Virus Production in Vero Cells Using a Serum-free Medium

K. Kolell, J. Padilla-Zamudio, B. Schuchhardt, S. Gilliland, S. McNorton, B. Dalton, S. Luo, K. Etchberger
JRH Biosciences, Inc., Lenexa, Kansas, USA

Abstract: The manufacture of viral vaccines has historically been accomplished using animal products such as chicken eggs, or cell cultures using fetal bovine serum. To reduce regulatory concerns in vaccine production, serum-free cell culture processes are being embraced by the vaccine industry. The Vero cell line initiated from the African green monkey is an excellent cell line for the production of animal and human prophylactic viral vaccines. We have developed a serum-free (SF) and animal-component free (ACF) medium for the production of viral vaccines using the Vero cell line. This medium supports growth of Vero cells on microcarriers in a controlled bioreactor environment and virus production equivalent to serum-containing cultures. These characteristics make this an ideal medium for vaccine production using the Vero cell line.

Key words: Serum-Free Medium, Vero, Virus, Microcarrier, Bioreactor

1. INTRODUCTION

The Vero cell line, isolated from the kidney of a normal adult African Green monkey (*Cercopithecus aethiops*), has been well characterized and is instrumental in the biotechnology sector for virus replication studies, viral plaque assays, $TCID_{50}$ determinations and production of viral vaccines.

JRH Biosciences has developed a new serum-free medium specifically for the Vero cell line. EX-CELL™ Vero is serum-free and free of animal-derived components. The medium contains a plant-derived hydrolysate and low levels of recombinant proteins, but does not contain phenol red or Pluronic® F-68. In these studies, we show that EX-CELL™ Vero supports

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high-density cell growth in both stationary flasks and on microcarriers in bioreactor culture.

2. RESULTS

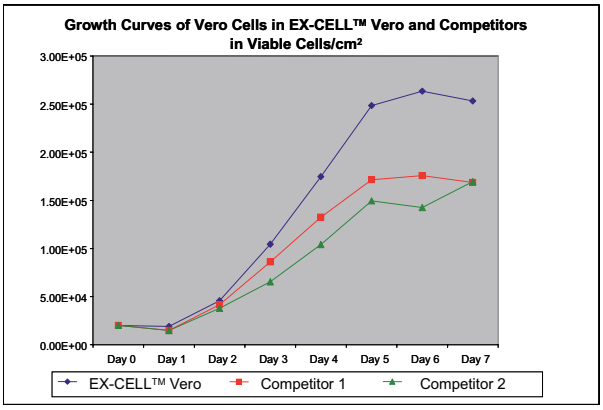

Figure 1. Vero cells were seeded in triplicate 25cm^2 T-flasks at 2x10^4 cells/cm^2. Cells were harvested from flasks and viable cells were counted daily for 7 days. Culture viabilities remained above 95% in all media during the study.

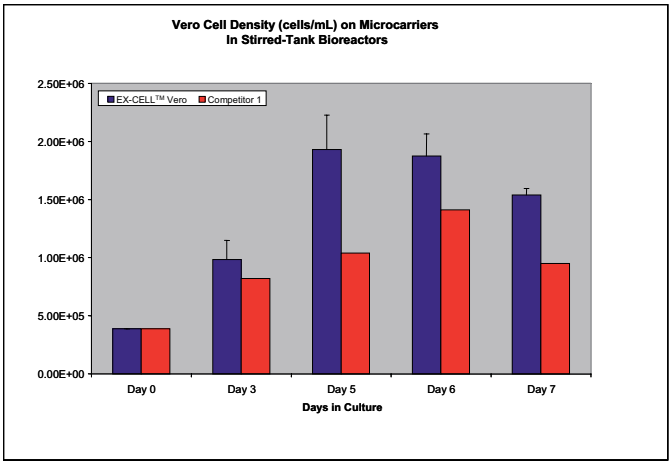

Figure 2. Vero cells were inoculated in Applikon stirred tank bioreactors at a 1L working volume. Reactor temperature was set to 37˚ C, the agitation speed was 70-85 rpm, dissolved O_2 was maintained at 50%, and pH was maintained at 7.0-7.3 with CO_2. Reactors were monitored and samples obtained daily for seven days; cell growth was determined by counting released nuclei using a crystal violet staining procedure. Cells grew to confluence

achieving maximum densities of approximately $1.9x10^6$ cells/mL (~$1.5x10^5$ cells/cm^2) in EX-CELL™ Vero and $1.4x10^6$ cells/mL (~$1.1x10^5$ cells/cm^2) in competitor's medium.

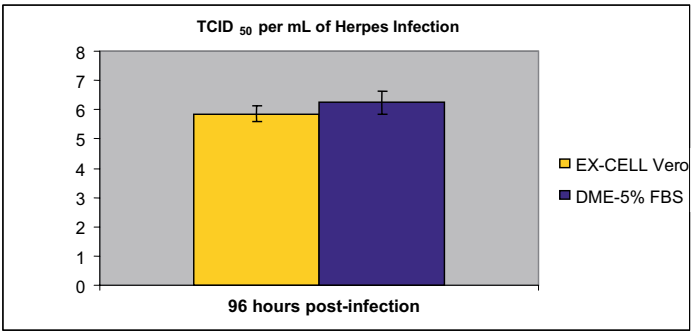

Figure 3. Vero cells were infected with Herpes Simplex Virus II (HSV-II) 96 hours post-seeding at a MOI of 0.01. Flasks displayed greater than 75% cytopathic effect 96 hours post-infection. Flasks were harvested and results represent $TCID_{50}$/ mL at harvest point.

3. CONCLUSION

EX-CELL™ Vero is a new serum-free medium designed and optimized for high-density Vero cell growth in adherent-stationary and adherent-suspension conditions. EX-CELL™ Vero is a regulatory-compliant medium, free from all animal-derived components, and contains only recombinant proteins. These studies indicate that adaptation to EX-CELL™ Vero from basal medium with serum can easily be accomplished and EX-CELL™ Vero supports high-density Vero cell growth, superior to competitor formulations.

EX-CELL™ Vero supported HSV-II production in Vero cells. The cells exhibited classic CPE in culture and produced HSV-II titers in the range of 106.0 $TCID_{50}$/mL, comparable to serum-supplemented cultures.

For further information regarding EX-CELL™ Vero, please contact Technical Services.