

Focus on Alternatives: Replacing Animal Serum in Biomedical Research

Website: www.focusonalternatives.org.uk



FoA comprises representatives from British non-profit organisations involved in replacing animal experiments. We work towards this goal by lobbying, facilitating access to information, educating animal users and organising workshops and meetings on specific topics of concern.

The Serum-Free Initiative Why is Replacement Important?

- *In vitro* techniques have replaced some types of animal experiments
- However, most cell culture work still relies on serum use



- Particularly fetal calf serum (FCS)
- In 2002 FoA began the Serum-Free Initiative to eliminate animal-based serum from cell culture research

Scientific Disadvantages of FCS

- Price and availability of FCS fluctuates due to
 - > Variation in worldwide stock numbers
 - > Importation regulations
 - > Beef and dairy prices
 - > Livestock feed costs
 - > Weather conditions
- Batch-to-batch variation means each batch has to be tested before purchase
 - > Can effect growth and culture composition
- Many substances in FCS have not been identified or their affects on cultured cells remain unclear so
 - > Optimal cell growth doesn't correspond to proper cellular function
 - > It can interfere with genotypic and phenotypic cell stability
 - > It can influences experimental outcomes
- FCS can suppress
 - > Cell spreading
 - > Cell attachment
 - > Embryonic tissue differentiation
- FCS can be contaminated with a variety of micro-organisms and substances, which can affect bulk production of proteins

Animal Welfare: Serum Collection



Courtesy of VIVA at: www.viva.org.uk

- Time between death of mother and cardiac puncture 5-30 mins
- Bleeding procedure 2-5 mins
- Annual global harvest of fetal calves is 1,000,000-2,000,000
- Fetus is expected to be ALIVE during blood collection

Calf is expected to experience pain and/or suffering at moment of cardiac puncture and thereafter until death occurs

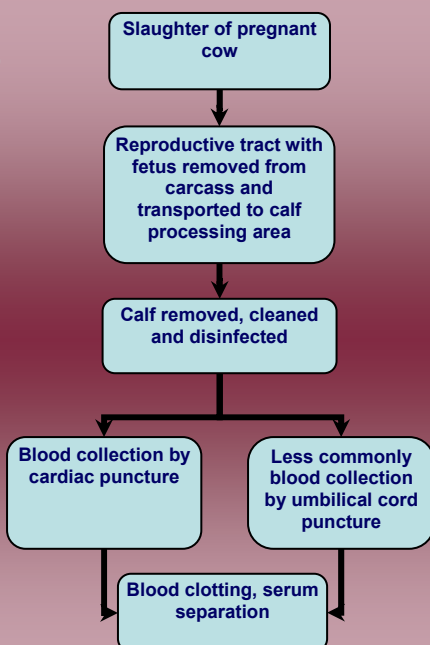


Chart adapted from Jochems CEA *et al.* ATLA 30(2): 219-227 (2002)

What Are The Alternatives?

- Preferably chemically defined (synthetic) media
 - Cell-type specific
 - Most cells can be successfully cultured (except for endothelial cells)
 - Reduced-serum media
 - Volume of FCS varies between 5 and 20%
 - Growth factors should usually be added
 - Cells need to be given a weaning period to adapt to different composition
- Several institutions & companies offer the development of serum-free media on request



Resources

Serum Questionnaire:
<http://www.focusonalternatives.org.uk/Word%20Documents/FOA%20Questionnaire%20final%20copy.doc>
 FoA Serum Free Table:
<http://www.focusonalternatives.org.uk/Word%20Documents/Serum-FreeTable.doc>



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Frequently Asked Questions

- 1. Can the collection of FCS be made 'humane'?**

Collection of blood from the living fetal calf is preferred by companies and stunning or euthanizing avoided because:
"Effective electrical stunning causes cardiac arrest and so electrical stunning is not suitable for harvesting of blood from the heart".
Blood coagulates upon death, making blood collection difficult.
There are humane protocols (van der Valk, J. *et al* [2004]. *Toxicology in Vitro* 18, 1-12) but it is difficult to regulate their implementation, especially as collection occurs in slaughter houses not laboratories and the scientific problems still remain.
- 2. Serum Free media may need the addition of animal-sourced components, so why is this preferable to FCS?**

The collection of FCS inevitably involves animal suffering and death. Other animal-sourced components can be obtained by more humane procedures. However, the use of a chemically defined medium that is entirely free from animal-derived components is preferred.
- 3. What are the comparable costs of FCS and SF media?**

Serum-free media can be perceived as costly, but good quality FCS is also expensive and periodic shortages in supply occur. Each batch of FCS must be screened, and some batches are rejected, adding further to costs. Cost-effective large-scale culture for the production of biopharmaceuticals has helped to drive the conversion to serum-free media.
- 4. How do I convert to serum-free media?**

Cells need to be adapted to growing in serum-free media by a gradual weaning process. Companies selling serum-free media can provide guidance and advice on this process. The initial effort involved in switching is recouped by the improved consistency and quality of results. Some cell culture suppliers can now offer a range of cell lines already adapted to serum-free media.
- 5. Will my cells be compatible with serum-free media?**

The range of cell types compatible with serum-free culture is large and increasing. FoA's serum-free media resource offers an index of cell types supported by the commercially available serum-free media. It is worth checking directly with companies for the latest advice on the compatibility of their products.
- 6. Will my cells behave differently in serum-free media?**

Changes in culture conditions may affect how cells behave, so it will be necessary to monitor cellular functions during the weaning to serum-free. Serum-free conditions can result in improvements, such as better growth of cells, at higher densities and higher yields of end products. Manufacturers and suppliers can offer advice on their own products and feedback on the experiences of other users.
- 7. Will serum-free media help to standardise my procedures?**

Absolutely. Once cells have been adapted to serum-free media, researchers can benefit from improved control over culture conditions, elimination of contaminant interference, improved reproducibility and consistency of results.
- 8. Where can I find published literature on using serum-free media?**

Currently there is limited published literature relating to the use of serum-free media. It would be helpful if more scientists reported and published their experiences with serum-free cell culture. FoA is seeking to gather evidence from researchers of their experiences, both positive and negative, with serum-free media via a questionnaire, with the intention of publication. Please contact nirmala@frame.org.uk for a copy of the questionnaire or visit the website above.