Virus expression

Adherent Kidney Media Panel rapidly identifies serum-free growth and productivity media for enhanced viral vaccine production

Introduction

The viral vaccine industry continues to shift to the use of mammalian cell culture–based production processes in lieu of chicken egg–based processes that can pose allergen, scale-up, and supply risks [1]. According to the CDC, mammalian cell culture has been successfully used for production of many vaccines approved in the US and Europe, including those for influenza, rotavirus, polio, smallpox, hepatitis, rubella, and chickenpox viruses [2]. However, industry efforts to improve productivity and scale up vaccine production in mammalian processes still pose several challenges. The use of suspension cell lines for viral vaccines is in development, yet production levels fall short of those attained with adherent cell lines such as Vero [3]. Additionally, the viral vaccine industry faces challenges in eliminating the use of serum and switching to animal origin–free (AOF) media and supplements. Lastly, the diverse and unique cell- and virus-specific nutritional requirements of a process can add complexity to development and extend timelines.

The Gibco™ Adherent Kidney Media Panel was developed to support the viral vaccine industry's needs for an efficient and effective screening tool to identify higher-performing, AOF, serum-free growth and production media for adherent kidney cell–based production processes. The panel contains four AOF, serum-free growth media—two that are peptone-free (Growth Medium No. 1 and Growth Medium No. 2) and two that contain AOF peptones (Growth Medium No. 3 and Growth Medium No. 4)—and two AOF, serum-free production media (Production Medium No. 1 and Production Medium No. 2) (Table 1).

Table 1. Composition of Adherent Kidney Medium Panel.

Panel medium	Animal origin–free (AOF)	Serum-free	Peptone-free	Contains AOF peptone
Growth Medium No. 1	•	•	•	—
Growth Medium No. 2	•	•	•	—
Growth Medium No. 3	•	•	—	•
Growth Medium No. 4	•	•	—	•
Production Medium No. 1	•	•	•	—
Production Medium No. 2	•	•	•	—

The Adherent Kidney Media Panel was evaluated for its capability to provide stronger media candidates rapidly and effectively with Vero cells. Panel media were evaluated for the ability to support cell growth and vesicular stomatitis virus (VSV) production, in comparison to commonly used commercially available media. A study with Vero cells directly adapted from a serum-containing bank evaluated the panel's growth and production media, comparing them against Gibco™ VP-SFM and Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS for growth and 2% FBS for production. A later study using Vero cells directly adapted from a serum-free bank evaluated Growth Medium No. 1 or Growth Medium No. 3 with Production Medium No. 1, comparing them against another supplier's growth and production medium and VP-SFM. Both studies assessed viable cell density (VCD) and cumulative population doublings (CPD), as well as VSV titers by plaque assay or median tissue culture infectious dose ($TCID_{50}$) assay.

Materials and methods

Serum-containing banked cell study

Cell culture

- Vero cells (ATCC CCL-81) banked in DMEM (Cat. No. 11965092) tested with 10% FBS were recovered for two passages and maintained in banking medium conditions. After recovery, cells were directly adapted to growth media test conditions for 6 passages. The panel growth media and VP-SFM (Cat. No. 11681020) were tested with 4 mM L-glutamine.
- Cells were cultured in duplicate T-75 flasks with 20 mL of media with passaging at a seeding density of 2 x 104 cells/cm2 for a 4-day culture and 3 x 104 cells/cm2 for a 3-day culture. VCD and viability were measured using a Vi-CELL™ BLU cell viability analyzer (Beckman Coulter).

Infection and harvest

- On the day of infection, a medium exchange was performed for each production medium condition. Production Medium No. 1 and Production Medium No. 2 were tested with 4 mM L-glutamine, and DMEM was tested with 2% FBS. Cells were infected on day 4 of passage 6 at a multiplicity of infection (MOI) of 0.0001 in triplicate T-75 flasks with VSV, Glasgow (Indiana) (ATCC VR-1415).
- Cells were harvested approximately 48 hours post-infection with a cytopathic effect (CPE) of >90%. After harvest, cells were lysed by freezing and thawing the cells to release any remaining virus. The thawed culture was centrifuged to remove the cell debris and the supernatant collected for viral quantification.

Virus quantification by plaque assay

- Vero cells were seeded at 1.0 x 105 cells/well in a 24-well plate. Cells were washed and infected after 3 days of growth. Plates were fixed and stained 48 hours after infection, and the foci in each well were counted manually. The sample titer was calculated based on the foci counted and the dilution factor.

Serum-free banked cell study

Cell culture

- Vero cells (ATCC CCL-81) banked in VP-SFM supplemented with 6 mM L-glutamine were recovered for 2–3 passages and maintained in banking medium conditions. After recovery, cells were directly adapted to each test medium for 4 passages in singlet T-75 flasks. On the 5th passage, cells were expanded to triplicate T-75 flasks in each medium condition for infection. Growth Medium No. 1, Growth Medium No. 3, and another supplier's growth medium were tested with 4 mM L-glutamine.
- Cells were cultured in 20 mL of media in T-75 flasks at 37°C and 5% CO_2 with passaging every 3–4 days at a seeding density of 2 x 104 cells/cm2. Cell counts and viability were measured using a Vi-CELL™ XR cell analyzer (Beckman Coulter).

Infection and harvest

- On the day of infection, a complete medium exchange was performed for each production medium condition. Production Medium No. 1 and another supplier's production medium were tested with 4 mM L-glutamine, and VP-SFM was tested with 6 mM L-glutamine. Cells were infected on day 3 of passage 5 at an MOI of 0.0001 in triplicate T-75 flasks with VSV, Glasgow (Indiana).
- Cells were dislodged by shaking, and the total volume was harvested approximately 48 hours post-infection. Cells were centrifuged and the supernatant was collected, aliquoted, and stored frozen.

Virus quantification by $TCID_{50}$

- Vero cells were seeded in 96-well plates at 5.0 x 10^4 cells/cm^2 in VP-SFM supplemented with 6 mM L-glutamine. The next day, cells were washed with a medium exchange to fresh VP-SFM, and infected. After 48 hours, the plates were scored and the data quantified using the Spearman–Kärber method.

Results

Vero cells adapted from serum-containing banks demonstrated the strongest growth performance using Growth Medium No. 1 and Growth Medium No. 3, with a 2.0-fold improved average VCD and higher average CPD compared to VP-SFM. Growth Medium No. 1 and Growth Medium No. 3 supported comparable average VCD and CPD relative to DMEM supplemented with 10% FBS (Figure 1). Cell viability remained high at 94–97% for all media conditions (data not shown). Using Growth Medium No. 1 and Production Medium No. 1, results indicated a stronger relative average VSV titer of 3.6-fold compared to VP-SFM. Additionally, Growth Medium No. 2 and Production Medium No. 2 supported a 2.6-fold relative average VSV titer compared to VP-SFM, with productivity comparable to that of DMEM supplemented with FBS at 10% for growth and 2% for production (Figure 2).

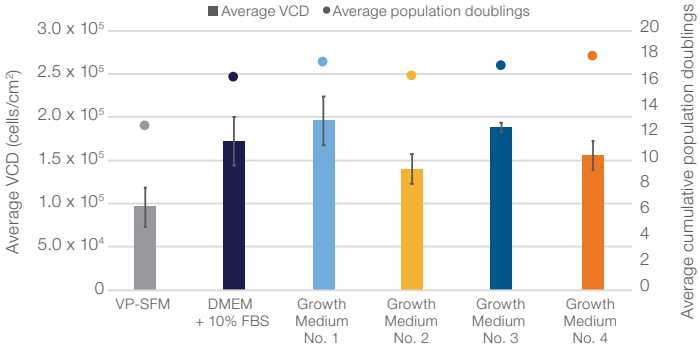

Figure 1. Adherent Kidney Media Panel formulations increase growth of serum-banked Vero cells. On passage 6, Vero cells adapted from a serum-containing bank yielded an improved 2-fold average VCD with Growth Medium No. 1 and Growth Medium No. 3, and higher CPDs, relative to VP-SFM. Comparable average cell growth was seen with Growth Medium No. 1 and Growth Medium No. 3, relative to DMEM supplemented with 10% FBS. Cell viability remained high at 94–97% for all media conditions (data not shown).

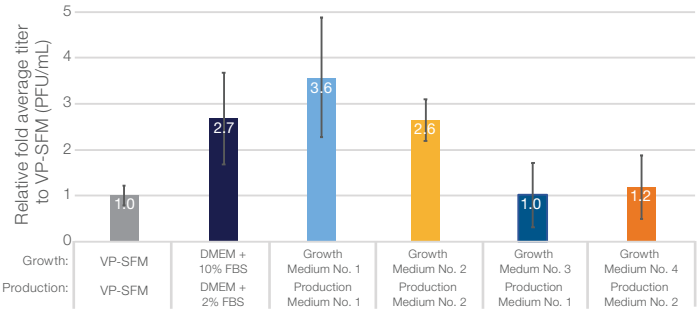

Figure 2. Panel formulations boost VSV productivity of Vero serum-banked Vero cells. Vero cells adapted from serum containing banks showed an enhanced 3.6-fold relative average titer with Growth Medium No. 1 and Production Medium No. 1 compared to VP-SFM. Growth Medium No. 2 and Production Medium No. 2 demonstrated a stronger 2.6-fold relative average titer to VP-SFM. Comparable productivity was observed relative to DMEM supplemented with 10% FBS for growth and 2% FBS for production.

With Vero cells directly adapted from a serum-free bank, Growth Medium No. 1 supported a 3.4-fold average VCD and increased CPD, compared to medium from another supplier and an average 1.4-fold growth and CPD relative to VP-SFM. Using Growth Medium No. 3, the average VCD was 2.2-fold with increased CPD relative to another supplier's medium and comparable results to VP-SFM (Figure 3). Cell viability remained high at 93%– 96% for all media conditions (data not shown). A $TCID_{50}$ viral production assay with cells using Growth Medium No. 3 with Production Medium No. 1 resulted in an improved 10.6-fold average VSV titer relative to another supplier's media and 7.0-fold relative to VP-SFM. Cells with Growth Medium No. 1 and Production Medium No. 1 produced an enhanced 5.0-fold relative average titer to another supplier's media and 3.3-fold relative to VP-SFM (Figure 4).

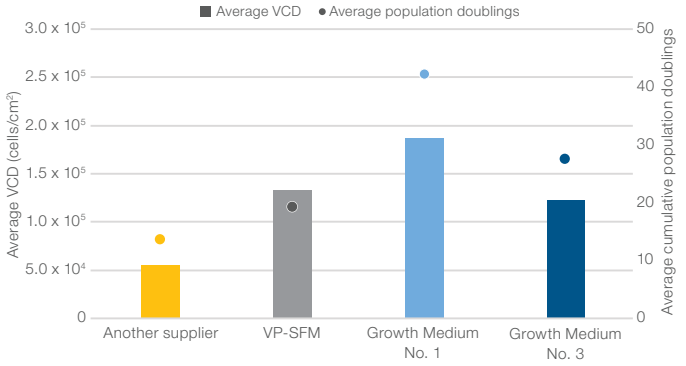

Figure 3. Adherent Kidney Media Panel identifies formulations to enhance growth of serum-free banked Vero cells. On passage 6, Vero cells adapted from a serum-free bank with Growth Medium No. 1 demonstrated an average 3.4-fold VCD and higher CPD relative to another supplier's medium, and a 1.4-fold average VCD and increased CPD relative to VP-SFM. Using Growth Medium No. 3, a 2.2-fold VCD and increased CPD was seen relative to another supplier's medium, and cell growth comparable to that of VP-SFM. Cell viabilities remained high at 93–96% for all media conditions (data not shown).

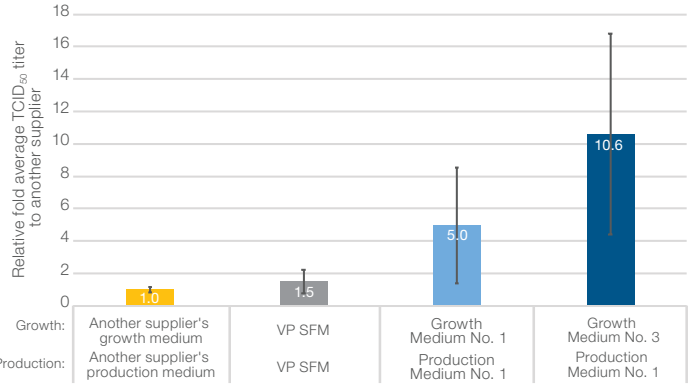

Figure 4. Panel formulations increase VSV productivity of serum-free banked Vero cells. Virus production of serum-free banked Vero cells using Growth Medium No. 3 with Production Medium No. 1 showed a 10.6-fold relative average VSV titer compared to another supplier's media, and a 7.0-fold improved average titer relative to VP-SFM. Additionally, Growth Medium No. 1 and Production Medium No. 1 produced a 5.0-fold improved average titer relative to another supplier's media and a 3.3-fold relative titer to VP-SFM.

Discussion and conclusions

The results presented show that the Adherent Kidney Media Panel can provide options for viral vaccine manufacturers to rapidly identify AOF and serum-free growth and production media formulations that support comparable or improved cell growth up to 3.4-fold and enhanced productivity up to 10.6-fold. Evaluations of the panel with Vero cells effectively identified improved media for cells directly adapted from a serum-containing bank and from a serum-free bank. In addition to Vero cells, media panel formulations can support comparable or stronger MDCK and BHK-21 cell growth relative to other commercially available serum-free and serum-supplemented media (data not shown).

The Adherent Kidney Media Panel provides rapid access to diverse, ready-to-use liquid formulations, protocols, and field support to easily and effectively identify improved AOF, serum-free media candidates for adherent virus production. The panel provides access to flexible media formats—from liquid format to the scalable and easy-to-reconstitute Gibco™ Advanced Granulation Technology (AGT™) dry format. Additionally, options are available to maximize candidate media optimization and processes with subject matter experts in the Gibco™ Media by Design™ Services. The Adherent Kidney Media Panel, together with the provided technical support and optional optimization services, can enhance productivity and accelerate vaccine development to help manufacturers meet global demand.

References

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Find out more about how utilizing the Adherent Kidney Media Panel can elevate your vaccine processes at **thermofisher.com/akmp**

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