Fit from development to manufacturing – A successful rAAV9 scalability study in a suspension process using HEK293

Abstract 522

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Background

Design of experiment (DoE) approaches performed in robust scale-down devices such as small-scale bioreactors for upstream process development allow product specific optimization of modular platform processes at speed and with reasonable development costs.

However, scaling up the process to larger bioreactors can be challenging. To stay in a single provider's system for all scales can be a good starting point, as many of them already deliver a homologue vessel height to diameter ratio as well as respective impeller, baffles, and sparger designs. But as often in sensitive biological systems, this might not be sufficient. Several process specific and defining conditions like mixing time, power input, impeller tip speed or oxygen transfer rate (OTR) cannot be kept constant simultaneously throughout all scales. Also, factors independent of the bioreactor setup but dependent on scale, such as process parameters and volumes of the cell seed train, must be considered, as they can impact the cell's performance and therefore product yield and quality, respectively. The aim of this study is to demonstrate scalability of AAV productivity and consistency of product quality across all different bioreactor scales of Ascend's AAV manufacturing platform offering.

Many critical quality attributes related to product impurities including full/empty ratio, residual plasmid and host cell DNA (HCD) packaging are strongly determined by the design and choice of the biological starting materials and the upstream process. Therefore, we consider it essential to scale-up processes with the target of comparable quality and productivity across all scales to be able to support our clients across a product's life cycle. This in turn necessitates robust, fit for purpose analytics allowing in-depth product characterization. Here we present AAV batch data from 250 mL to 200 L bioreactor size using several proprietary analytical methods to assess both quantity and quality of the material generated.

Used scales*

Scale	Nominal volume	Working volume
Ambr® 250	0.25 L	> 0.21 L
Univessel® SU 2L	2 L	> 1.68 L
Univessel® Glass 5 L	5 L	> 4.2 L
BIOSTAT STR® 50	50 L	> 42 L
BIOSTAT STR® 200	200 L	> 168 L

Ambr® 250

Univessel® SU 2L

Univessel® Glass 5 L

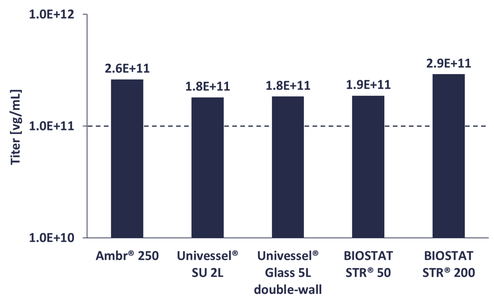
BIOSTAT STR® 50

BIOSTAT STR® 200

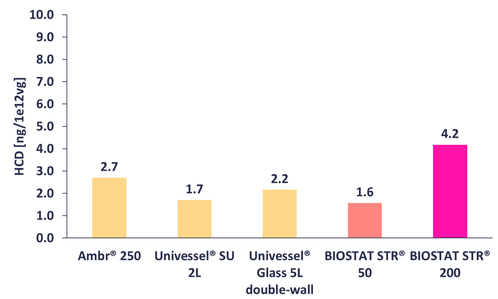
*Image source: "Sartorius" Catalog and Brochure

Scale-up results for a 3kb AAV9-SEAP vector excluding a specific full/empty enrichment step.
Key critical quality attributes mainly defined by plasmid design and upstream process parameters have been analyzed

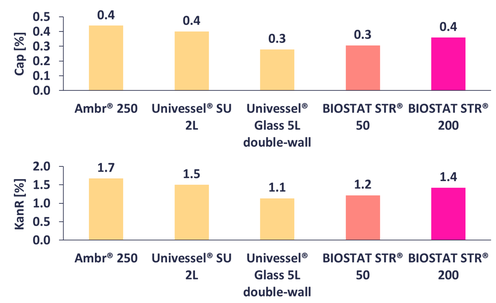
Vector genome titer by SEAP ddPCR



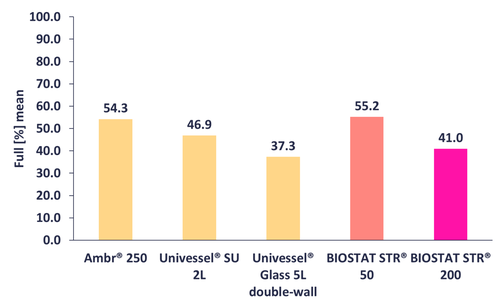
Host cell DNA impurities by 18S ddPCR



Residual Plasmid impurities by duplex ddPCR



% Full Capsids by Refeyn Two™ Mass Photometry



1.00E+11 vg/mL
Specified titer minimum

Raw lysate
POROS™ AAVX spin purified
ffBDS (final filtered bulk drug substance)
Affinity purified

A SEAP ddPCR showed a vg yield variation of maximum +32% to the average with the highest yield obtained at 200L scale.

Host cell DNA (HCD) impurities varied between 1.6 and 4.2ng / 1.0E12vg and are overall low compared to industry standard.

The plasmid-derived impurities capsid (cap) and kanamycin resistance (kanR) gene showed comparable results between as little as 0.3-0.4% impurity for cap, respectively 1.1-1.7% for kanR over all scales.

Refeyn Two™ Mass Photometry analysis reveals a high percentage of full capsids, ranging from 37% to 55% across all scales. Variation could be attributed to the analytical method variance or differences in the purification method.

The present graphs show the whole workflow variation including seed train to seed train, run to run and assay variance for n=1 for each scale.

Summary

Here, we demonstrate the successful scale-up of the upstream process of our modular AAV manufacturing platform using our split two plasmid platform, from bench-scale to 200 L production scale meeting our stringent development target of comparable quality at expected volumetric yield increase. Our commitment to our clients is to support development of their products through all stages, from early preclinical and tox studies to Phase 3 and commercial manufacturing with a de-risked CMC regulatory pathway.
For more information on applicability of our platform on different serotypes, see poster #1498.

Aim higher

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