



Development of a Novel Sf9 Rhabdovirus-Negative Cell Line (Sf-RVN®) and Companion Chemically Defined Medium

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Introduction

The baculovirus-insect cell system (BICS) is widely used for production of recombinant proteins due to its flexibility, speed, simplicity and eukaryotic protein processing capacity. This platform is leveraged to produce several biologics approved for use in humans (Cervarix®, Provenge®, Glybera® and Flublok®) and several veterinary applications (Porcilis® Pesti, Circumvent® PCV, Ingelvac CircoFLEX® and Porcilis® PCV). The BICS is also used for production of virus-like particles (VLP) used in vaccines and adeno-associated viruses (AAV) used in gene therapies.

Baculovirus is a rod-shaped virus that exclusively infects insects and therefore has relatively low safety concerns. It has a genome of 134 kilobase pairs with 154 open reading frames, which allows enough space for the gene insertions necessary to produce recombinant proteins, adeno-associated viruses (AAV) and virus-like particles (VLP).

Cell lines derived from ovarian tissue of the fall armyworm, *Spodoptera frugiperda* (Sf), are widely used as hosts for recombinant proteins, virus-like particles (VLP) and viral vectors such as AAV. The original Sf cell line, designated IPLB-SF-21 (Sf21), was derived from pupal ovaries in 1977. Other commonly used Sf cell lines include Sf9, a subclone of Sf21.

A novel rhabdovirus, known as Sf-rhabdovirus, was discovered to be a common and persistent contaminant of Sf9 cell lines. Sf-rhabdovirus is rod-shaped and contains single-stranded RNA. While its exact host range is not fully defined, it is believed that Sf-rhabdovirus is unlikely to be harmful for humans as it cannot replicate in mammalian cells. Despite this finding, the presence of this adventitious agent in a process used to manufacture biologics for human use must be addressed. Removal of the virus can be achieved through use of appropriate

downstream applications and its clearance must be confirmed by validated assays.

This whitepaper describes the development of a novel Sf9 Rhabdovirus-negative cell line, named Sf-RVN®, with companion chemically defined medium, the EX-CELL® CD Insect Cell Medium. Combined, these two products form the Sf-RVN® Platform and offer a Sf-Rhabdovirus free alternative for the BICS production of biologics.

Data demonstrated that Sf-RVN® Insect Cells cultivated with the optimized chemically defined EX-CELL® CD Insect Cell Medium provide robust growth and also a high productivity of the secreted alkaline phosphatase (SEAP), the Rudolph red, erythropoietin (EPO) and the porcine parvovirus (PPV).

Characteristics of the Sf-RVN® Insect Cell Line

An Sf9 cell line that is free from rhabdovirus was initially described in 2016 by Dr. Jarvis and the GlycoBac lab (Maghodia et al. 2016). They demonstrated that the Sf-RVN® Insect Cell Line shows similar density, size, doubling time and morphology when compared to Sf9 cell lines infected with rhabdovirus (**Figure 1**). The absence of any viral sequences, including rhabdovirus, in the Sf-RVN® Insect Cell Line has been confirmed using the TBLASTN program to search massively parallel sequencing assemblies (Geisler et al. 2018). The nearly identical growth and morphology characteristics, along with this sequencing study, confirms that the Sf-RVN® Insect Cell Line is one of the best-characterized cell lines in the field of Sf9. It also proves that the Sf-RVN® insect cells are negative for rhabdovirus while maintaining other characteristics of the standard Sf9 cell line.

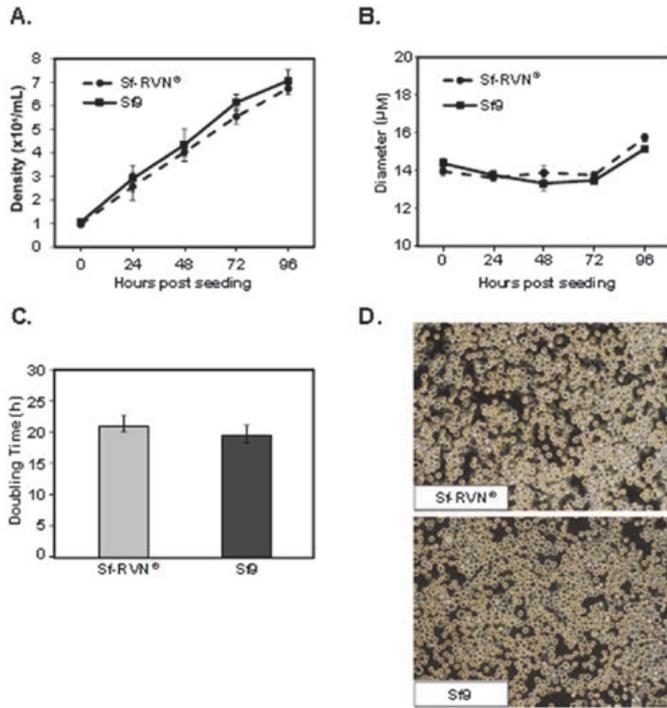


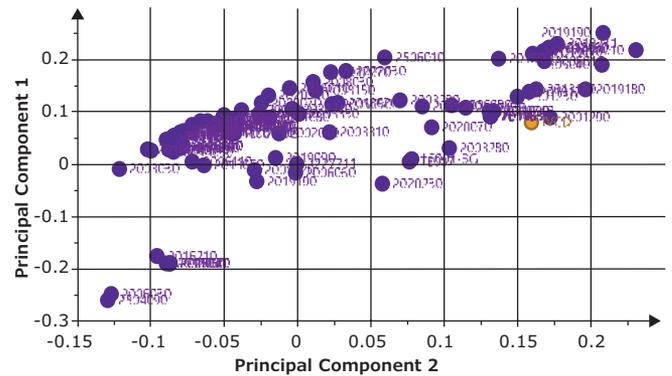
Figure 1. Comparison of Sf-RVN[®] insect cells and Sf9 cells infected with rhabdovirus revealed similarity in density (A), diameter (B), doubling time (C) and morphology (D) (Adapted from Maghodia et al. 2016).

Development of Optimized EX-CELL[®] CD Insect Cell Medium

Grace's medium, supplemented with serum, is the earliest formulation developed for insect cell culture. The desire to move to a serum-free medium led to the addition of peptones, hydrolysates such as yeast extract, lipids and Poloxamer 188. These media are not chemically defined and variation in the hydrolysates results in lot-to-lot inconsistencies in the media and cell culture.

We developed a chemically defined medium optimized for insect cell culture (EX-CELL[®] CD Insect Cell Medium). Cell culture optimization technology was used to determine the best balance of components to deliver the desired cell growth and productivity. A key consideration during development of the specialty medium was the cell density effect. While Sf9 cells can reach peak densities of up to 10^7 cells/mL, if the density exceeds 4×10^6 cells/mL, there is a severe loss in protein yield. Ideally, infection should take place when the cells are at $2-3 \times 10^6$ cells/mL to achieve the highest protein yield.

Several media formulations were used as a starting point to allow for the evaluation of a diverse range of components and concentrations. Unique components of insect cell media were then added to these formulations. One of these starting formulations was our EX-CELL[®] 420 Serum-Free Medium for insect cells with the yeast extract removed. A design of experiments (DOE) approach was used to identify those biochemical components that supported growth and productivity of the Sf-RVN[®] Insect Cell Line.



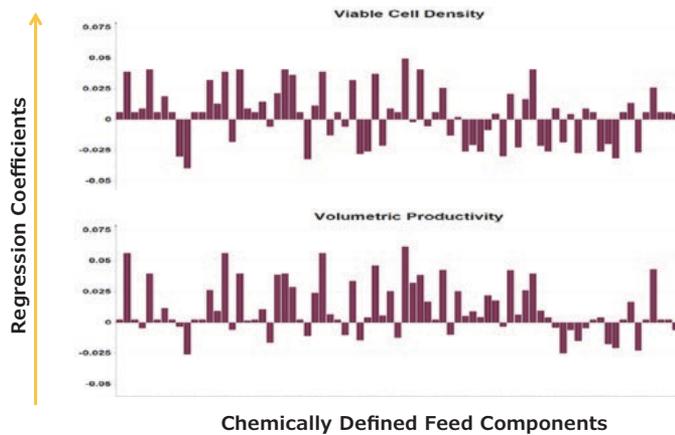


Figure 4. Regression coefficients reflect positive and negative correlations of biochemical components to individual responses.

A graph of regression components indicated if the components had a positive or negative correlation to individual responses compared to all other components (**Figure 4**). Bars that are above zero have a positive effect while those below zero have a negative effect. The top graph represents components critical for cell growth while the bottom graph shows critical components for productivity. It is obvious there are components that are important for growth and not for productivity and vice versa.

These three sets of data indicated which components should be included in the medium and which can be eliminated from consideration. The data also provided concentration ranges to test for both growth and productivity to create EX-CELL® CD Insect Cell Medium. While EX-CELL® CD Insect Cell Medium is optimized for the Sf-RVN® Insect Cell Line, this medium is compatible with other insect cell lines such as Sf9, Sf21, Tni, S2 and C636.

Sf-RVN® Platform Performance

Performance of the Sf-RVN® Platform, composed of the Sf-RVN® Insect Cell Line with the specifically formulated EX-CELL® CD Insect Cell Medium, was evaluated using several assays:

- Cell growth assay
- Secreted Alkaline Phosphatase (SEAP) production
- Rudolph Red and human erythropoietin (EPO) production
- Porcine Parvovirus (PPV) production

Cell Growth Assay

Figure 5 shows a comparison of cultivation stability of the Sf-RVN® Insect Cell Line when grown in the EX-CELL® CD Insect Cell Medium, competitor medium and EX-CELL® 420 Serum-Free Medium containing yeast extract. The chemically defined medium consistently reached $6-8 \times 10^6$ cells/mL by day 4 when seeded at $0.7-1 \times 10^6$ cells/mL and reached more than 10×10^6 cells/mL by day 7 when seeded at 0.5×10^6 cells/mL. Viability remained consistently high throughout the culture period. Competitor medium varied from $3-5 \times 10^6$ cells/mL during passing with a maximum viable cell density of 5×10^6 cells/mL on day 7 of the growth curve.

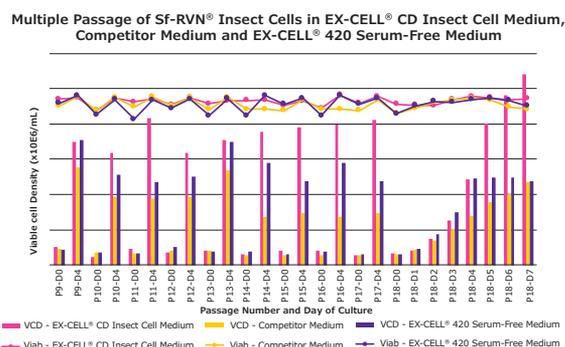


Figure 5. Stable growth of the Sf-RVN® Insect Cell Line over multiple passages.

The EX-CELL® 420 Serum-Free Medium showed slightly better growth as compared to the competitor medium but not as good as the chemically defined medium. EX-CELL® 420 Serum-Free Medium, which contains hydrolysates, showed some passage to passage variability. This variability emphasizes the advantage of culturing with a chemically defined medium as it provides improved consistency during the cell culture process.

Secreted Alkaline Phosphatase Production

Figure 6 shows the production of SEAP by the Sf-RVN® Insect Cell Line in the same three media. The baculovirus producing SEAP is an occlusion-negative virus, which means it is secreted directly into the supernatant. The growth curve reflects uninfected cells; cells were plated at 1×10^6 cells/mL (day zero) and infected on day 1 when the cultures were at approximately 2×10^6 cells/mL. The Sf-RVN® insect cells were infected at a multiplicity of infection (MOI) of 5 and harvested on day 3 post-infection. All the baculovirus used for these experiments had been passaged twice in the EX-CELL® CD Insect Cell Medium prior to the final infection.

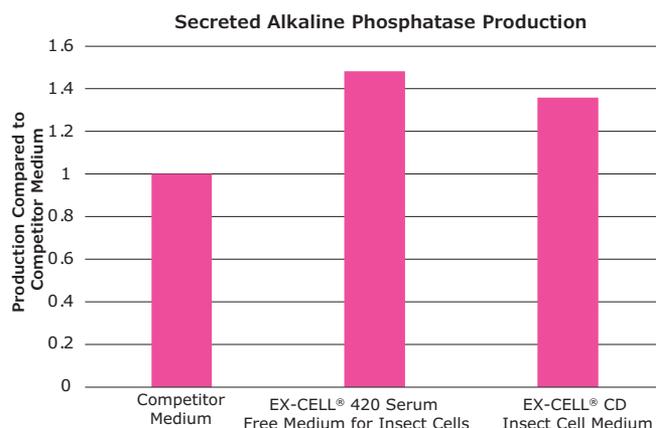


Figure 6. Comparison of SEAP production by Sf-RVN® insect cells in three different media formulations.

As shown in **Figure 6**, production of SEAP was 36% higher with the EX-CELL® CD Insect Cell Medium than with the competitor medium. Production in EX-CELL® 420 Serum-Free Medium was slightly higher (48%).

Rudolph Red and Human EPO Production

In this study, the baculovirus was co-infected with Rudolph Red and human Erythropoietin (hEPO). Human EPO is secreted into the supernatant and can be directly measured while the Rudolph Red remains intracellular and once released from the cells, is quantified with a fluorescent plate reader. The infection was done at an MOI of 5 and harvested on day 3 post-infection.

As shown in **Figure 7**, production of Rudolph Red was 45% higher with the EX-CELL® CD Insect Cell Medium than with the competitor medium.

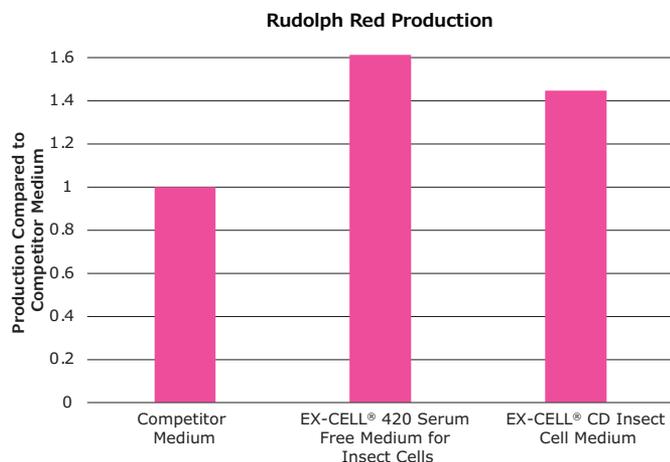


Figure 7. Comparison of Rudolph Red production in Sf-RVN® cells in three different media formulations.

Porcine Parvovirus Production

Similar to AAV, PPV is a capsid virus in which the polyhedral nuclear capsid will remain within the cell. Both AAV and PPV are small, non-enveloped single-stranded DNA viruses that are assembled from three viral proteins: VP1, VP2, and VP3.

As with the previous studies, infection was at an MOI of 5 with approximately 2×10^6 cells/mL on day 1 of the culture and harvest was on day 3 post-infection. The EX-CELL® CD Insect Cell Medium outperformed both the competitor medium and EX-CELL® 420 Serum-Free Medium by 22% (**Figure 8**).

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Porcine Parvovirus Production

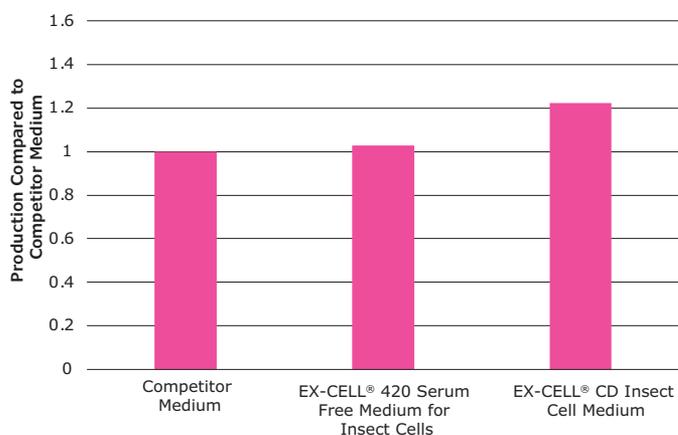


Figure 8. Comparison of PPV production in Sf-RVN® cells in three different media.

Conclusion

Despite the discovery that Sf9 cells are contaminated with Sf-rhabdovirus, these cell lines are widely used as hosts for the production of recombinant proteins, virus-like particles (VLP) and adeno-associated viruses (AAV) used for gene therapy applications. While it is believed that this virus is unlikely to be harmful to humans, removal of rhabdovirus, as with any adventitious agent, is advised to ensure patient safety.

Data confirms that the EX-CELL® CD Insect Cell Medium not only supports robust growth of the Sf-RVN® Insect Cell Line, it also produces as well or better than EX-CELL® 420 Serum-Free Medium containing hydrolysate.

The Sf-RVN® Insect Cell Line is demonstrated to be free of the contaminating virus and improves the safety profile of bioprocesses. The combination of this cell line with the EX-CELL® CD Insect Cell Medium forms the Sf-RVN® Platform and provides robust growth and productivity.

References:

1. Maghodia AB, Geisler C, Jarvis DL. Characterization of an Sf-rhabdovirus-negative Spodoptera frugiperda cell line as an alternative host for recombinant protein production in the baculovirus-insect cell system. *Protein Expr Purif*. 2016 Jun; 122:45-55.
2. Geisler, C. A new approach for detecting adventitious viruses shows Sf-rhabdovirus-negative Sf-RVN® cells are suitable for safe biologicals production. *BMC Biotechnology*, 2018, 18:1, Page 1

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