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Serum-free media contribute to better reproducibility in in vitro research

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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

Toxicology in Vitro 24 (2010) 1053–1063

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Review

Optimization of chemically defined cell culture media – Replacing fetal bovine serum in mammalian *in vitro* methods

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ABSTRACT

Quality assurance is becoming increasingly important. Good laboratory practice (GLP) and good manufacturing practice (GMP) are now established standards. The biomedical field aims at an increasing reliance on the use of *in vitro* methods. Cell and tissue culture methods are generally fast, cheap, reproducible and reduce the use of experimental animals. Good cell culture practice (GCCP) is an attempt to develop a common standard for *in vitro* methods. The implementation of the use of chemically defined media is part of the GCCP. This will decrease the dependence on animal serum, a supplement with an undefined and variable composition. Defined media supplements are commercially available for some cell types. However, information on the formulation by the companies is often limited and such supplements can therefore not be regarded as completely defined. The development of defined media is difficult and often takes place in isolation. A workshop was organised in 2009 in Copenhagen to discuss strategies to improve the development and use of serum-free defined media. In this report, the results from the meeting are discussed and the formulation of a basic serum-free medium is suggested. Furthermore, recommendations are provided to improve information exchange on newly developed serum-free media.

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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 3rd 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.

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 (hPLs)

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

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

- But: *undefined – batch to batch differences*

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

- *Selective for specific cell types*

- *As yet, no universal chemically-defined medium*

Identification of available serum-free media

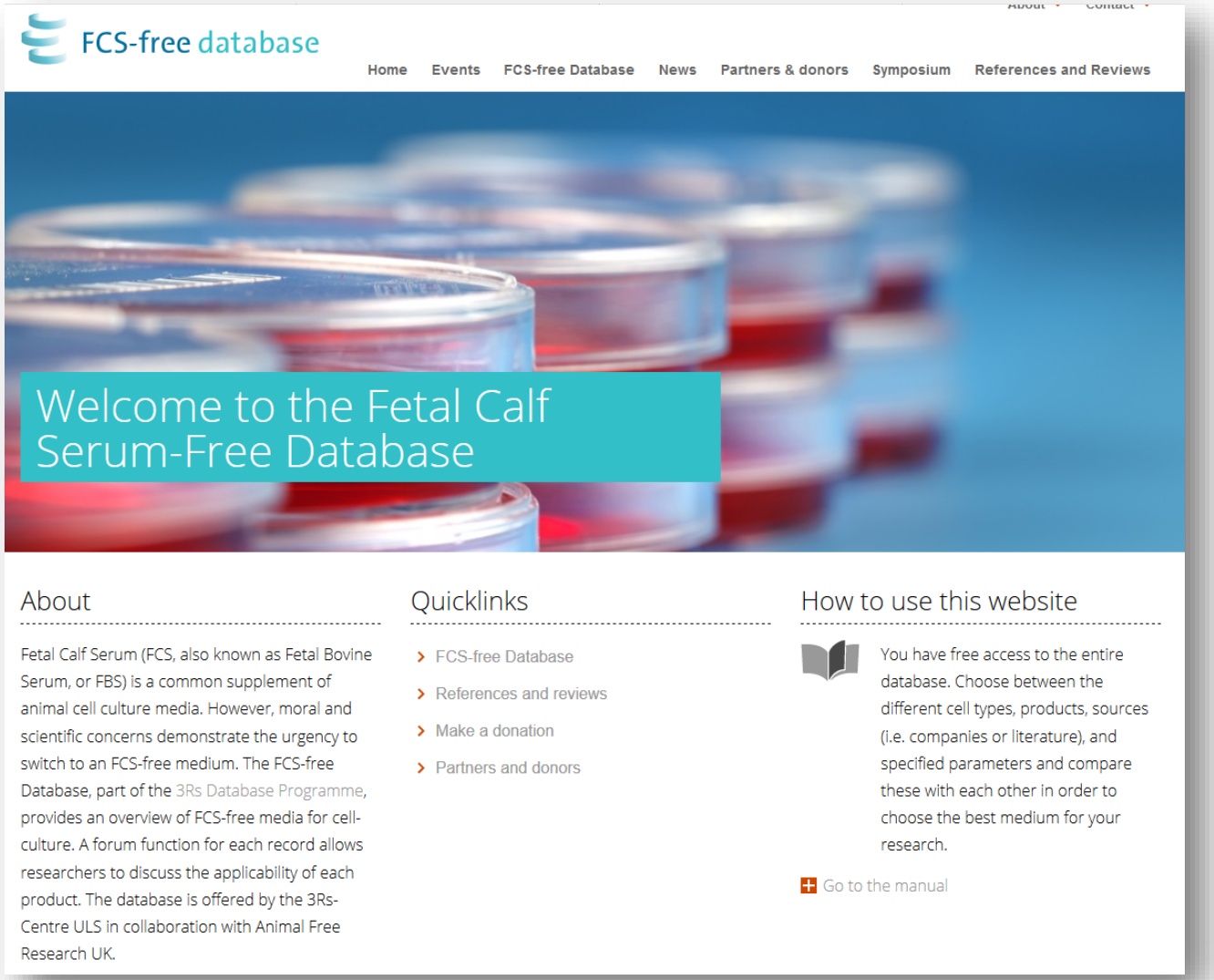
Photo by Annie Theby on Unsplash

FCS-free database

fcs-free.org

260 different cell types.

531 different media



FCS-free database

fcs-free.org

At least 530 formulations available for 260 cell types.

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

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

Source: Fcs-free.org

Issues with commercially available supplements

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

Developing your own serum-free medium

Photo by Louis Reed on Unsplash

Developing your own serum-free medium

1. Basal medium

Van der Valk et al, 2010

- 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*

Van der Valk et al, 2010

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

Design of Experiments (DoE)

The methodology of statistical design of experiments (DoE) was originally developed by Fisher (1926). DoE analysis is based on the variation of multiple components in the same experiment according to a design matrix, which allows identification of factor interactions and thereby enabling more efficient adjustment of culture medium additives.

Cytotechnology (2010) 62:557–571
DOI 10.1007/s10616-010-9307-8, Knöspel et al.

Developing your own serum-free medium

3. Factorial design approach

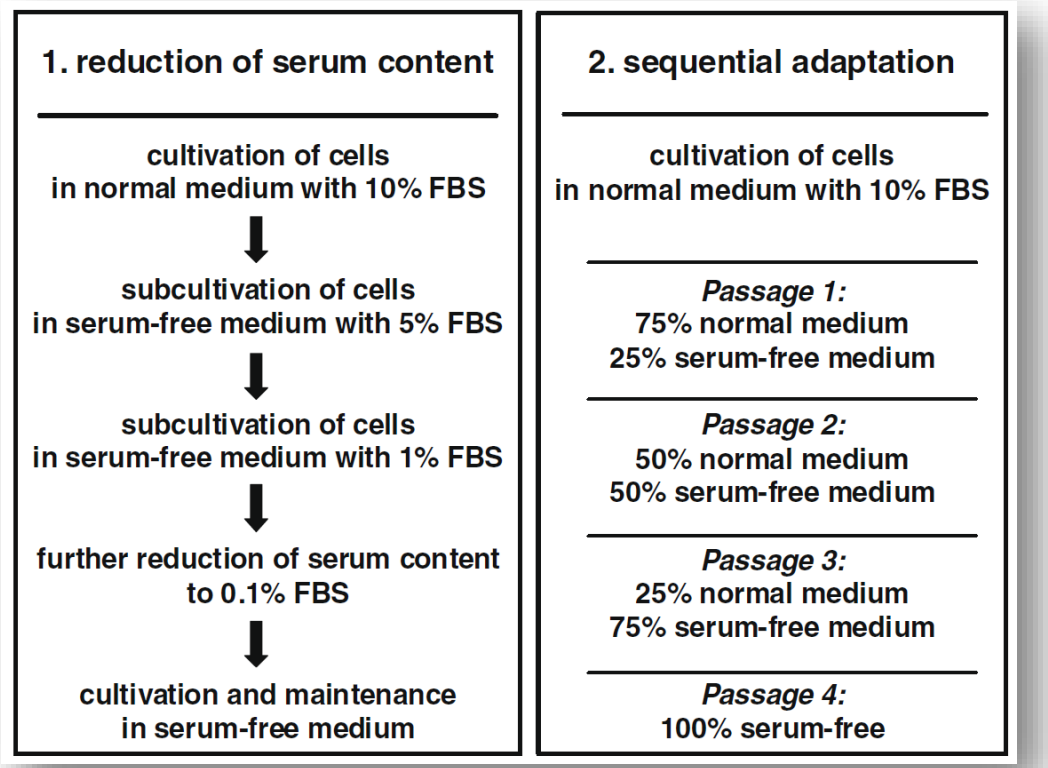
This statistical method *(factorial experimental design and analysis)*, aided with a commercially available specialized computer program,

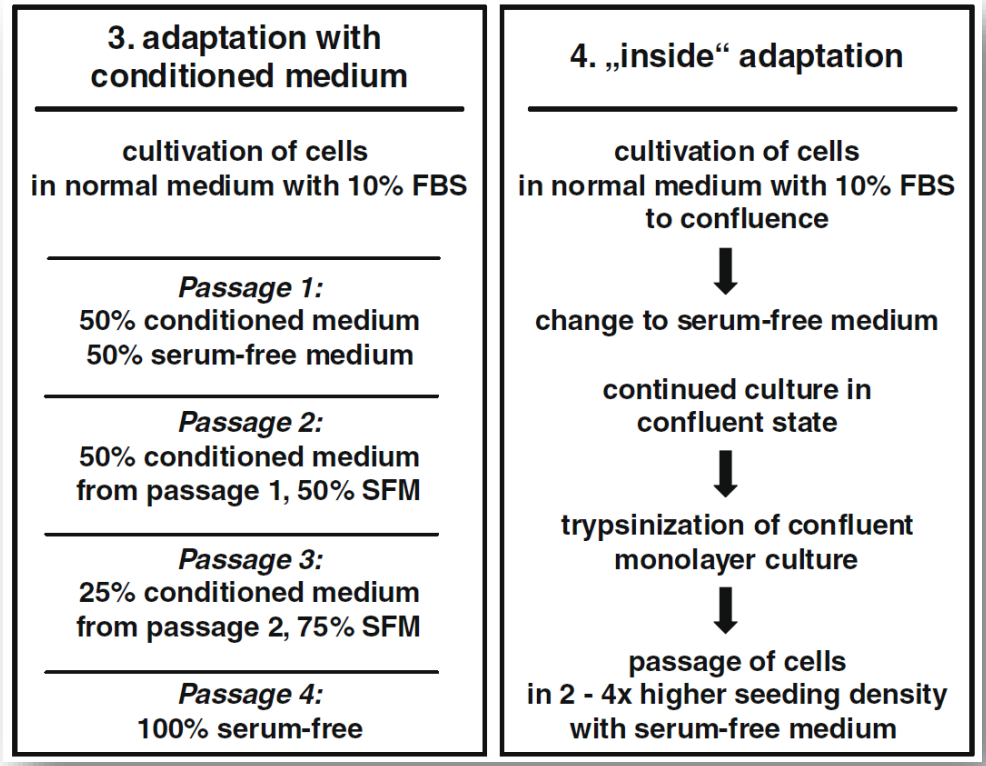
- enables the systematic study of complex components,
- identifies significant factors,
- and, most importantly, their interactions.

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

Developing your own serum-free medium
4. Cell adaptation

Van der Valk et al, 2010





Developing your own serum-free medium
4. Cell adaptation

Some cell lines support better the adaptation process. In some cases, it is possible to start the serum withdraw directly from 50% of serum-free medium (5% of serum supplementation).

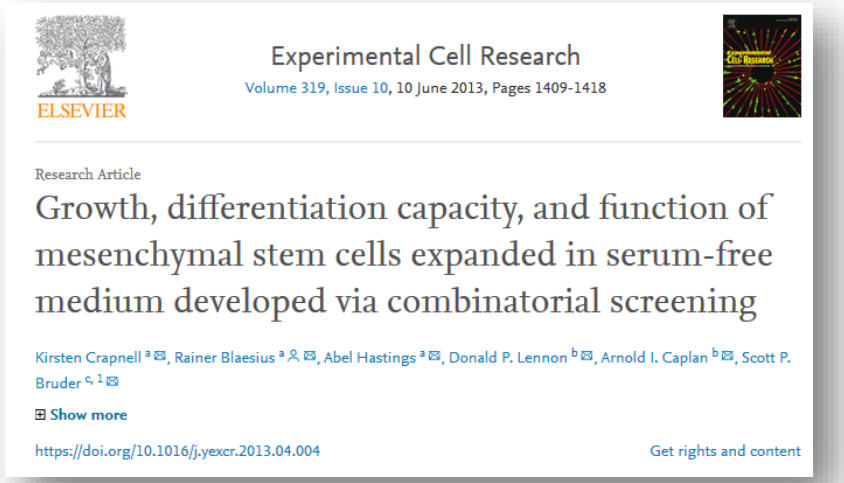
Caron et al. (2018) Strategies to Suspension Serum-Free Adaptation of Mammalian Cell Lines for Recombinant Glycoprotein Production. https://doi.org/10.1007/978-1-4939-7312-5_6

Developing your own serum-free medium

5. Share/publish your results

- Meetings
- Journals
- Fcs-free.org database

FCS-free database



ELSEVIER

Experimental Cell Research

Volume 319, Issue 10, 10 June 2013, Pages 1409-1418

Research Article

Growth, differentiation capacity, and function of mesenchymal stem cells expanded in serum-free medium developed via combinatorial screening

Kirsten Crapnell [a], Rainer Blaesius [a], Abel Hastings [a], Donald P. Lennon [b], Arnold I. Caplan [b], Scott P. Bruder [c, 1]

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https://doi.org/10.1016/j.yexcr.2013.04.004

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Summary

- Preference for chemically-defined media
- hPL's undefined, but universal application
- Serum-free database (fcs-free.org)
- Strategies to develop chemically-defined media

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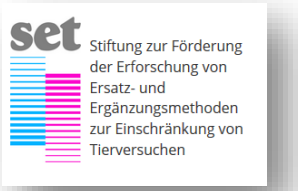

Thank You!

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