The Case for Serum-Free Media

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Use of serum-free media and the related genera of animal-free and protein-free media has increased significantly over the past 15 years. This is particularly true in industrial applications, in which the use of serum presents a safety hazard and a source of unwanted contamination in the production of biopharmaceuticals (1). As the name suggests, serum-free media are prepared without animal serum, but it may contain serum constituents or substitutes thereof. Animal-free media are similar to serum-free except that the components are derived from nonanimal sources. Recombinant proteins replace native proteins, and the nutrients are obtained from synthetic, plant, or microbial sources. By contrast, protein-free (or chemically defined) media are devoid of protein, although few formulations can be 100% protein free without loss of function. A more accurate definition of such a medium would describe it as low in protein, with minimal quantities of small mass proteins used.

The three types of media can be thought of in a gradient of complexity. After true serum-based media, serum-free media are the most complex compositions designed for universal use in culturing mammalian cell lines. Animal-free and protein-free media formulations are less complex and more defined but limited to the cultivation of specific cell types. Thus, selection of one medium over another depends on its intended use. For most research applications, serum-free medium is the best choice because it provides broad-spectrum utility and enables good control of the composition. By contrast, in the manufacture of protein-based drugs, potential contaminants and production costs play a more significant role in the selection of more defined medium compositions.

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PROCESS FOCUS: PRODUCTION

WHO SHOULD READ: RESEARCH, MANUFACTURING, QUALITY ASSURANCE AND CONTROL, PROCESS DEVELOPMENT

KEYWORDS: CELL CULTURE, GROWTH MEDIUM, CONTAMINATION CONTROL

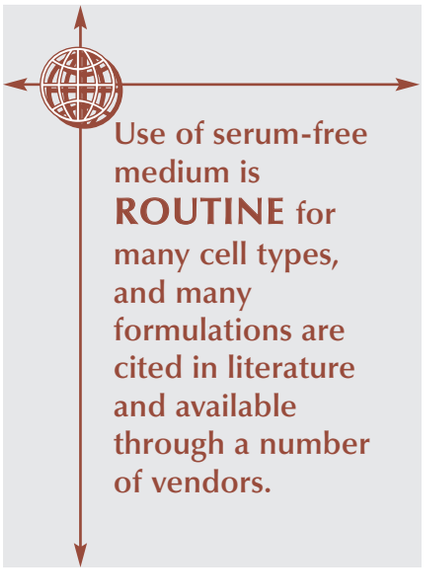
LEVEL: BIOTECH BASICS

ADVANTAGES

Serum-free media offer several basic advantages over serum-containing media. The simplified composition is better defined than that of serum-based media. Contaminants are reduced, and a potential source of infectious agents is eliminated. Serum-free media can be a more economical choice (2).

Simplified and Defined Composition. Serum, by nature, is an ill-defined component of medium. Typically used at 5% to 10% v/v, it provides a range of factors that have proved necessary for cultivating mammalian and insect cells. Serum, however, can be derived from different sources, and its composition can vary greatly. Advances in the manufacture and processing of serum have reduced that variability, but many researchers continue to find it necessary to qualify new lots of serum by comparing the growth of their cell lines in each new serum relative to that of a lot currently in use. With typical acceptance criteria at ±20%, the variance of any given physiological function can be quite high.

That variation leads to poor reproducibility of results between and within laboratories. Stories abound of irreproducible results that can be attributed directly to the use of serum as a medium component. A quantitative understanding of cellular physiology



cannot be obtained in ill-defined and variable conditions.

Serum-free medium, on the other hand, is a more defined medium. Although it is also composed of many constituents, those constituents are known, and the level of each component is precisely defined. Therefore, the variance seen with serum-containing media is eliminated, which creates a more controlled environment in which cells can grow.

Reduced Range and Level of Contaminants. The protein concentration of media containing 10% serum is 6,200–10,000 mg/L. The concentration for a typical recombinant protein produced in mammalian cells is anywhere from a few mg/L to 1,000 mg/L. For native proteins, the level of accumulation can be even lower. Serum proteins thus become a major contaminant of any crude supernatant in which a target protein accumulates. Further, if the target protein is functionally, biochemically, or physically related to a serum protein, it may be difficult to separate them.

Such contamination could be disastrous for the production of a protein-based drug, but it is equally troubling to researchers trying to study the functional and physical properties of a target protein. The protein concentration of serum-free media ranges between 50 mg/L and 1,000 mg/L. Unlike serum, the medium composition is known. Typically, three proteins (albumin, transferin, and insulin) make up 80–90% of the proteins present. Consequently, the relative level of a target protein produced will be significantly higher, and the supernatant will be less contaminated than when using serum-containing medium. Thus, the target protein should be easier to purify and require using fewer steps, which should lead to higher recovery values.

Drug discovery, physiological, or gene expression studies are simpler to perform with serum-free than with serum-containing media. Serum components bind, degrade, or otherwise interact with chemicals added to the medium. Complex associations are possible among the serum, the added effectors, and the cells. Thus, an effect elicited by added chemicals can be altered or eliminated by the serum factors. Serum-free media incorporate fewer possible interferents, and any interactions observed should be more readily controlled.

Elimination of Potential Source of Infectious Agents. Viral, bacterial, and fungal contamination of serum has for some years been a concern for biopharmaceutical manufacturers. The concern has been a driving force behind the adoption of serum-free, animal-free, and protein-free media formulations in the manufacturing process. Recently, transmissible spongiform encephalopathies described in animal-derived materials has created a desire to eliminate all animal products from the manufacture of pharmaceutical products.

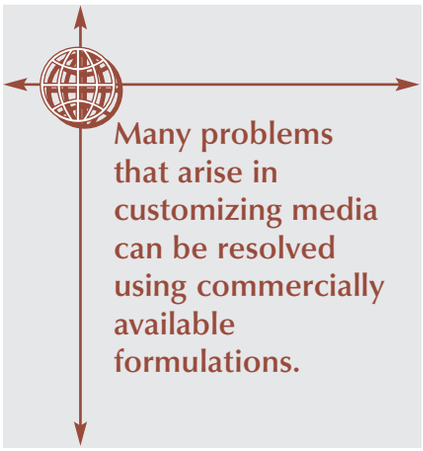
In a research setting, where more fastidious cell lines are used, the complete elimination of animal-derived components is impractical. Recombinant forms of some key components remain expensive, if they are at all available. Many commercially available animal-free and protein-free media were designed for specific cell types, so they do not support the growth of many cell types. Because the materials used in production of serum-free media are for the most part highly purified, the risk of contaminating cultures with adventitious agents is greatly reduced or eliminated. For nonpharmaceutical applications, serum-free medium remains a good balance between safety and efficacy.

Cost and Availability. In US dollars, serum can cost between $7 and $50 per liter of medium, depending on the type and percentage of serum used. Fetal bovine serum, the most commonly used source, is also the most costly, with prices averaging $456/L of serum (as of August 2002). Therefore, serum can contribute significantly to the cost of a medium. Serum-free medium averages about $120/L, with a range from $26/L for a Dulbecco's modified Eagle's medium (DMEM) derivative to $692/L for highly specialized bone marrow medium. Most serum-free media are supplied ready-to-use, so no multicomponent reagent preparation is necessary.

Disadvantages

The use of serum-free medium is routine for many cell types, and many formulations are cited in literature and available through a number of vendors. Nevertheless, a few drawbacks should be considered before making use of serum-free medium. First, it takes time to adapt a particular cell line to growth in serum-free medium. Cells must be weaned from serum slowly. Moreover, some cell lines require growth factors specific to their cell type to overcome deficiencies in a particular medium. It is advisable to begin with a serum-free medium that includes growth factors, such as pituitary extract, which can be removed incrementally if necessary.

A second limitation is that the low protein concentration of serum-free medium — an advantage that reduces potential sources of contamination — removes proteins that play a role in shear protection and attachment to growth substrata. The bovine serum albumin (BSA) present in serum protects cells grown in suspension from shear



damage. Addition of Pluronic F68 or polyethylene glycol may fill that role. BSA also provides other transport functions, and the replacement of native sources of albumin with recombinant sources is not straightforward. Alternative supplements may be needed. Many problems that arise in the process of developing a custom medium can be resolved by using commercially available formulations.

Attachment-dependent cell lines require an extracellular matrix on the growth substratum. Serum provides some components for this matrix. Therefore, if serum-free medium is used, the substratum (e.g. plasticware or carrier beads) should be precoated with fibronectin, laminin, or another suitable alternative such as FNC Coating Mix coating (a fibronectin/collagen mixture manufactured by AthenaES of Baltimore, MD), Pronectin (a synthetic fibronectin polymer manufactured by Sanyo Chemical Industries of Kyoto, Japan) or Matrigel (available from BD Biosciences, Speaks, MD).

A Viable Alternative

Serum-free medium presents an alternative to serum-containing media for the cultivation of cells in the laboratory or manufacturing suite. It offers several advantages, which include better definition of the composition, reduced contamination by adventitious and infectious agents, and lowered costs. The commercial availability of many variations of serum-free medium has made it easy to obtain and employ. Consequently, its use has now become routine in many laboratories and biopharmaceutical manufacturing processes for the culture of a wide variety of cell types.

Selected Guidance Documents

The following selected guidance documents dealing with the use of cell culture media in the manufacture of biologics can be found online at the URLs provided.

www.fda.gov/cber/gdlns/ptcsomat.pdf
Guidance for Human Somatic Cell Therapy

www.fda.gov/cber/gdlns/cmcdna.txt
Guidance for Industry: For the Submission of Chemistry, Manufacturing, and Controls Information for a Therapeutic Recombinant DNA-Derived Product or a Monoclonal Antibody Product for In Vivo Use

www.fda.gov/cber/gdlns/mab032901.htm
Monoclonal Antibodies Used as Reagents in Drug Manufacturing

www.ich.org/pdfICH/q5a.pdf
Q5A: Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin

www.ich.org/pdfICH/q5b.pdf
Q5B: Quality of Biotechnological Products — Analysis of the Expression Construct in Cells Used for Production of r-DNA Derived Protein Products

www.ich.org/pdfICH/q6bstep4.pdf
Q6B: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products

www.emea.edu.int/pdfs/human/bwp/032899en.pdf
CPMP/BWP/328/99: Development of Pharmaceuticals for Biotechnological and Biological Products — Annex to Note for Guidance on Development Pharmaceutics

www.emea.edu.int/pdfs/vet/regaffair/041001en.pdf
EMEA/410/01 Rev. 1: Note for Guidance on Minimizing the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products

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2 Froud, SJ. The Development, Benefits and Disadvantages of Serum-Free Media. In *Animal Sera, Animal Sera Derivatives and Substitutes Used in the Manufacture of Pharmaceuticals: Viral Safety and Regulatory Aspects*. Vol. 99, *Dev. Biol. Stand*. Brown, F et al., eds. Karger: Basel, Switzerland, 1999; 157–166.

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