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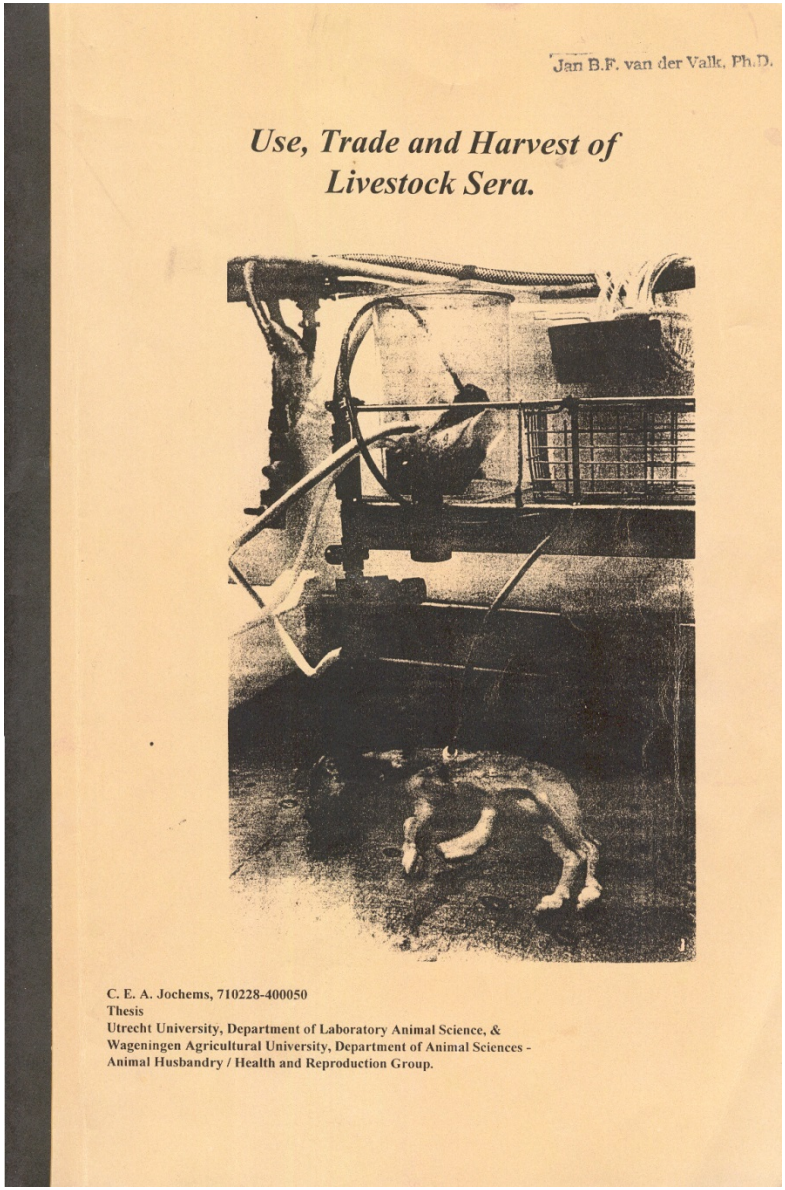
Serum-free Media and Serum Alternatives

Jan van der Valk,

3Rs-Centre ULS, DASS, Fac. Vet. Med, UU/ U-AIM



Use, Trade and Harvest of Livestock Sera.

C. E. A. Jochems, 710228-400050

Thesis, 1997

Utrecht University, Department of Laboratory Animal Science, &

Wageningen Agricultural University, Department of Animal Sciences -

Animal Husbandry / Health and Reproduction Group.

ATLA 30, 219-227

The use of fetal bovine serum: ethical or scientific problem?

Carlo E. A. Jochems[1], Jan B.F. van der Valk[2], Frans R. Stafleu[3] and Vera Baumans[1]

[1]Department of Laboratory Animal Science, Faculty of Veterinary Medicine, Utrecht University, PO Box 80166, NL-3508 TD Utrecht, The Netherlands; [2]Department of Animals and Society, Netherlands Centre Alternatives to Animal Use (NCA), Utrecht, The Netherlands; [3]Center for Bio-Ethics and Health Justice, Utrecht, The Netherlands.

Summary - Fetal bovine serum (FBS) is a common component of animal cell culture media. FBS is harvested from bovine fetuses taken from pregnant cows during slaughter. FBS is commonly harvested by means of a cardiac puncture without any form of anaesthesia. Fetuses are likely exposed to pain and/or discomfort and therefore current practice of fetal blood harvest is inhumane. Apart from moral concerns, several scientific and technical problems exist regarding the application of FBS in cell culture. Efforts should be made to reduce and preferably replace FBS by synthetic alternatives.

2002

Conclusions Jochems et al.

Policy makers should note that vertebrate fetuses can feel pain, and that different opinions exist amongst scientists as to the possible onset of pain experience in vertebrate fetuses. (…)

…….. a relevant guideline may be issued.

Conclusions Jochems et al.

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

possible onset of pain experience in vertebrate fetuses. (…)

…….. a relevant guideline may be issued.

Workshop “Towards Better In Vitro Methods, The Replacement of Fetal Bovine Serum”

Universiteit Utrecht

ELSEVIER

Toxicology in Vitro 18 (2004) 1–12

Toxicology in Vitro

www.elsevier.com/locate/toxinvit

Workshop report

The humane collection of fetal bovine serum and possibilities for serum-free cell and tissue culture

J. van der Valk[a,*], D. Mellor[b], R. Brands[c], R. Fischer[d], F. Gruber[e], G. Gstraunthaler[f], L. Hellebrekers[g], J. Hyllner[h], F.H. Jonker[i], P. Prieto[j], M. Thalen[k], V. Baumans[l,m]

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[j] *ECVAM, Institute for Health & Consumer Protection, Joint Research Centre, European Commission, 21020 Ispra (Va), Italy*

[k] *Laboratory for Process and Product Development, P.O. Box 457, Netherlands Vaccine Institute, Bilthoven, NL-3720 AL, The Netherlands*

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Abstract

Fetal bovine serum (FBS) is a common supplement to in vitro culture media. A workshop was organized to discuss whether or not fetuses might suffer when blood is withdrawn, and to discuss serum replacement methods. When bovine fetuses are exposed after slaughter of the dam, they can suffer only if they inflate their lungs with air and increase their blood oxygen to levels compatible with awareness. Preventing fetuses from breathing air or killing them by an efficient method, according to clearly defined safeguards, ensures that fetal blood collection is humane. Since serum is a supplement of unknown composition, which could be contaminated with unwanted factors, there are scientific and safety reasons for omitting FBS from culture media. Several media have been developed in which minimal or no animal derived components are present. Also, different cell types have been adapted to serum-free media. As yet, no standard serum free media are present, and each cell type requires its own medium composition. Among other recommendations, the establishment of a public database with information on cell types and their serum-free medium composition is proposed.

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Statement

on May 30th 2017 the EFSA published a scientific opinion on “Animal welfare aspects in respect of the slaughter or killing of pregnant livestock animals”:

http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2017.4782/full

“It is very likely to extremely likely (i.e. with 90–100% likelihood) that livestock fetuses in the last third of gestation have the anatomical and neurophysiological structures/correlates for experiencing pain and/or other forms of discomfort.

Recommendations to avoid foetal suffering

- Blood collection must not begin until at least 5 min after slaughter
- Fetus must not be allowed to breathe air after uterus removal
- Otherwise, fetus must be killed by stunning
- Fetal Calf Slaughter Welfare Protocol (Mellor & Gregory, 2003)

Van der Valk et al (2004) Toxicology In Vitro 18 (1), 1-12

In vitro method: alternative?

FCS should NOT be used in an *in vitro* method when that method is developed to replace experimental animals!!

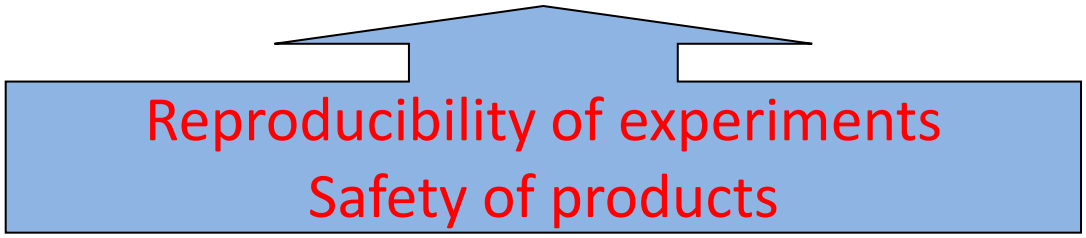
Legal consequences
Dir 2010/63/EU

- Procedure: (art. 3.1): *…any use, invasive or non-invasive, of an animal for experimental or other scientific purposes, with known or unknown outcome…*
- Experimental animals: art. 1.3.a *(ii) foetal forms of mammals as from the last third of their normal development*

Project review

Scientific problems

- 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, mycoplasms and prions



cellasys

The elephant in the room:

The use of FBS may be a driver for the reproducibility problem in biomedical science

www.forbes.com

Conclusions

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.

Van der Valk et al, 2009

"For methods forwarded to ECVAM for validation/prevalidation where [the use of nonanimal alternatives to FBS] is not fulfilled a justification for future use must be provided, including measures taken to seek non-animal alternatives to fetal calf serum"

(ECVAM Scientific Advisory Committee (ESAC), 2008)

OECD

Draft GUIDANCE DOCUMENT ON GOOD *IN VITRO* METHOD PRACTICES (GIVIMP) FOR THE DEVELOPMENT AND IMPLEMENTATION OF *IN VITRO* METHODS FOR REGULATORY USE IN HUMAN SAFETY ASSESSMENT

…it is recommended to develop new *in vitro* methods with a serum-free, chemically-defined medium, to avoid potential sources of uncertainty that may be introduced by using animal serum (Jochems *et al*., 2002; Pamies *et al*., 2016).

http://www.oecd.org/env/ehs/testing/OECD%20Final%20Draft%20GIVIMP.pdf

Development of (fc) serum-free media

Workshop Copenhagen

November 2009

Organisers

ESTIV
European Society of Toxicology *In Vitro*

INVITROM
Dutch-Belgian Society for in Vitro Methods

The Danish *In Vitro* Toxicology Network

Sponsors

DOERENKAMP-ZBINDEN Foundation

ZonMw

Landsforeningen Forsøgsdyrenes Værn

Toxicology in Vitro 24 (2010) 1053–1063

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

Review

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

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ARTICLE INFO

Article history:
Received 10 March 2010
Accepted 25 March 2010
Available online 31 March 2010

Keywords:
In vitro methods
Fetal bovine serum
Serum-free
Good cell culture practice
Tissue culture
3Rs

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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Serum free medium

Serum free media
animal/human tissue or plant extracts

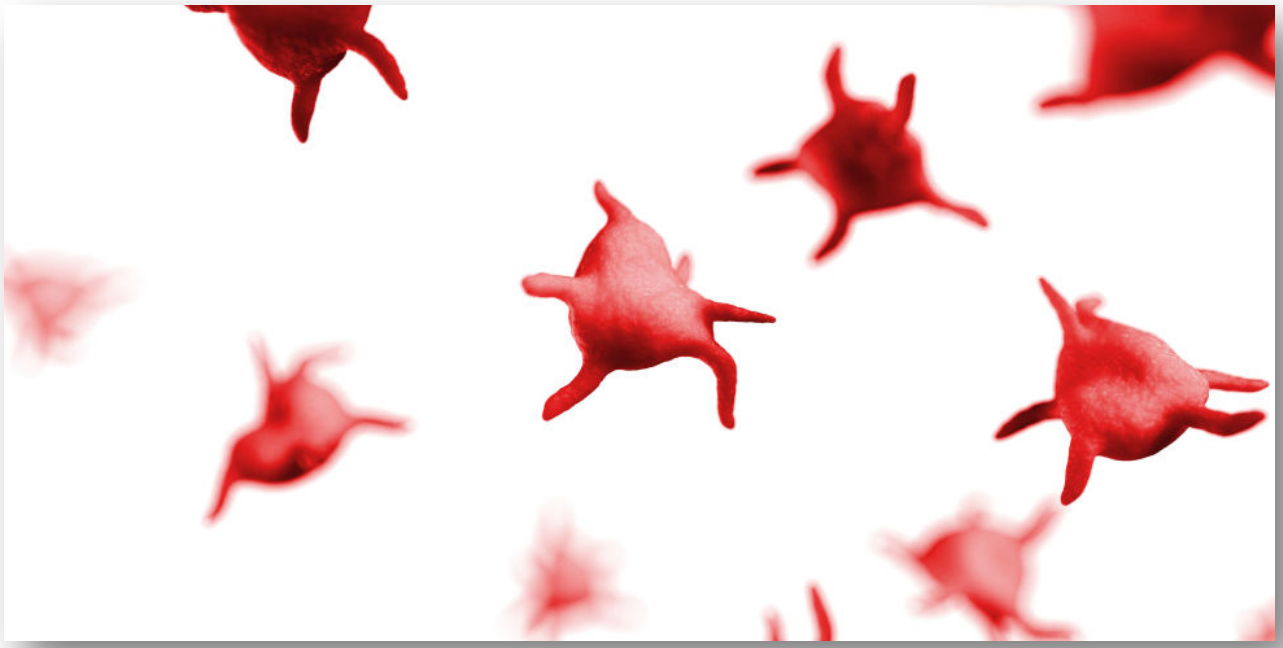
Protein free media
peptide fractions. Not defined.

Animal-derived component free
plant, bacteria or yeast components

Chemically defined
fully defined

Van der Valk et al, 2004

Platelet lysates



Chemically-defined media

Advantages of serum-free cell culture media

→ Chemically defined/controlled

→ Low qualitative and quantitative variability

→ Simplified isolation of synthetic products/metabolites

→ Avoids animal use (3R principles)

✓ Medium is selective for specific cell types.

but: not suitable for every cell type

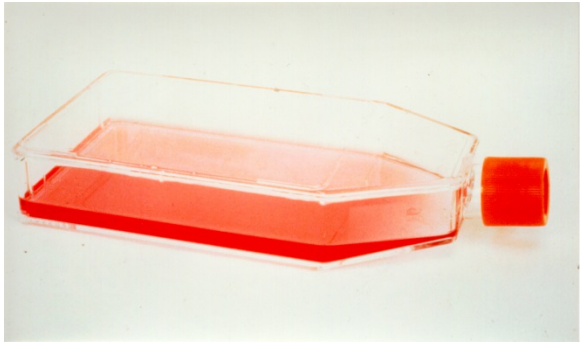
Commercially available supplements

- Limited information on ingredients
- Change of composition without notice
- Expensive
- Formulations may vary between suppliers
- Terminology: “xenogen-free," "animal-free", "serum free" and “chemically defined”

Basal Medium

- 50:50 (v/v) mixture of DMEM and Ham's nutrient mixture F-12

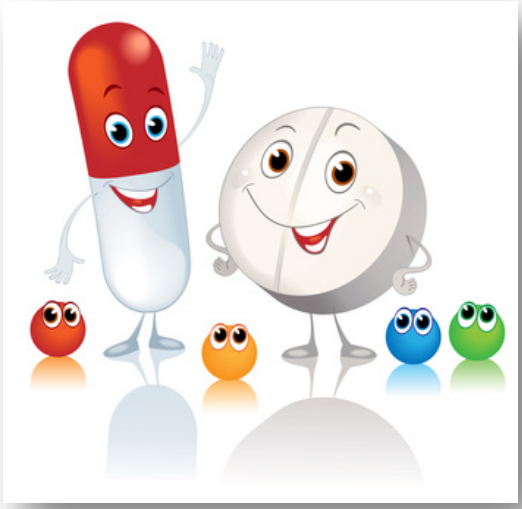
- ITS supplement (insulin, transferrin and selenium)



Van der Valk et al, 2009

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, 2009

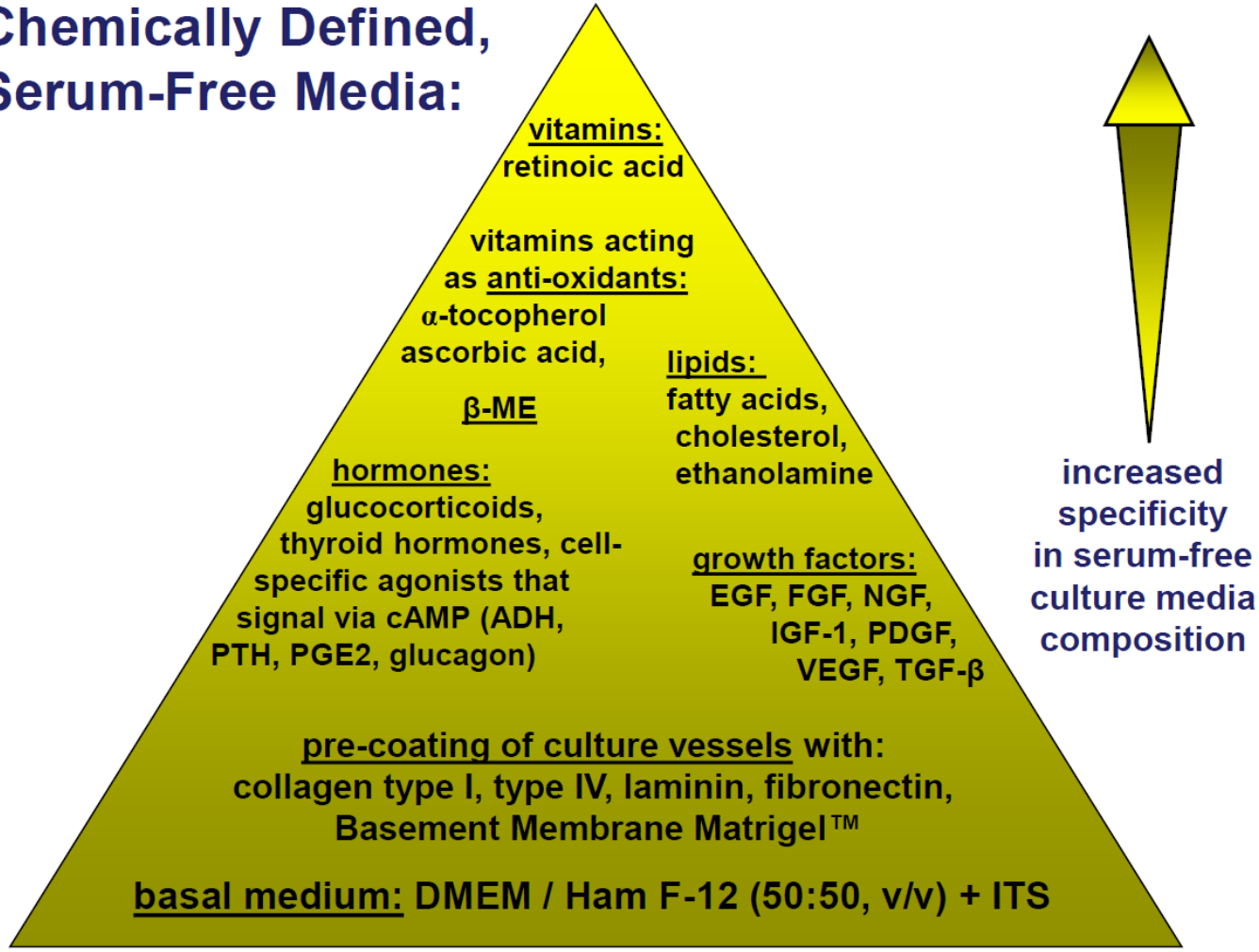
Factorial design

"Applying factorial design approaches have been shown to minimise the screening time, allow prediction for best medium formulation and can be used as a high throughput medium optimisation platform".

Plackett-Burman design, Response Surface Methodology

www.oecd.org/env/ehs/testing/OECD%20Final%20Draft%20GIVIMP.pdf

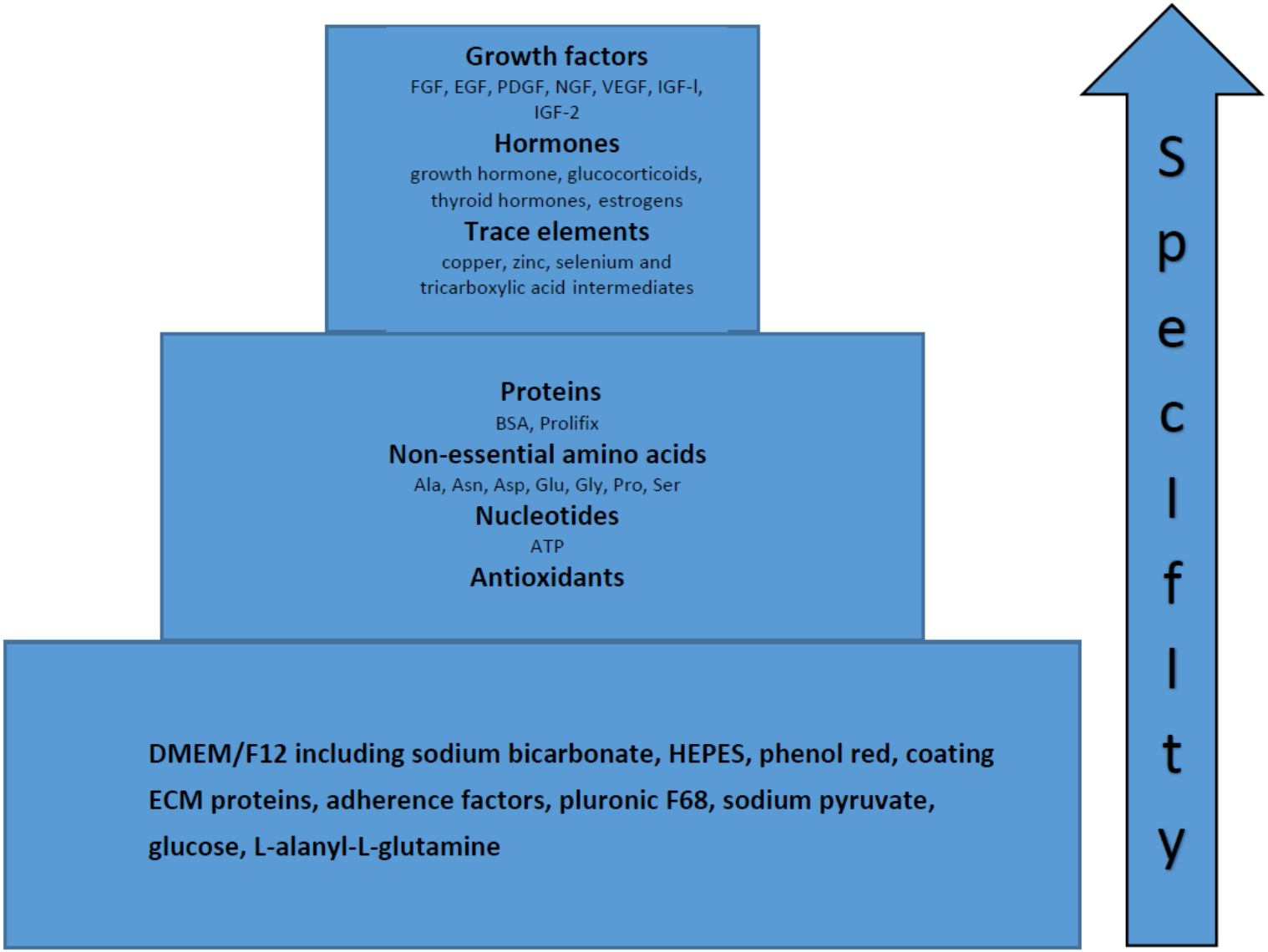
Chemically Defined, Serum-Free Media:



increased specificity in serum-free culture media composition

Gstraunthaler und Lindl, 2013

Fig 1. Schematic for developing serum free media



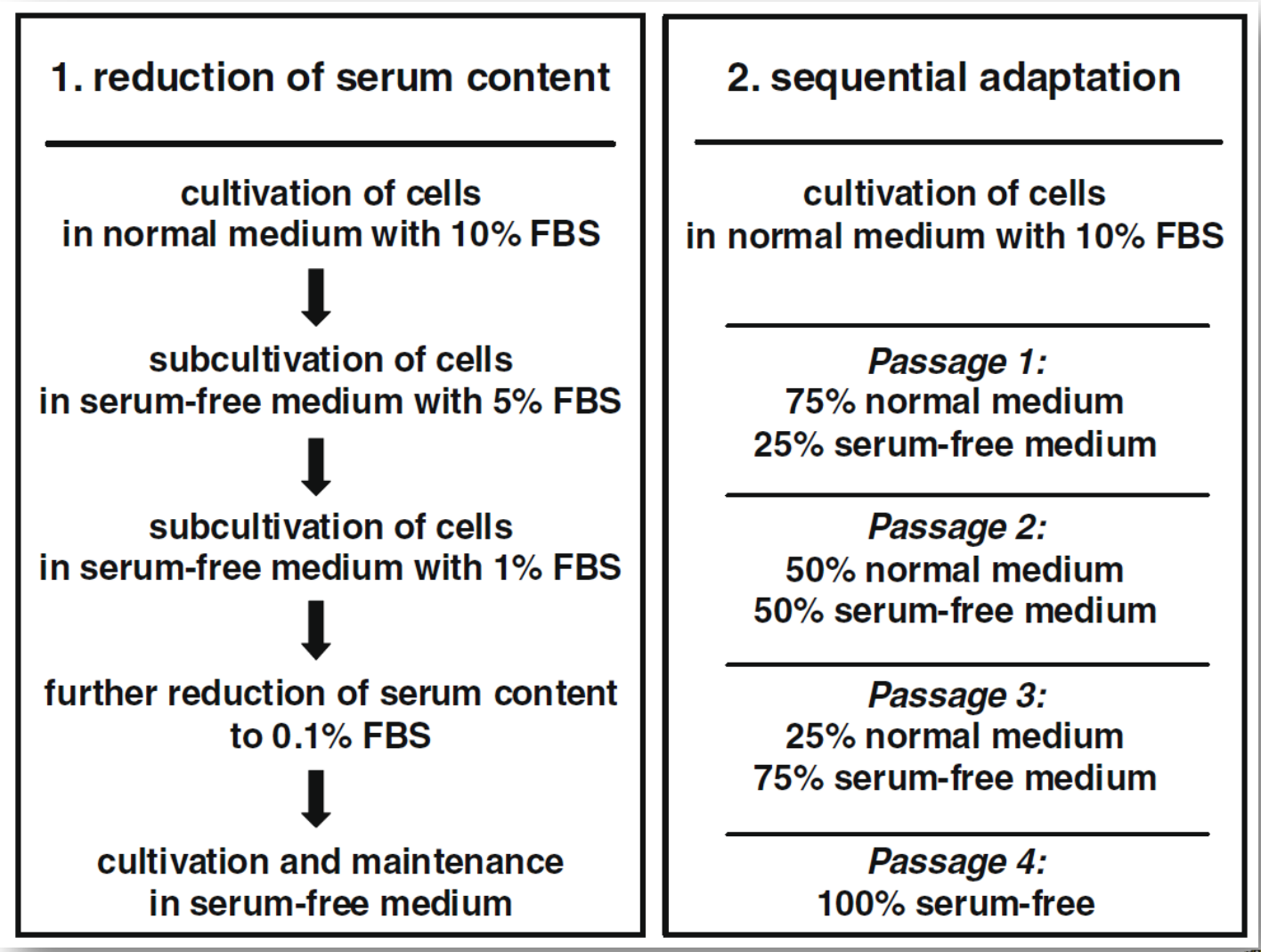
Expand pyramid

- Other media formulations
- Surface – pre-coating
- Fluidic systems

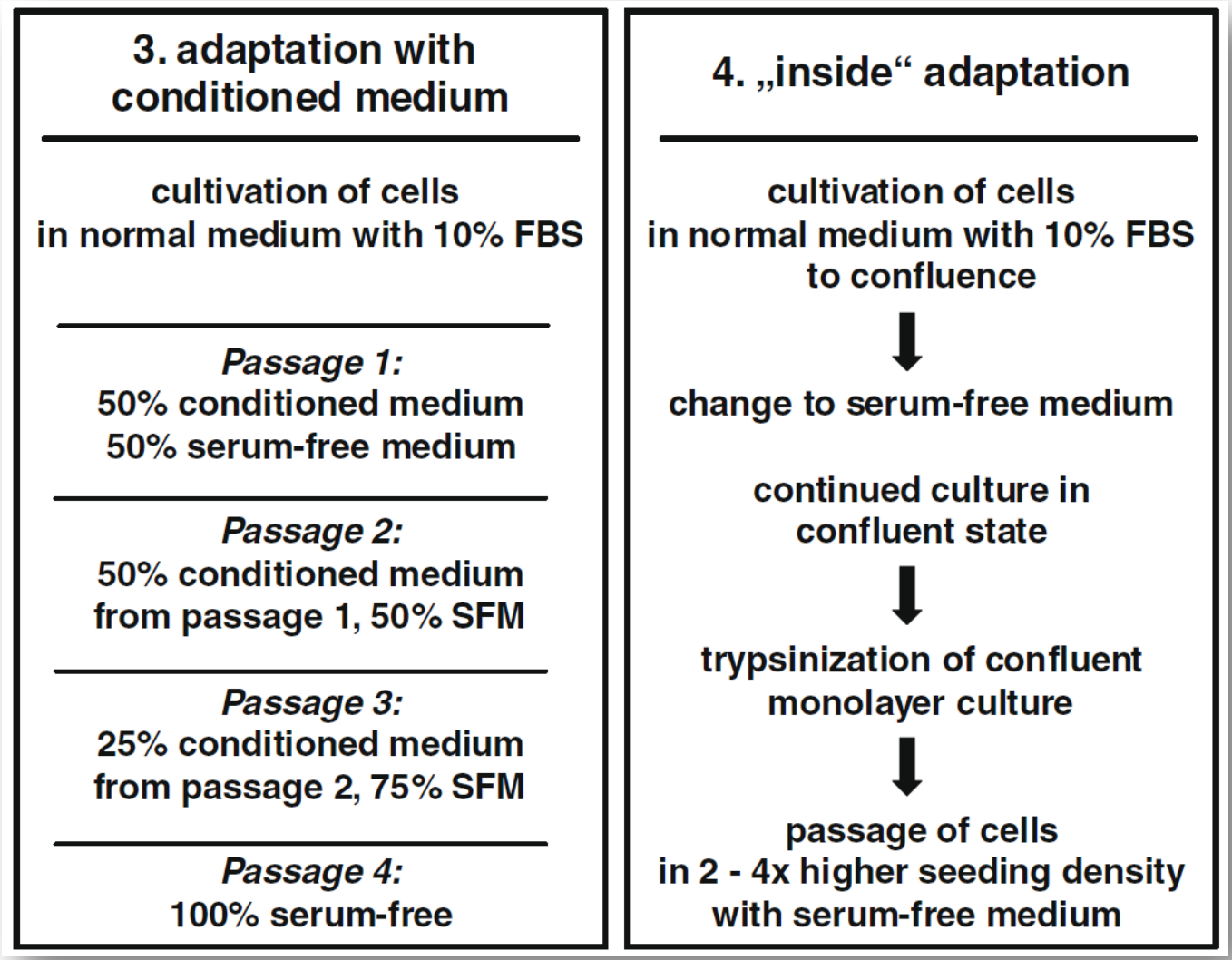
Media: scientific basis?

The numerous available (commercial) media of today are still based on the early experimental work of G. Sato. Little fundamental research, on the development of serum-free media and the analysis of serum components, has been done since.

Adaptation

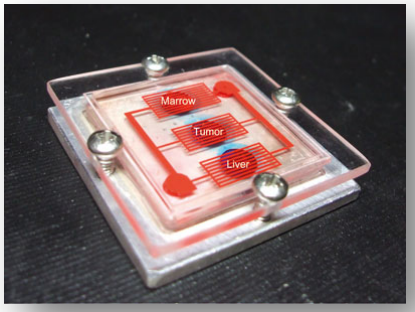

1. reduction of serum content
cultivation of cells
in normal medium with 10% FBS
subcultivation of cells
in serum-free medium with 5% FBS
subcultivation of cells
in serum-free medium with 1% FBS
further reduction of serum content
to 0.1% FBS
cultivation and maintenance
in serum-free medium
2. sequential adaptation
cultivation of cells
in normal medium with 10% FBS
Passage 1:
75% normal medium
25% serum-free medium
Passage 2:
50% normal medium
50% serum-free medium
Passage 3:
25% normal medium
75% serum-free medium
Passage 4:
100% serum-free

Adaptation


3. adaptation with conditioned medium
cultivation of cells in normal medium with 10% FBS
Passage 1:
50% conditioned medium
50% serum-free medium
Passage 2:
50% conditioned medium
from passage 1, 50% SFM
Passage 3:
25% conditioned medium
from passage 2, 75% SFM
Passage 4:
100% serum-free
4. „inside“ adaptation
cultivation of cells
in normal medium with 10% FBS
to confluence
change to serum-free medium
continued culture in
confluent state
trypsinization of confluent
monolayer culture
passage of cells
in 2 - 4x higher seeding density
with serum-free medium

The way forward

Each cell type has its own unique media supplement requirements to promote cell adhesion, differentiation, proliferation, and growth. One of the challenges in the development of a body-on-a-chip system is the establishment of a suitable universal medium that enables retention of cellular phenotype and function, and provides effective humoral communication between the multiple cell and tissue types.



Sung et al, 2014, Experimental Biology and Medicine, 0: 1–15

The way forward

The complexity of body-on-chip systems will continue to increase with the addition of more organ types and will increase the demand for a universal cell culture medium or system.

Sung et al, 2014, Experimental Biology and Medicine, 0: 1–15

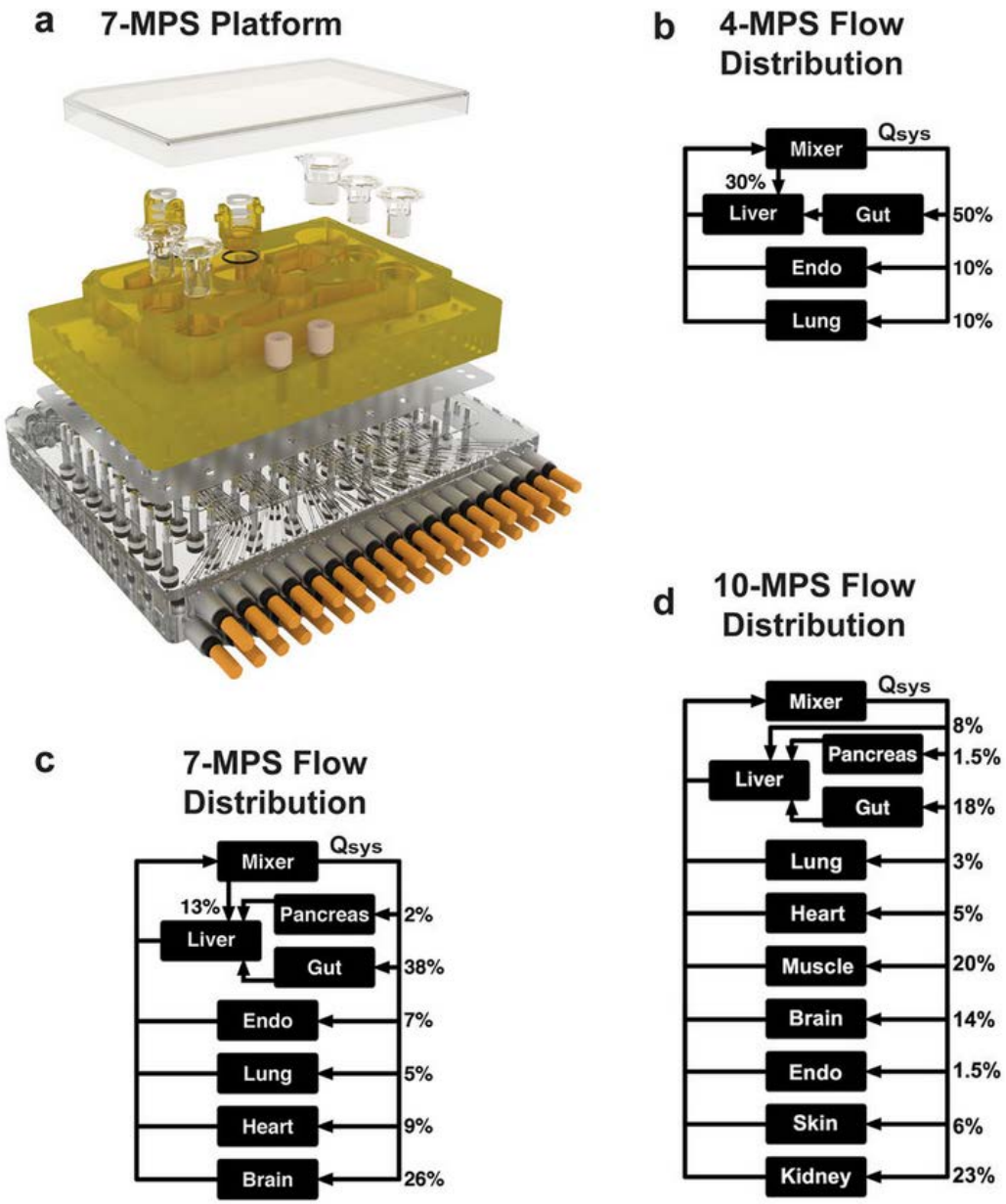
Multi-Organ toxicity demonstration in a functional human *in vitro* system composed of four organs

We report on a functional human model to evaluate multi-organ toxicity in a 4-organ system under continuous flow conditions in a serum-free defined medium utilizing a pumpless platform for 14 days.

*Oleaga et al, 2016, Scientific **Reports** | 6:20030*

Figure 2

From: Interconnected Microphysiological Systems for Quantitative Biology and Pharmacology Studies



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Artificial meat

- Using conventional culturing methods, but with relatively high serum concentrations of 300 mL L^{-1}, the cells divide in up to 50 population doublings over a period of a 7–8 weeks. Once sufficient cell numbers are obtained, they are divided in portions of 1.5 million cells and each batch is submerged in a collagen/Matrigel™ gel that is displayed in a culture dish around a central hub of agarose gel. Over the course of the following days, the cells will self-organize into a donut-shaped muscle fiber of 1 mm diameter. The tension that is developed in the ring structure by contraction of the muscle fiber is a strong stimulus for muscle maturation and protein production.[8] The muscle fiber is harvested after 3 weeks. For the 85 g hamburger that was presented, cooked and sampled on 5 August 2013 in London, we used 10 000 of these muscle strips.

http://onlinelibrary.wiley.com/doi/10.1002/jsfa.6474/full

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http://onlinelibrary.wiley.com/doi/10.1002/jsfa.6474/full, 2013

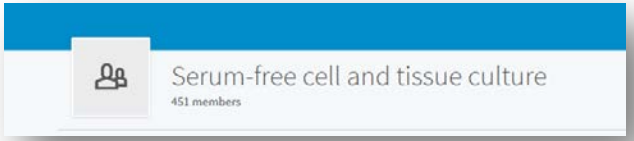
Elsewhere, the artists have estimated that growing 10 g of tissue would require ‘serum from a whole calf (500 ml), which is killed solely for the purpose of producing the serum’ (Catts and Zurr, 2008: 133)

Available resources for serum-free media

- **SEFREC**
  http://www.sefrec.com/

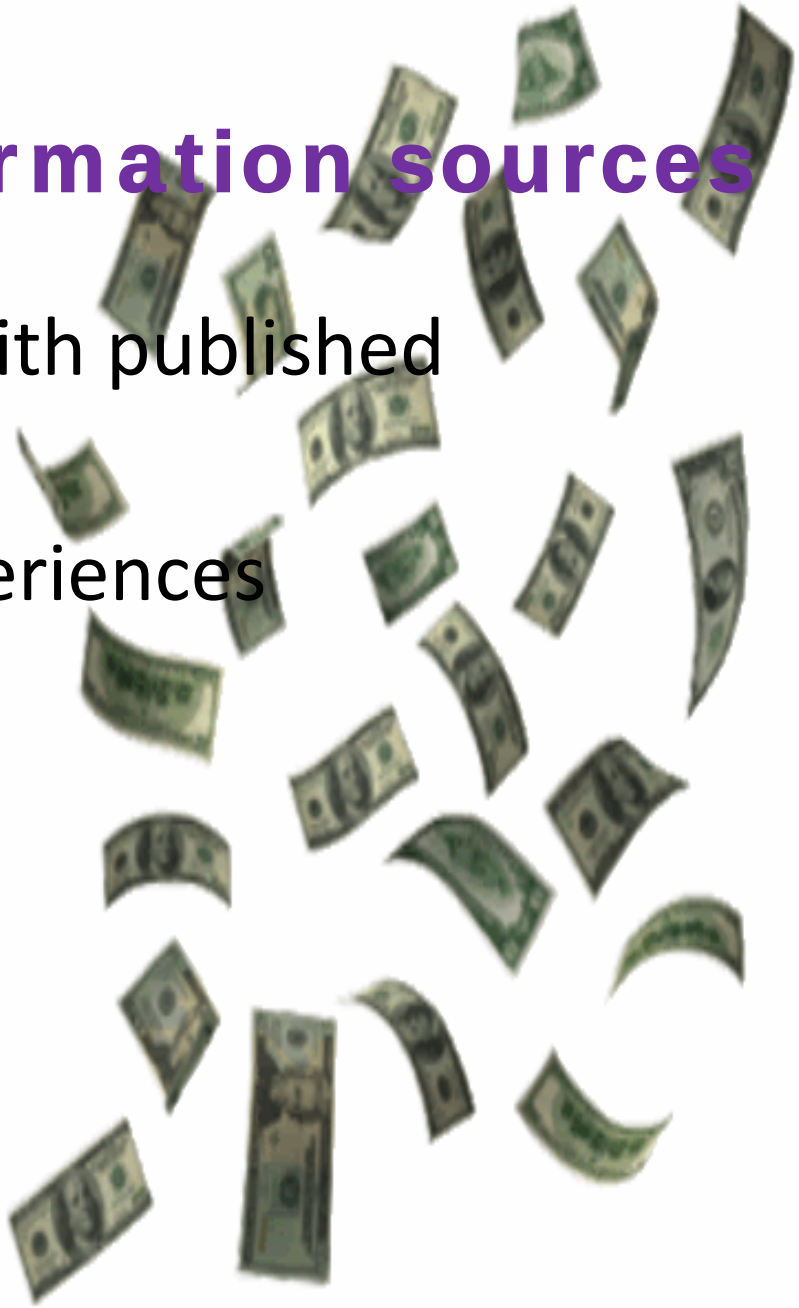
- **Dr Hadwen Trust**
  http://www.drhadwentrust.org/science-and-education/serum-free-media

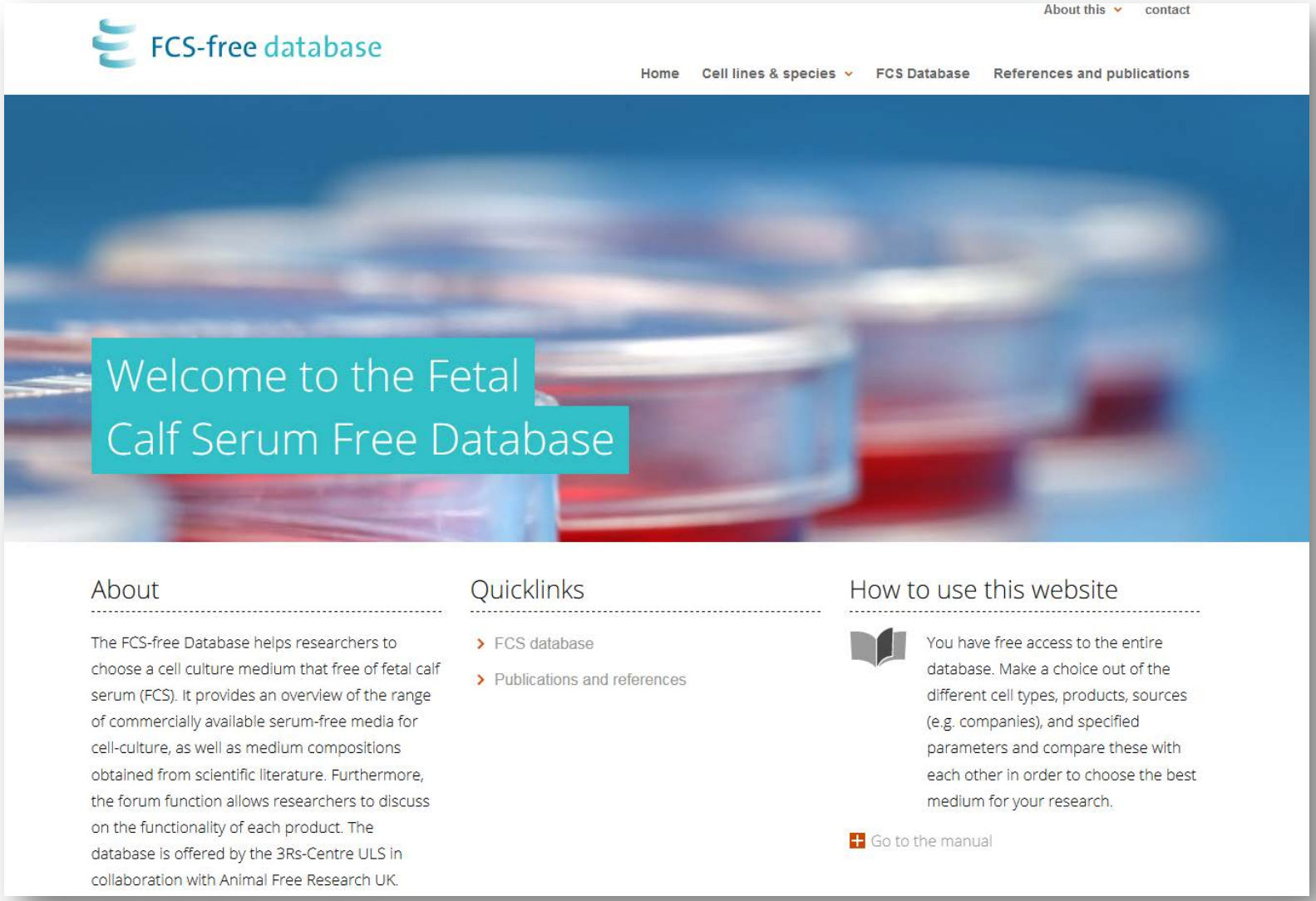
- **LinkedIn Group Serum-free cell and tissue culture**



Available information sources

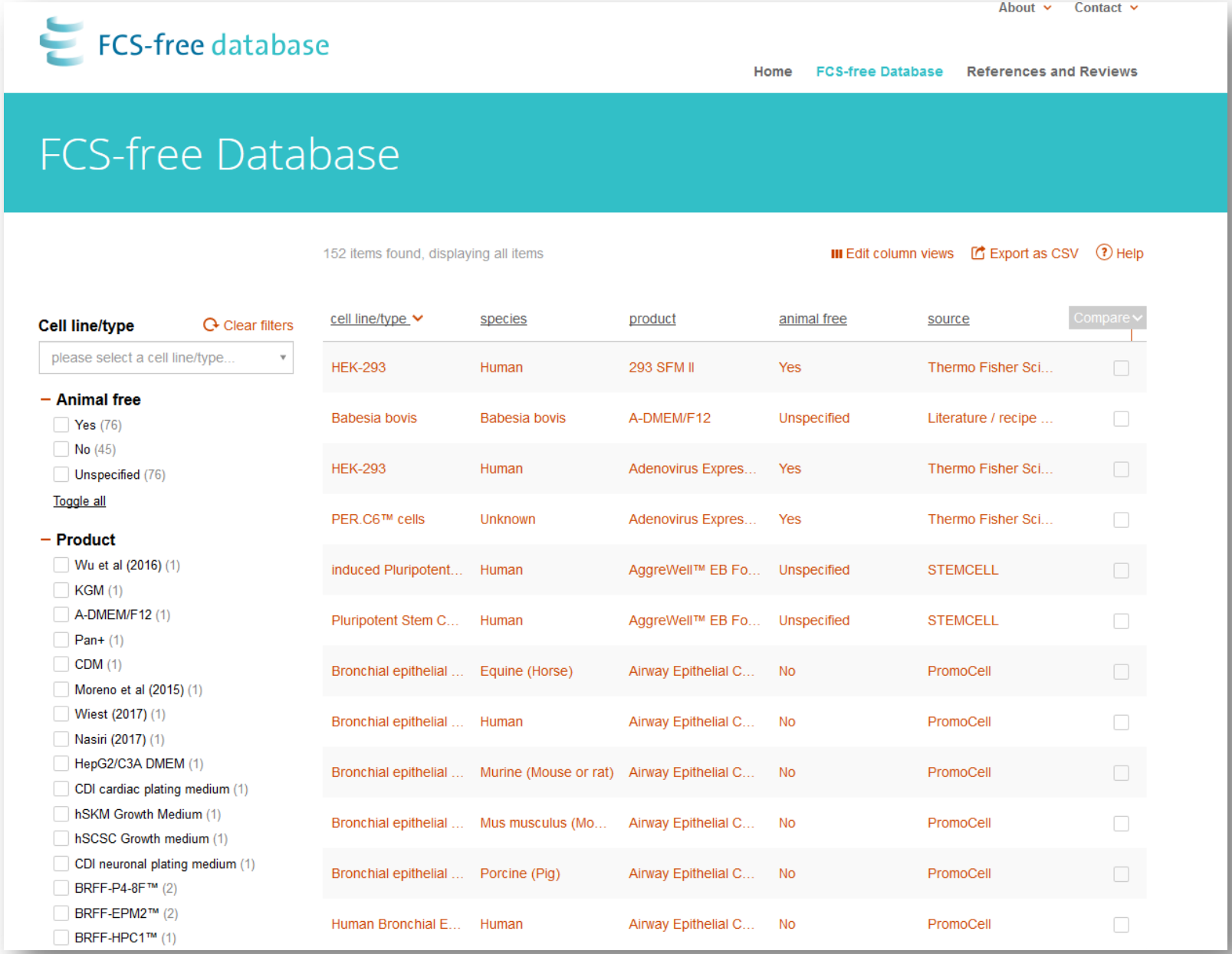
- Expand commercial with published information
- Expand with user experiences
- Free use
- Funding required





FCS-free.org

ANIMAL FREE RESEARCH UK



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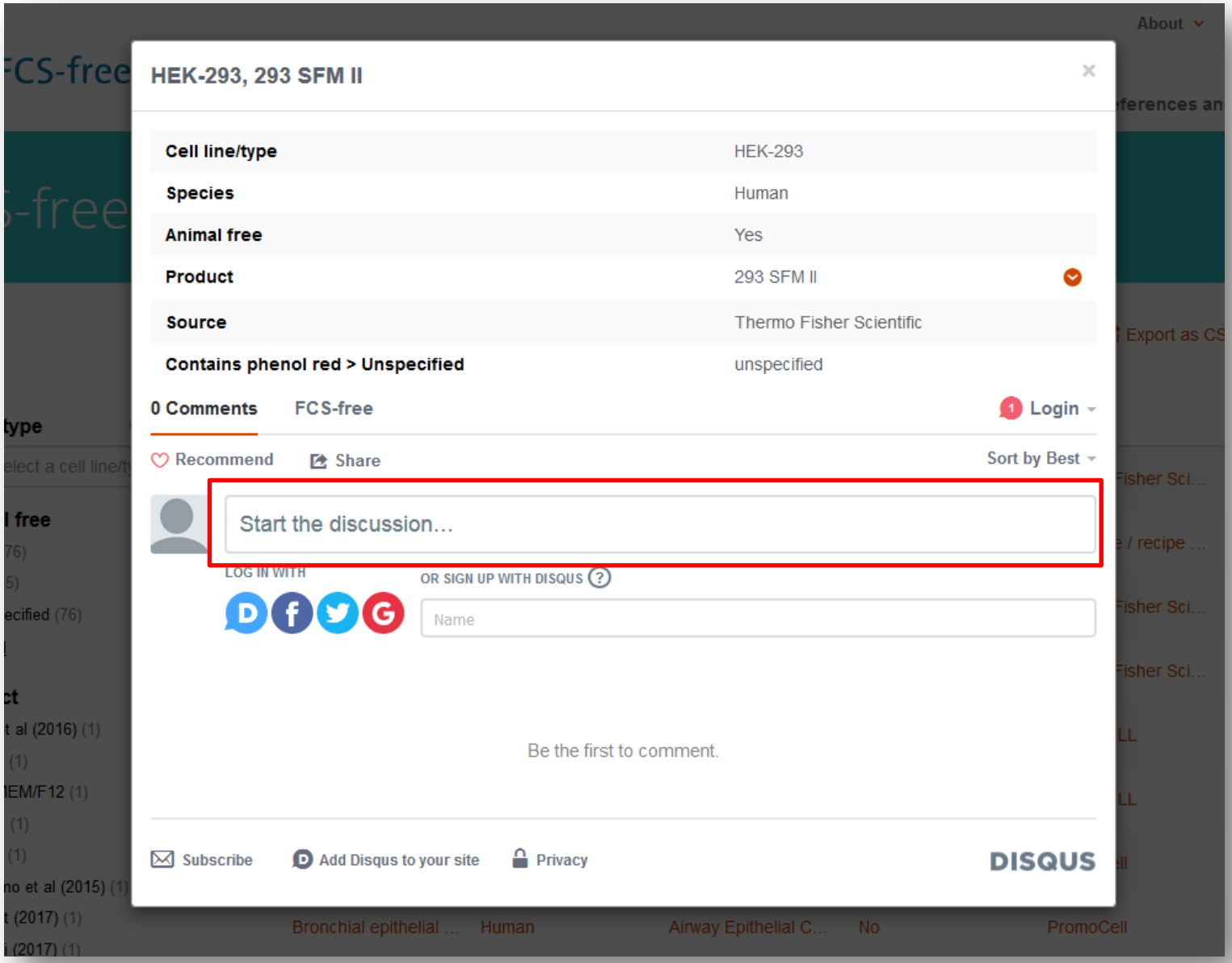
About
HEK-293, 293 SFM II
Cell line/type
HEK-293
Species
Human
Animal free
Yes
Product
293 SFM II
Source
Thermo Fisher Scientific
Contains phenol red > Unspecified
unspecified
0 Comments
FCS-free
Login
Recommend
Share
Sort by Best
Start the discussion…
LOG IN WITH
OR SIGN UP WITH DISQUS
Name
Be the first to comment.
Subscribe
Add Disqus to your site
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DISQUS

Actions

Raise awareness:

- scientists
- funding organisations
- regulatory authorities
- National Committees

- Complete database

Recommendations

1. Always check whether the performance of the cells has changed and whether the endpoints of the study are affected.
2. When successful, share your formulation with colleagues, and through existing cell culture databases.

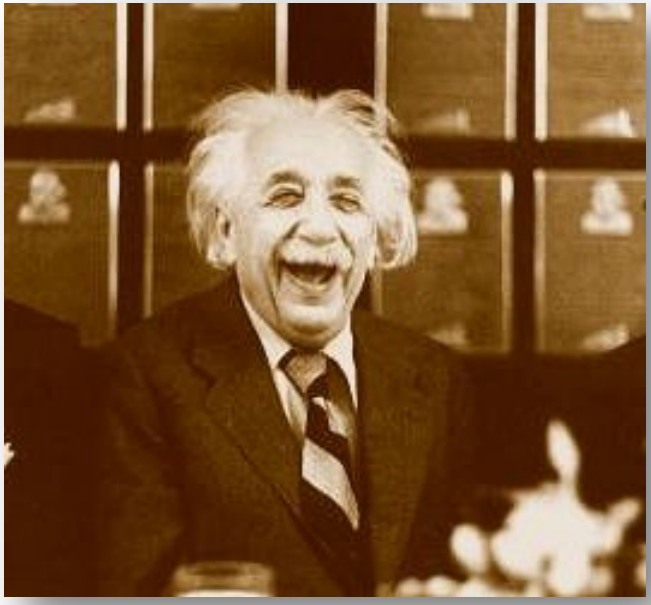
Van der Valk et al, 2009

Final conclusion

Serum free media are:

- ***Better for the animals***

- ***Better for research***



Thank You!

OECD

Draft GUIDANCE DOCUMENT ON GOOD *IN VITRO* METHOD PRACTICES (GIVIMP) FOR THE DEVELOPMENT AND IMPLEMENTATION OF *IN VITRO* METHODS FOR REGULATORY USE IN HUMAN SAFETY ASSESSMENT

Batches of serum can differ dramatically in their ability to support the growth of cell lines due to variation in the concentration of growth factors and hormones, therefore, new batches should be tested on the appropriate cell line(s) for cell attachment, spreading, cloning efficiency, growth rates and activity in functional assays (Geraghty *et al.*, 2014).

These effects can be overcome by adaption to serum-free culture conditions (Section 4.3.3) using specific protocols (Beltran Paschoal *et al.*, 2014; Leong *et al*., 2017; Sinacore *et al.*, 2000) for a gradual weaning of cells (van der Valk *et al*., 2010).

…it is recommended to develop new *in vitro* methods with a serum-free, chemically-defined medium, to avoid potential sources of uncertainty that may be introduced by using animal serum (Jochems *et al*., 2002; Pamies *et al*., 2016).

http://www.oecd.org/env/ehs/testing/OECD%20Final%20Draft%20GIVIMP.pdf