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Development of Animal Component-Free Medium for Virus Production

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Abstract

The use of wild-type reovirus has been developed as a cancer treatment by Oncolytics Biotech (Reolysin™). We developed an animal component-free cell culture medium to facilitate the production of this virus as a pharmaceutical agent. The development was initiated with an intensive media screen. From this screening phase, we were able to identify not only a formulation for further optimization but also some critical components for virus productivity. More over, in the optimized formulation SAFC Biosciences Item No. 65719, the cells generated less "metabolic waste" ammonia and utilized "metabolic wastes" glutamate and lactate when the energy sources such as glutamine and glucose were depleted. Formulation Item No. 65719 supported viable cell densities near 9E6cells/mL and Tissue Culture Infecting Dose (TCID50/mL) value greater than 5E10/mL were achieved after medium and process optimization.

Introduction

Reovirus is manufactured in a serum-free medium cultured HEK 293 cells. To meet regulatory requirements for clinical material, an animal component-free manufacturing process is essential. Cell culture medium components that increase productivity can vary for different virus. A low ammonia level may also be important for maximizing virus productivity and stability. With these objectives in mind, we initiated studies to develop an animal component-free medium to enhance cell growth of HEK 293 and the production of reovirus. Additionally, we investigated the effects of process optimization using the newly developed medium formulation.

Results

	7 Days Total VCD (E6cell/mL)	Viability on Day 7 (%)	Ammonia Level on Day 7 (mM/L)	Average Doubling Time (hr)	TCID 50/mL
Control medium	9.38	67.6	3.1	33.48	3.49E+09
Item No. 65620	15.21	92.1	2.13	28.85	2.78E+09
Item No. 65621	18.85	89.5	2.27	24.83	1.1E+10
Item No. 65650	12.57	78.6	3.23	34.47	4.4E+09
Item No. 65651	14.40	83.3	2.91	26.86	5.53E+09
Item No. 65654	15.63	65.7	1.32	24.82	6.99E+08
EX-CELL™ 14561	27.70	94.5	2.22	20.32	1.39E+10
EX-CELL™ 14571	15.58	96.2	2.39	27.51	6.96E+09
Item No. G9916	16.20	87.7	3.52	24.28	3.49E+09
Item No. M-G9916	10.13	96	3.74	30.29	6.99E+08

Table 1. Medium Screening

Eleven formulations were screened for cell growth and virus productivity. Cells were infected when the viable cell density reached 1E6cells/mL. The multiplicity of infection (MOI) was 0.5. Virus samples were harvested on day 4 based on the protocol developed for the control medium. Data from Table 1 shows that EX-CELL™ 14561 supported cell growth at 8-9E6/mL with a TCID50 of 1E10/mL. Cells in EX-CELL™ 14561 grew to three-fold higher density but generated less ammonia than in control medium. Therefore, formulation EX-CELL™ 14561 was chosen for further optimization.

Medium	Total Cell Density (E6cell/mL)	TCID 50/mL
EX-CELL™14561	27.701	1.39E+10
Item No. 65716	26.911	2.78E+09
Item No. 65720	18.779	3.49E+09

Table 2. Media without critical components

Item No. 65716 is EX-CELL™ 14561 without insulin and LongR3IGF-I (LongR3)

Item No. 65720 is EX-CELL™ 14561 without hydrolysate

Data in Table 2 shows that the formulations without insulin, LR3 or hydrolysate, produced less infectious virus.

Medium	Total Cell Density (E6cell/mL)	TCID 50/mL
Control Medium	12.638	4.4E+09
Item No. 65719	32.909	1.75E+10

Table 3. Medium Optimization

To support downstream purification and regulatory requirements, components that are not critical for virus production were removed from EX-CELL™ 14561. Infection was performed by following the same protocol in medium screening phase. Data in Table 3 demonstrates that Item No. 65719, which is leaner version of EX-CELL™ 14561, supported a cell density of 8–9E6/mL and virus productivity of 1.75E10/mL. This is similar to previous data using EX-CELL™ 14561.

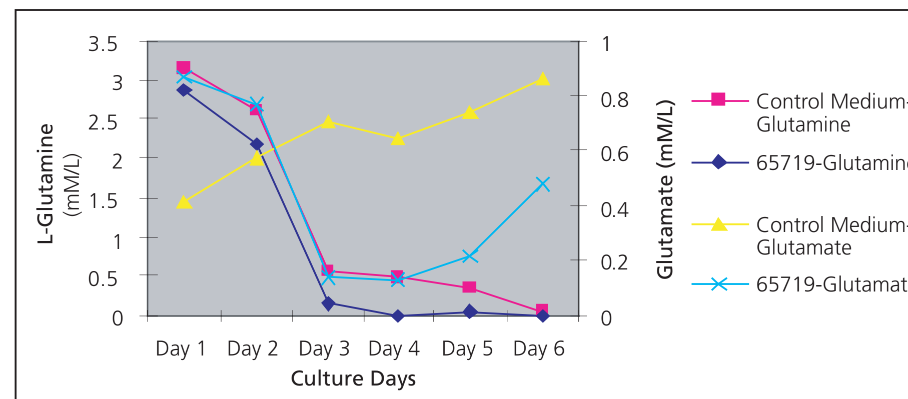

Figure 1A: L-glutamine and glutamate metabolism in Item No. 65719 and control medium

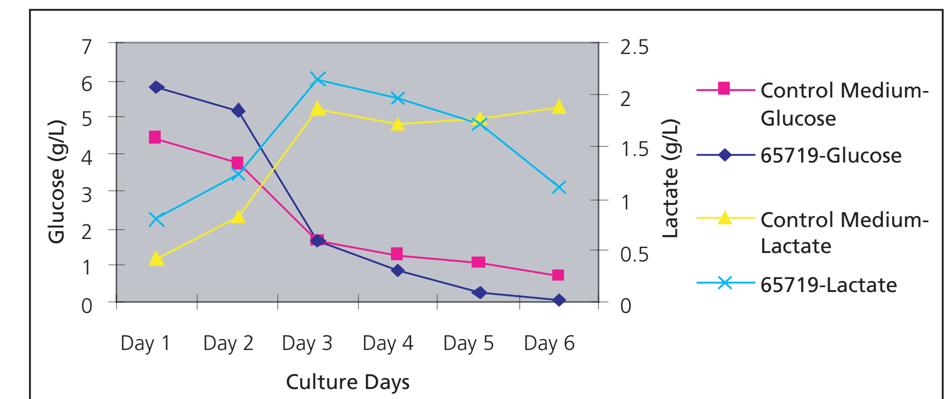

Figure 1B: Glucose and lactate metabolism in Item No. 65719 and control medium

Cells were seeded at 1E6cell/mL. Glucose, L-glutamine, glutamate and lactate levels were monitored every day during culture. Data in Figure 1A and 1B demonstrate that glutamate and lactate decreased when glucose and glutamine were depleted.

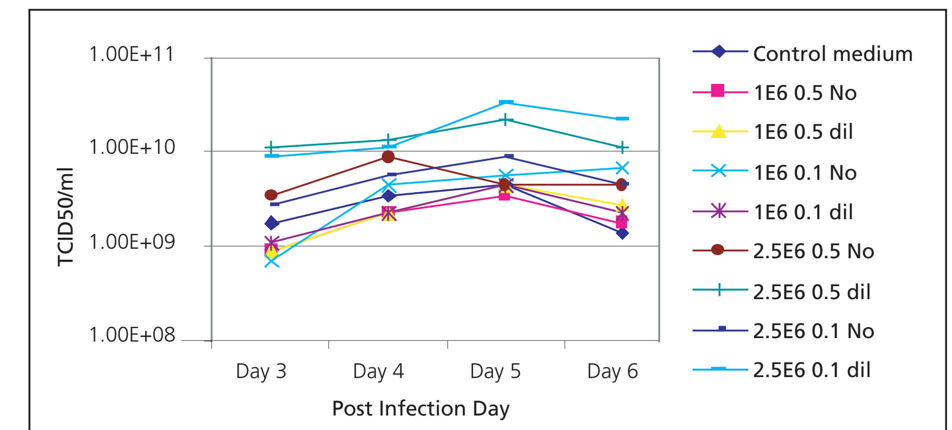

Figure 2. Infection optimization for Item No. 65719

Infection conditions includes cell density, MOI, fresh medium addition and harvest time were tested for best virus production. Data from Figure 2 shows that virus titer was 10 times higher than control medium at 2.5E6/mL cell density with 0.1 MOI. Furthermore, adding fresh medium at time of infection improved virus titer.

Conclusions

- Growth factor and hydrolysates are critical for the production of this non-enveloped virus.
- Cells in Item No. 65719 generate less ammonia and utilize glutamate and lactate when L-glutamine and glucose are depleted.
- Final medium supports 8–9E6/mL viable cell density. A three-fold increase in cell density when compared to control formulation.
- A cell density of 2.5E6/mL at the time of infection with 0.1 MOI significantly improves virus titer.
- The combination of improved basal medium and process optimization allows us to achieve viral titer greater than 5E10 TCID 50/mL, which is a ten-fold increase over control medium and process.

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