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Achieving sensitivity of 0.1% variant allele frequency (VAF) in plasma and urine with the nRichDX revolution sample prep system evaluated on the MassARRAY ® system

Nafiseh Jafari[1], Mayer Saidian[1], Jason Saenz[1], Lauren Lee[1], Andrew Dunnigan[1], Carlos Hernandez[1], Jessica Mendoza[2], Darryl Irwin[2]
[1]nRichDX | [2]Agena Bioscience njafai@nrichdx.com, Darryl.Irwin@agenabio.com

INTRODUCTION

This study focuses on evaluating the effectiveness of the nRichDX Revolution Sample Prep System when used in conjunction with the MassARRAY® System. It aims to investigate how this combination can augment sensitivity levels, mainly aiming to achieve a detection limit of 0.1% Variant Allele Frequency (VAF).

Using plasma and urine samples underscores the versatility and relevance of the methodology in non-invasive or minimally invasive sampling methods, where obtaining tissue samples might be challenging or impractical. Detecting cancer variants at such low frequencies, represented by a 0.1% VAF, holds immense potential to improve clinical practice and patient outcomes.

By pushing the boundaries of sensitivity, this study explores the technical capabilities of the employed systems and addresses a critical need in cancer diagnostics. Achieving the ability to detect low-frequency variants opens new possibilities for early detection, monitoring disease progression, and tailoring treatment strategies to individual patients.

MATERIALS & METHODS

Whole Blood from healthy donors was collected in K2EDTA tubes, and plasma was isolated and then pooled together in preparation for cfDNA extraction. 5mL and 20mL plasma samples were extracted using the nRichDX Revolution Max20 cfDNA Isolation Kit and the Qiagen QIAamp Circulating Nucleic Acid Kit. All samples were spiked prior to extraction with a cfDNA Reference Standard containing KRAS p.G12V mutation at a 0.1% allele frequency (VAF) concentration. The nRichDX cfDNA extraction followed the cfDNA Isolation IFU for 5mL and 20mL samples and was eluted in 50µL. The QIAamp kit is limited by its 5mL sample input volume. To perform a 20mL sample extraction, four 5mL samples were extracted following the manufacturer's instructions and eluted in 50µL. The eluants were pooled and concentrated to 50µL using the Amicon Ultra Centrifugal filter device.

First-void urine was collected from healthy donors using the Novovsanis Colli-Pee device and pooled together in preparation for cfDNA extractions. After collection, urine was centrifuged at 16,000xg for 10 minutes at 4˚ C to obtain cf-urine. Extractions from 10mL (3) and 20mL (3) cf-urine were performed using the nRichDX cfDNA Isolation Kit and the MagMax Cell-Free DNA Isolation Kit. All samples were spiked prior to extraction with a cfDNA Reference Standard containing KRAS p.G12V mutation at a 0.1% allele frequency (VAF) concentration.

Samples extracted using the nRichDX platform followed the nRichDX Max20 cfDNA Isolation Kit IFU for 10mL and 20mL samples and then eluted in 50µL. MagMax Cell-Free DNA Isolation Kit is limited to a 10 mL sample volume. To perform a 20mL sample extraction, two 10mL samples were extracted, eluted in 50µL, pooled together, and concentrated to 50µL using the Amicon Centrifugal filter device.

The amplifiable cfDNA copies, high/low molecular weight dynamic, and overall cfDNA quantity and quality (QC) were assessed using the LiquidIQ® panel on the MassARRAY system. The recovery and frequency of the cfDNA Reference Standard were determined with the UltraSEEK™ Lung V2 Panel.

RESULTS

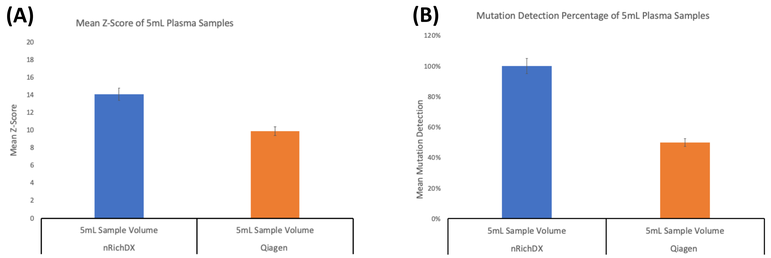

Figure 1. (A) In the plasma experiment, all 5mL plasma samples extracted with nRichDX passed the initial QC with notably higher Z-scores than Qiagen ($p<0.03$). However, the mutation at the lower variant allele frequency (VAF) of 0.1% was not detected in half of the samples extracted with Qiagen. (C) Both methods exhibited similar amplifiable copies.

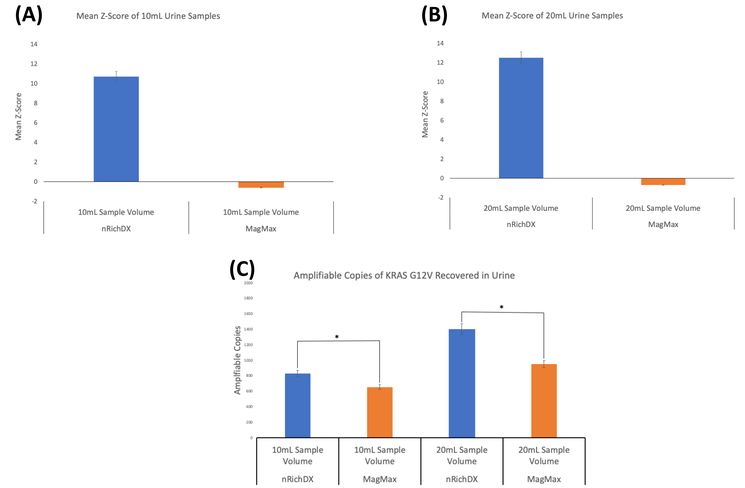

Figure 2. MagMax failed the initial QC in the urine experiment for all samples and did not recover mutations at the lower VAF compared to nRichDX. Conversely, all urine samples extracted with nRichDX passed the initial QC, with all low VAF mutations detected in every sample. (A, B) The nRichDX system had significantly higher z-scores than the MagMax isolation kit in all sample volumes. (C) Significantly more amplifiable copies were recovered by the nRichDX system in all sample volumes than in the MagMax isolation kit.

CONCLUSION

This study effectively assessed the extraction efficiency of cfDNA amplifiable copies using three distinct kits across two sample types: the nRichDX cfDNA Isolation Kit (for plasma and urine), the MagMax Cell-free DNA Isolation Kit (specifically for urine), and the Qiagen QIAmp Circulating Nucleic Acid Kit (for plasma), considering Z-scores and quality control (QC) parameters. The results indicate that the nRichDX cfDNA Isolation Kit surpasses the MagMax and Qiagen kits in extracting significantly higher amplifiable copies, particularly notable in urine samples across various volumes tested (p-value < 0.05). Notably, while the nRichDX Revolution system and Qiagen Kit met all QC parameters, the MagMax kit failed QC assessment for both urine sample volumes investigated. Furthermore, the nRichDX extraction kit exhibited significantly higher Z-scores than the Qiagen or MagMax kit across 5mL plasma samples, 10mL urine samples, and 20mL urine samples.

The powerful extraction capabilities of nRichDX's Revolution system, especially evident in handling larger sample volumes, in conjunction with Agena Bioscience's highly sensitive MassARRAY system, could substantially improve early-stage cancer detection.