How to limit the use of serum in viral processes: a Gibco perspective

Céline R. Martin, Margarito Rojas, Anna-Barbara Hachmann, Serena Fries Smith, Abhijeet Kohli and Steve Gorfien
Thermo Fisher Scientific, 3175 Staley Rd, Grand Island, NY 14072

ABSTRACT

Vaccine manufacturing targeting animal and human prophylaxis has been relying heavily on the use of sera to reach adequate titers in mammalian cell culture processes. Safety concerns, lack of process robustness, costs of qualification and storage are some of the main challenges faced while using serum. Taking into account these drawbacks, serum has remained one of the principal raw material in vaccine manufacturing, but with limited supply worldwide and increased demands, notably from the cell therapy industry, serum's poor economic predictability might become a major issue on cost of good models in the future.

INTRODUCTION

Using Gibco™'s expertise in designing and manufacturing cell culture media for the past 50 years, we identified 4 approaches to limit the use of serum in viral processes. The first strategy to put in place and requiring limited process rework is to reduce the amount of serum by using enriched basal media and/or bovine serum albumin as a substitute. The second option is to identify which step of the manufacturing process actually requires serum supplementation. While cell growth in adherent conditions may require serum for expansion, production phase sometimes does not require FBS or albumin supplementation, thus simplifying processes and compliance to regulatory guidelines. The third option, providing viral transfer is not adherence-dependent, is to adapt adherent cell lines to suspension, thereby removing the need to provide adhesion factors present in serum. Finally, a completely controlled process can be developed using cells adapted to CD media (chemically-defined and protein-free). The Gibco perspective on vaccine technology is that implementing strategies to remove serum from current processes is an effective way to provide large scale solutions for vaccine manufacturers.

MATERIALS AND METHODS

Medium and supplements used: OptiMEM™ I, Advanced DMEM, AlbuMAX™ I & II, Transition Medium 1 & 2, CD BHK-21 Production Medium, VP-SFM, OptiPRO™ SFM, E-MEM, Glasgow MEM, H-BME, E-BME, FBS, GMEM/TPB (Gibco™ catalog or custom media).
Cell lines: VERO (ATCC CCL-81), BHK-21 (ATCC CCL-10), MDCK (ATCC CCL-34), PK 15 (ATCC CCL-33), MRC-5 (ECACC 05072101), BEK , HEK (ATCC CRL-1573), CEF, COS-7 (ATCC CRL-1651), MDBK (ATCC, CCL-22).
TCID50 Assay: Virus production was determined with a TCID50 assay and titers were calculated following the method by Reed and Muench [1].

Figure 1. Growth obtained in VP-SFM

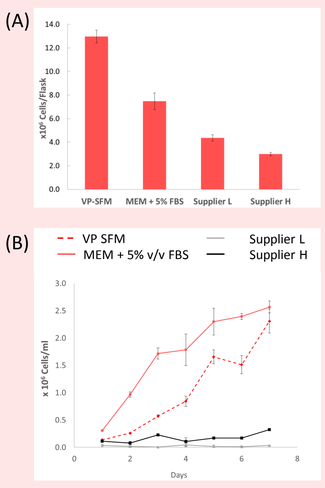
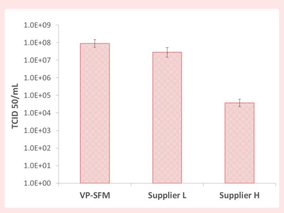
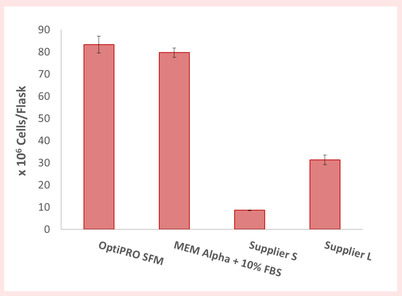


Figure 2. Titers obtained in VP-SFM



Adherent vero cells infection with Vesicular Stomatitis Virus (VSV, Glasgow, Indiana, ATCC® VR-1415™). Cells grown in serum free media and infected in duplicate (±sd) at MOI 0.1. Virus was harvested after 120h and freeze-thawed twice before quantification.

Figure 3. Growth obtained in OptiPRO SFM



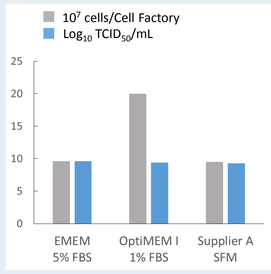
Comparative growth of adherent MDCK cells after adaptation to 3 serum-free media from α–MEM + 10% v/v FBS. MDCK cells were adapted to SFM according to manufacturer's recommendations. MDCK cells were cultured for 5-days in T-225 Flasks. All media were supplemented with 6mM glutamine. Shown are averages of triplicates (±sd).

Table 1. FBS reduction using OptiMEM-I

Cell Type	Control medium + 10 % v/v FBS	OptiMEM I + [FBS] % v/v	
		Optimal concentration via direct adaptation	*Minimal concentration via sequential adaptation*
VERO	E-MEM	2	0.5
BHK-21	Glasgow MEM	2	1
MDCK	E-MEM	1	0.5
PK 15	E-MEM	3	1
MRC-5	H-BME:E-BME	2-4	1
BEK	H-BME	4	2
HEK	H-BME	4	2
CEF	Glasgow MEM	4	2

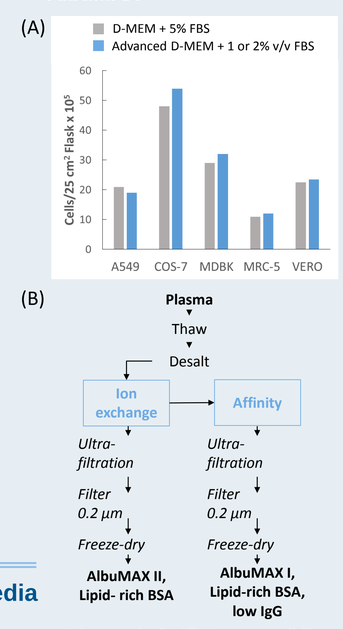
Comparable growth rates obtained between 3 conditions on several adherent cell lines: Condition 1, control medium with 10% v/v FBS; Condition 2, OptiMEM I with reduced FBS (1-4% v/v) via direct adaptation; Condition 3, OptiMEM I with reduced FBS (0.5-2% v/v) via sequential adaptation. For low serum supplementation (<1% v/v) with adherent cells, calcium chloride should be supplemented (0.5-1 g/L).

Figure 4. Growth and titers obtained in OptiMEM-I



MDBK cell growth and bovine herpesvirus 1 (BoHV-1) production in Nunc Cell Factory systems. Cell Factory systems were plated with 9.6 x 10^6 MDBK cells. Cell counts were performed on day 3, and cultures were inoculated with BoHV-1 at an MOI of 0.1 TCID50/cell. High cell counts were due to the lack of contact-inhibition with MDBK cells in OptiMEM-I. Both OptiMEM-I and Supplier A media contained animal-derived proteins.

Figure 5. FBS reductions using Advanced DMEM and AlbuMAX



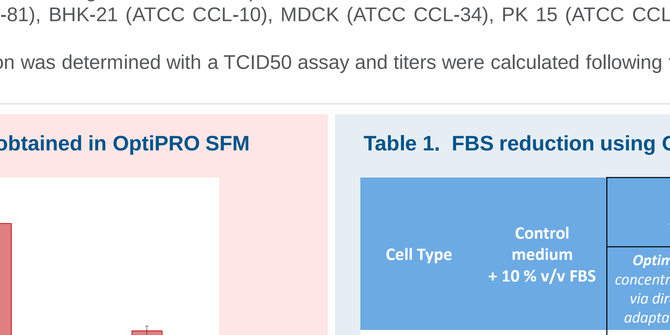
Advanced DMEM compatibility with different cell lines. Advanced DMEM is supplemented to allow for serum reduction, notably with AlbuMAX I. (A) Viable cell densities ranging from 1.0-5.0x10^5 cells/T25 in duplicate. Results over 3 passages, 4 days passage cycle. (B) AlbuMAX I and II manufacturing process.

Removal of serum can be achieved using VP-SFM or OptiPRO SFM.

- **VP-SFM and OptiPRO SFM are serum-free media with no components from animal-origin.** VP SFM is an utra-low protein medium (<5 µg/ml) while OptiPRO SFM is a low protein media (<10 µg/ml).

- **Direct adaptation can be achieved using both media in multiple kidney derived cell lines:** OptiPRO SFM supports BHK-21, MDCK, MDBK, PK-15, COS-7 and HeLa cells. VP-SFM supports Vero, BHK-21 and HEp2 cells.

Comparative growth of adherent Vero cells after adaptation to 3 serum-free media from MEM + 5% v/v FBS. Vero cells were adapted to SFM according to manufacturer's recommendations. (A) Vero cells were cultured for 5-days in T-225 Flasks, all media were supplemented with 6mM glutamine. Cells were detached using TrypLE. Shown are averages of triplicates (±sd). (B) Vero cells grown on Cytodex-1 microcarriers for 7 days in 500ml spinner flasks. Seeding density of 2.5 x 10^5 cells/ml with 3g/l Cytodex 1. Medium was exchanged on day 3 and day 5. (C) Cell phenotype observed after 7 days of cultures.

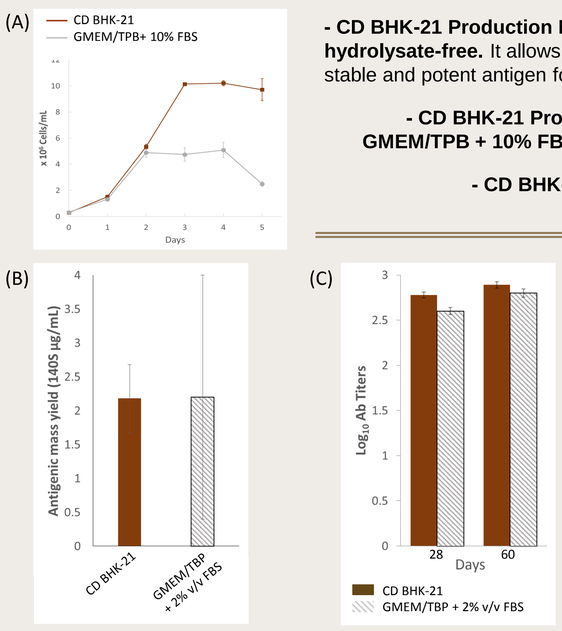


Strategies for serum reduction includes optimized basal media and more defined supplementations.

- **Opti-MEM I helped reduce serum concentration from 10% to 0.5-2%.** It contains insulin, transferrin, hypoxanthine, thymidine, and trace elements.

- **Advanced media are enhanced basal media formulations** (DMEM, DMEM/F-12, MEM, and RPMI 1640). They contain supplementations in ethanolamine, glutathione, ascorbic acid, insulin, transferrin, trace elements and AlbuMAX I lipid-rich bovine serum albumin (IgG content ≤0.1%).

Figure 6. Growth and titers obtained in CD BHK-21 Production Medium



Comparative growth and titers of BHK-21 cells adapted to suspension in either CD BHK-21 Production Medium or GMEM/TPB + 2 or 10% v/v FBS. (A) BHK-21 adapted to suspension were cultured for 4 days in shake flasks. Results representative of at least 3 experiments, (B) After FMD infection, CD BHK-21 Production Medium achieves comparable antigen yields to classical media with serum with greater consistency. Results are representative of at least 2 independent experiments (±sd), (C) FMD serotype O monovalent vaccines were manufactured at 5000L scale (n=1). No statistical difference was detected in neutralizing antibody titers in vaccinated animals (n=16, ±sd) at the two time points evaluated

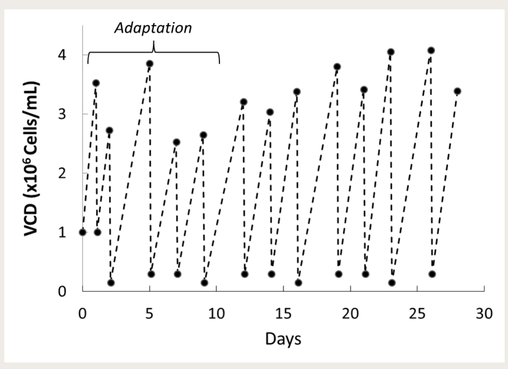
Vaccine cell lines can be adapted to fully chemically-defined conditions after adaptation to suspension.

- **CD BHK-21 Production Medium is Chemically Defined, protein-free, hydrolysate-free.** It allows for cleaner downstream processing and yields stable and potent antigen for animal vaccine formulation.

- **CD BHK-21 Production Medium is more nutritionally dense than GMEM/TPB + 10% FBS.** It enables infection of cultures at higher cell density

- **CD BHK-21 Production Medium allows for lower operational cost compared to using 2% v/v FBS and TPB.**

Figure 7. BHK-21 cells adaptation to CD BHK-21 Production Medium



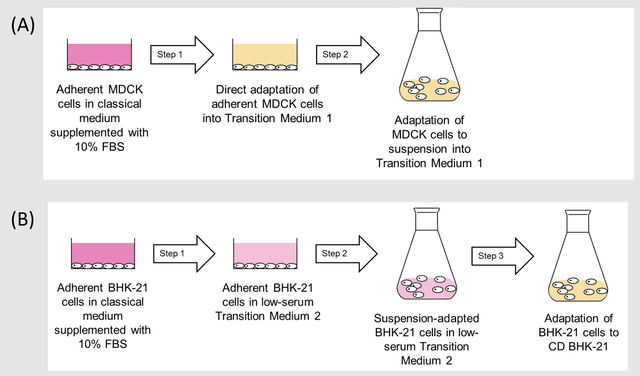
Adaptation method. Direct adaptation of suspension BHK-21 cells grown and banked in GMEM/TPB + 10% v/v FBS into serum-free CD BHK-21 Production Medium in 5 passages (~10 days) with consistent growth in subsequent passages. Suspension-adapted BHK-21 cells were cultured in 125 mL shake flasks with vented caps (125 RPM, 37˚ C, 8% CO2) and subcultivated every two days.

Many vaccine cell lines can be adapted to suspension.

- **Adaptation to suspension can be performed** [2] **:**
directly or over the course of several passaging,
with sequential serum reduction from passage to passage,
with the use of a transition media,
with Minimal amount of FBS to increase VCD.

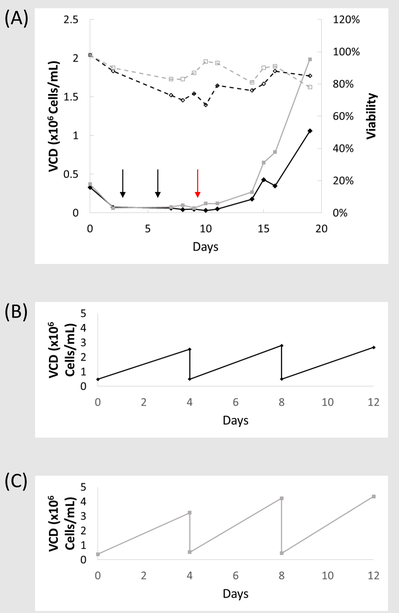
- **Strategies to favor single-cell suspension formation includes:**
Use of cell strainer, differential sedimentation [3], single-cell or addition of trypsin or TrypIE at low concentration,
Reduction of Mg^{2+} and Ca^{2+} concentrations or increased concentration of Pluronic or Anti-Clumping Agent [4].

Figure 8. Step-wise adaptation to suspension using Transition Media



Adaptation methods. (A) MDCK were directly transferred to Transition medium 1 (chemically defined, animal-origin free) to be cultured in static conditions until fully adapted (20 days) then transferred to shake flask. (B) BHK-21 cells were first transferred in low serum Transition medium 2 in adherence then suspension. The full process took 15 weeks for BHK-21 cells (steps 1, 2 and 3). For step 3, cells were considered adapted to chemically-defined media after 5 passages in CD BHK-21 Production Medium (Figure 7).

Figure 9. Adaptation of MDCK cells to suspension



MDCK adaptation to suspension in Transition Medium (black lines) or Transition Medium + 0.5% FBS v/v (grey lines). (A) MDCK cells grown in MEM 10% FBS were harvested and 3.0x10^5 cells were seeded directly into 20 ml of the indicated medium, black arrows represents fresh media additions and red arrows transfer to larger T-flask with fresh medium addition. (B and C) Passaging schedule after adaptation to suspension in 125 mL shake flask (125 RPM, 37 ˚ C, 8% CO2).

REFERENCES

1. Reed L.J. and Muench H. A simple method of estimating fifty per cent endpoints (1938) *American Journal of Epidemiology* 27:493-497.
2. Caron, Angelo Luis, Rafael Tagé Biaggio, and Kamilla Swiech. "Strategies to Suspension Serum-Free Adaptation of Mammalian Cell Lines for Recombinant Glycoprotein Production." Recombinant Glycoprotein Production. Humana Press, New York, NY, 2018. 75-85.
3. Lohr, V., et al. "A new MDCK suspension line cultivated in a fully defined medium in stirred-tank and wave bioreactor." Vaccine 28.38 (2010): 6256-6264.
4. Wu, Jianyong, et al. "Adaptation of insect cells to suspension culture." Journal of Fermentation and Bioengineering 70.2 (1990): 90-93.

ACKNOWLEDGEMENTS

We would like to thank Angel Varela-Rohena, Chance Lai and all teams involved in generating these data for their work at Thermo Fisher Scientific. Some data presented in the study on CD BHK-21 Production Medium were generated by the National Institute of Agricultural Technology, Argentina (INTA).

TRADEMARKS/LICENSING