Diploid Serum-Reduced Medium system supports viral production and outperforms in specific productivity

Introduction

The Gibco™ Diploid Serum-Reduced Medium (SRM) system was developed for diploid cells in order to minimize the use of fetal bovine serum (FBS) in vaccine production and overcome cultivation challenges by supporting cell growth without medium adaptation. The system consists of Gibco™ Diploid Growth SRM for cell culture and Gibco™ Diploid Production Serum-Free Medium (SFM) for virus production. In a recent application note [1], Diploid Growth SRM was shown to allow recovery and expansion of diploid cells without adaptation, while reducing serum use up to 90%.

Here we present a study that was conducted to evaluate the growth and production capabilities of the Diploid SRM system with MRC-5 cells. MRC-5 cell expansion was compared in Diploid SRM and three alternative media from other suppliers over a cultivation period of eight passages. In addition, varicella zoster virus (VZV) and vesicular stomatitis virus (VSV) titer and specific productivity were evaluated with the Diploid SRM system and with classical media.

Materials and methods

Cell culture to evaluate growth performance

As outlined in Table 1, MRC-5 cells were recovered in banking medium in vented T-75 flasks (Corning). After recovery, cells were transferred to flasks with 25 mL of each growth medium, in triplicate, and subcultured every 4 days for a total of eight passages. Cell counts were performed using a Vi-CELL™ XR analyzer (Beckman Coulter).

Table 1. Test conditions for growth performance of MRC-5 cells.

Parameter	Description
Cells	MRC-5 pd19 (ECACC, Cat. No. 05072101)
Seeding density	1.5 x 10^4 cells/cm^2
Banking and recovery medium	Gibco™ MEM α, Nucleosides* (Cat. No. 12571063), supplemented with 4 mM Gibco™ L-glutamine (Cat. No. 25030149), 2 g/L Gibco™ glucose (Cat. No. A2494001), and 10% Gibco™ FBS (Cat. No. A3160901)
Media A and B	Supplemented with 6 mM L-glutamine and 1% FBS
Medium C	Supplemented with 2% of the included supplement, 6 mM L-glutamine, and 1% FBS
Diploid Growth SRM	Gibco™ Diploid Growth SRM kit (Cat. No. A3968901): Diploid Basal Medium* supplemented with 1% of the included Diploid Growth Supplement and 1% FBS
Incubation conditions	37°C with 5% CO_2

* Diploid Basal Medium contains 6 mM L-glutamine. MEM α, Nucleosides contains 2 mM L-glutamine, so it is supplemented with 4 mM L-glutamine to obtain the same concentration as in Diploid Basal Medium.

Cell culture and infection to evaluate viral production performance

As outlined in Table 2, MRC-5 cells were recovered in banking medium with scale-up over three passages. Cells were seeded in 25 mL of each growth medium in vented T-75 flasks (Corning) and subcultured every 4 days. For infection, cell counts were determined in representative flasks, and the growth medium was replaced in triplicate flasks with 20 mL of each production medium. Three

different lots of each production medium were tested. VZV or VSV was added to the appropriate flasks at the given multiplicity of infection (MOI). Flasks were incubated and observed daily for cytopathic effect (CPE). Once CPE was visible in one of the culture flasks, all flasks were harvested for that same virus. VZV cultures were harvested at 3 days post-infection (dpi) and VSV cultures at 2 dpi. Viral titers were determined using a tissue culture infectious dose 50% ($TCID_{50}$) endpoint method.

Results

Comparing growth performance of Diploid Growth SRM to alternative media

When MRC-5 cells were cultured for 8 passages, based on the last three passages (6, 7, and 8), cultures grown in Diploid Growth SRM demonstrated 119% higher average viable cell density (VCD) compared to the combined average growth with the alternative media A, B, and C (Figure 1). While cells achieved better growth with medium A than with media B and C, cell expansion with Diploid Growth SRM yielded 65% higher average growth than with medium A. Additionally, neither medium B nor C supported sufficient cell growth to passage 8.

Table 2. Test conditions for viral productivity performance of MRC-5 cells.

Parameter	Description
Cells	MRC-5 pd19 (ECACC)
Seeding density	1.5 x 10^4 cells/cm^2
Banking and recovery medium	MEM α, Nucleosides,* supplemented with 4 mM L-glutamine, 2 g/L glucose, and 10% FBS
Control growth medium: VZV and VSV	MEM α, Nucleosides,* supplemented with 4 mM L-glutamine, 2 g/L glucose, and 10% FBS
Control production medium: VZV	MEM α, Nucleosides,* supplemented with 4 mM L-glutamine, 2 g/L glucose, and 2% FBS
Control production medium: VSV	Gibco™ Advanced MEM (Cat. No. 12492013) supplemented with 4 mM L-glutamine and 2 g/L glucose
Diploid Growth SRM: VZV and VSV	Diploid Growth SRM kit: Diploid Basal Medium* supplemented with 1% of the included Diploid Growth Supplement and 1% FBS
Diploid Production SFM: VZV and VSV	Gibco™ Diploid Production SFM kit (Cat. No. A3969001): Diploid Basal Medium* supplemented with 1% of the included Diploid Production Supplement
MOI	0.01
Incubation conditions	37°C with 5% CO_2

* Diploid Basal Medium contains 6 mM L-glutamine. MEM α, Nucleosides contains 2 mM L-glutamine, so it is supplemented with 4 mM L-glutamine to obtain the same concentration as in Diploid Basal Medium.

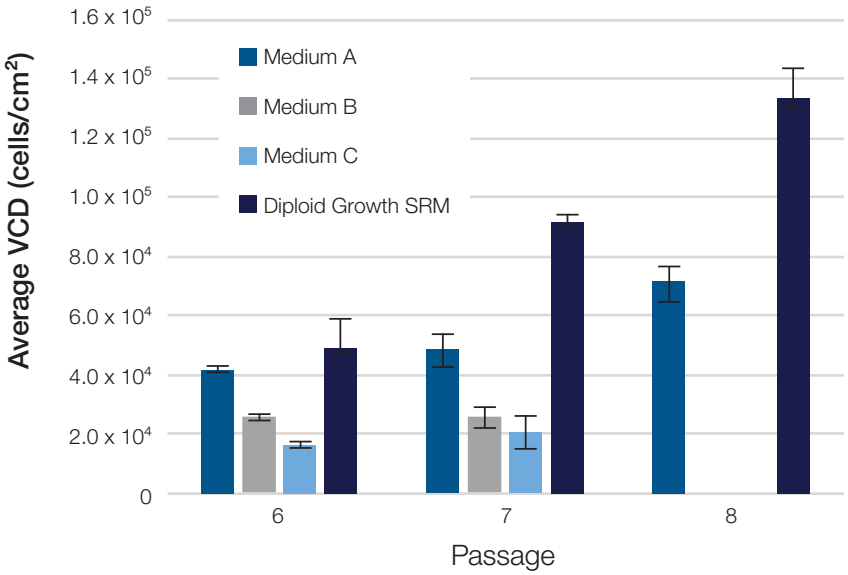

Figure 1. MRC-5 growth. Based on the last three passages (6, 7, and 8), Diploid Growth SRM demonstrated 119% higher average VCD than the combined average of three alternative media. Diploid Growth SRM outperformed the leading alternative, medium A, by 65%.

Evaluating VZV and VSV titer and specific productivity using the Diploid SRM system

When MRC-5 production of VZV and VSV was evaluated, the Diploid SRM system, without FBS supplementation during virus production, supported average titers comparable to the control media (Figures 2 and 3 for VZV and VSV, respectively).

In addition, viral production with the Diploid SRM system demonstrated 147% higher specific productivity for VZV and 73% higher for VSV, compared to the control media (Figures 4 and 5 for VZV and VSV, respectively).

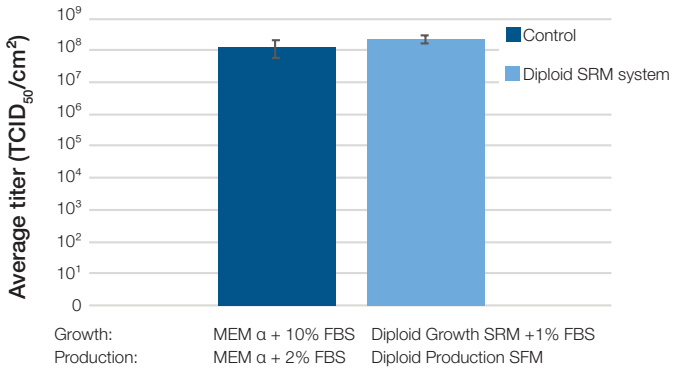

Figure 2. MRC-5 VZV titer. The Diploid SRM system, with 1% FBS for growth and serum-free production, enabled average VZV titers comparable to the control media, MEM α with 10% FBS for growth and 2% FBS for production.

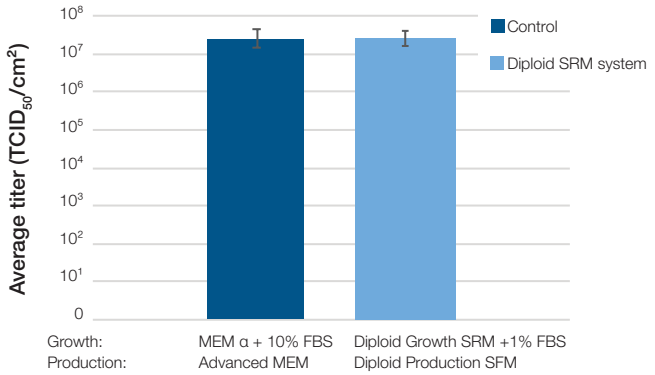

Figure 3. MRC-5 VSV titer. MRC-5 cells cultured with the Diploid SRM system, with 1% FBS for growth and serum-free production, supported average VSV titers comparable to the control media, MEM α with 10% FBS for growth and Advanced MEM for production.

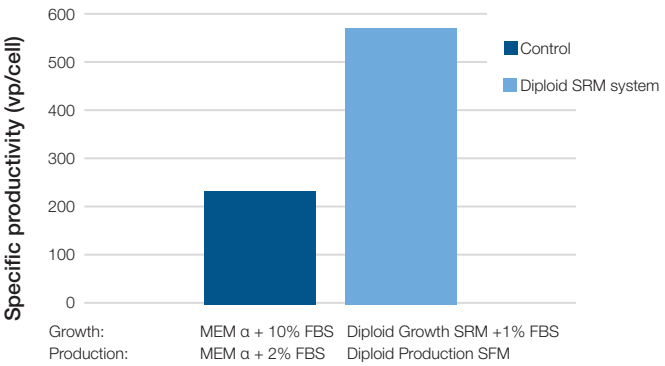

Figure 4. MRC-5 VZV specific productivity. The Diploid SRM system, with 1% FBS for growth and serum-free production, enabled 147% higher average specific productivity than the control media, MEM α with 10% FBS for growth and 2% FBS for production.

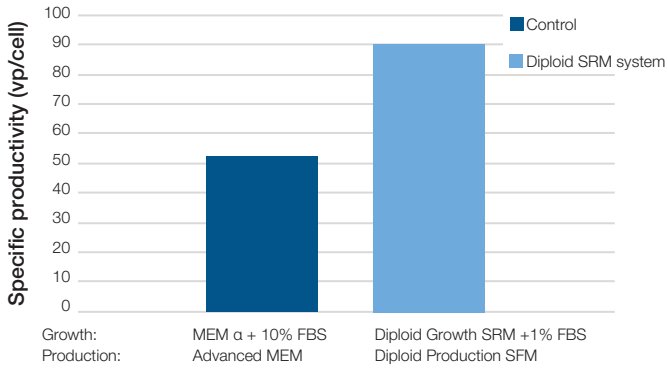

Figure 5. MRC-5 VSV specific productivity. MRC-5 cells cultured with the Diploid SRM system, with 1% FBS for growth and serum-free production, supported 73% higher average specific productivity than the control media, MEM α with 10% FBS for growth and Advanced MEM for production.

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Conclusions

This study evaluated MRC-5 cell growth and virus production performance with the Diploid SRM system and selected alternative media. Under comparable 1% serum-supplemented conditions, MRC-5 cells demonstrated higher average VCD with Diploid Growth SRM, substantially exceeding the combined average growth with the alternative media. The Diploid Growth SRM enabled 65% higher average growth compared to the best-performing alternative, medium A.

Additionally, VZV and VSV productivity in MRC-5 was evaluated with the Diploid SRM system to determine its capabilities compared to control media. Cells cultured with the Diploid SRM system demonstrated comparable average titers and higher VZV and VSV average specific productivities.

Overall, the comparative growth and viral production results demonstrate that the Diploid SRM system can enable vaccine manufacturers to considerably reduce the use of FBS while achieving potential increases in viral specific productivity with diploid cells. Previous studies, along with these results, demonstrate the potential and flexibility for vaccine manufacturers to integrate the Diploid SRM system into their existing process, with the potential to increase productivity and reduce overall production costs.

Reference

1. Thermo Fisher Scientific (2021) Diploid Growth Serum-Reduced Medium can reduce or eliminate serum use in human and animal vaccine production. Application note available at thermofisher.com/diploid

Find out more at **thermofisher.com/diploid**

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