

# Practical pathways to address reagent source as a variable in study reproducibility

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There is a strong desire within the scientific community to optimize the reproducibility of studies, and fetal bovine serum (FBS) and antibodies are two of the most commonly used reagents in *in vitro* research and testing. Both are recognized to have batch-to-batch variability, which can not only lead to irreproducibility but also to misinterpretation of test results. According to a recent survey<sup>1</sup> of scientists by Oltre la Sperimentazione Animale, the Joint Research Centre of the European Commission, Technical University of Denmark, Eurogroup for Animals, Deutscher Tierschutzbund e.V., and the Centre for Predictive Human Model Systems, most respondents felt that they had inadequate information on animal-free reagents. Here, we elucidate how to optimize research reproducibility by addressing the types of antibodies and cell culture media, and we provide practical information on the process and feasibility of purchasing and using animal-free reagents.

## ANTIBODIES

**Recombinant antibodies (rAbs)** have a defined DNA sequence and are infinitely reproducible.

**Animal-free rAbs**  
Developed from recombinant libraries derived from human or synthetic sources, animal immunisation is not used at any phase of their production or to generate a library. Animal-free rAbs can be either monoclonal or multivalent.

**rAbs made with no new animal use**  
Developed from existing animal libraries or sequenced from hybridomas that were in storage, animal immunisation is not used at any phase of their ongoing production.

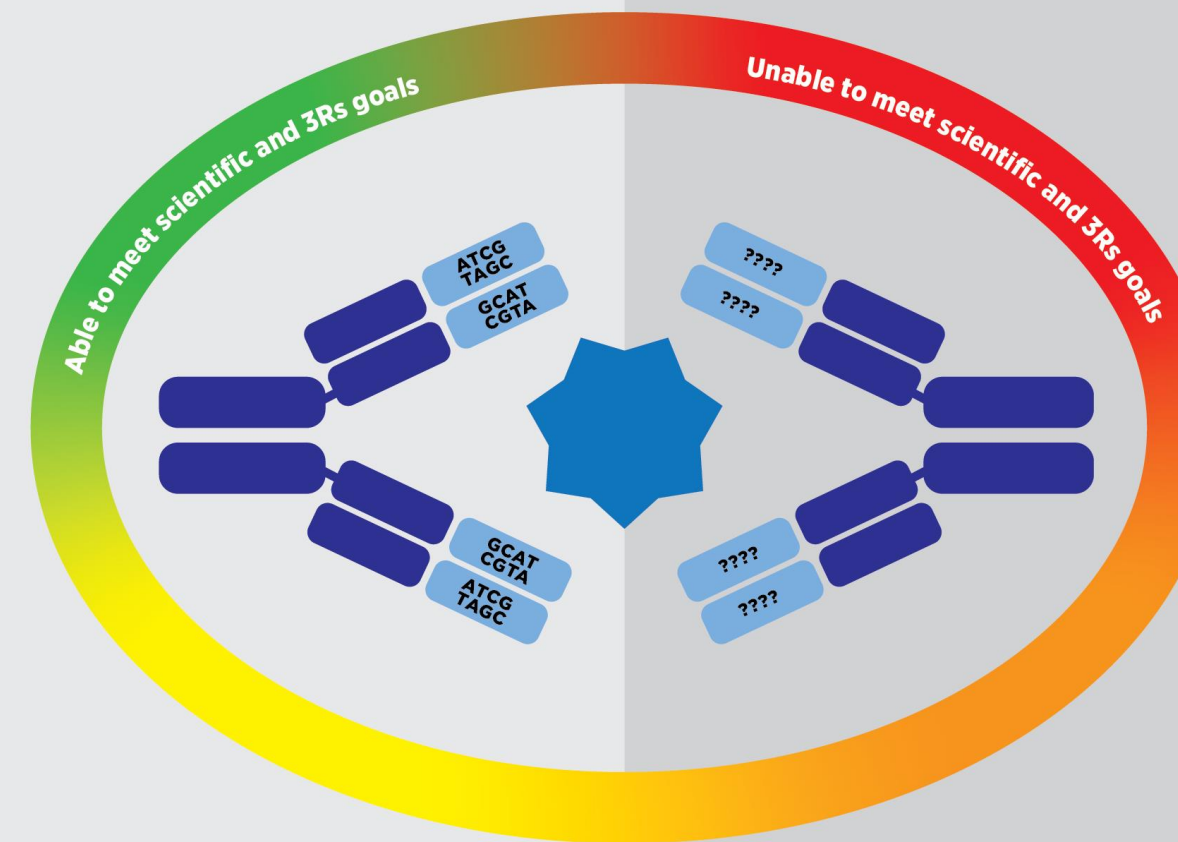
**rAbs made with new animal use**  
These antibodies are developed from newly produced naïve or immunised animal libraries or rAbs sequenced from hybridomas developed for that purpose.

**Animal-derived antibodies** are produced without a known sequence.

**Animal-derived polyclonal antibodies**  
Derived from multiple lines of antibody-producing cells, these antibodies bind to more than one target. Dependent on an individual's immune system for production, they are not reliably reproducible.

**Ascites-derived monoclonal antibodies**  
Derived from a single line of antibody-producing cells, these antibodies are intended to bind to one target and are developed and expanded in animals. Dependent on an individual's immune system for production, they are not reliably reproducible.

**Monoclonal antibodies produced via hybridomas**  
Derived from a single line of antibody-producing cells, these antibodies are intended to bind to one target. They are developed in an animal, and expanded *in vitro*. Dependent on an individual's immune system for development as well as on the stability of a hybridoma, they are not reliably reproducible.



Recombinant antibodies can be used in all applications in which animal-derived antibodies are used. Regardless of the type, all antibodies should be validated in their specific contexts of use.

## ANTIBODY SUPPLIERS

Abcalis offers animal-free recombinant monoclonal antibodies and multivalent antibodies produced in animal-serum free media.



Abcam produces a selection of antibodies using phage display recombinant antibody technology.

Bio-Rad uses its Human Combinatorial Antibody Libraries (HuCAL) library to offer animal-free recombinant monoclonal antibodies.



GENEVA  
ANTIBODY  
FACILITY

The Geneva Antibody Facility develops animal-free recombinant antibodies at the University of Geneva. The University of Geneva's ABCD (AntiBodies Chemically Defined) database is a manually curated depository of sequenced antibodies developed at the University of Geneva and available for academic purposes. All antibodies discovered and/or produced by the facility are made available to the scientific community.



Additional companies, such as Absolute Antibody, sequence existing monoclonal and polyclonal antibodies so that they can be made recombinantly moving forward.

More companies offer custom animal-free recombinant antibody production. See [www.thePSCI.eu/antibody-availability](http://www.thePSCI.eu/antibody-availability)

## CELL CULTURE MEDIA

FBS is often used as a supplement for cell culture media. It provides an undefined mixture of macromolecules that maintains cell viability and facilitates cell metabolism, growth, proliferation, and spreading in culture. Collected from the fetus when a pregnant cow is slaughtered, the collection and use of FBS poses significant ethical, safety, and reproducibility challenges. Notably, undefined and variable components result in the risk of significant batch-to-batch variation and unexpected and undesired interactions in studies.



### Replacements for FBS

Serum-free media or human platelet lysates can replace FBS when culturing cells. For optimal definition and reproducibility, the goal should be to use chemically-defined animal-free medium, thereby avoiding the use of all animal-derived supplements.

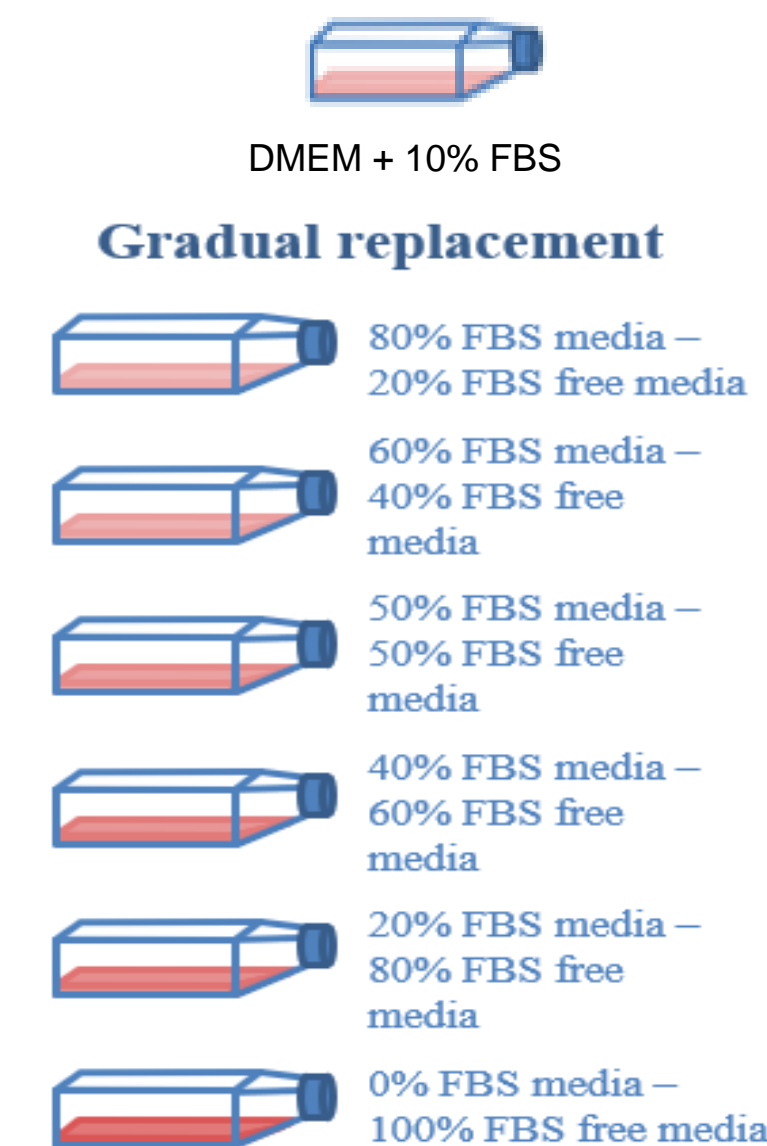
### Transitioning cells

While some cell lines can be directly transferred to a different medium, cells normally must be gradually transitioned to FBS-free conditions to reduce stress and provide time for cells to adjust to the environment.

Because the medium may affect cell morphology and functionality and can favor certain cell pheno- and genotypes, cells should be characterized after any changes to the culture conditions.

Case studies demonstrating the transition of cells to serum-free media include:

- Weber *et al.* Case studies exemplifying the transition to animal component-free cell culture. *ATLA*. 2022;50(5):330-338.
- Chary *et al.* Maximizing the relevance and reproducibility of A549 cell culture using FBS-free media. *Toxicol In Vitro*. 2022;83:105423.
- Schmidt and Wiest. Three-stage approach for evaluation of chemically defined cell culture medium for the caco-2 cell. *ALTEX Proceedings*. 2021;9(1):215.



From Chary et al. 2022.

## ANTIBODY AND MEDIA RESOURCES

### Webinars

Cell culture media webinars are available at:

- [www.thepsci.eu/fbs](http://www.thepsci.eu/fbs)
- [www.the3rs.uni-tuebingen.de/en/3r-webinar](http://www.the3rs.uni-tuebingen.de/en/3r-webinar)
- [www.ascctox.org/webinar/87](http://www.ascctox.org/webinar/87)
- [bioscience.lonza.com/lonza\\_bs/US/en/webinar-cell-culture-media-why-go-serum-free](http://bioscience.lonza.com/lonza_bs/US/en/webinar-cell-culture-media-why-go-serum-free)

Recombinant antibody webinars are available at:

- [www.thepsci.eu/antibody-webinars](http://www.thepsci.eu/antibody-webinars)
- [www.bio-rad-antibodies.com/antibody-webinars.html](http://www.bio-rad-antibodies.com/antibody-webinars.html)
- [www.antibodysociety.org/learningcenter/antibody-validation-webinar-series/](http://www.antibodysociety.org/learningcenter/antibody-validation-webinar-series/)

### Funding



The Recombinant Antibody Challenge offers grants for free catalogue recombinant antibodies for use in research and testing.

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### Types of media

Media type	Description
Serum-free	Does not contain human or animal serum, but may contain discrete proteins or bulk protein fractions (e.g., human or animal tissue or plant extracts) and are thus regarded as chemically undefined (see: chemically defined media)
Protein-free	Does not contain high molecular weight proteins or protein fractions, but may contain peptide fractions (protein hydrolysates)
Xeno-free	If containing animal-derived components, they are only from the same species as the cells used. Medium intended to be used with human cells does not contain components of animal origin, but may contain human components, such as human derived growth factors
Animal-component-free	Does not contain components of animal or human origin
Chemically defined	The chemical structures of all components are known. Does not contain proteins, hydrolysates, or any other components of unknown composition. Components of either animal or plant origin can be added when highly purified, characterized, and quantified, including recombinant products
Chemically defined recombinant	The media is chemically defined as described above but any components of animal, plant, or human origin are recombinant

From Chary et al. 2022.

## MEDIA SUPPLIERS

- For some cell types, serum-free media have already been developed, and they are available commercially or their components are published in the literature.
- For other cell lines, researchers will need to optimize the concentration of supplements for a new media or test the cell line in a commercially-developed media used for other cell types.

The Fetal Calf Serum-Free Database contains media formulations and products. See [www.fcs-free.org](http://www.fcs-free.org)



<sup>1</sup>Cassotta *et al.* A worldwide survey on the use of animal-derived materials and reagents in scientific experimentation. *Eng Life Sci*. 2022;22(9):564-583.

