VAC_04 - Assessing mRNA Integrity using Capillary Electrophoresis: Insights into Scientific Parameters

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Introduction: The rise of mRNA-based vaccines through via *in vitro* transcription (IVT) has seen rapid development and widespread acceptance due to their potential in the combating of diseases. Ensuring mRNA integrity in these therapies is critical for efficacy and safety. Evaluating mRNA purity using capillary gel electrophoresis is crucial, involving a meticulous analysis of mRNA degradation and abortive mRNA species, directly impacting vaccine effectiveness and safety.

Objectives: Due to insufficient parameters in scientific literature for mRNA integrity analyses via IVT in biopharmaceutical development, our study aimed to explore two critical parameters. We investigated the impact of user-controlled variables: adjusting the minimum RFU (Relative Fluorescence Units) threshold for signal processing and selecting the 'smear analysis' function (specific areas) in capillary electrophoresis.

Methodology: We evaluated three IVT mRNAs, each about 4,000 nucleotides long, using capillary electrophoresis on the Agilent 5200 Fragment Analyzer with DNF-471 RNA kit, following the manufacturer's guidelines. Sample preparation and analysis assessed IVT mRNA size and integrity.

Results: Our analysis involved three IVT mRNA samples, with 'smear analysis' set at 10% on both sides. We tested minimum RFU values of 5, 10, 20, and 100. At 5 RFU, peak percentages were 91.1%, 88.3%, and 67.1%. With 10 RFU, the values were 91.9%, 88.7%, and 68.8%, while at 20 RFU they increased to 93.7%, 89.6%, and 71.9%. Employing 100 RFU yielded 100%, 96.8%, and 92.9%. Higher RFU and broader 'smear analysis' windows led to less stringent quality control for IVT mRNA samples.

Conclusion: The gap in standardized parameters in the current literature highlights the potential for diverse estimations of mRNA integrity in IVT samples. Addressing this is crucial for the rigorous analysis of IVT-based immunotherapeutic products. Maintaining a standardized minimum RFU value enhances the reliability and comparability of the results. Selecting a fixed peak window percentage, like 10-20% on both sides of RNA size, enables a comprehensive evaluation of mRNA integrity. Implementing these factors bolsters scientific integrity, instilling confidence in findings' accuracy and reproducibility.

Keywords: mRNA-based vaccines; mRNA integrity; Standardized parameters