

Coleen McCormick, Jeanette Hartshorn, Chas Hernandez, Sandy McNorton,  
Gaurav Chauhan, Elisa Atarod, Majid Mehtali<sup>1</sup>, and Matt Caple

## Abstract

Many human and animal vaccines are currently being developed and produced in eggs or primary chicken embryonic fibroblasts (CEF). However, there are known limitations to these platforms, including challenges in both handling and scaling. Consequently, continuous cell lines and the use of animal-component free media show exciting promise for vaccine manufacturers. EB14 is a novel suspension cell line derived from avian embryonic stem cells and has been identified as a permissive host for pox and influenza viruses in cell culture. EB14 is a well characterized, genetically stable cell line which has been adapted to serum-free media and can be cultured using industrial processes including stirred tank bioreactors. The present study identifies optimized serum-free media formulations, feed strategies and cell culture processes that support robust growth and viral productivity in the EB14 cell line.

## Introduction

SAFC Biosciences ([www.safcbiosciences.com](http://www.safcbiosciences.com)) and Vivalis ([www.vivalis.com](http://www.vivalis.com)) formed a scientific partnership to develop a novel cell culture-based vaccine platform. Vivalis has taken advantage of its expertise in avian biology and embryonic stem cells to develop fully characterized and documented cell lines that are permissive to a variety of viruses. SAFC Biosciences has drawn on its expertise in media development to generate an offering of serum-free media that support robust growth and viral production in the EB14 cell line. The EX-CELL™ EBx™ media allows consistently high growth and viral titers in scales ranging from flasks to bioreactors. This cell line provides a novel serum-free, animal component-free, cell culture-based platform for vaccine production.

## Materials and Methods

### Cells

EB14 is a novel suspension cell line that was derived from avian embryonic stem (ES) cells using a proprietary process by Vivalis. EB14 cells maintain some of the unique features of ES cells, such as a strong constitutive expression of telomerase. Furthermore, EB14 cells are diploid, undifferentiated, non-tumorigenic and genetically stable. Stock cultures were maintained in a 37 °C, 7.5% CO<sub>2</sub> humidified incubator. EB14 cells were cultured in 125 mL Erlenmeyer flasks (25 mL culture volumes) at 90 rpm.

### Cell Culture Media

Stock cultures were maintained in EX-CELL™ EBx™ (SAFCB Item No. 63066) and Medium X. Both media were supplemented with 2.5 mM L-glutamine (Catalog No. 59202C). Base medium (SAFCB Item No. 65421) and complete medium (SAFCB Item No. 65946) were used for viral production. All items except Medium X are from SAFC Biosciences, Lenexa, Kansas.

### Process Development

Bioreactor runs were conducted in 3L stirred tank bioreactors (Applikon® Biotechnology, Sciencedam, Holland). Bioreactors were seeded at 0.1, 0.2, and 0 cells/mL in EX-CELL™ EBx™ and Medium X. DO, pH and temperature were monitored and controlled. Uninfected samples were collected daily to monitor cell density, viability and metabolic consumption/production (data not shown). Infected samples were collected daily to monitor viral production by TCID<sub>50</sub> endpoint dilution, hemagglutinin production, and fluorescent microscopy.

### Viral Infection

EB14 cells were seeded at 0.1 to 0.4E6 cells/mL forty eight to seventy two hours prior to infection in 125 mL Erlenmeyer flasks or 3L stirred tank bioreactors. Cells were inoculated with a multiplicity of infection (M.O.I.) of 10<sup>2</sup> for MVA and 10<sup>4</sup> for the indicated strains of human influenza and allowed to adsorb for 1 hour at 37 °C or 34 °C, respectively. After 1 hour, production media (with or without feeds) was added and the cultures were incubated at the appropriate temperature for an additional 5 to 9 days.

### Titration of Viruses by TCID<sub>50</sub> Endpoint Dilution

TCID<sub>50</sub> analysis was performed following standard protocols (Reed and Muench, 1938). Briefly, 100 µL of viral sample serial dilutions (10<sup>-4</sup> to 10<sup>-11</sup>) were dispensed in replicates and one row was left as a control in which no virus was added. The titer was then calculated by the Reed-Muench method. DF-1 and MDCK cells were used for MVA and human influenza strains, respectively.

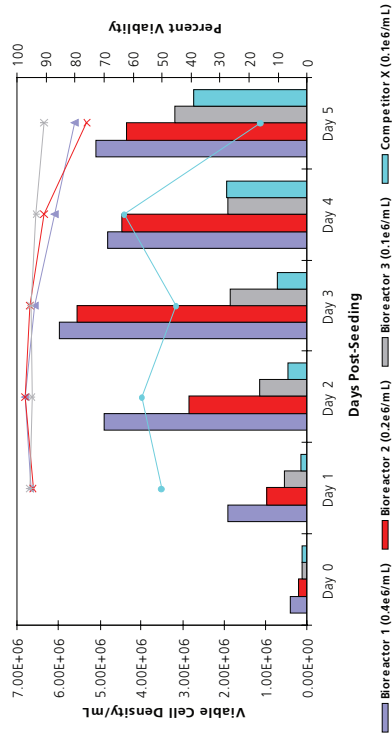
### Single Radial Immunodiffusion Assay (SRID)

SRID analysis was performed following standard protocols to determine hemagglutinin (H.A.) concentration (Wood, et. al., 1977). Human influenza anti-sera was obtained and used at concentrations recommended by NIBSC. Dose-response curves of antigen dilutions against the surface were constructed and the results were calculated according to standard slope-ratio assay methods.

### Analytical Methods

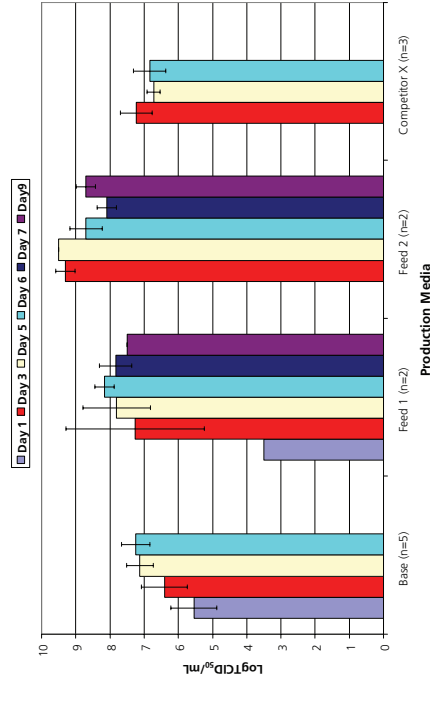
Cell density and viability were determined by Cedex (Innovatis, Bielefeld, Germany) and by trypan blue exclusion method with hemacytometer. Metabolic consumption and production in uninfected cultures was monitored offline with the BioProfile® 100 (Nova Biomedical Corporation, Waltham, MA; data not shown).

## Results



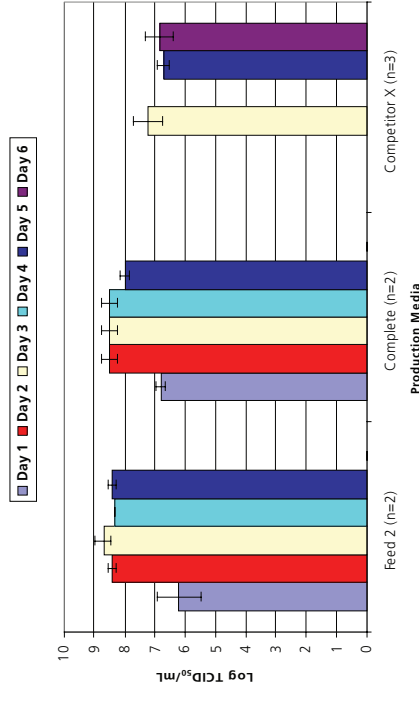
**Figure 1: Growth and Viability of EB14 Cells in EX-CELL™ EBx™ and Competitor X Medium in Stirred Tank Bioreactors.**

EB14 cells were seeded into 3L Applikon stirred tank bioreactors at 0.1E6, 0.2E6, and 0.4E6 cells/mL. Cell density and viability were monitored for five days following seeding. EB14 cells reached a significantly higher maximum cell density and demonstrated greater longevity in EX-CELL™ EBx™ when compared to Medium X. EB14 cells seeded in competitor Medium X had a significant drop in cell viability by day 5 (followed by a drop in cell density on day 6, data not shown).



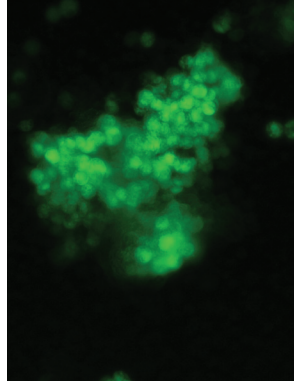
**Figure 2: Infectious MVA Production in EX-CELL™ EBx™ with and without Feeds Compared to Competitor X Medium.**

EB14 cells were seeded at 0.2E6 cells/mL in EX-CELL™ EBx™ or competitor X media. After 48 hours, the cells were infected with MVA at an M.O.I. of 10<sup>2</sup> and production medium was added. The feeds were also added at the time of infection. Feed 1 increased the viral titer over the base formulation titer by 2 logs.



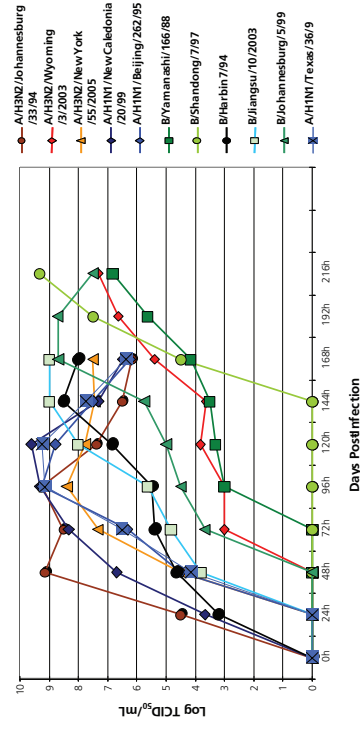
**Figure 3: Infectious MVA Production in EX-CELL™ EBx™ as a Complete Medium Compared to Competitor X Medium.**

EB14 cells were seeded at 0.2E6 cells/mL in EX-CELL™ EBx™ or competitor X media. After 48 hours, the cells were infected with MVA at an M.O.I. of 10<sup>2</sup> and production medium was added. The supplements contained in Feed 2 can be incorporated directly into the production medium at the time of manufacture, resulting in a similar increase in viral titers.



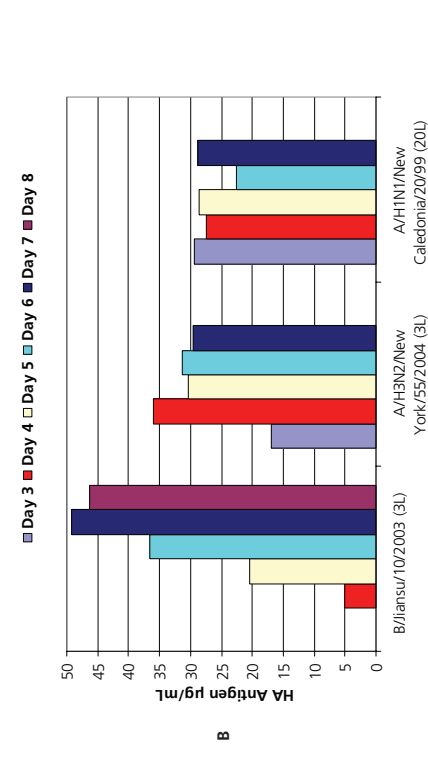
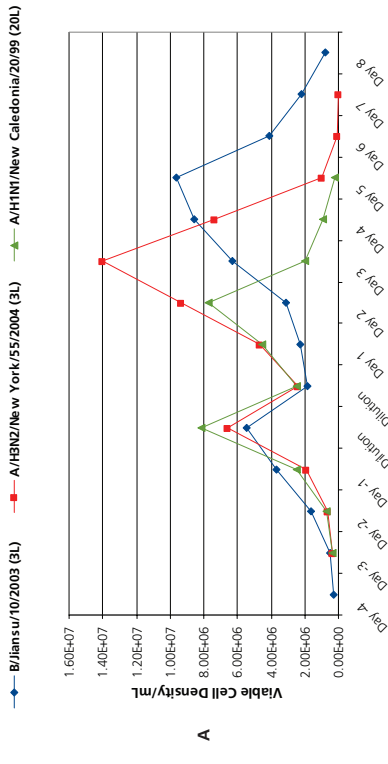
**Figure 4: MVA-GFP Infected EB14 Cells in EX-CELL™ EBx™ in Flasks.**

EB14 cells were seeded at 0.4E6 cells/mL in EX-CELL™ EBx™. After 48hrs, cells were infected with MVA-GFP at an M.O.I. of 10<sup>2</sup> and production medium was added. A sample was removed and examined for fluorescence at 10X magnification 4 days post-infection.



**Figure 5: Infectious Human Influenza Production in EX-CELL™ EBx™ in Flasks.**

EB14 cells were seeded at 0.4E6 cells/mL in EX-CELL™ EBx™. After 48 hrs, cells were infected with the indicated strains of human influenza at an M.O.I. of 10<sup>4</sup>. Feed 2 was added 1 hour later and samples were pulled daily to monitor viable cell density (data not shown) and production of infectious virus. High viral titers were obtained with all strains of human influenza tested.



**Figure 6: Human Influenza Hemagglutinin Production in EX-CELL™ EBx™.**

EB14 cells were seeded at 0.4E6 cells/mL in EX-CELL™ EBx™ in 3L or 20L bioreactors as indicated in the legends. After 72 to 96 hours, cells were infected with the indicated strains of human influenza at an M.O.I. of 10<sup>4</sup>. Feed 2 was added 1 hour later and samples were pulled daily to monitor viable cell density (Panel A) and production of H.A. antigen (Panel B). All three bioreactors reached at least 8E6 cells/mL following infection and produced at least 30 µg/mL of HA as determined by SRID.

## Conclusions

- EX-CELL™ EBx™ is a serum-free, animal-component free medium for EB14 cells in suspension culture.
- EX-CELL™ EBx™ supports higher cell density, improved viability and increased culture longevity when compared with a competitor medium.
- EX-CELL™ EBx™ supports MVA and Influenza Virus production
- Optimization of key components, feeds, and metabolic analysis has led to the development of media that exhibit robust growth and viral productivity.

## Acknowledgements

The authors would like to thank Cell Sciences and Development (SAFC Biosciences) and the Research and Development (Vivalis) teams, for without their dedicated efforts and expertise this project would not have been possible.

## References

EX-CELL™ is a trademark of SAFC Biosciences.  
EBx™ is a trademark of Vivalis.

## Footnote

1. Vivalis, Nantes, France