From Development to Commercialization: Late-Stage Process Characterization for rAAV Manufacturing

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Late-Stage, Process characterization, PRA, Scale Down Model, Risk priority number, CQA, QTPP, control strategy, Risk assessments

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Introduction

Background

The FDA drug development process comprises four major phases, each designed to progressively evaluate the safety, efficacy, and manufacturability of a product. Early clinical phases (Phase I and Phase II) primarily emphasize establishing initial safety profiles, determining appropriate dosage ranges, and assessing preliminary efficacy. In contrast, late-stage phases (Phase III and Phase IV) focus on validating long-term safety, process robustness and therapeutic benefit in larger patient populations, and compliance with regulatory requirements. Following successful Phase III outcomes, drug products undergo a comprehensive review and approval process by regulatory authorities. To ensure readiness for this critical transition, developers must enter late-stage development with robust, well-characterized manufacturing processes and a deep understanding of product and process performance.

At Forge Biologics, we partner with clients across all stages of development to accelerate readiness for regulatory review through integrated process development, manufacturing, quality control, and analytical expertise. In early development, efforts center on designing high-yielding and scalable upstream and downstream processes that consistently meet product quality specifications. As programs progress to late-stage development, these processes must evolve into highly controlled, commercial-ready systems supported by rigorous data, robust analytics, and risk-based justification.

PLATFORM APPROACH [A-GENE FRAMEWORK]

1. **IPO Mapping** — Define critical process relationships and parameter ranges
2. **Parameter Risk Assessment (PRA)** — Identify relationship between process parameters and critical quality attributes; classify CPPs
3. **Scale-Down Models (SDM)** — Platform-informed; proactively identified early in development
4. **Process Characterization (PC)** — DoE, OFAT, and SVEM studies to define design space
5. **FMEA** — Identify failure modes; confirm control strategy post-PC

Strategic platform advantage: Forge proactively identifies scale-down models early in the program lifecycle, enabling rapid iteration and significantly compressing late-stage timelines — reducing comparability risk at scale-up.

Lifecycle of Late-Stage Process Development

Aspect	Early-Stage Development	Late-Stage Development (Forge Focus)
Primary Goal	Feasibility; speed to First-in-Human trials	Scalability, robustness, regulatory compliance for commercial use
Timeline	Preclinical → IND/Phase 1/2	Phase 3 → BLA submission
Process Characterization	Minimal; enough to produce material, more focus on process optimization and scale-up	Extensive DoE, critical parameter mapping, SDM, BLA-enabling studies
Product Quality Attributes	Defined but loosely controlled	Tightly controlled; CQAs/CPPs confirmation
Analytics	Fit-for-purpose (sometimes still in development)	Qualified/validated methods required for commercial release
Quality Oversight	Minimal to none	R&D Quality oversight
Regulatory Risk	High if not documented; more flexibility given	Lower tolerance for variation; critical for approval
Process Changes	Many; process still evolving	Minimal to none- unless required to improve process robustness

PC WORKFLOW AT FORGE [A-GENE FRAMEWORK]:

Early Stage → Provides input for later PRA

CQAA → Identify CQAs from QTPP; determined by effect on safety & efficacy

PRA/MRA → Assess parameters & materials with greatest risk of affecting CQAs;

SDM + PC → PC studies for higher-risk parameters (from PRA) using representative and/or qualified SDM

FMEA → Post-PC: confirm critical/key/non-critical process parameters; identify failure modes

CQAA: Critical Quality Attribute Assessment
QTPP: Quality Target Product Profile
PRA: Parameter Risk Assessment
MRA: Material Risk Assessment
PAR: Proven Acceptable Range
SDM: Scale-down model
PC: Process Characterization
FMEA: Failure modes and effects analysis

Risk Classification Framework

Parameters are classified following PC studies. FMEA informs final control strategy. Parameters may be classified into the following categories based on impact and availability of monitoring strategies:

- **Critical:** Impact to CQA; must stay within narrow PAR; tight control required.
- **Key:** Does not impact CQA, impacts process performance indicators; monitored with defined acceptable ranges.
- **Non-Critical:** Negligible impact to CQA or process performance within studied range; routine monitoring

Quality Attributes	Bulk Drug Substance (BDS) Target	Drug Product (DP) Target	CQA? (Y/N)
Vector Purity/Identity	N/A	≥ 90% VP1/2/3 With No Single Impurity > 5%	Y
Capsid Identity	Fingerprint consistent with AAVX	N/A	Y
pH	N/A	7.2 ± 0.2	Y
Bioburden	TBD ≤ 10 CFU/mL	TBD N/A	Y
Sterility	N/A	No growth	Y
Endotoxin	TBD	≤ 0.5 EU/mL	Y
Residual Host (HEK293) Cell Protein	< LOQ	N/A	Y
Residual Endonuclease	<LOQ	N/A	Y
% Empty Particles	< 30% Empty Particles	N/A	Y

Figure 1: *Example of quality attributes and classifications*

RISK PRIORITY NUMBER (SEVERITY x OCCURRENCE x DETECTION)

Based on the risk priority number (RPN) assigned, the following are called for:

- **High RPN:** Extensive DoE; tightest PAR; process lock-down recommended
- **Medium RPN:** OFAT screening; defined NOR; enhanced monitoring
- **Low RPN:** Documented assessment; periodic monitoring acceptable
- **Post-PC FMEA:** Confirms classification; identifies residual failure modes

	CQAs	pH	Titer, ug/mL	Yield, %	Bioburden, CFU/mL	Endotoxin, EU/mL	HCP ELISA, ug/mL	Residual Ligand, ng/mL	Osmolality, mOsm/kg	Residual Host Cell DNA, ng/mL	
	Units										
Elution buffer pH	N/A	9	3	3	1	1	3	3	1	3	42
Elution hold temperature	°C	1	1	1	1	1	1	1	1	1	9
Residence time (product contact: Load, Washes, Elution)	minutes	1	3	9	1	1	3	1	1	3	24
Load ratio	kg/mL-resin	1	3	3	1	1	3	1	1	1	27
Wash 2 buffer conductivity	mS/cm	1	1	1	1	1	3	1	1	1	11
Peak collection start	AU/cm	1	3	3	1	1	3	3	1	1	17
Peak collection stop	AU/cm	1	3	3	1	1	3	3	1	1	17
Asymmetry	N/A	1	1	3	1	1	1	1	1	1	11
Load volume	L	1	3	1	1	1	1	1	1	1	11
Wash volume	CV	1	1	1	3	3	1	1	1	1	13
Post-use sanitization volume	CV	1	1	1	3	3	1	1	1	1	13
Elution buffer conductivity	mS/cm	1	1	1	3	1	1	1	1	1	9
EQ buffer pH	N/A	1	1	1	1	1	1	1	1	1	9
EQ buffer conductivity	mS/cm	1	1	1	1	1	1	1	1	1	9
Wash 2 buffer pH	N/A	1	1	1	1	1	1	1	1	1	9
Strip buffer pH	N/A	1	1	1	1	1	1	1	1	1	9
Pre-use EQ volume	CV	1	1	1	1	1	1	1	1	1	9
Strip volume	CV	1	1	1	1	1	1	1	1	1	9
Elution volume	CV	1	1	1	1	1	1	1	1	1	9

Figure 2: *Example of risk priority number assignments for an affinity chromatography process*

REGULATORY ALIGNMENT

Applicable standards: **ICH Q8**, **ICH Q9**, **ICH Q10**, **ICH Q5 (R2)**, **ICH Q11**, **FDA Process Validation**

Scale Down Model Establishment

Enabling Commercial-Scale Confidence

SDMs need to account for scale effects and be representative of the manufacturing scale. A qualified SDM also considers robustness and worst-case scenarios (e.g. viral clearance). Forge proactively identifies SDMs early in the program lifecycle, enabling:

- **Earlier risk assessment and accelerated IPO, PRA, SDMQ, and PC activities**
- **Reduced development timelines through rapid iteration of PC studies**
- **Platform benefit from shared learnings across multiple rAAV serotypes**

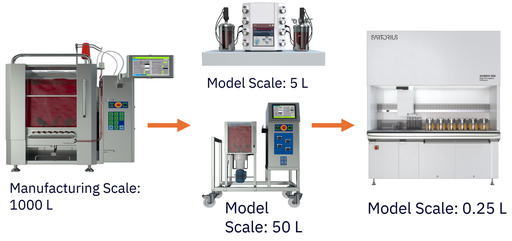

Figure 3: *Schematic of upstream scale down model qualification (SDMQ)*

REGULATORY REQUIREMENT

ICH Q8 · ICH Q11 · FDA Process Validation Guidance

SDM-based PC studies are only as defensible as the at-scale data behind them.

FDA expects sponsors to demonstrate SDM representativeness and/or equivalence using actual manufacturing-scale data, not engineering rationale alone.

1. **Demonstration, not assumption** — ICH Q8 / Q11 require SDMs to be shown representative of the commercial process , not merely designed to be.
2. **PAR defensibility at BLA** — PARs established solely at lab scale carry regulatory risk. At-scale data anchors them to the commercial process.
3. **Comparability as the bridge** — At-scale GMP runs confirm, via analytical methods and pre-defined criteria, that SDM outputs are predictive of commercial performance.

Forge: At-scale GMP runs are integrated into SDM qualification from the outset - ensuring PC conclusions are backed by manufacturing-scale evidence before BLA.

SDM Case Study: Affinity Chromatography

Figure 4 (left): *Schematic of downstream process scale down qualification. From left to right: Repligen KRM10, ÄKTA Pure, TECAN Fluent. Compare with Figure 3.*

- Purification of adeno-associated virus (AAV) by affinity chromatography requires scalable, high-throughput screening tools to accelerate process development; here we evaluate CaptureSelect™ AAV9 resin across five column formats spanning a 50-fold scale range (0.2–10 mL), operated on both automated liquid handling (Tecan) and FPLC (ÄKTA) platforms, to assess whether miniaturized RoboColumn™ formats can serve as predictive models for larger-scale purification processes.
- Consistent purification performance was demonstrated across all scales, with recovery ranging from 59–90% and no statistically significant difference detected by one-way ANOVA, supporting the use of miniaturized RoboColumn™ formats on automated liquid handling platforms as predictive scale-down models.
- Particle quality attributes assessed by dynamic light scattering were statistically equivalent across all formats (% mass > 98% for all formats), confirming that neither column scale nor instrument platform introduces measurable perturbation to AAV capsid integrity.

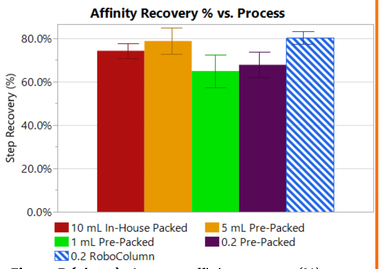

Figure 5 (above): *Average affinity recovery (%) across column volumes, n=≥3 per condition. Solid bars represent runs performed on AKTA system. Dashed bar represents run performed using TECAN system. Equivalence testing confirms that 5mL columns are representative of manufacturing scale (1-2L columns)*

Conclusions

Late-stage process characterization at Forge Biologics is built as an integrated, end-to-end framework -- not a series of isolated studies -- and is deliberately designed to scale seamlessly from 250 mL bench systems to 1000L manufacturing reactors. By connecting critical quality attribute assessment, parameter risk ranking, scale-down model qualification, and failure mode analysis into a single continuous workflow, Forge ensures that every process knowledge gap is closed before it reaches the regulatory reviewer. The result is a regulatory submission package grounded in manufacturing-scale evidence, a design space that is defensible under scrutiny, and a commercial process that performs as characterized -- with fewer deviations, fewer surprises, and a shorter path from Phase III to launch.

Integrated Framework
- CQAA→ PRA → SDM+PC → FMEA
- Single continuous workflow
- No knowledge gaps prior to regulatory submission

Regulatory Alignment
- ICH Q8/Q11, FDA-ready from day 1
- Audit-ready docs at every milestone
- Design space defensible at BLA

Commercial Readiness
- Lab scale → GMP, no rework
- Deviations controlled pre-launch
- Post-approval changes minimized

Compressed Timelines
Proactive SDM enables earlier risk assessment and faster PC iteration

Audit-ready regulatory package
PC reports, risk assessments and validated methods ready at submission

Successful Commercial Process
Risk identified and controlled before PPQ and commercial manufacturing begins

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