The Performance of Serum-Free and Animal Component-Free Media for Multiple Hybridoma Cell Lines and Culture Systems

Heather N. Loke, Steven C. Peppers, Daniel W. Allison, Damon L. Talley, and Matthew V. Caple
Sigma-Aldrich Corporation, P.O. Box 14508, St. Louis, MO 63178

Abstract

Recently, there has been a greater interest in a cell culture medium completely free of serum and other animal components. Serum and animal components are poorly defined, can include unwanted contaminants, have inter-batch variations causing standardization problems, and have numerous regulatory issues. To meet this demand, we have developed a serum-free medium (H4281) and an animal component-free medium (H4409) for the culture of hybridoma cells. Data was generated to reflect their applicability to multiple hybridoma cell lines in various growth systems. Seven different hybridoma cell lines were used compared our media products to the eight best commercially available products (four serum-free and four protein-free). The ability of these media to sustain cell growth and promote IgG production in these cell lines was examined and compared. Both our serum-free and animal component-free media performed better or equivalent in both cell growth and IgG production than the other commercially available products. We were also interested in studying the growth and production of hybridoma cells in various culture systems. The hybridoma cell lines were grown in spinners, roller bottles, fed-batch stirred-tank bioreactors, and fixed-batch stirred-tank bioreactors. Data generated from these assays demonstrated the ability of our media to support cell growth and production in a variety of hybridoma cell lines in numerous culture systems.

Introduction

With the recent interest in the use of antibodies or antibody fragments as *in vivo* therapeutic agents, greater emphasis has been put on the methods and materials used to manufacture these products.[1] The traditional method for producing antibodies from hybridomas involves culturing the cells in medium supplemented with serum. The use of serum and other animal-derived components however pose numerous problems when used to produce therapeutic agents and have recently come under great scrutiny.[2] The composition of serum is poorly defined and may have inter-batch variations causing standardization problems in manufacturing. Adventitious agents and toxins, such as viral particles and endotoxins, can potentially be introduced into the growth system, causing numerous downstream processing issues. Regulatory concerns must also be considered when using animal- or human-source materials in manufacturing biopharmaceuticals. These factors all contribute to the higher cost involved with using serum or animal-derived components in media employed for the production of antibodies.

To avoid these problems, there has been significant interest in media devoid of serum and animal-derived components. To meet these needs, we developed two hybridoma media. An animal component-free medium (H4409) was formulated with low protein content, containing only recombinant human insulin. For more problematic cell lines or for cultures not requiring absence of all animal components, a serum-free medium (H4281) containing bovine insulin, human transferrin, and bovine serum albumin (BSA) was also formulated.[3]

A variety of culture systems to produce antibodies are employed throughout the biopharmaceutical industry, depending on the manufacturing capabilities and purpose of production. In addition, variation in the growth and expression characteristics of the individual hybridoma clones must be taken into consideration. To optimize a single medium to meet these requirements necessitates a comprehensive formulation able to meet the many needs of a manufacturer. The media we have developed were evaluated using several hybridoma cell lines and a variety of growth systems. Both cell proliferation and antibody production were assessed to demonstrate the applicability of these media to support the growth of hybridoma cells for antibody production.

Materials and Methods

All materials were obtained from Sigma-Aldrich Corporation, unless otherwise stated.

Cell Lines: HFN 7.1 cells (CRL-1606), derived from mouse spleen cells fused with P3X63Ag8 myeloma cells, and M-2E6 cells (HB138), derived from mouse spleen cells fused with Sp2/0-Ag14 myeloma cells, were acquired from ATCC cell bank. TP4 9.2.1A anti-Taq cells and TP6 25.3D anti-Taq cells are proprietary Sigma-Adrich cell lines used in the production of antibodies. Three hybridoma cell lines were provided by Abbott Laboratories and given the designations “Cell Line H,” “Cell Line M,” and “Cell Line P.” Cells were grown in H4281 serum-free medium until frozen at 1.2×10^6 cell in DMSO-containing freezing medium (Sigma product code: C6164). Cell stocks were routinely thawed into H4281 with 5% FBS in flasks and weaned within 3 to 6 days into H4281 without serum in a 500 ml spinner flask. The cells were subcultured every 2-3 days and were not allowed to exceed 20 passages. Cell densities were consistently maintained between 5×10^4 and 1×10^6 cells/ml in these stock cultures.

Spinner Flask Assays: Cells were seeded into 125 ml

or 250 ml capacity spinner vessels at $5x10^4$ or $1x10^5$ cells/ml on day zero and grown in a humidity-controlled incubator at 37°C and 5% CO_2. In each spinner, viable cells/ml were determined using trypan blue exclusion and a hemacytometer for percent viability and a Casy® 1 Schärfe System cell counter for the number of total cells. Cell counts were averaged from two spinner flasks unless otherwise noted. Cell-days, the integral area under the cell growth curve, was approximated using a trapezoidal point-to-point summation method.

Roller Bottle Assays: Cell culture stocks were grown in H4281 in 500 ml spinners. Cells were seeded at $5x10^4$ cells/ml on day zero into duplicate 490 cm^2 roller bottles and rotated at 4 rpm in a humidity-controlled incubator at 37°C and 5% CO_2. Cell counts and IgG productivity were determined using the same methods as the spinners flasks.

Fed-Batch and Fixed-Batch Stirred-Tank Bioreactor Assays: Cells culture stocks were grown in H4281 in 500 ml spinners. Cells were seeded at $5x10^4$ cells/ml on day zero in a 5.0 L tank B.Braun Biotech International bioreactor maintained at 37°C. The bioreactor culture was stirred at 85 rpm with a pO_2 of approximately 50% air. The pH was kept between 6.9 and 7.2 and controlled by a 1 N NaOH base pump. Glucose and glutamine levels were monitored daily by using a YSI 2700 bioanalyzer. In the fed-batch bioreactor runs, glucose and glutamine levels were maintained at 15-mM glucose and 5-mM glutamine in the 5.0 L culture by fed additions of a 45% glucose solution and a 200-mM L-Gln solution. In the fixed-batch bioreactor runs, no supplements were added to the culture. In each bioreactor, culture samples were taken once or twice daily and the cell counts were determined using the same methods as the spinners flasks.

IgG Concentrations: The concentration of immunoglobulin G secreted into the medium by the hybridoma cells was determined by HPLC using a Protein-A binding column for capture and 280 nm absorbence for quantitation.

Results and Discussion

Our serum-free medium (H4281) and animal component-free medium (H4409) were developed and optimized for cell growth and antibody production based on the performance of HFN 7.1 cells. This cell line is an excellent model because of its similarities to many of the cell lines currently being used in biopharmaceuticals. HFN 7.1 cells are robust, have high productivity, and are readily available through ATCC or other cell banks.

HFN 7.1 cells obtained from ATCC were first thawed into DME/F-12 medium supplemented with 2% FBS. The HFN cells were then gradually weaned over ten days into H4281, H4409, and sixteen other commercially available media marketed as serum-free, protein-free, or animal component-free. Eight of these media were not able to support the growth of the HFN cells through the weaning process, leaving ten adapted cell populations. These cell populations weaned into H4281, H4409, and eight other media, four serum-free media and four protein-free media, were used for further investigation to examine the ability of the media to support cell growth and antibody production. A spinner flask assay was started using the weaned HFN cell populations and the viable cells and IgG concentrations were determined. A second spinner flask assay was started eight days later using the same HFN cell population stocks maintained in their respective media. An additional weaning procedure was later performed, followed by a spinner flask assay to confirm the previous results. Figure 1 illustrates the average growth and antibody productivity resulting from the three experiments with standard error bars (n=3 for H4281, H4409, A, B, C, and E; n=2 for F, G, and H; and n=1 for D).

HFN Cells Weaned into Various Hybridoma Media: Means with Standard Errors (n=3)

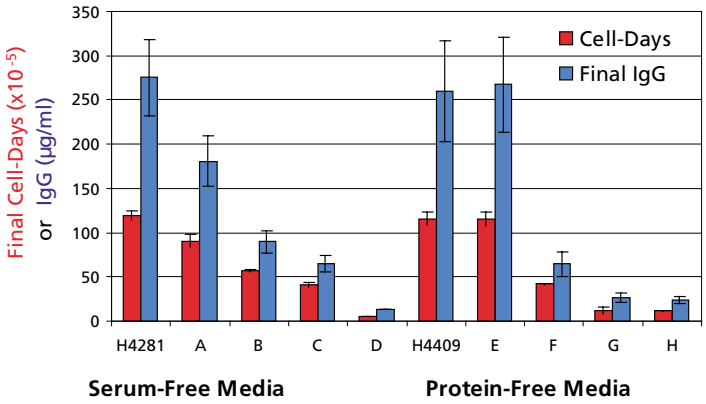

Figure 1

M-2E6 Cells Weaned into Various Hybridoma Media: Average of Duplicate Spinner Flasks.

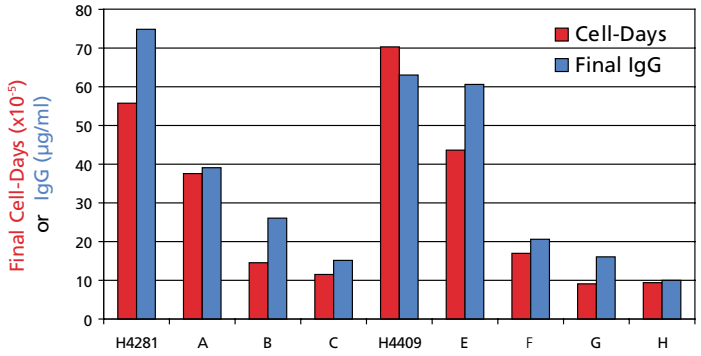

Figure 2

Based on these results, M-2E6 cells were weaned in a similar manner into H4281, H4409, and seven of the best performing competitor media from the HFN weaning assays. Media D was unable to grow the M-2E6 cells and was eliminated. Averages of duplicate spinners were used to determine final cell-days and IgG production for the M-2E6 cell line in these media. Figure 2 shows that H4281 and H4409 outperforms all the media tested using M-2E6 cells.

Variations in the genetic composition of individual hybridoma clones results in different growth and expression characteristics. Because of this, the requirements of the different clones can vary. A comprehensive hybridoma growth medium must meet the needs of numerous hybridoma clones with minimal modifications. To further explore this issue, various media were compared using seven different hybridoma cell lines. HFN 7.1, M-2E6, TP4 9.2.1A, and TP6 35.3D cell lines were all grown in duplicate spinner flasks in the nine hybridoma media previously tested. Cell Line H, cell line M, and cell line P were grown in duplicate spinner flasks in H4281, H4409, and media A, E, and F. Figure 3 illustrates the final cell-days and final IgG concentrations for these seven cell lines in the various media.

Cell Days of 7 Cell Lines in Spinner Flasks in Sigma Hybridoma Media and the Best Commercially Available Competitor Media

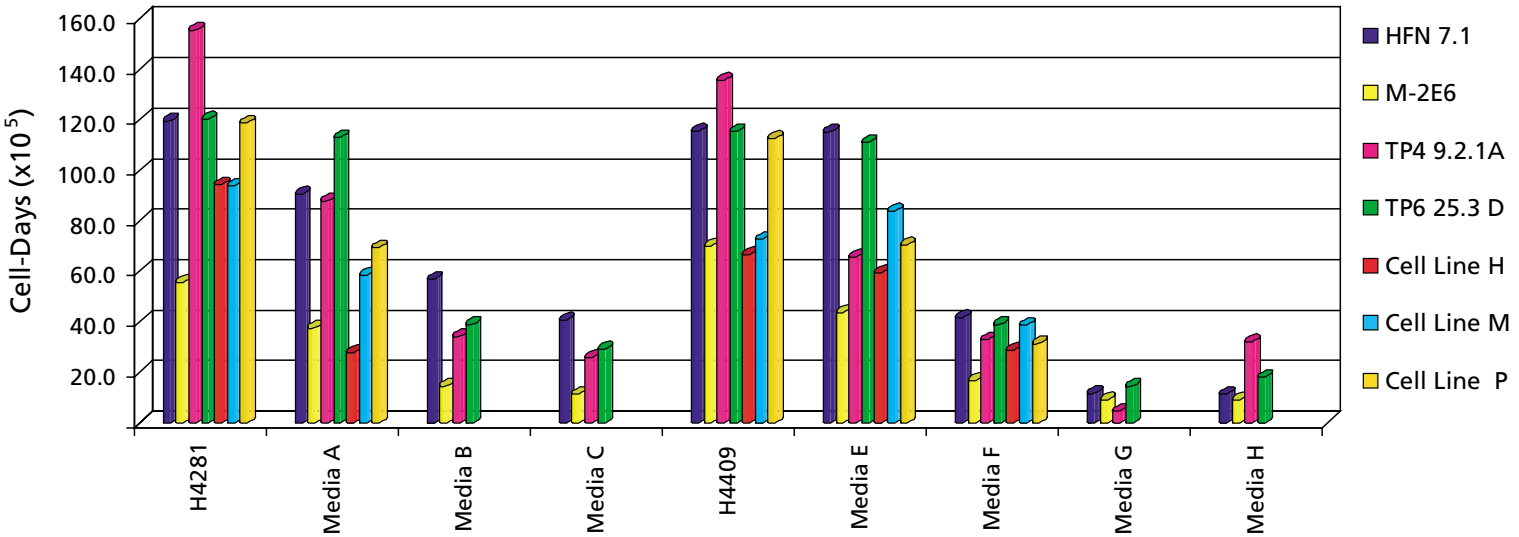

Figure 3a

IgG Production of 7 Cells Lines in Pinner Flasks in Sigma Hybridoma Media and the Best Commercially Available Competitor Media

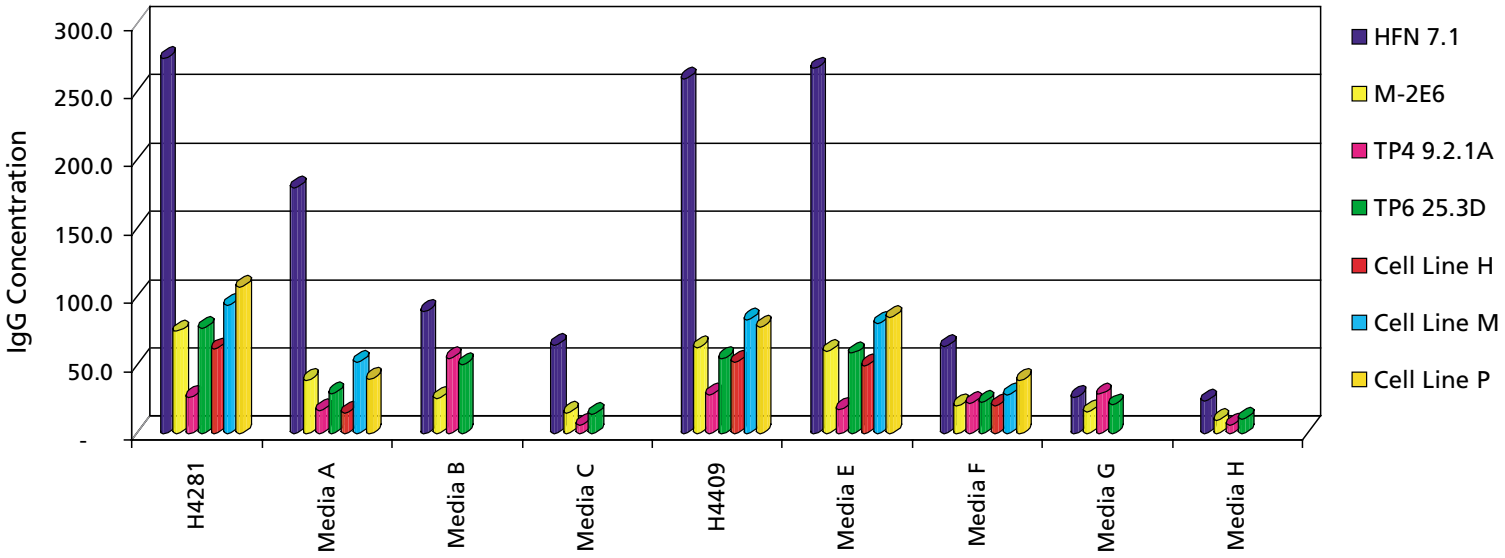

Figure 3b

Final cell-days and IgG production of these cell lines in the various hybridoma media are again illustrated in Figure 4. The data is normalized to the levels of H4409 and expressed as percentages of H4409. For media B and G, data for IgG was omitted because of interference in the HPLC reading causing an abnormal curve for the IgG peak with some of the cell lines. When averaging the data from the seven tested cell lines, H4409 and H4281 rank at the top of the commercially available hybridoma media tested.

Different Hybridoma Lines in Various Hybridoma Media: Normalized to H4409 Results (Means with standard errors)

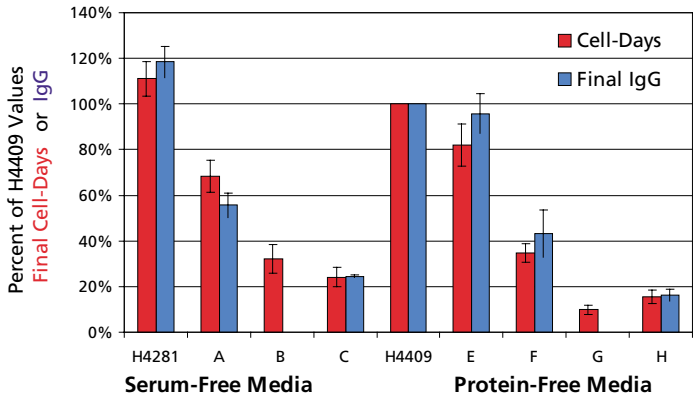

Figure 4

The cell culture system used, as well as the medium in which the cells are grown, are known to profoundly affect cell growth and antibody production. To better understand this interaction, the ability of the various media to support cell growth and productivity was examined in different culture systems. Roller bottles are a convenient scaled-up culture system readily used for manufacturing biopharmaceuticals. Although labor intensive, they provide an ideal system for producing intermediate quantities of the desired products with minimal investment of capital resources. Cell growth and antibody production in roller bottles were examined using HFN 7.1 cells grown in H4281, H4409, and the other seven best performing commercially available media. The data from this assay is depicted in figure 5. Again, H4281 outperformed the other serum-free media and H4409 outperformed the other protein-free media.

Roller Bottle Assay with Serum-Free and Protein-Free Media Using HFN Cells

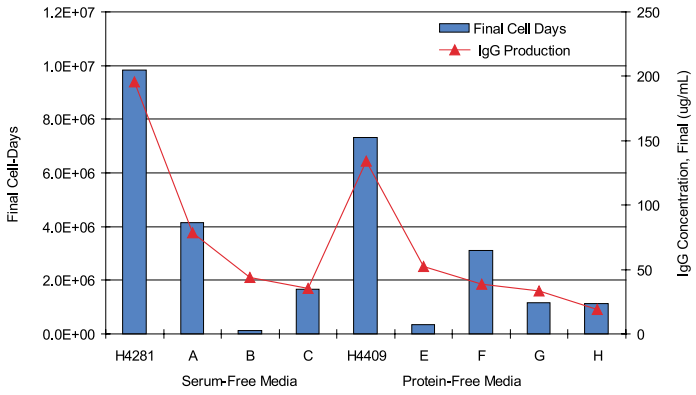

Figure 5

To further explore the effects of culture systems on cell growth and antibody production in hybridoma cells, HFN 7.1 cells were grown in a fed-batch stirred-tank bioreactor in H4281, H4409, and another serum-free medium. Fed-batch stirred-tank bioreactors allow for a larger culture volume, a more controlled environment, and better agitation, enabling cells to reach higher cell density and productivity. Viable cell density, cumulative cell-days, and IgG concentrations were analyzed in all three media. Figure 6 shows similar results to those observed in the spinner flasks and roller bottles.

Fed-Batch Stirred-Tank Bioreactor with HFN 7.1 Cells Viable Cells/ Cell-Days/ IgG Concentration

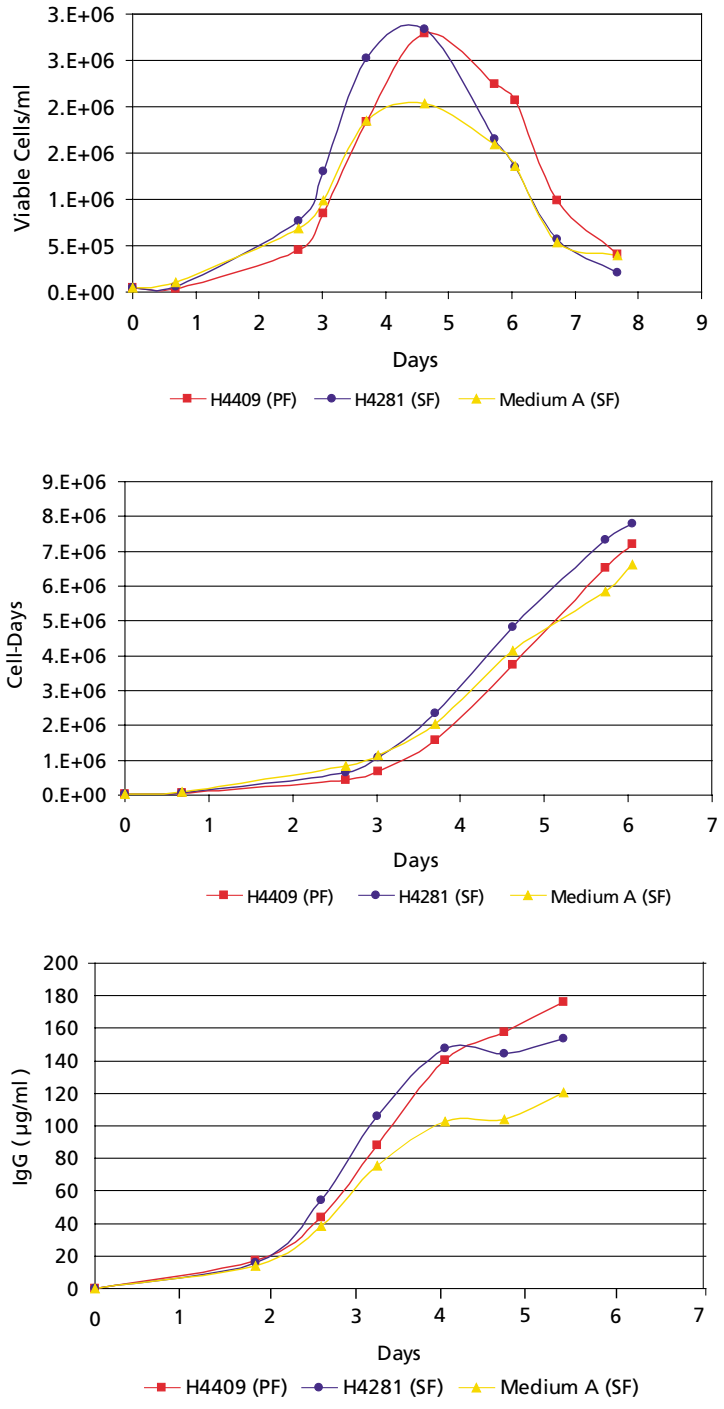

Figure 6

To confirm these results, HFN 7.1 cells were also grown in a fixed-batch stirred-tank bioreactor in H4281, H4409, and the same serum-free medium. Similarly, fixed-batch bioreactors have many of the same assets as fed-batch bioreactors, but without nutrient supplementation. Viable cell density, cumulative cell-days, and IgG concentrations for the fixed-batch bioreactor were also analyzed in all three media. Again, similar results were found and presented in figure 7.

Fixed-Batch Stirred-Tank Bioreactor with HFN 7.1 Cells Viable Cells/ Cell-Days/ IgG Concentration

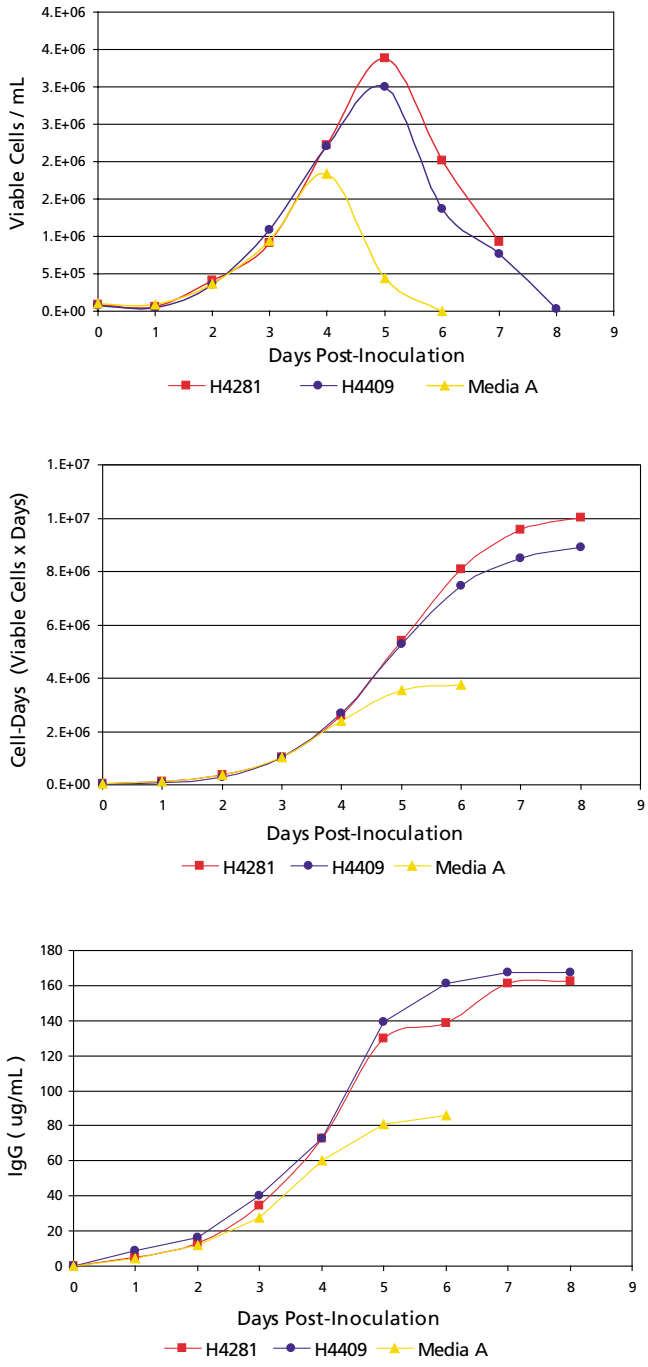

Figure 7

Conclusion

With the impetus for the elimination of serum and animal-derived components from media for various reasons, a hybridoma medium devoid of these components that is able to support robust cell growth and antibody production is desired for biopharmaceutical applications. A hybridoma growth medium must be comprehensive to work with a range of cell lines and in a variety of culture systems. Although no single medium will be optimal for all cell lines and culture systems, the serum-free medium (H4281) and the animal component-free medium (H4409) reported here have demonstrated excellent cell growth and antibody production. Additional culture systems, such as perfusion stirred-tank bioreactors and hollow-fiber bioreactors, are currently being tested to further expand the data presented here. To date, the H4281 and H4409 media have proven to be equivalent or superior to other commercially available serum-free and protein-free hybridoma media, respectively, for supporting cell growth and antibody production.

Acknowledgements

We would like to acknowledge Abbott laboratories for providing us with three hybridoma cell lines, and Bill Pieczynski and Bill Zeck for their consultations regarding these cell lines. We would also like to thank Lori Schultz for providing us with three hybridoma cell lines and advice on culturing these cell lines, and Terrell Johnson for discussing and reviewing this poster.

References

1. Merten, O-W., Safety issues of animal products used in serum-free media, in Animal Sera, Animal Sera Derivatives and Substitutes Used in the Manufacture of Pharmaceutical: Viral Safety and Regulatory Aspects. Dev. Biol. Stand. Basel, Karger, **99**, 167-180 (1999).
2. Wrotnowski, C., Cell culture media trends mirror bioindustry. Gen. Eng. New, **20(8)**, 8 (2000).
3. Peppers, S., Allison, D., Johnson, T., Talley, D., Caple, M., Replacing the animal component in serum: evaluating raw materials of inclusion in opimized hybridoma media. BioPharm. May 2001, in print.
4. Steven C. Peppes, Terrell K. Johnson, Damon L. Talley, and Matthew V. Caple. Three-Stage Approach of Hybridoma Media Optimization. Poster presented at 40th American Society for Cell Biology Annual Meeting. December 9-13, 2000.