



Utrecht University

# ***Foetal Bovine Serum Replacement***

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## Reasons to replace Fetal Bovine Serum

### *Animal welfare*

**Fetuses are likely exposed to pain and/or discomfort and therefore current practice of fetal blood harvest is inhumane.**

**Do we have evidence?**

**Animal deserves the benefit of the doubt!**

*Jochems et al., (2002) ATLA 30, 219-227*



## Reasons to replace Fetal Bovine Serum

### *Fraud 1*

*Jochems et al., 2002*

<https://doi.org/10.1177/026119290203000208>

Since scientists would like 'clean' FBS, New Zealand sourced FBS has preference.

There, is at least, twice as much NZ serum on the market than is produced there.



# Reasons to replace Fetal Bovine Serum

## *Fraud 2*

Since the demand is greater than the supply, prices have gone up by 300%.

ATLA 42, 207–209, 2014

Gstraunthaler, Lindl, van der Valk, 2014

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### **Comment**

*“These products may contain added adult bovine serum albumin (BSA) of United States origin, water, and/or cell growth promoting additives. For*

In 2011, GE Healthcare (a unit of General Electric Co.) acquired PAA Laboratories, Linz, Austria. In April 2013, GE Healthcare published a product information to customers, stating that batches of fetal bovine serum (FBS) produced at PAA facilities from March 2008 to March 2013 are subject to label non-conformances, i.e. that:

From this, it can be concluded that the use of serum in cell culture may involve a number of disadvantages: a) serum in general is an ill-defined supplement in culture media, with high qualitative and quantitative, geographical and seasonal batch-to-batch variations; b) FBS may contain adverse factors, like endotoxins, mycoplasma, viral contaminants or prion proteins; c) there are animal

# Reasons to replace Fetal Bovine Serum

## *Scientific problems*

*Van der Valk et al, 2010*

- Composition of FBS unknown
- Qualitative and quantitative variations between serum batches
- May contain different amounts of endotoxins, haemoglobin and other adverse factors
- May be contaminated with viruses, bacteria, fungi, mycoplasmas and prions



Reproducibility of experiments  
Safety of products

# Conclusions

*Van der Valk et al, 2010*

When considering supplementing cell and tissue culture media with animal serum the **“Not, unless....”** principle should be applied.

Preferentially, the medium should **not** contain any *animal-derived* component, unless it was proved to be an absolute requirement.



*Replacing FBS*



ELSEVIER

Review

Optimization of fetal bovine serum

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## Consensus Report

# Fetal Bovine Serum (FBS): Past – Present – Future

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### Summary

The supplementation of culture medium with fetal bovine serum (FBS, also referred to as ‘fetal calf serum’) is still common practice in cell culture applications. Due to a number of disadvantages in terms of quality and reproducibility of *in vitro* data, animal welfare concerns, and in light of recent cases of fraudulent marketing, the search for alternatives and the development of serum-free medium formulations gained global attention. Here, we report on the 3<sup>rd</sup> Workshop on FBS, Serum Alternatives and Serum-free Media, where (a) regulatory aspects, (b) the serum dilemma, (c) alternatives to FBS, (d) case-studies of serum-free *in vitro* applications, and (e) the establishment of serum-free databases, were discussed.

The whole process of obtaining blood from a living calf fetus to using the FBS produced from it for scientific purposes is *de facto* not yet legally regulated, despite the existing EU-Directive 2010/63/EU on the use of animals for scientific purposes. Together with above mentioned challenges, several strategies have been developed to reduce or replace FBS in cell culture media in terms of the 3Rs (Refinement, Reduction, Replacement). Most recently, releasates of activated human donor thrombocytes (human platelet lysates) have been shown to be one of the most promising serum alternatives when chemically defined media are not yet an option. Additionally, new developments in cell-based assay techniques, advanced organ-on-chip and microphysiological systems are covered in this report. Chemically-defined serum-free media are shown to be the ultimate goal for the majority of culture systems, and examples are discussed.

ALTEX Online first  
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# Serum-free media

*Van der Valk et al, 2010*

## Serum-free media

animal/human tissue or plant extracts

## Protein-free media

peptide fractions. Not defined.

## Animal-derived component free

plant, bacteria or yeast components

## Chemically defined

fully defined

# Human platelet lysates (hpl's)

*Van der Valk et al, 2010 and  
Rauch et al., 2011*

- Growth factors
- Expired donated human blood
- Obtained by freeze/thawing
- Safe /clinically tested, high quality
- Human based xeno-free system
- Universal application



Photo by [LuAnn Hunt](#) on [Unsplash](#)

# Chemically-defined medium

*Van der Valk et al, 2010*

## Advantages:

- Chemically defined and controlled
- Low qualitative and quantitative variability
- Simplified isolation of (synthetic) products/metabolites
- Avoids animal use
- *As yet, no universal chemically-defined medium*
- *Selective for specific cell types*

# Chemically-defined medium

At least 530 formulations available for 260 cell types.

- Commercially available
- Modifications of (commercially) available media
- Formulation available from literature

*Many cell types have not yet a chemically-defined medium.*

# Commercially available supplements

- Limited information on ingredients
- Change of composition without notice
- Expensive

*Developing your own serum-free medium*

# Developing your own serum-free medium

*Van der Valk et al, 2010*

## *1. Basal medium*

- 50:50 (v/v) mixture of DMEM and Ham's nutrient mixture F-12
- ITS supplement (insulin, transferrin and selenium)

# Developing your own serum-free medium

## 2. *Supplements*

- *Hormones*
- *Growth factors*
- *Protease inhibitors*
- *Protein hydrolysates*
- *Shear force protectors*
- *Proteins*
- *Vitamins*
- *Amino acids*
- *Glutamine*
- *Trace elements*
- *Lipids*
- *Antibiotics*
- *Attachment factors*
- *Osmolarity*

*Van der Valk et al, 2010*





# Factorial design approach

## **Development of a serum-free medium using computer-assisted factorial design and analysis**

Lao, MS. & Schalla, C. Cytotechnology (1996) 22: 25. <https://doi.org/10.1007/BF00353921>

## **Use of real-time cellular analysis and Plackett-Burman design to develop the serum-free media for PC-3 prostate cancer cells**

Zhao et al, *PLOS one* September 25, 2017, <https://doi.org/10.1371/journal.pone.0185470>

**“In summary, this high-throughput scheme minimized the screening time and may thus provide a new platform to efficiently develop the serum-free media for adherent cells”.**

# Factorial design approach

This statistical method (*factorial experimental design and analysis*), aided with a commercially available specialized computer program,

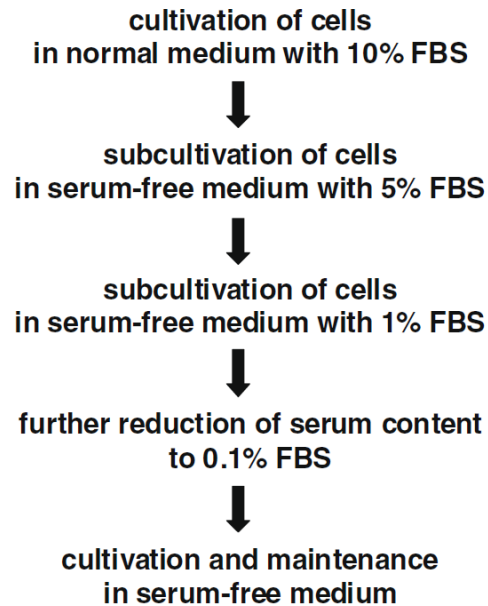
- enables the systematic study of complex components,
- identifies significant factors,
- and, most importantly, their interactions.

*Lao, MS. & Schalla, C. Cytotechnology (1996) 22: 25. <https://doi.org/10.1007/BF00353921>*

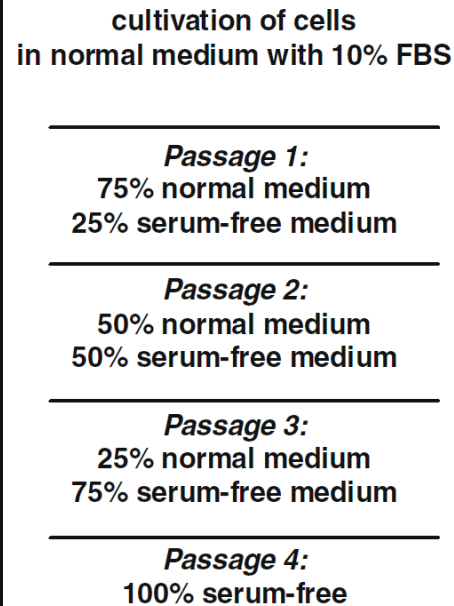
# Cell adaptation

*Van der Valk et al, 2010*

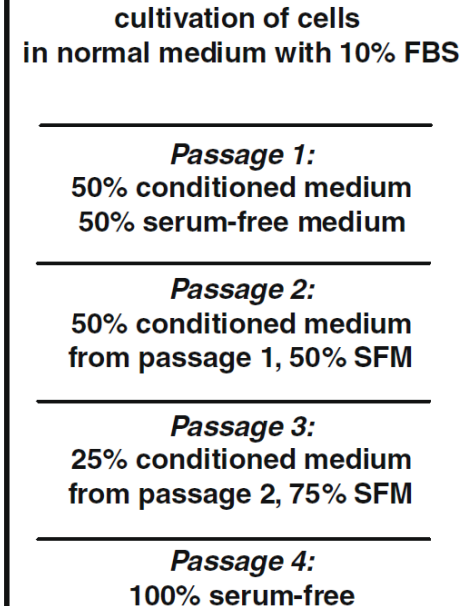
## 1. reduction of serum content



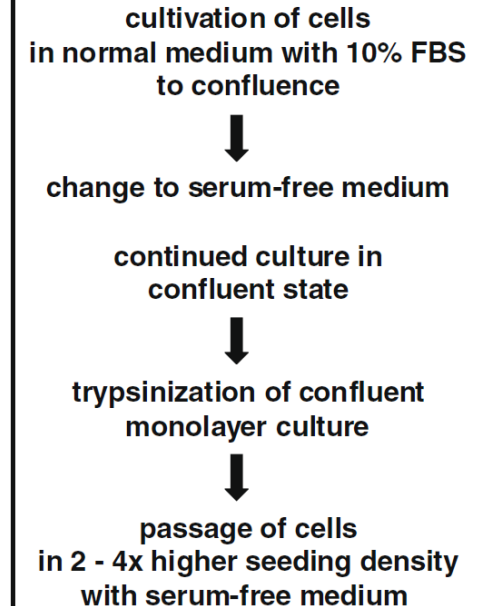
## 2. sequential adaptation



## 3. adaptation with conditioned medium



## 4. „inside“ adaptation



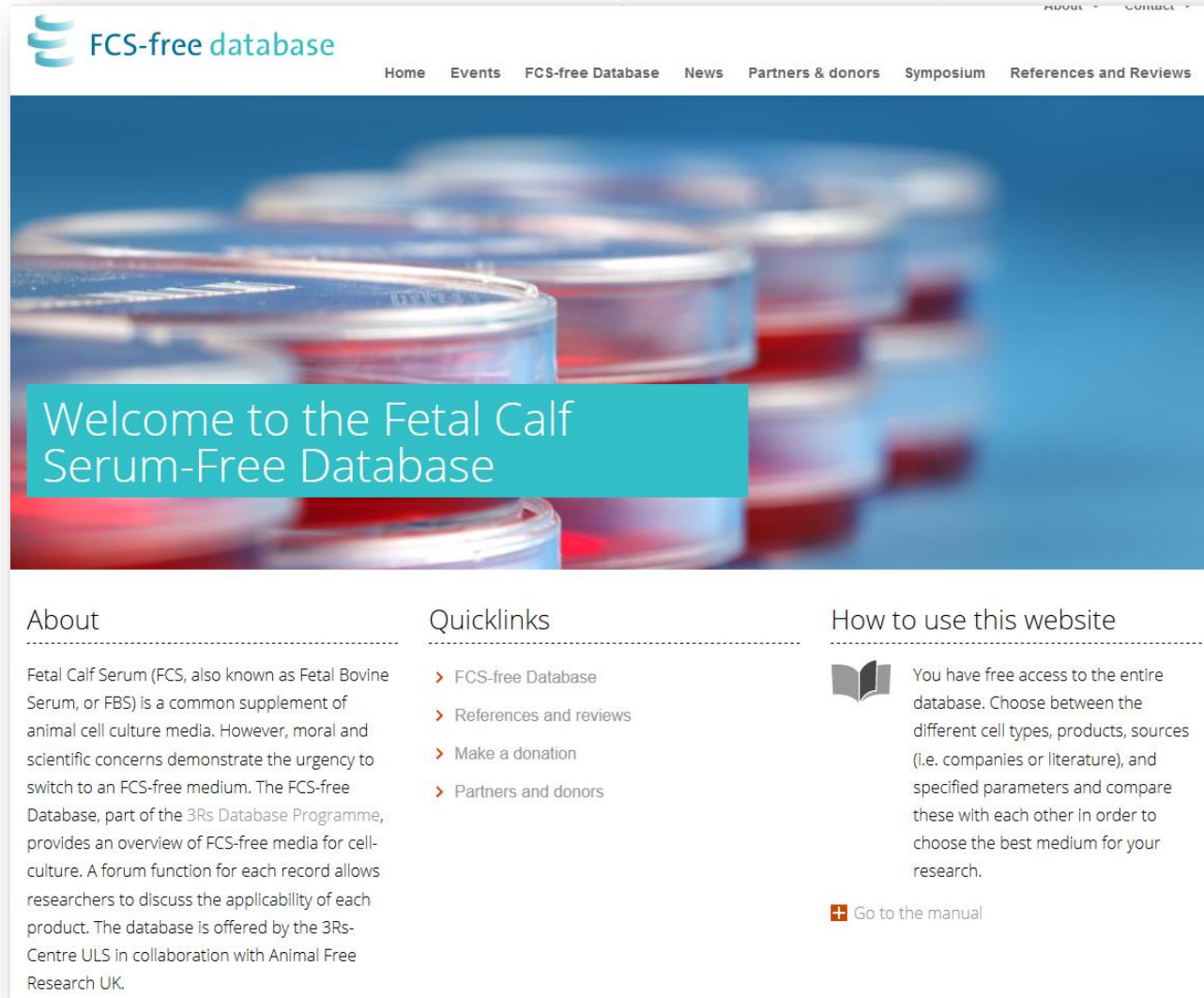
*Identification of available serum-free media*

# FCS-free database

*fcs-free.org*

260 different cell types.

531 different media



The screenshot shows the homepage of the FCS-free database website. The header features the logo and navigation links. A large banner image of petri dishes with red media is at the top. Below the banner, there are three columns of content: 'About', 'Quicklinks', and 'How to use this website'.

**FCS-free database**

Home Events FCS-free Database News Partners & donors Symposium References and Reviews

## Welcome to the Fetal Calf Serum-Free Database


### About


Fetal Calf Serum (FCS, also known as Fetal Bovine Serum, or FBS) is a common supplement of animal cell culture media. However, moral and scientific concerns demonstrate the urgency to switch to an FCS-free medium. The FCS-free Database, part of the 3Rs Database Programme, provides an overview of FCS-free media for cell-culture. A forum function for each record allows researchers to discuss the applicability of each product. The database is offered by the 3Rs-Centre ULS in collaboration with Animal Free Research UK.

### Quicklinks

- FCS-free Database
- References and reviews
- Make a donation
- Partners and donors

### How to use this website

 You have free access to the entire database. Choose between the different cell types, products, sources (i.e. companies or literature), and specified parameters and compare these with each other in order to choose the best medium for your research.

 [Go to the manual](#)

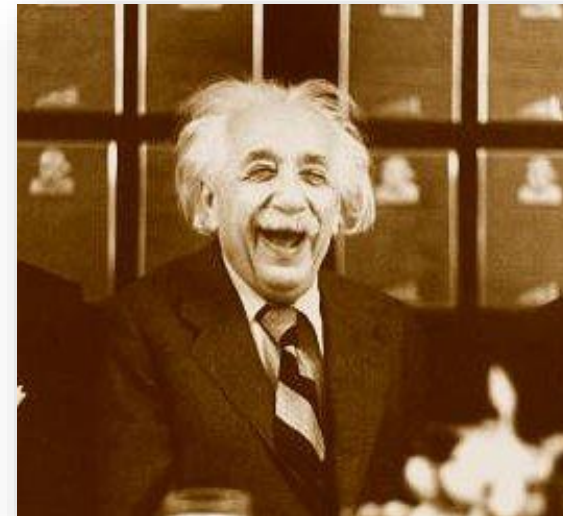
## Summary

- **Animal welfare is possibly at stake when fetal calf blood is collected for production of FBS**
- **Fraudulous practices**
- **Scientific problems**
- **Preference for chemically-defined media**
- **Serum-free database ([fcs-free.org](http://fcs-free.org))**

# Final conclusion

Serum free media are:

- *Better for the animals*
- *Better for research*



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