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Whitepaper

Application of the Sf-Rhabdovirus-Negative (Sf-RVN®) Platform for AAV Production

Thibaut Deschamps, Sandy McNorton, Kim Schrag and Charlotte Javalet

Introduction

The Baculovirus Expression Vector System (BEVS) has been used for more than thirty years as a research tool. In the last two decades, the number of viral vaccines and gene therapy vectors produced using this system has increased from two commercial products in the mid-2000 to a dozen currently. The initial commercial use of BEVS was for the production of recombinant protein or virus-like particles (VLPs) for veterinary and human vaccines. Most recently, the BEVS is being utilized by biopharmaceutical companies to produce recombinant adeno-associated virus (AAV) to treat genetic diseases. The resurgence of gene therapies is providing lifesaving options to patients with otherwise untreatable diseases, leading to increased demand for large amounts of high-quality viral vectors. Compared to stable chromosomal integration or transient transfection, BEVS production relies on a viral carrier to transport the foreign genetic information offering higher flexibility, manufacturing speed, cost reduction and competing product titers.

Spodoptera frugiperda (Sf) cell lines are widely used as hosts for BEVS. However, the majority of Sf-9 and Sf-21 cell lines contain an Sf-rhabdovirus which is considered a contaminant and must be eliminated during the downstream purification process (Hailun Ma et al. 2014). Because viral safety is essential in the manufacture of biopharmaceuticals and required to ensure patient safety, we offer a proven Sf-9-rhabdovirus-negative (Sf-RVN®) Insect Cell Line (Maghodia et al. 2016 and 2017) that improves the safety profile of baculovirus insect cell bioprocesses. To get excellent growth and productivity of the Sf-RVN® Insect Cell Line, we specifically developed a chemically defined medium: the EX-CELL® CD Insect Cell Medium. Combined, these two products form the Sf-RVN® Platform and provide a high performant rhabdovirus-free BEVS alternative for the BEVS production of biologics.

In this study, we explored the performances of the EX-CELL® CD Insect Cell Medium for growth and AAV2 production in both Sf-RVN® and Sf-9 cells. Then, we optimized the baculovirus infection process for AAV2 production with the Sf-RVN® Platform in Ambr® microbioreactor. Data demonstrated that EX-CELL® CD Insect Cell Medium is the best medium tested to support the growth of both Sf-RVN® and Sf-9 cells and enables high AAV2 productivity in the two cell lines. In regards to infection optimization in Ambr® microbioreactor, we found that the optimal conditions are a MOI of 0.01 and an equal ratio of AAV2RepCap:ITR-GFP.

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Results

Growth performances evaluation of the EX-CELL® CD Insect Cell Medium

To evaluate performances of the EX-CELL® CD Insect Cell Medium, we compared the growth of two *Spodoptera frugiperda* (Sf) cells, our rhabdovirus-free Sf-RVN® Insect Cell Line and its parent, the Sf-9 cell line, both cultivated in four non-chemically defined media and two chemically defined media, including the EX-CELL® CD Insect Cell Medium. As presented in **Figure 1**, we confirmed high performances of the EX-CELL® CD Insect Cell Medium and demonstrated that this medium outperforms all media tested, including non-chemically defined media, for both Sf-RVN® and Sf-9 cells.

Since the EX-CELL® CD Insect Cell Medium was developed for the Sf-RVN®, we tested whether this medium could support the growth of a variety of insect cell lines. Thus, we found that the EX-CELL® CD Insect Cell Medium supports the growth of Sf-21, S2, *Tni* and C636 cells (**Figure 2**) whereas C636 and *Tni* cell lines did not grow in any of the other media tested, chemically defined of not (data not shown).

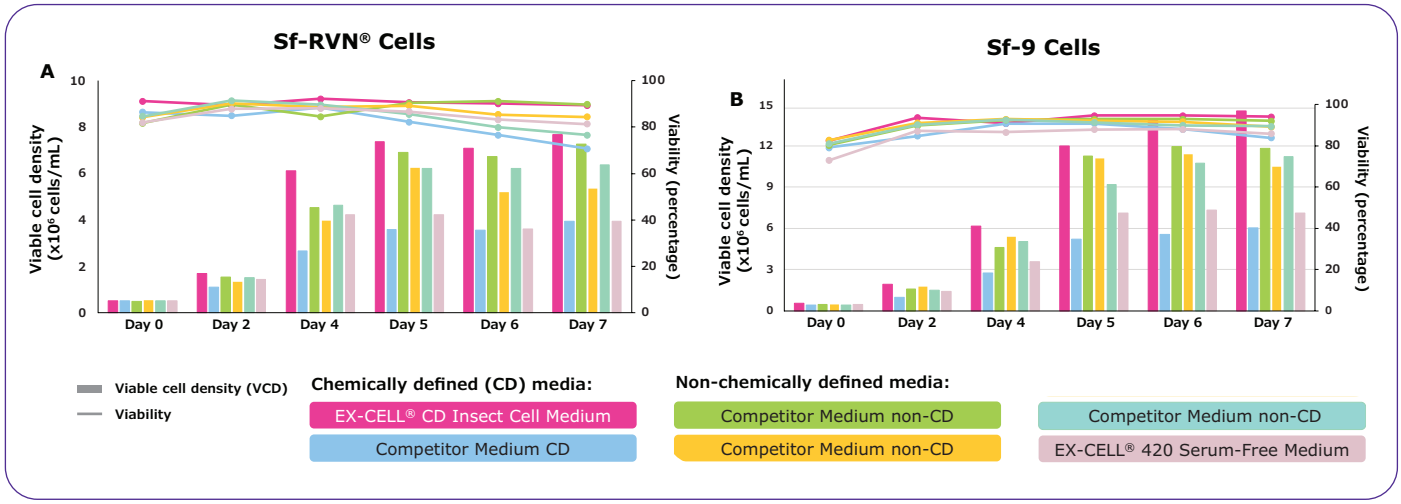

Figure 1: EX-CELL® CD Insect Cell Medium is the best medium tested to support Sf-RVN® (A) and Sf-9 (B) cells growth. Cells were adapted for at least five passages in six different cell culture media. Two of them are chemically defined (including the EX-CELL® CD Insect Cell Medium) and the four others are not chemically defined and contains hydrolysates. After adaptation, cells were seeded 0.5x10⁶ cells/mL on day zero. Viable cell density (VCD) and viability were followed for 7 days.

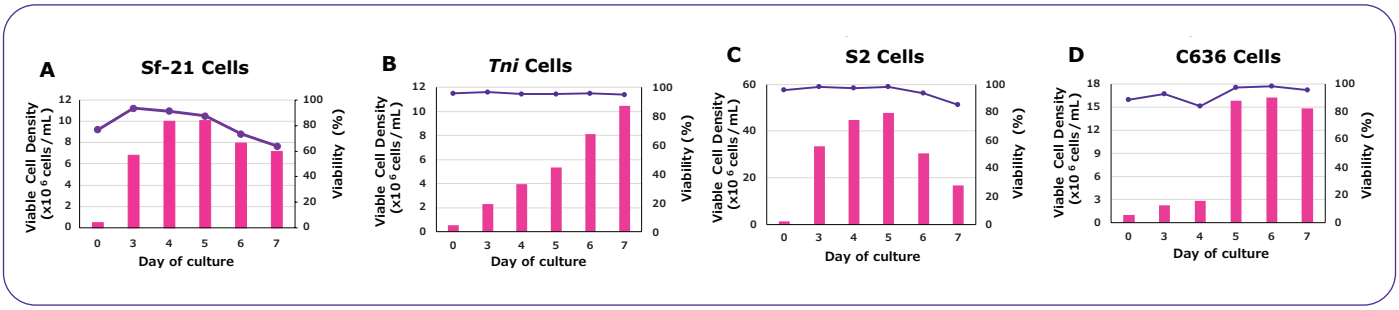

Figure 2: EX-CELL® CD Insect Cell Medium supports the growth of multiple insect cells. Sf-21 (**A**), *Tni* (**B**), S2 (**C**) and C636 (**D**) cells were adapted for at least three passages in the EX-CELL® CD Insect Cell Medium. After adaptation, cells were seeded at 0.5x10⁶ cells/mL on day zero. Viable cell density (VCD) and viability were followed for 7 days.

Generating baculovirus stock

The first step for BEVS production is to establish a Sf-rhabdovirus negative baculovirus stock. Indeed, most of the available baculovirus stock available have been established in a rhabdovirus positive cell line. In addition, bacmids transfection yields a lower transfection efficiency than smaller plasmids hence the necessity to optimize the transfection step.

To produce AAV2, we used in this study, the two baculovirus system based on a combined AAV2Rep-Cap baculovirus and a transgene baculovirus (ITR-GFP). In order to optimize the transfection process, Sf-RVN® cells were transfected with the Escort™ IV transfection reagent, in parallel with the two bacmids (ITR-GFP and AAV2-RepCap), two concentrations of bacmids and four different ratios of bacmid: Escort™ IV.

Upon transfection, cell growth was inhibited, and cell diameter increased. The effect of transfection is exacerbated with a bacmid concentration of 1 µg/mL compared to the lowest concentration at 0.1 µg/mL. No difference between the ratio of bacmid: Escort™ IV was observed in cell growth, diameter or viability (**Figure 3**).

Harvested P0 baculovirus production was used to produced P1 stock using a 1/50 dilution of P0 (**Figure 3**). The baculovirus titer was routinely measured by plaque assay with a titer ranging from 10^5-10^6 pfu/mL for P0 and 10^7-10^8 pfu/mL for P1 and subsequent passages.

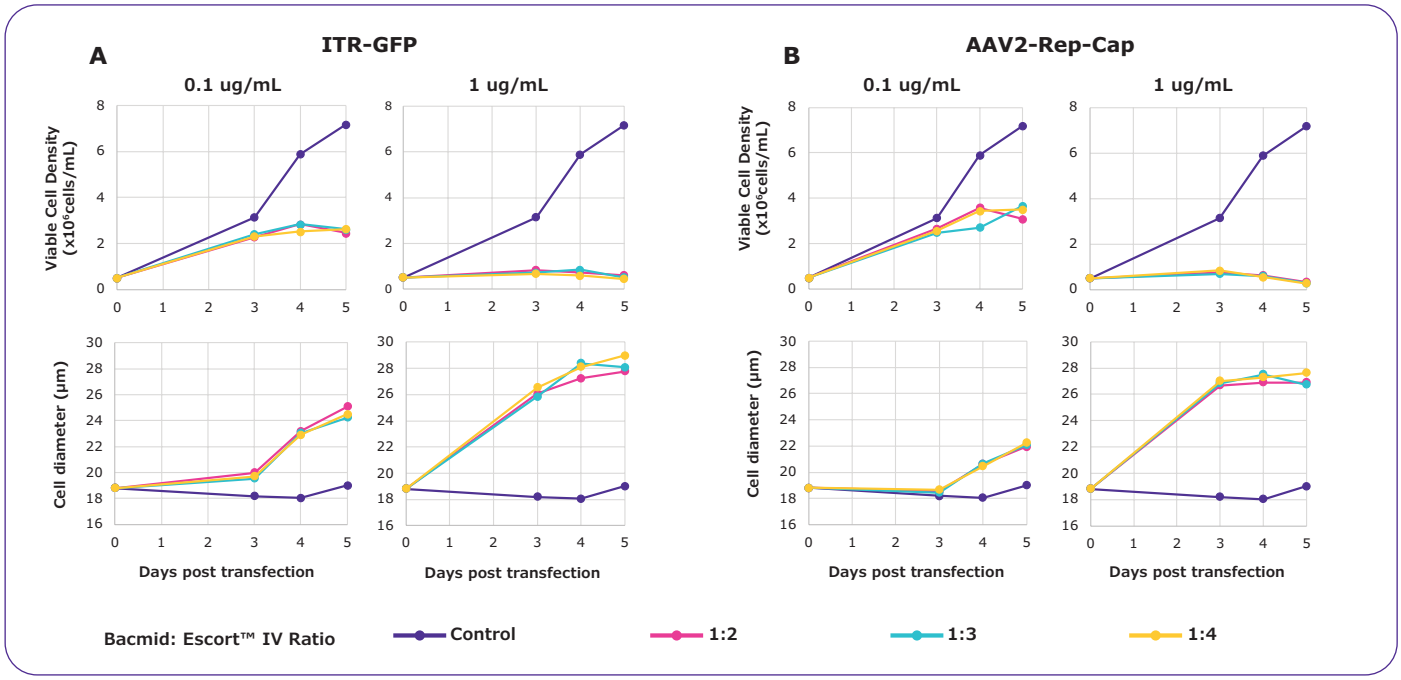

Figure 3: Bacmid transfection of Sf-RVN® cells inhibits cell growth and increases cell diameters. Sf-RVN® cells were cultivated in the EX-CELL® CD Insect Cell Medium and transfected in parallel with two bacmids, ITR-GFP (**A**) and AAV2-RepCap (**B**) with two different concentrations of bacmids (either 0.1 or 1 µg/mL) and three different ratios of bacmid: Escort™ IV transfection reagent (1:2, 1:3 and 1:4). Viable cell density (VCD) and cell diameter were followed for 5 days.

Infection optimization

The produced ITR-GFP and AAV2-RepCap baculovirus stocks (P1) were then used to produce AAV2 using the BEVS system in both Sf-RVN® and Sf-9 cells. The infection process was optimized by testing different multiplicity of infection (MOI). As shown in **Figure 4**, the highest titers were obtained with an MOI of 0.01 at 120 hours post infection for both the Sf-RVN® and the Sf-9 cells. We observed that the MOI used was inversely proportional to AAV2 titer with an MOI 1 producing the lowest titer. The decrease in productivity between 96 to 120 hours post infection with the MOIs of 0.1 and 1 is likely due to a degradation of the culture and an accumulation of cellular debris.

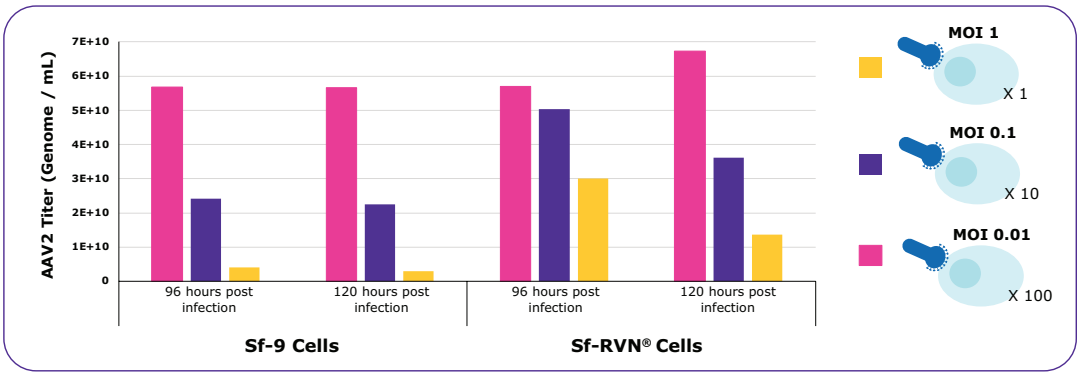

Figure 4: Low MOI infection enables high AAV2 titer in both Sf-RVN® and Sf-9 cells. Cells were cultivated in the EX-CELL® CD Insect Cell Medium, seeded at 2x10^6 cells/mL and co-infected by two baculoviruses (ITR-GFP and AAV2-RepCap) at an equal ratio (1:1) and three different MOIs: 0.01, 0.1 or 1. Cells were harvested at 96 hours and 120 hours post-infection and AAV2 productivity was measured by ddPCR.

Evaluation of the EX-CELL® CD Insect Cell Medium performances for AAV Production

We compared the production of AAV2 in the Sf-RVN® and the Sf-9 cultured in the EX-CELL CD® Insect Cell Medium and the same five competitors' media from **Figure 1**. **Figure 5** shows that the EX-CELL® CD Insect Cell Medium outperforms the chemically defined medium tested by a factor 10 and outperforms or competes with the non-chemically defined media for both AAV2 titer measurement methods, ELISA (**Figure 5A)** and ddPCR **(Figure 5B**) for both Sf-RVN® and Sf-9 cells.

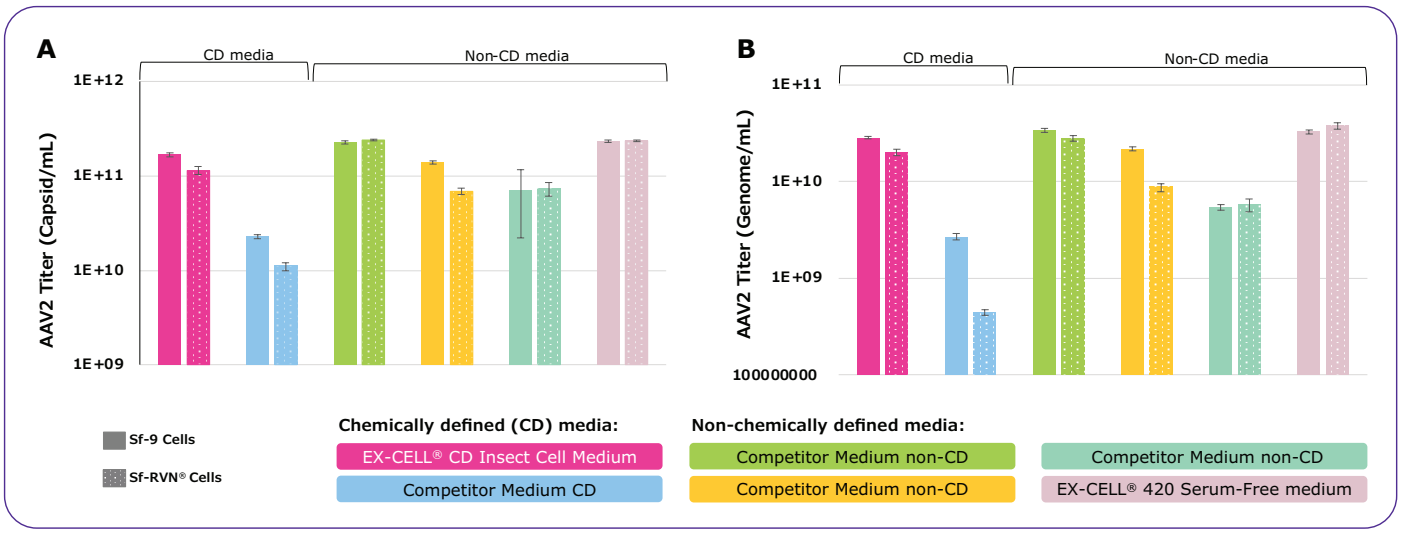

Figure 5: EX-CELL® CD Insect Cell Medium is the best chemically defined medium tested for AAV2 production in Sf-RVN® and Sf-9 cells. Cells were adapted for at least five passages in six different cell culture media. Two of them are chemically defined (including the EX-CELL® CD Insect Cell Medium) and the four others are not chemically defined and contains hydrolysates. Cells were seeded at 2x10^6 cells/mL, infected with both ITR-GFP and AAV2-RepCap baculoviruses at an MOI of 0.01 and an equal ratio. Samples were harvested at 120 hours post infection (hpi) and the AAV2 productivity was assessed by ELISA (**A**) and ddPCR (**B**).

Scalability Assessment to Microbioreactor

Finally, we tested the production of AAV2 with the Sf-RVN® Platform using the BEVS in the Ambr® 15 microbioreactor with parameters described in **Figure 6A**. This allowed us to test a first set of bioreactors conditions prior to a larger scale. Similar to the previous experiment, we optimized the infection process by testing different MOIs as well as different ratio between the two baculoviruses (ITR-GFP and AAV2-RepCap). As we previously found, a low MOI (0.01) infection enables high AAV2 titer (**Figure 4**), we tested the effect of a lower MOI on the production of AAV2. Data in **Figure 6B** confirms that optimal infection conditions for Ambr® 15 microbioreactor culture are with an MOI of 0.01 and an equal ratio of AAV2RepCap:ITR-GFP.

A

Ambr®15 Parameters				
Final Volume	DO	RPM	Temperature	Vessel Type
15 mL	40%	900	27.5°C	Sparge-less

AAV2-Rep-Cap	ITR-GFP MOI	Baculovirus ratio AAVRep-Cap:ITR-GFP
Mock		0:0
0.01	0.001	10:1
0.01	0.01	1:1
0.001	0.001	1:1

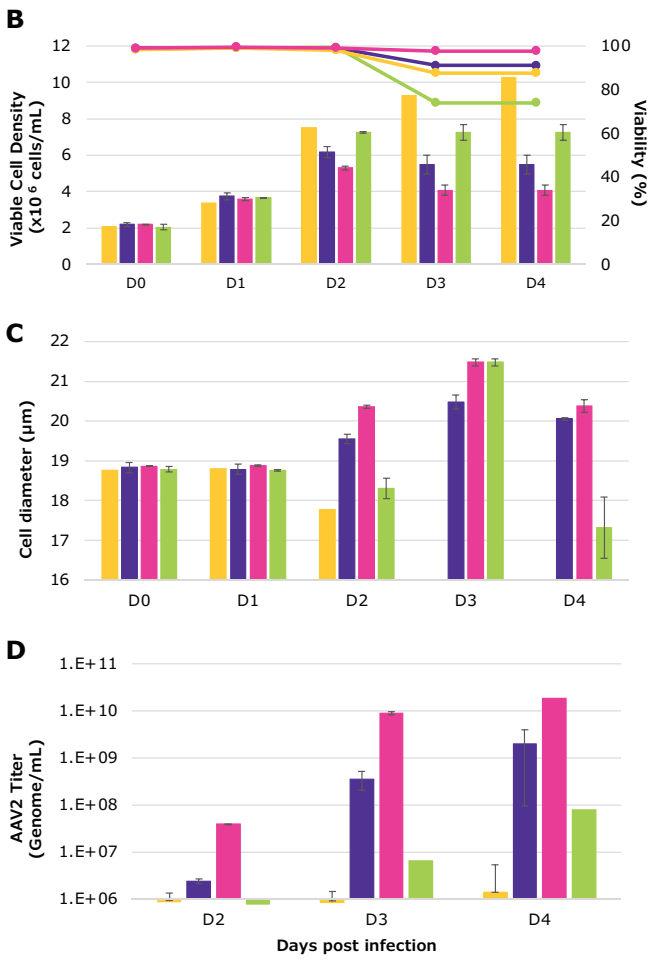

Figure 6: Low MOI and equal ratio of the two baculoviruses yields high AAV2 production of Sf-RVN® cells in Ambr® 15 microbioreactor. Cells were seeded at 2x10⁶ cells/mL in 15 mL EX-CELL® CD Insect Cell Medium and co-infected by two baculoviruses (ITR-GFP and AAV2-RepCap) as described (**A**). Cells were harvested at 48, 72 or 96 hours post-infection. VCD (**B**) and cell diameter (**C**) were measured as well as the AAV2 productivity, evaluated by ddPCR (**D**).

Conclusions

The Sf-RVN® Platform is a two-part system comprising a proven Sf-9 cell line devoid of Sf-rhabdovirus (the Sf-RVN® cell line) and a chemically defined medium engineered for the cell line, the EX-CELL® CD Insect Cell Medium.

The Sf-RVN® Platform was designed to improve the safety profile of baculovirus processes and to provide high production of recombinant protein, virus-like particles (VLPs) but also AAV. Through this work we showed that the EX-CELL® CD Insect Cell Medium outperforms all media tested to support the growth of both Sf-RVN® and Sf-9 cells and provides high AAV2 productivity in the two cell lines.

The optimization of the production of recombinant AAV, whether it is in an HEK or insect expression system, is at the core of the current research performed in the field of viral gene therapy. When it comes to upstream development, medium and process development occupy a center spot for the improvement of AAV critical attributes such as productivity and the ratio of empty versus full capsid. For the production of AAV2 using the BEVS system, we evaluated several starting MOI and found that an MOI of 0.01 achieved the highest viral titer. Indeed, this low MOI allows for the culture to double post-infection increasing the amount of producing cells and allow for an infection through cell-to-cell contact. Work is currently being done to improve the ratio of empty versus full while maintaining a high viral titer. The next step for AAV2 production in the Sf-RVN® Platform is the scalability. Our initial work in the Ambr15® system showed good titer correlation with our small-scale system, further work is in progress to provide optimal AAV production condition in bioreactor.

Overall, this study demonstrates that the Sf-RVN® Platform offers a safe, efficient, robust and consistent system for the production of AAV using the Baculovirus Expression Vector System.

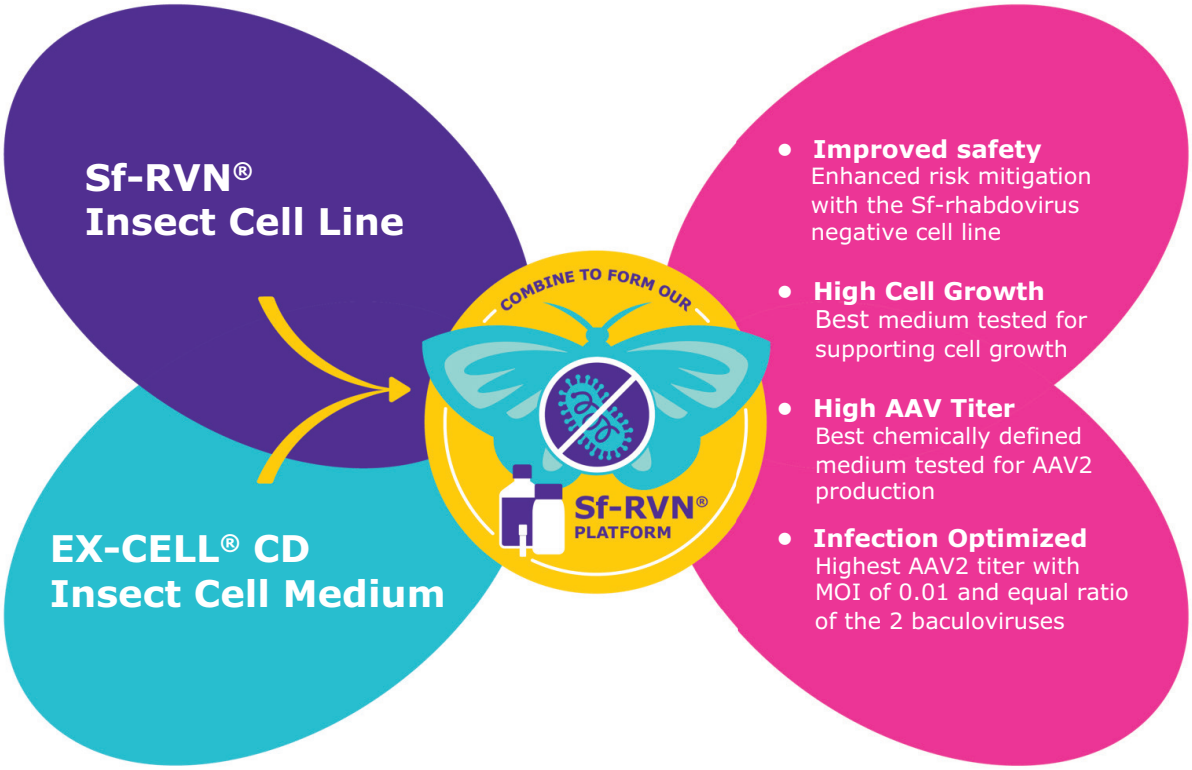

Figure 7: The Sf-RVN® Platform offers a safe, efficient, robust and consistent system for the production of AAV using the Baculovirus Expression Vector System.

References

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