Use of Nunc Cell Factory equipment to accelerate workflows and increase product consistency

Introduction

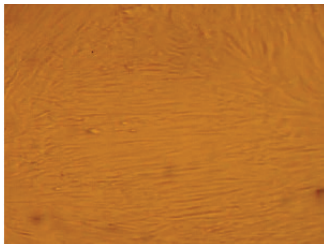
Scaling up adherent cell culture can be a difficult process for many vaccine, cell therapy, gene therapy, and viral vector manufacturers. Some cells can alter their properties if they are not cultured in specific conditions. Using multiple tissue culture flasks with the same culture conditions comes with a host of its own concerns, including batch-to-batch inconsistency, increased probability of contamination, and labor intensification. The simplest way to mitigate all these risks is by using a multilayered vessel, such as the Thermo Scientific™ Nunc™ Cell Factory™ system, in a closed system with automation equipment. The Nunc Cell Factory equipment automates the filling, emptying, harvesting, and trypsinization steps of adherent cell culture to help realize consistency, optimization, and standardization. This combination of equipment ensures a quick scale-up solution for quality product.

One company upgraded to the Nunc Cell Factory system to lower their risk of contamination in their production of varicella vaccine; however, doing so at the desired scale introduced pronounced inter-batch differences. Manually operated cell factories can be difficult to use in a scale-up process, leading to slow workflows and inconsistent results. The company integrated Nunc Cell Factory equipment to solve these problems through automation.

Methods and discussion

First, the company compared the use of the Thermo Scientific™ Nunc™ Automated Cell Factory Manipulator (ACFM) to a manual operation workflow. Both methods showed a dense monolayer of MRC-5 cells through virus infection (Figure 1), but using the Nunc Cell Factory equipment significantly cut the time required to complete each process (Figure 2). Technicians harvested the varicella cultures and compared product titers and serum albumin residues for the manual operation and the automatic operation. The automatic operation led to a more consistent virus titer that was also slightly more concentrated (Table 1). The albumin residue was also more consistent in the automatic operation as compared to the manual operation, indicating very little difference between batches made using the Nunc ACFM (Table 2).

Manual operation of cell factory (4 days)



ACFM operating cell factory (4 days)

Figure 1. Growth status of MRC-5 cells in manual (left) and automatic (right) modes of operation.

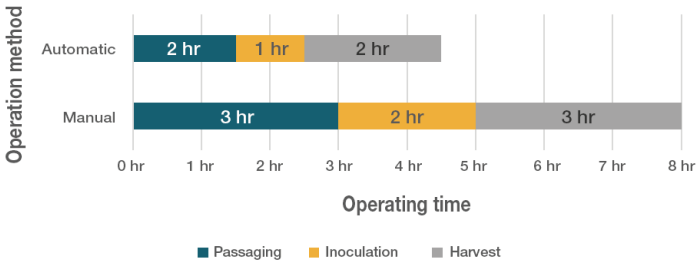


Figure 2. Time to complete workflow using manual and automatic modes of operations.

Table 1. Virus titer in stock culture.

Operation method	Lot No.	Virus titer (log PFU/mL)
Manual	S20140301	5.1
	S20140302	4.9
	S20140303	5.0
Automatic	S20140304	5.1
	S20140305	5.1
	S20140306	5.1

PFU: plaque forming unit
σ automatic = 0.00, σ manual = 0.10

Table 2. Bovine serum albumin residues in stock culture.

Operation method	Lot No.	Raw liquid bovine serum albumin residue (ng/mL)
Manual	S20140301	32
	S20140302	33
	S20140303	36
Automatic	S20140304	33
	S20140305	32
	S20140306	32

σ automatic = 0.58, σ manual = 2.08

Consistency is affected not only by manipulation of the cell factories and cells but also by incubation conditions. Temperature fluctuation, either in different parts of a warm room or within an incubator, can increase batch-to-batch variation. The company completed two experiments in which the Nunc Cell Factory incubator was compared first with a traditional warm room, and then later with another common incubator.

The company performed the same vaccine development workflow but used either a warm room or a Nunc Cell Factory incubator for the incubation steps. Compared to a warm room, the Nunc Cell Factory incubator cultured more consistently with a slightly higher live-cell count (Figures 3, 4). In addition to cell count, the Cell Factory incubator showed the same results for virus titer (Table 3). Using the incubator also saved on-floor space and power usage, compared to a warm room (Table 4). An average warm room takes up more space than four Cell Factory incubators, and additionally, four Nunc Cell Factory incubators use less power (5,600–6,000 W) and can hold more Cell Factory systems (64 40-layer to 48 40-layer).

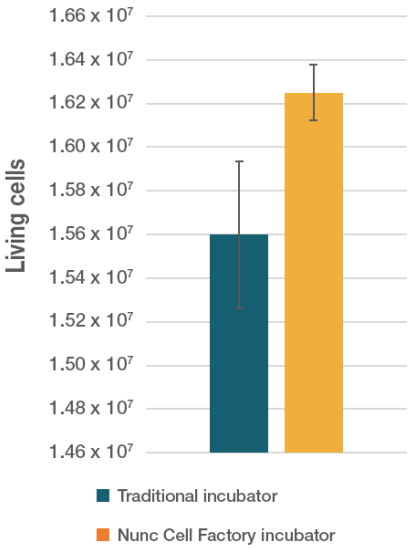
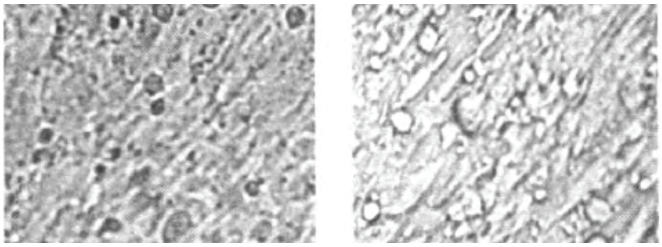


Figure 3. Comparison of MRC-5 cell counts cultured in different incubators.



Traditional warm room cultivation | Incubator culture

Figure 4. Comparison of viruses cultured in a traditional warm room and the Nunc Cell Factory incubator.

Finally, the company compared the Nunc Cell Factory incubator with another common incubator. Test results showed that the temperature fluctuation range of the Nunc Cell Factory incubator (36.8–37.3°C) was smaller than that of the common incubator (36.3–37.8°C) (Figure 5). Consequently, they found once again that the Nunc Cell Factory incubator led to more consistent virus titer (Table 5). Furthermore, the Nunc Cell Factory incubators reduced labor costs by requiring only one operator, whereas the other incubator required at least two. The Nunc Cell Factory system also increased capacity from eighteen 10-layer Cell Factory systems to sixteen 40-layer Cell Factory systems while reducing operating time from 30 minutes to 10 minutes.

Table 3. Comparison of virus titer of stock culture obtained from culture of traditional warm room and the Nunc Cell Factory incubator.

Cultivation method	Lot No.	Virus titer (log PFU/mL)
Traditional warm room	S20160201	5.1
	S20160202	4.9
	S20160203	5.0
	S20160204	4.9
Incubator	S20160205	5.1
	S20160206	5.1
	S20160207	5.2
	S20160208	5.2

PFU: plaque forming unit
σ incubator = 0.058, σ traditional warm room = 0.096

Table 4. Comparison of the floor area and energy consumption of traditional incubator and the Nunc Cell Factory incubator.

Cultivation method	Floor area (m^2)	Power (W)	40-layer Cell Factory system load quantity	Power used by a single 40-layer Cell Factory system (per unit)
Traditional warm room	12.00	6,000	48 units	125.0
Incubator	2.12	1,400	16 units	87.5

Table 5. Virus titer (log PFU/mL) of bulk of varicella vaccine.

Incubator	Lot No.	No. 1 No. 2 No. 3	No. 1 No. 2 No. 3	No. 1 No. 2 No. 3
Common	S2014021	4.9	4.8	4.8
	S2014022	5.1	5.0	5.1
	S2014023	5.0	4.9	4.9
Nunc Cell Factory	S2014024	5.2	5.2	5.2
	S2014025	5.2	5.1	5.1
	S2014026	5.2	5.2	5.2
PFU: plaque forming unit.		σ ordinary = 0.10 σ Cell Factory = 0.00	σ ordinary = 0.10 σ Cell Factory = 0.058	σ ordinary = 0.15 σ Cell Factory = 0.058

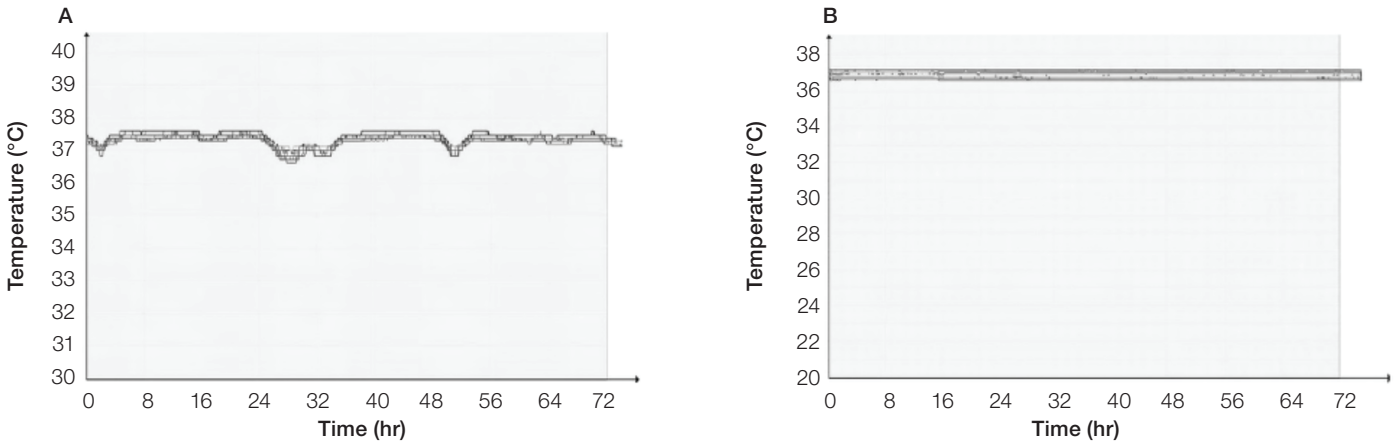


Figure 5. Temperature curve of (A) ordinary incubator and (B) Nunc Cell Factory incubator.

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Conclusion

Adding Nunc Cell Factory equipment to the varicella virus production workflow increased consistency between batches while lowering costs. It also helped this company reduce labor costs by saving time and manpower without sacrificing quality or worker safety. Technicians noted that "cleaning, maintenance, and replacement of the incubator is also more convenient" with the Nunc Cell Factory incubator, leading to a more efficient workflow. These advantages make Nunc Cell Factory equipment the perfect solution for scale-up of adherent cell culture.

References

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