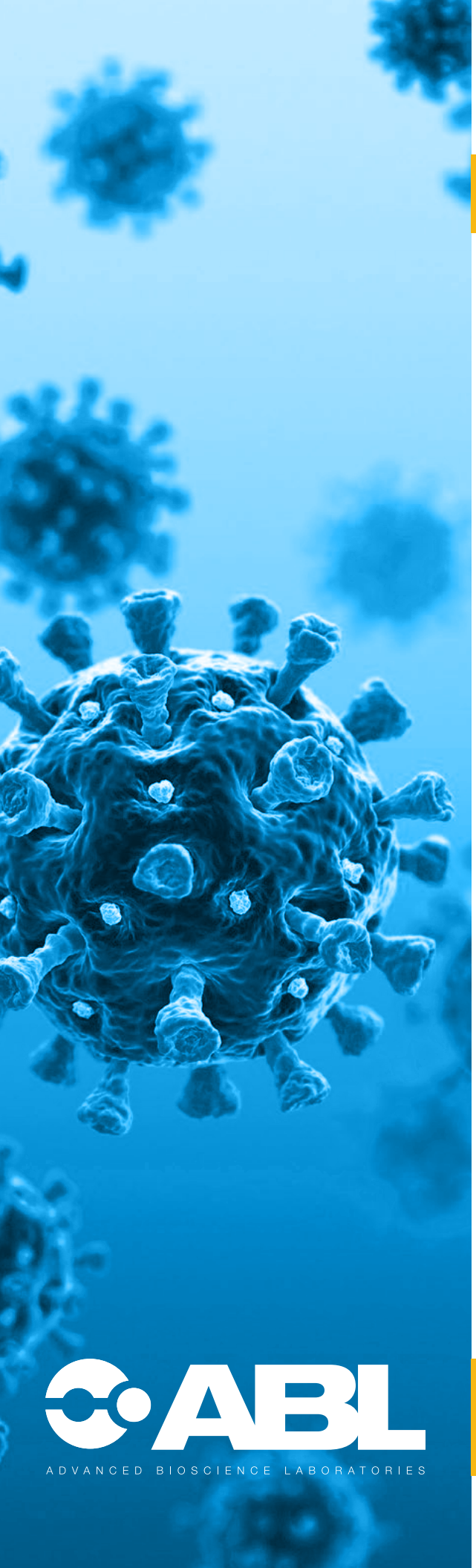

LENTIVIRUS PURIFICATION

DEVELOPING A DOWNSTREAM PROCESS

ABL is a CMO with a history of producing and purifying biomolecules for applications including oncolytics, vaccines, and cell and gene therapies.

ABL has over forty years of experience in HIV research and development, including development of the first HIV diagnostic blood test. Lentivirus is derived from HIV and we utilized our HIV expertise to develop a process for lentivirus purification. Test conditions and parameters at each step of the process were thoroughly vetted to attain a purification strategy that can be easily adapted to any client lentivirus product.

Lentivirus has several unique properties that make it a suitable vector for cell therapy, such as large genetic capacity, host genome integration and reduced chance of eliciting an immune response in the host. However, there are significant challenges when working with lentivirus, as listed below.

1. The susceptibility to external factors such as shear stress, temperature, pH and salt concentration.
2. Attaining sterility of the final product while achieving high titers required for clinical use.
3. Removal of host cell debris, proteins and nucleic acid while maintaining the integrity of the virus during the purification process.

To address these challenges, ABL has developed the following purification strategy:

ABL leverages decades of experience with human retroviruses into our lentivirus process.

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PROCESS DEVELOPMENT

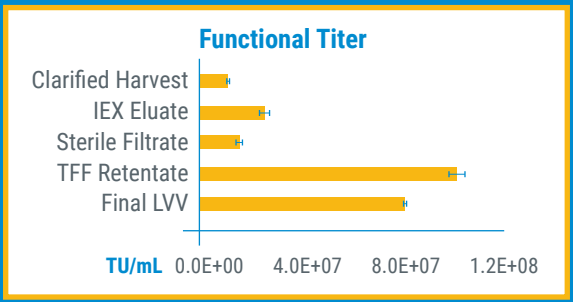

Figure 1: Functional Titers at each step

	Phys. Titer	Func. Titer	p24 Conc
Chromatography	64%	55%	30%
Sterile Filtration	82%	76%	85%
TFF	68%	85%	80%
Final Filtration	90%	86%	72%
Overall	**32%**	**31%**	**14%**

Table 1: Recoveries at each step of the process

	HCP Log Reduction	hcDNA Log Reduction
Clarified Harvest	-	1.82
IEX Eluate	1.85	1.89
Sterile Filtrate	1.86	2.67
TFF Retentate	2.31	2.73
Final LVV	**2.32**	**3.12**

Table 2: Cumulative Log Reduction in Impurities

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LENTIVIRUS PURIFICATION

FINDING SOLUTIONS TO CHALLENGES IN LENTIVIRUS PURIFICATION

Challenge 1: Lentivirus is inherently unstable during processing.

Solution: ABL developed a downstream process that minimizes loss of titer due to external factors. This purification strategy results in a physical to functional particle ratio consistently below 270 throughout the process. As shown in Figure 1, we achieved a functional titer of 1E+08 TU/mL. Table 1 presents the percent recoveries at each step.

Conclusion: The optimized process steps allow for gentle and efficient purification of the virus with minimal loss in titer.

Challenge 2: Sterile filtration of high titer virus is difficult and thus limits final virus titer concentration.

Solution: When ABL incorporated a sterile filtration step before concentration, high virus recovery was observed. The TFF can be processed aseptically and the final sterile filtration which may result in great loss of virus can be optional.

Conclusion: Incorporating a sterile filtration before TFF allows for concentrating the final product to a titer of 5E+08 TU/mL.

Challenge 3: Removal of residuals before use in clinical applications.

Solution: ABL optimized each step of the process to decrease host cell protein (HCP) and host cell DNA (hcDNA) levels. A significant reduction in impurities in the final product was seen as shown in Table 2 (*left*).

Conclusion: ABL's purification conditions allow for drastic reduction in HCP and hcDNA impurity levels.

Conclusion: ABL has established a downstream purification strategy that yields lentivirus at high titer with low impurity levels.

ABL's flexibility & agility is key to your success.
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