Improvement of Bovine Parainfluenza 3 Virus Production Yields and Capacities Using the Corning® HYPER*Flask*® Cell Culture Vessel

Application Note

CORNING

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Introduction

Parainfluenza 3 (PI3V) is a major virus that causes respiratory diseases, ranging from common cold to advanced pneumonia, in calves. This results in a high susceptibility to secondary infections that could be potentially fatal for the infected animals. Virus transmission occurs through respiratory aerosols or direct contact with infected animals, which results in a highly contagious disease that needs to be carefully monitored to reduce incidence and enable disease control.

Diagnostic kits are widely available to control the serological status of cattle related to PI3V. In these kits, viruses of interest are captured by exposing specific monoclonal antibodies to bovine serum. Specific antibodies from seropositive animals bind viruses, which then are detected with a horseradish peroxidase (HRP)-conjugated anti-bovine immunoglobulin antibody. Reproducible batches of PI3V are required to manufacture these kits. Virus productions are performed through *in vitro* methods in which Madin-Darby Bovine Kidney (MDBK) cells are grown and infected with a small quantity of virus from a standard bank to produce a highly concentrated batch. In view of the virus quantities required to produce these kits, the production is generally performed using multiple layer culture vessels such as the competitor vessel.

However, in large scale production, the manipulation of bulky stacked vessels can be labor-intensive, which not only increases the chance of contamination during the production process but also requires a significant amount of incubator space. With its revolutionary design, the Corning HYPER*Flask* vessel provides ten compact interconnected layers of growth surfaces. This vessel utilizes a gas-permeable film to provide oxygen and carbon dioxide exchange, resulting in gas exposure to a large surface area within the flask. By eliminating the headspace, the HYPER*Flask* vessel provides a total area of 1,720 cm^2, which is equivalent to ten T-175 flasks within the same footprint of one T-175, greatly increasing the viral production for a given amount of incubator footprint.

In an attempt to simplify the production of viral diagnostic reagents, we determined the suitability of the HYPER*Flask* cell culture vessels by comparing production yields and practical considerations against a competitor vessel, the traditional production vessel for this process. Conclusions were greatly in favor of the use of the HYPER*Flask* in terms of production yields, capacities, and practical aspects.

Materials and Methods

Cell Culture

Madin-Darby Canine Kidney (MDBK) cells were grown and maintained in 1X Dulbecco's Modified Eagle Medium (DMEM) containing 2 mM L-Glutamine (Lonza Cat. No. BE17-605E), 0.075% Sodium Bicarbonate (Thermo Fisher Cat. No. 25080-094), 50 µm 2-mercaptoethanol (Thermo Fisher Cat. No. 31350-010), 100U/100 µg Penicillin and Streptomycin (Lonza Cat. DE17-602E), and 10% heat-inactivated fetal bovine serum (FBS, GE Healthcare Cat. No. SV30160.03), and incubated at 37°C, 5% CO^2.

Viruses

The virus used in this study was the Loverix PI3V strain. Virus stocks were previously produced according to CER Groupe SOP, i.e. by preparing master and working viral banks that were both stored at -80°C and characterized by viral titration and immunolabelling with specific antibodies.

Virus Production

The day before virus inoculation, all cells from two T-175 flasks were seeded into one HYPER*Flask* vessel (Corning Cat. No. 10024) and incubated at 37°C, 5% CO_2. The same operation was performed with a competitor vessel, starting from six T-175 flasks to achieve the same cell number/surface ratio as in the HYPER*Flask* vessel, which has approximately one-third the total surface area as the competitor vessel. The next day, 1.2 x 10^6 $TCID_{50}/cm^2$ of PI3V working virus bank was inoculated into the HYPER*Flask* and the competitor vessel. Following an incubation period of 3 days, supernatants were harvested, aliquoted and frozen at -70°C.

Virus Titration

In order to quantify the viral productions, one cryogenic vial of each virus supernatant was used to perform the virus titration by immunolabeling. Briefly, MDBK cells were seeded in 96-well microplates, and serial dilutions of the viral productions were added to the wells, two microplates by virus sample. Following an incubation period of 4 days in an incubator at 37°C and 5% CO_2, wells were fixed with a solution of PBS-formol, rinsed with PBS, incubated with anti-PI3V monoclonal antibodies conjugated to HRPO (CER Groupe Cat. No. I7D4G6-HRP), washed with PBS, revealed with AEC substrate, washed with PBS, and analyzed under an inverted microscope. Viral titers were calculated based on the number of infected wells at the dilution of interest.

Results

Virus Growth in the Corning® HYPER*Flask*® Vessel

A compact cell culture flask with multiple layers provides a significant advantage for large scale virus production. Providing an easier manipulation and saving incubator space. Due to its innovative design, the HYPER*Flask* vessel was tested for the production of Parainfluenza 3 virus.

MDBK cells were grown in a 10-layer competitor vessel and the HYPER*Flask* vessel, after which the culture was infected with PI3V to proceed with the virus amplification. At the end of the production period, supernatants were collected and frozen to release viral particles remaining into host cells by disruption of cell membranes. The amount of virus produced after a 3-day incubation period was compared for each vessel.

We observed that both systems enabled viral infection. Results of subsequent virus titrations are summarized in Table 1 and represented in Figures 1 and 2. In Table 1, a column summarizing results obtained with the eight last batches of PI3V was also included in the analysis to give an overview of mean productivities.

Concerning PI3V production (Figure 1), we observed that titers were slightly higher in the competitor vessel compared to the HYPER*Flask* vessel. However, we noted that production levels observed in the HYPER*Flask* vessel were in the range of previous batches produced recently, with results slightly above mean log $TCID_{50}$. In addition, it's important to highlight that only two HYPER*Flask* vessels were required to produce the same quantity of virus compared to the competitor 10-layer vessel. Which is a great advantage in view of the space required in an incubator to maintain a HYPER*Flask* vessel compared to the competitor vessel. So, bigger batches can be produced easily. Moreover, when we compared the quantity of virus produced by cm^2 (Figure 2), we observed that this parameter was twice the value in the HYPER*Flask* vessel as in the competitor vessel.

The higher productivity observed in the HYPER*Flask* vessel may be related to the improved metabolism conditions within the culture, achieved by the improved design of this vessel. The proprietary design of the HYPER*Flask* vessel enables a better gas exchange in comparison to a traditional stacked vessel, where the gas exchange occurs via the vented cap and headspace. In terms of the manufacturing process, it's also possible to manage several HYPER*Flask* vessels in parallel in an incubator, resulting in a clear advantage of the HYPER*Flask* vessel for viral production. It is also worth noting that no preliminary optimization was performed to reach these production levels.

Finally, we can also mention that the HYPER*Flask* vessel was also preferred by our technicians from a practical point of view, in the sense that these vessels were easier to manipulate, to place under the laminar flow hood, to perform medium exchange, and to maintain compared to the competitor vessel.

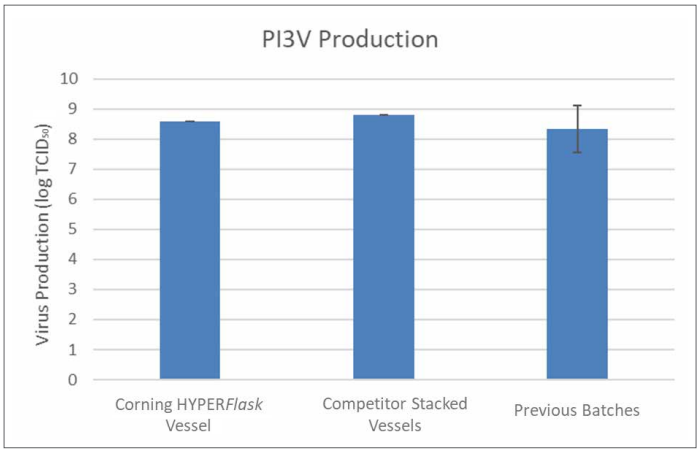

Figure 1. Virus Production per mL of PI3V in the Corning HYPER*Flask* vessel and competitor vessel.

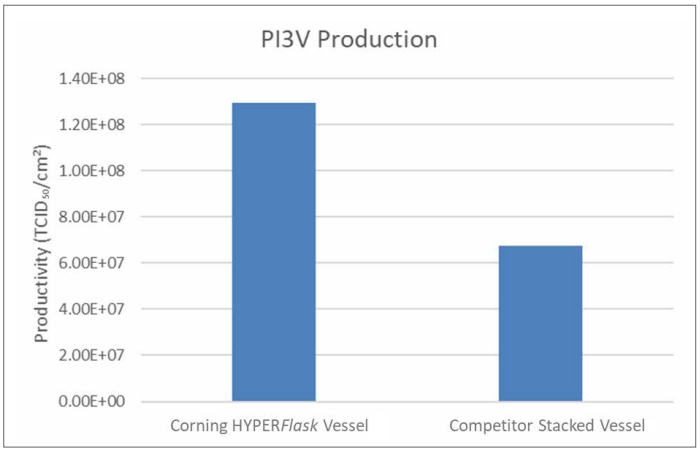

Figure 2. Productivity per cm^2 of PI3V in Corning HYPER*Flask* vessel and competitor vessel.

Table 1. Production Results of PI3V in Corning HYPER*Flask* and competitor vessel.

Target Virus	**PI3V**		
Vessel	Corning HYPER*Flask* Cell Culture Vessel	Competitor Vessel	Previous Batches (Competitor Vessel) Mean Value
Titer (log $TCID_{50}$/mL)	8.60	8.80	8.35 (SD = 0.78)
Volume (mL)	560	675*	675
Total production (log $TCID_{50}$)	11.35	11.63	11.17
Surface (cm^2)	1720	6320	6320
Productivity ($TCID_{50}/cm^2$)	1.30×10^8	6.74×10^7	2.39×10^7

*This reflects a modified protocol routinely used in virus production.

Conclusions

- The Corning® HYPER*Flask*® vessel was demonstrated to be an effective method of producing PI3V for diagnostic kits.
- This method requires fewer flasks to produce the same amount of virus with less manipulation, resulting in significant time savings and reduction of contamination risk.
- The small footprint of this vessel also allows for a large amount of virus to be produced in a limited incubator space.
- In addition, the HYPER*Flask* vessel achieved higher productivity than the competitor vessel in terms of yield per cm^2, thus constituting an economic solution for PI3V production.

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