Diploid Growth Serum-Reduced Medium can reduce or eliminate serum use in human and animal vaccine production

Introduction

Human diploid cells have played a key role in the development of vaccines since the 1960s when they were quickly adopted to help combat devastating infectious agents, including measles, mumps, rubella, polio, hepatitis A, and rabies viruses [1]. Supplementing classical media with 10% fetal bovine serum (FBS) is a traditional culture method in the production of most vaccines. Over the years, human and animal vaccine production processes have used FBS as a media supplement and have had long-established safety profiles in conjunction with availability of premium FBS sources. However, the global supply of premium FBS is currently challenged by the increase in demand to support manufacturing of existing vaccines and new therapies. The increase in demand for FBS has led to additional challenges for vaccine manufacturers, such as increased frozen storage requirements and higher production costs, which ultimately have a negative impact on vaccine pricing.

While these difficulties and high industry demands have driven the need to reduce or eliminate serum in vaccine production, the longevity of human diploid cells in culture has posed a critical challenge. Diploid cells have a limited capacity to divide, approximately 40–60 population doublings, before they reach the Hayflick limit and become senescent [2]. This limitation requires the development of a serum-reduced or serum-free medium that supports cell growth without adaptation.

To help vaccine manufacturers using diploid cells meet their production forecasts while reducing production costs and serum use, Thermo Fisher Scientific launched the Gibco™ Diploid Growth Serum-Reduced Medium (SRM) System.* Human diploid cells, including MRC-5, WI-38, KMB-17, and 2BS, and chicken embryo fibroblasts (CEFs) can be used with this system. Once cells are recovered and expanded in Diploid Growth SRM, virus production is seamlessly performed with a simple medium exchange into Diploid Production SFM, without requiring cell washes or supplementation with serum or albumin.

To assess the potential of Diploid Growth SRM for cell recovery and expansion, viable cell density (VCD) was evaluated using MRC-5 cells. To help ensure comparability, MRC-5 cells banked in a control medium with FBS or in Diploid Growth SRM were directly recovered under different conditions and monitored over several passages. Long-term expansion of MRC-5 cells in Diploid Growth SRM was compared to the control medium with FBS. In addition, the potential to culture CEFs in Diploid Growth SRM without the use of serum was investigated.

* The Diploid Growth SRM System is composed of two kits designed to be used in conjunction: the Gibco™ Diploid Growth SRM kit for cell expansion (Cat. No. A3968901) and the Gibco™ Diploid Production Serum-Free Medium (SFM) kit for viral production (Cat. No. A3969001). Each kit contains a dry powder basal medium and a concentrated 100X liquid supplement designed for growth or production. The growth supplement contains a purified animal-origin component from a BSE/TSE-free source that has been tested to be free of viruses (9 CFR guidelines). The basal media and the production supplement are free of animal-origin components.

Materials and methods

Expansion of MRC-5 cells

Growth (VCD) was evaluated using MRC-5 cells grown in a control medium or in Diploid Growth SRM (Table 1). Four different cell recovery and growth conditions (A–D) were tested using the Diploid Growth SRM and the control medium (Figure 1). Cell expansion was evaluated for each condition over a total of four post-recovery passages.

Cells in all four conditions were cultured in vented T-75 flasks (Corning). For passaging, cells were washed with Gibco™ Dulbecco's Phosphate-Buffered Saline (DPBS), no calcium, no magnesium (Thermo Fisher Scientific, Cat. No. 14190144), then dissociated with Gibco™ Trypsin-EDTA (Thermo Fisher Scientific, Cat. No. 25300054) and quenched with Gibco™ Defined Trypsin Inhibitor (Thermo Fisher Scientific, Cat. No. R007100). The cells were then centrifuged for 5 min at 200 x *g* and resuspended in their respective media. Cell counts were performed using a Vi-CELL™ XR analyzer (Beckman Coulter). Cells were seeded at a density of 1.5 x 10^4 cells/cm^2 in 25 mL of the control medium or Diploid Growth SRM and cultured for four 3-day passages followed by four 7-day passages.

MRC-5 cells that were transferred at passage 1 (P1) to Diploid Growth SRM following recovery (Figure 1, condition B) were cultured further over multiple passages for nearly 50 population doublings over a period of 45 days, alongside cells recovered and grown in the control medium (Figure 2).

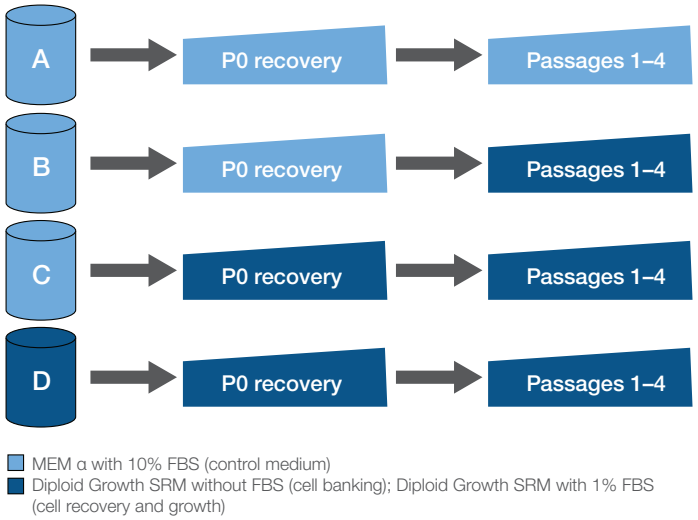

Figure 1. Recovery and growth conditions tested for MRC-5 cells. **(A)** Cells banked in the control medium were recovered and grown in the control medium. **(B)** Cells banked in the control medium were initially recovered at passage 0 (P0) in the control medium and subsequently passaged at P1 into Diploid Growth SRM. **(C)** Cells banked in the control medium were directly recovered and passaged in Diploid Growth SRM. **(D)** Cells banked in Diploid Growth SRM (without FBS) were recovered and grown in Diploid Growth SRM with 1% FBS.

Culture of CEFs without serum

The feasibility of culturing CEFs without the use of serum was evaluated by growing them in Diploid Growth SRM without serum and comparing the results to a control medium (Table 2).

Table 1. Conditions for experiments evaluating growth (VCD) of MRC-5 cells.

Parameter	Description
Cells	MRC-5 pd19 (ECACC, Cat. No. 05072101)
Seeding density	1.5 x 10^4 cells/cm^2
Control medium	Gibco™ MEM α, Nucleosides* (Cat. No. 12571063), supplemented with 4 mM Gibco™ L-glutamine (Cat. No. 25030149), 2 g/L Gibco™ Glucose Solution (Cat. No. A2494001), and 10% Gibco™ FBS (Cat. No. A3160401)
Diploid Growth SRM	Diploid Basal Medium* supplemented with 1% Diploid Growth Supplement (both included in the Diploid Growth SRM kit) and 1% Gibco FBS
Incubation conditions	37°C with 5% CO_2

* MEM α, Nucleosides contains 2 mM L-glutamine. Diploid Basal Medium contains 6 mM L-glutamine.

Table 2. Conditions for experiments evaluating growth of CEFs without serum.

Parameter	Description
Cells	CEFs harvested from embryonated specific pathogen–free (SPF) chicken eggs sourced from Lohmann Brown and Lohmann LSL
Seeding density	1.5 x 10^5 cells/cm^2
Control medium	Gibco™ MEM, Hanks' Balanced Salts (MEM-H, Cat. No. 11575032)** supplemented with 2 mM L-glutamine, 10% FBS, and 1% Gibco™ penicillin-streptomycin (Cat. No. 15140148)
Diploid Growth SRM	Diploid Basal Medium** supplemented with 1% Diploid Growth Supplement (both included in the Diploid Growth SRM kit) and 1% penicillin-streptomycin
Incubation conditions	37°C with 5% CO_2 and 90% humidity

** MEM-H contains 2 mM L-glutamine. Diploid Basal Medium contains 6 mM L-glutamine.

CEFs were washed with DPBS without calcium and magnesium and treated with 0.25% trypsin-EDTA. The cells were then centrifuged, resuspended, and seeded in complete MEM-H containing 10% FBS, or in complete Diploid Growth SRM without serum supplementation.

Cells in both the control medium and Diploid Growth SRM were cultured for 4 days in 15 mL of growth medium in Thermo Scientific™ Nunc™ EasYFlask™ Cell Culture Flasks, Nunclon™ Delta treated (Cat. No. 156499). Cell counts were performed using a NucleoCounter™ NC-200™ Automated Cell Counter (ChemoMetec A/S).

Results

MRC-5 cells demonstrate strong adaptation-free expansion in Diploid Growth SRM

The VCD values for MRC-5 cells cultured in Diploid Growth SRM with 1% FBS showed growth results comparable to the control condition (Figure 2, condition A) following four passages. Interestingly, all recovery scenarios showed comparable growth: control medium–banked cells recovered at P0 into control medium, with subsequent P1 transfer into Diploid Growth SRM (Figure 2, condition B), control medium–banked cells directly recovered into Diploid Growth SRM at P0 (Figure 2, condition C), and cells banked in Diploid Growth SRM without FBS directly thawed into Diploid Growth SRM with 1% FBS (Figure 2, condition D). These results demonstrate the flexibility of Diploid Growth SRM to allow multiple methods of recovery and still achieve comparable growth.

Additionally, MRC-5 cells from condition B were repeatedly passaged in Diploid Growth SRM for a cumulative period of 45 days (nearly 50 population doublings) for comparison with the control medium. The results demonstrate similar expansion with Diploid Growth SRM supplemented with a final concentration of 1% FBS, compared to the control medium (Figure 3).

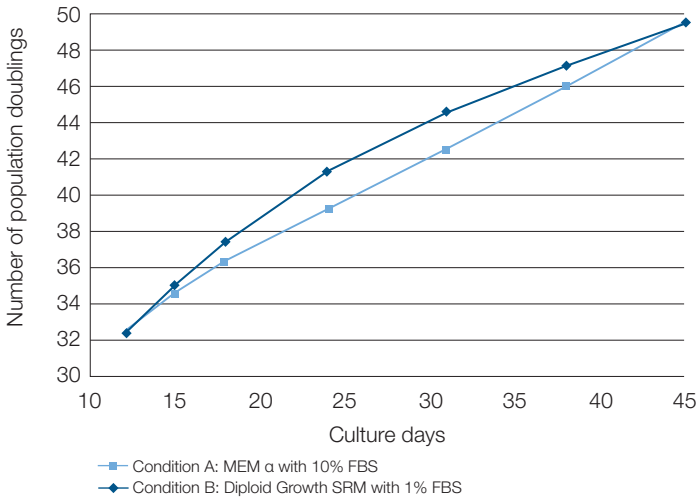

Figure 3. MRC-5 cumulative population doublings. MRC-5 cells cultured for a total of 45 days and nearly 50 population doublings in Diploid Growth SRM (condition B) demonstrated growth similar to cells grown for the same time period in the control medium (condition A).

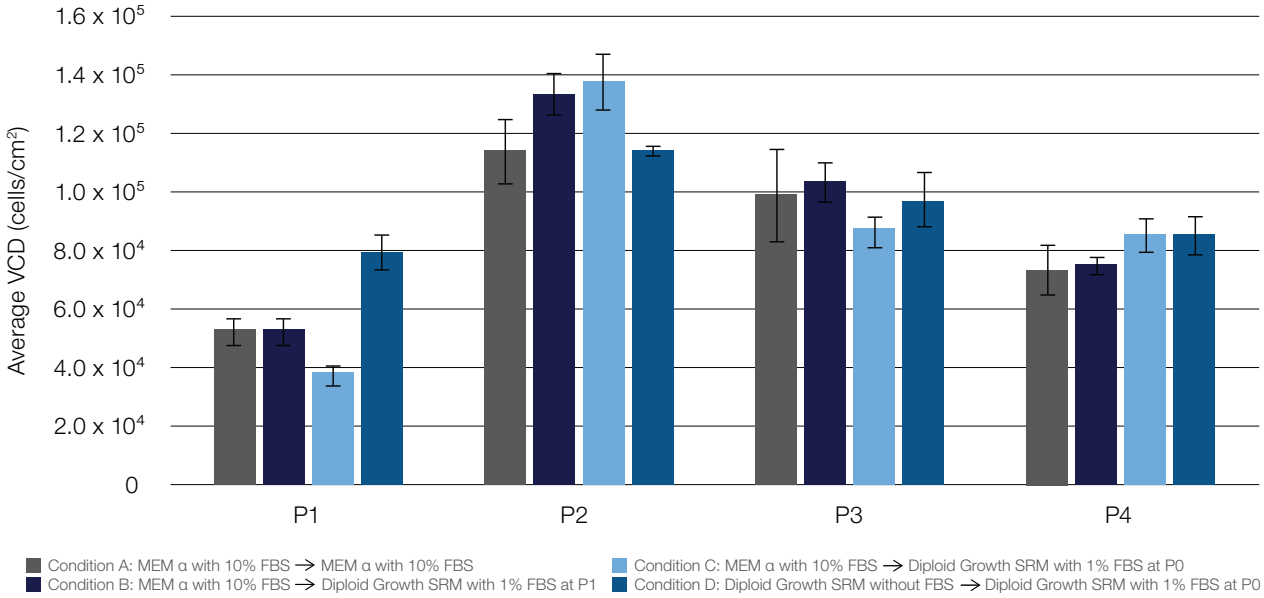

Figure 2. VCD of MRC-5 cells after different recovery and growth conditions. By passage 4, MRC-5 cells banked and recovered by different methods demonstrated similar growth in Diploid Growth SRM supplemented with a final concentration of 1% FBS (conditions B–D) compared to the control medium with a final concentration of 10% FBS (condition A).

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Diploid Growth SRM supports the culture of CEFs without serum

As shown in Figure 4, Diploid Growth SRM without serum supplementation demonstrated 13% higher CEF growth compared to the control medium.

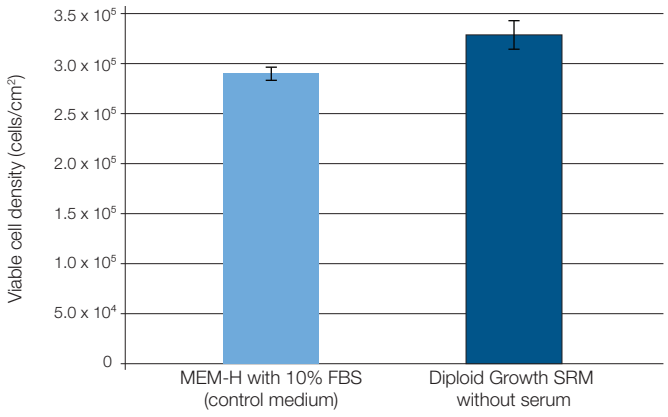

Figure 4. CEF growth results. CEFs demonstrated 13% higher growth in Diploid Growth SRM than in the control medium.

Diploid Growth SRM not only allows for cell banking without serum but also enables serum-banked cells to be directly recovered from frozen conditions without adaptation or adverse effects on the limited population doubling capacity of human diploid cells. The data presented here demonstrate these capabilities with MRC-5 cells, as well as the medium's ability to robustly support long-term culture with 1% FBS over 45 days and nearly 50 population doublings. In addition to human diploid cells, Diploid Growth SRM also was demonstrated to support better growth of CEFs without the need for serum supplementation. The use of Diploid Growth SRM has the potential to reduce serum usage while supporting the production of vaccines worldwide.

References

1. Human cell strains in vaccine development. The history of vaccines: An educational resource by the College of Physicians of Philadelphia. https://www.historyofvaccines.org/content/articles/human-cell-strains-vaccine-development
2. Hayflick L (1965) The limited *in vitro* lifetime of human diploid cell strains. *Exp Cell Res* 37(3):614–636.

Conclusions

Vaccine manufacturers are increasingly faced with challenges from the use of serum on top of the high industry demand. Mitigating these challenges through reduction or elimination of serum is key to a healthy vaccine industry. For users of diploid cells this change has proven to be difficult, as diploid cells divide only a finite number of times before they become senescent. This constraint has created a need for serum-reduced or serum-free media that support cell growth without adaptation. The data presented show that Diploid Growth SRM enables an 80–90% reduction in serum usage, from 10% to 1–2% for diploid cells.

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