic kidney)				
Freestyle 293 Expression Medium supplemented with 0.1% Pluronic F-68.	Durocher *et al*, 2007: Scalable serum-free production of recombinant adeno-associated virus type 2 by transfection of 293 suspension cells[29]	www.thermofisher.com	https://www.thermofisher.com/order/catalog/product/12338018	Medium developed to support the growth and transfection of 293-F cells under suspension-type culture conditions. Animal-free.
Tumour cells				
EX-CELL® NS0 – without L-glutamine. Available with or without sodium bicarbonate. With Synthechol supplement.	Raja *et al*, 2011: Adaptation of cholesterol requiring NS0 cells to serum free culture conditions[30]	www.sigmaaldrich.com	http://www.sigmaaldrich.com/catalog/product/sigma/14650c?lang=de&region=US	Medium developed for the long-term growth of NS0-related cells in suspension culture. Animal-free.
Stem cells				
KnockOut™ DMEM supplemented with 15% KnockOut™ Serum Replacement XenoFree (KSR XF), 1% GlutaMAX™-I, 1X KnockOut™ SR XenoFree GF Cocktail, 0.1 mM 2-mercapto-ethanol, and 20 ng/ml basic fibroblast growth factor.	Boucher *et al*, 2011: Xenofree culture systems for stem cells[31]	www.thermofisher.com	www.thermofisher.com/order/catalog/product/10829018	Medium for the growth of human embryonal stem (ES) cell lines I-6, I-3, and H-9. Animal-free.

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