Successful validation of capsid titer and host-cell derived DNA impurity assays extends our rAAV batch release QC portfolio

F Thoennissen, M Magerl, C Zach, M Gora, T Kloetzler, P Zanotti, S Beer, R Kober, A Schoberth, S Schermann, S Geiger

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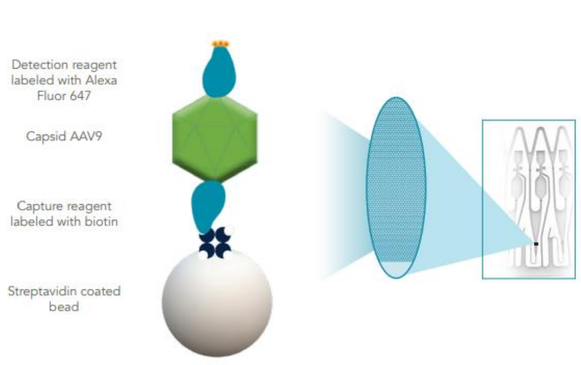
Abstract

Quantification of the capsid titer and packaged host-cell derived (HCD) impurities are critical quality attributes for rAAV batch release.
AAV9 capsid titer determination was established on the automated immunoassay system Gyrolab xPlore. Commercially available AAV9 empty capsids from two different manufacturers were used as standard and trending control, respectively. In robustness studies, usage of different lots of empty capsids for standard and trending control and kit components was addressed.
For HCD determination, we established a droplet digital PCR (ddPCR) targeting the 18S ribosomal RNA gene locus serving as a surrogate gene for packaged DNA impurities in rAAV. Before analytical validation, the method was qualified and tested for several parameters like droplet lifetime and sample dilution storage in intensive robustness testing.
Robustness studies of both methods were carried out in accordance with ICH Guideline Q14 – Analytical Procedure Development.
In our GMP laboratories, analytical validation of both methods was performed according to ICH Guideline Q2(R2) – Validation of Analytical Methods. Here we present the results of robustness testing and analytical validation addressing specificity, working range including suitability of calibration model and lower range limit verification, precision, and accuracy of the methods.
Since both platforms include the possibility of analyzing different serotypes (capsid titer) and rAAV batches from different production cell lines (HCD impurities) by making minor adaptions to the protocols, they offer high potential to extend our portfolio for customer rAAV batch release testing.

Platform 1: Gyrolab xPlore ™

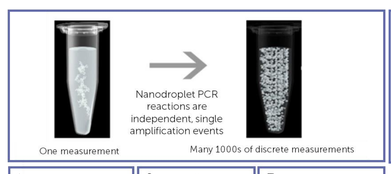
Automated immunoassay for AAV9 capsid titer determination

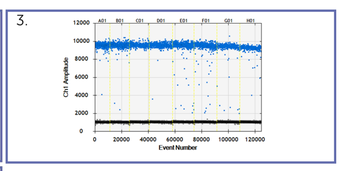


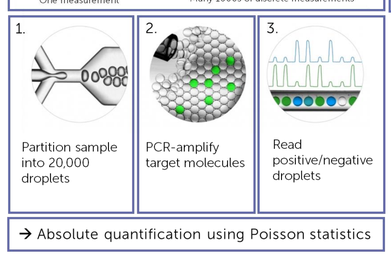
Pictures taken from Gyrolab Product Information Sheets D0023564/E and D0039753/C

Platform 2: QX200 ™ ddPCR system

18S ddPCR for quantification of HCD impurities in rAAV







In consecutive steps, the rAAV sample dilution is partioned in about 20,000 droplets in a Droplet Generator (1), amplified in a Thermal Cycler (2) and positive and negative droplets are read on a QX200 Droplet Reader (3) followed by absolute quantification by Poisson statistics.
18S ddPCR amplifies a 202 bp fragment of the 18S rRNA gene (surrogate for HCD) using a FAM-labelled probe.

Adapted from Bulletin_6407.pdf (bio-rad.com)

Robustness studies for AAV9 capsid titer determination

Robustness Parameters			Results		
Run No.	Kit	Standard/ Samples	Std	TC	AAV9 sample
1	Lot I	Std lot I TC lot I TC lot II AAV9 sample	Std working range (Std 2-7): • Recoveries: 95% – 109% • CV≤ 7% Anchor points (Std 1 and 8): • Recoveries: 93-107% • CV≤ 8% Blanks: • Values < Std 7 all runs	Recoveries both lots single runs: 78%-113% TC lot I: CV: 8% TC lot II: CV: 8% TC lot I+II: CV: 9%	CV single runs ≤4% CV all runs: 4%
2	Lot I	Std lot II TC lots I TC lot II AAV9 sample			
3	Lot II	Std lot I TC lots I TC lot II AAV9 sample			

Std: Standard, AAV9 empty capsids (supplier lot I+II)
TC lot I: Trending control, AAV9 empty capsids supplier 1
TC lot II: Trending control, AAV9 empty capsids supplier 2
AAV9 sample: Inhouse rAAV production batch

Robustness studies for 18S ddPCR

Robustness Parameter	Results			
40, 60 and 90 min between droplet generation and cycling	**Sample**	**Time**	**Recovery (%)**	**CV (%)**
	rAAV sample 1	40 min	100 (reference)	4
		60 min	108 (pass)	5
		90 min	103 (pass)	4
66 h between cycling and readout	**Sample**	**Time**	**Recovery (%)**	**CV (%)**
	rAAV sample 1	immediately	100 (reference)	4
		after 66 h	101 (pass)	2
Storage of rAAV sample dilutions at 2-8°C for 24 h	**Sample**	**Dilution**	**Recovery (%)**	**CV (%)**
	rAAV sample 1	fresh	100 (reference)	4
		24 h	74 (fail)	10
	rAAV sample 2	fresh	100 (reference)	3
		24 h	58 (fail)	21
Preparation of rAAV sample dilutions in DLBT	**Sample**	**Tube Type**	**Recovery (%)**	**CV (%)**
	rAAV sample 1	PLBT	100 (reference)	4
		DLBT	91 (pass)	6

PLBT: Protein LoBind Tubes
DLBT: DNA LoBind Tubes

Analytical method validation for AAV9 capsid titer determination

Parameter	Acceptance Criteria	Test Result	Status
Specificity (Matrix)	• Recovery 80%-120% to highest dilution of AAV9 sample • CV≤ 15%	• Recovery (dilutional linearity confirmation): **95%-100%** • CV: **3%**	Pass
Specificity (Spike)	• Recovery 80%-120% to non-spiked AAV9 sample (spike: AAV8 sample) • CV ≤ 15% (dilution) • CV ≤ 15% (across all dilutions) • Spiked NTC (AAV8) < Std 7	• Recovery to non-spiked sample: **96%-100%** • CV≤ **7%** (dilution) • CV ≤ **5%** (across all dilutions) • Spiked NTC (AAV8) < **1.95E+08** (Std 7)	Pass
Working range	• Recovery 80%-120% (Std 2 to Std 7) • CV≤ 15% (3x 4 sample dilutions)	• Recovery: **97%-107%** (Std 2 to Std 7) • CV=**3%**	Pass
Response (calibration model, Std linearity)	• $r^2 \geq 0.98$ (six Std points all 4 runs) • Recovery 80%-120% (Std 2 to 7 all 4 runs) • CV ≤ 15% (Std replicates all 4 runs)	• r^2 =**1.00** • Recovery: **96%-107%** • CV ≤ **7%**	Pass
Response (sample linearity)	• $r^2 \geq 0.98$ • Recovery 80%-120% • CV ≤ 15%	• r^2 =**1.00** • Recovery: **95%-102%** • CV ≤ **6%**	Pass
Accuracy	• Inferred from specificity, linearity and precision	• Specificity (pass), linearity (pass) and precision (pass)	Pass
Precision	Repeatability: • CV≤ 15% across 3 sample replicates Intermediate precision: • CV ≤ 15% (on one plate) • CV ≤ 20% of MVs (all 4 runs).	Repeatability: • CV: **3%** Intermediate precision: • CVs on one plate (sample): **2% – 3%** CVs on one plate (TC): **4% – 6%** • CV of MVs (sample)= **4%** CV of MVs (TC) = **6%**	Pass

Analytical method validation of the 18S ddPCR assay

Parameter	Acceptance Criteria	Test Result	Status
Specificity (Spike)	• Recovery 80%-120% to non-spiked sample (plasmid spike) • CV ≤ 15% (dilution) • CV ≤ 15% (across all dilutions) • Spiked NTC < LLOQ	• Recovery to non-spiked sample: **100%** • Dilutional CVs: **0% – 4% w/o spike, 1% – 2% with spike** • Overall CVs: **2% w/o spike, 2% with spike** • Mean value spiked NTCs = **1.3 copies/reaction <LLOQ**)	Pass
Specificity (Matrix)	• Confirmed by dilutional linearity	• No interference from buffer components (Range)	Pass
Range (Response, Validation of Lower Range Limits)	• $r^2 \geq 0.98$ (at least five dilutions) • Recovery 80-120% to highest dilution • CV ≤ 15% (dilution) • CV ≤ 15% (across all dilutions)	• r^2 = **1.00** (across **10 dilutions**) • Recoveries in the range **89%-101%** • CVs of dilutions in the range: **1-14%** • CVs across all dilution in range: **8%** • LLOQ: 28 copies/reaction	Pass
Accuracy	• CV ≤ 15% (dilution) • CV ≤ 15% (overall) • Recovery 80-120% to orthogonal spectrophotometric measurement of 18S plasmid conc. (dilutions and overall)	• Dilutional CVs: **1% – 4%** • Overall CV: **1%** • Recovery individual dilutions: **98%-99%** • Recovery all dilutions: **98%**	Pass
Precision	Repeatability: • CV≤ 15% across 3 replicates Intermediate precision: • CV ≤ 15% (all sample/TC dilutions on one plate) • CV ≤ 20% of MVs all sample/TC measurements (3 runs).	Repeatability: • CV: **9%** Intermediate precision: • CVs on one plate (sample): **2% – 6%** CVs on one plate (TC): **3% – 4%** • CV of MVs (sample)= **5%** CV of MVs (TC) = **2%**	Pass

Summary

For AAV9 capsid titer determination, testing of different kit lots, trending control (TC) suppliers and standard (Std) lots confirmed robustness of the assay setup.
18S ddPCR showed good robustness concerning droplet stability between droplet generation and thermal cycling (up to 90 min) as well as between thermal cycling and droplet read-out (up to 66 h). Recombinant AAV sample dilutions were not impacted when prepared in DLBT instead of PLBT, however storage of sample dilutions for 24 h at 8°C led to impaired recoveries.
Both assays met all acceptance criteria during analytical method validation and are now available for GMP QC testing at Ascend. The platforms can be adapted for customer requirements concerning target sequence (ddPCR) or serotype/protein target type (Gyrolab) for further GMP testing.

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www.ascend-adv.com
felicia.thoennissen@ascend-adv.com