

Biodistribution and Transcript Quantification in Mouse Tissues Using ddPCR

STUDY OVERVIEW

A preclinical study was performed to characterize the biodistribution and transgene expression of an AAV vector-delivered therapeutic in mice. Using validated ddPCR and RT-ddPCR methods, vector copy number and vector-derived transcript copy number were quantified across multiple tissues.

The objective of this study was to support preclinical safety and efficacy assessments by determining tissue-specific distribution and transcriptional activity of the AAV vector.

STUDY DETAILS

- **Drug Class:** AAV vector-delivered gene therapy
- **Study Type:** Preclinical biodistribution and transcript quantification
- **Animal Model:** Mice
- **Analytes:** Vector DNA and vector-derived mRNA
- **Tissues Assessed:** Blood, bone marrow, brain, heart, kidney, liver, lung, lymph node, spleen, and gonads
- **Analytical Platforms:** Droplet Digital PCR (ddPCR) and Reverse Transcription ddPCR (RT-ddPCR)
- **Sponsor Collaboration:** Primers, probes, and plasmid reference standards supplied by sponsor
- **Site:** Altasciences, Seattle

METHODS

The sponsor provided primer and probe sequences as well as linearized plasmid reference standards. ddPCR and one-step RT-ddPCR assays were optimized on the **Bio-Rad Droplet Digital PCR system**.

Quality controls, positive and negative controls were prepared in a background of 1 µg genomic DNA using naïve mouse tissues provided by Altasciences. Both assays were validated to a **sensitivity of 50 copies of AAV vector target**.

All study samples were received from the sponsor's designated testing facility and processed on-site at **Altasciences, Seattle**, following site-specific SOPs.

Samples were **cut frozen**, homogenized using a **bead-beating procedure**, and subjected to nucleic acid isolation using **Qiagen DNeasy® (DNA)** and **RNeasy (RNA)** kits. Nucleic acids were quantified via **UV spectrophotometry** and diluted to working concentrations for ddPCR analysis.

Blood collected for RNA analysis was stabilized in **PAXgene reagent** and processed using the corresponding **PAXgene RNA isolation kit**, then quantified and diluted prior to RT-ddPCR analysis.

RESULTS

The validated range of detection extended up to **180,000 copies of vector target**.

No vector DNA or vector-derived transcripts were detected in vehicle control animals, confirming assay specificity.

At both assessed exposure timepoints, **all tissue types** demonstrated **quantifiable vector copy numbers** and **vector-derived transcript copy numbers**, confirming widespread biodistribution and active transcription of the AAV vector.

CONCLUSIONS

Validated ddPCR and RT-ddPCR methods enabled highly sensitive and specific quantification of AAV vector DNA and transgene RNA across multiple mouse tissues. These results provided the sponsor with critical biodistribution and transcriptional data supporting the preclinical evaluation of their AAV-based therapeutic.

This study demonstrates Altasciences' capability to support **gene therapy programs** through **robust digital PCR platforms**, validated bioanalytical methods, and precise tissue processing workflows.

ABOUT ALTASCIENCES

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