AAV8 Shedding Assay to Support Gene Therapy Clinical Trials

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INTRODUCTION

In the evolving landscape of gene therapy, understanding the phenomenon of "shedding" is paramount. Shedding refers to the release of gene therapy vectors or products from the patient into the environment through various biological routes such as excreta, secreta (including urine, saliva, and nasopharyngeal fluids), or through the skin (via pustules, sores, or wounds). This process not only has implications for the direct safety and monitoring of treated individuals but also holds significance for public health and environmental safety, as it involves the potential transmission of genetically modified vectors to third parties or ecosystems. Consequently, shedding studies are a critical component of gene therapy clinical trials, aimed at evaluating the risks associated with vector dissemination and guiding safety protocols to mitigate unintended exposures.

REGULATORY CONSIDERATIONS

FDA and EMA set guidelines for shedding assays, emphasizing their specificity, sensitivity, reproducibility, and accuracy. Replicate testing of clinical samples is encouraged to confirm reproducibility. Though full validation of shedding assays is not mandatory, it is essential that they are qualified to meet basic performance criteria.

Notably, these assays are not in vitro diagnostic (IVD) tests and are not subject to IVD or IVDR regulations. Instead, they are specialized tools integral to the safety assessment in gene therapy trials, aligning with EMA's preference for quantitative PCR methods and the FDA's safety and environmental protection standards.

CONCLUSION

Advanced Assay Strategy: Implemented a novel approach in gene therapy shedding analysis for enhanced precision.
Extraction Kit Screening: Thoroughly screened and tested multiple extraction kits to identify the most reliable for use.
Innovative Spiking Method: Pioneered spiking standards and quality controls before extraction, a bioanalytical assay with improved accuracy.
Impact on Gene Therapy: The improved shedding assay protocols contribute to more robust shedding analysis, benefiting patients and therapy development.

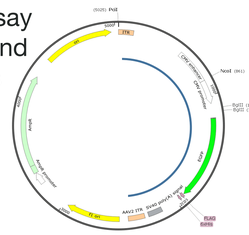
EXTRACTION KIT EVALUATION

In our comprehensive evaluation of extraction kits, we embarked on a comparative analysis designed to identify the most efficient kit for accurately detecting gene therapy vectors across various biological matrices. This comparison was meticulously conducted across a range of kits from leading manufacturers, assessing their performance in extracting nucleic acids from different sample types such as blood, plasma, saliva, urine, semen, and feces. Key performance metrics included recovery efficiency, limit of detection (LOD), and coefficient of variation (%CV), which collectively provided insight into each kit's ability to yield high-quality, quantifiable nucleic acids. The results revealed significant variability in kit performance, with certain kits showing superior efficiency and lower LODs in specific matrices. This detailed comparison not only highlighted the critical importance of selecting the appropriate extraction kit based on the sample matrix and assay requirements but also underscored the necessity for rigorous kit evaluation as an integral component of assay development in gene therapy clinical trials. Through this analysis, we were able to recommend the most suitable extraction kits for various matrices, thereby enhancing the reliability and accuracy of shedding assessments in the context of gene therapy safety evaluations.

Kit \ Matrix		Blood	Plasma	Saliva	Urine	Semen	Feces
Qiagen Blood and Tissue Kit	Recovery %CV	110% 13%CV	118% 14%CV	92% 13%CV		71% 46%CV	
	LOD (GC/µL)	2.7	1.3	1.6		4.5	
Qiagen Fast DNA Stool Mini Kit	Recovery %CV						85% 23%CV
	LOD (GC/mg)						7.3
Qiagen Viral RNA Mini Kit	Recovery %CV		19% 25%CV	52% 15%CV	37% 23%CV		
	LOD (GC/µL)		6.8	2.5	3.4		
Zymo Research Quick DNA Urine Kit	Recovery %CV				30% 48% CV		
	LOD (GC/µL)				1.1		
Zymo Research Quick DNA miniprep Plus Kit	Recovery %CV	59% 23%CV	45% 33%CV	6% 55.8%CV		28.4% 48%CV	No recovery
	LOD (GC/µL)	1.3	1.7	12.5		4.6	
Promega gDNA Extraction Kit	Recovery %CV	25% 20%CV	39% 27%CV	28% 18%CV	10% 38%CV	51% 38%CV	No recovery
	LOD (GC/µL)	6.0	3.9	5.3	15	10.2	

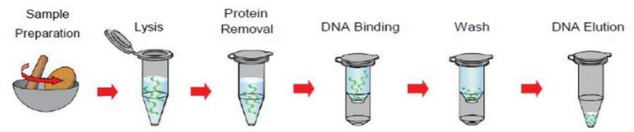
ASSAY DEVELOPMENT WORKFLOW

1. Design: Begin with designing the assay by selecting 3-5 primer/probe sets and considering 3 different master mixes (MM) for use in the quantitative PCR (qPCR) assays.



2. qPCR Optimization: Define the best primer and master mix combination. Determine the lowest limit of quantitation (LLOQ) and the limit of detection (LOD). Also, assess co-linearity between plasmid and viral DNA, which is critical for ensuring the assay can accurately quantify the virus/vector present in samples.

3. DNA Extraction: Test at least three different extraction kits for each biological matrix (such as blood, urine, or saliva). It's important to assess how well each kit recovers AAV viral particles, which is indicative of the kit's efficiency and suitability for the assay.



4. Final Optimization: Standard (STD) and quality control (QC) materials are spiked in prior to extraction to evaluate the entire process, including the recovery and detection limit within the context of the matrix. This final step is essential to confirm that the assay performs well with actual clinical samples.

Each step of this workflow is critical to developing a robust and reliable shedding assay. It's designed to ensure that by the time the assay is implemented, it has been thoroughly vetted and optimized for the best performance in detecting and quantifying the shedding of gene therapy vectors.