



Dramatic Time Savings for Cell Culture Vaccine Development

INTRODUCTION

Protein Sciences Corporation (PSC) in Meriden CT uses their patented baculovirus expression vector system to produce proteins for vaccines. The recombinant baculovirus is grown in insect cells in a serum free suspension cell culture system. PSC is poised to become the first vaccine manufacturer to produce an FDA approved influenza vaccine (FluBlok®) using cell culture.

Currently available influenza vaccines are produced in fertilized chicken eggs. However, egg-based production methods have several industry-wide problems, including long planning and procurement times for eggs, extensive purification to remove egg-based impurities, and antigenic variability relative to original clinical isolates due to the need to create a viral hybrid that grows well in eggs. Cell culture methods have the potential to overcome many of these limitations by significantly reducing production times thereby enabling a more rapid response to potential pandemic viral strains.

CHALLENGE

Quantification of virus levels during the fermentation process can be used to infer protein expression and to optimize harvest time. Traditional virus quantification methods such as plaque assays are too slow to allow for real-time monitoring. In fact, baculovirus quantification of viral stocks by the plaque titer assay requires approximately 6 days, which can significantly delay vaccine production. Scientists typically rely on indirect methods to judge fermentation success. One such indirect method is a measurement of host cell viability as a function of time during fermentation. While host cell viability is generally a reliable method, it can be compromised for many reasons and the measurement may or may not be indicative of virus production. Scientists at PSC sought a real-time method for virus quantification during fermentation as a means to enhance productivity by ensuring immediate feedback on growth conditions and virus production.

SOLUTION

The Virus Counter provides a direct measurement of virus particle concentration within minutes. To evaluate the potential of the Virus Counter to address their real-time monitoring needs, scientists at PSC collected a time-course sample set during several fermentation batches. In this case, the cell culture system was designed for generation of vaccine targeted influenza hemagglutinin (HA). Each sample was first analyzed for host cell viability using a CEDEX cell counting system and subsequently centrifuged to remove cells and excess cellular debris. The supernatant samples were shipped frozen and blinded to InDevR for analysis on the Virus Counter. InDevR reported average virus particle concentrations for each sample in each time course set based on three measurements. The HA content expressed by cells after extraction from each batch was also quantified by PSC with the laborious SRID method. PSC subsequently provided host cell viability values as well as representative protein yields for each batch.

RESULTS

The virus particle concentration (red) measured using the Virus Counter for samples collected as a function of time during fermentation are shown in the top panel of Figure 1 along with host cell viability measurements (green) for the same samples. Data points represent the average of measurements for three fermentation batches with error bars indicating $\pm 1\sigma$ from the average. The data show that as the fermentation progresses host cell viability decreases and virus-like particle content (vlp/mL) increases, as expected. The data are consistent and indicative of virus production. The strong and predictive inverse correlation between the Virus Counter results and host cell viability is shown in the bottom panel of Figure 1.

For each of the three fermentations protein expression levels were quantified as an independent assessment of the success of the fermentation. The average protein concentration was acceptable at 20 ± 4 mg/L, as determined by SRID analysis. In contrast, results from a fourth, unsuccessful, fermentation exhibited protein expression levels less than 6 mg/L and no statistically significant changes were observed in either host cell viability or total virus-like particle concentration as a function of time.

These initial studies indicate that the Virus Counter can provide a direct and real-time assessment of virus production in cell culture. Since results can be obtained in minutes, time-savings for PSC is a factor of ~ 250 relative to plaque assays. *“The Virus Counter provides real-time insight into virus production during cell culture fermentation, allowing for immediate use of virus stocks for protein expression,”* said Dr. Wafaa Mahmoud, Cell Culture Manager. PSC scientists now have a Virus Counter in-house and anticipate that such dramatic time savings could lead to enhanced productivity and shorter time to market in the challenging and time-critical vaccine industry.

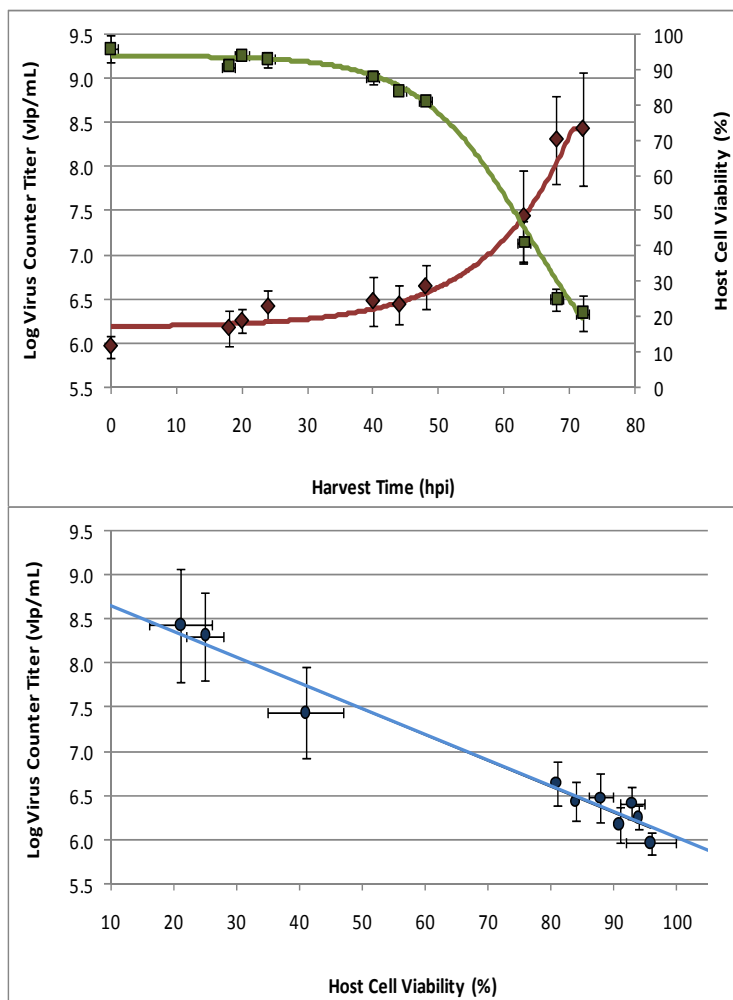


Figure 1. Results for three fermentation batches showing good product yield. Top panel shows reported host cell viability (expressed as a percentage, green squares) and Virus Counter results (vlp/mL, red diamonds) as a function of fermentation time (hours post infection). The inverse correlation of the log (vlp/mL) and host cell viability is shown in the lower panel. A linear regression fit to the data in the bottom panel yields an R^2 value of 0.97.