Comparative Evaluation of Whole Urine and Pellet-Based Extraction Workflows for Urine Liquid Biopsy

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INTRODUCTION

Urine contains multiple analyte compartments relevant to molecular testing: cell-free nucleic acids in the supernatant, as well as cellular material (e.g., exfoliated uroepithelial cells, immune cells, tumor cells) that can contribute DNA/RNA to downstream assays. While fractionation can be useful for some applications, it introduces additional processing, opportunities for sample loss, and potential bias in analyte recovery. The nRichDX Revolution Sample Prep System enables a single extraction from whole urine that captures nucleic acids from both the cell-associated and cell-free fractions in one workflow.

MATERIALS & METHODS

Whole urine was collected and preserved (nRich RNA Preservative). For fractionation, samples were centrifuged (16,000 x g, 10 min, 4°C), supernatant was transferred, and the remaining pellet was washed/resuspended (PBS) before extraction. For the DNA comparison, whole-urine single extraction was compared to separate extraction of the cell pellet and the corresponding supernatant, followed by comparison to the paired sum of pellet + supernatant yields. For the RNA study, urine was spiked with cells (target concentration 10,000 cells/mL) and an RNA reference standard (10 ng/mL). Extractions were performed using nRichDX Revolution IFU. All conditions were processed under comparable extraction parameters as recorded in the run sheets. Total nucleic acid yield was quantified by Qubit fluorometry (DNA or RNA as indicated). RT-qPCR was performed for KRAS G12V using a spiked-in standard input.

RESULTS

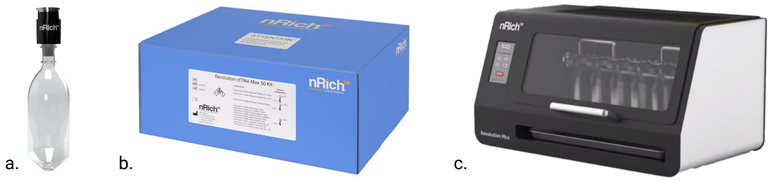
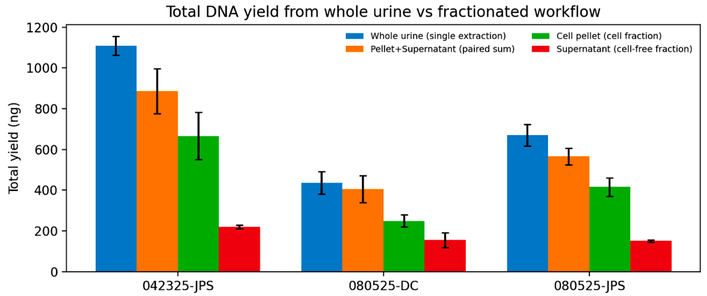
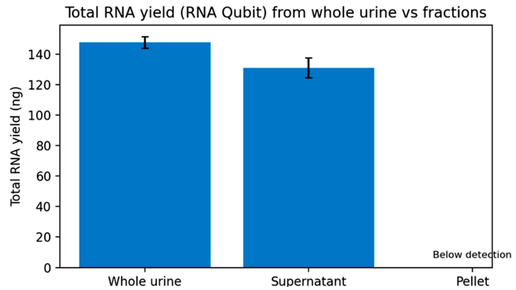


Figure 1. The nRichDX Revolution Sample Prep System. Comprising (a) the unique nRicher Cartridges included in Revolution Kits, (b) Revolution Kits (Revolution cfTNA Max 50 Kit shown), and (c) the Revolution Plus Processor.



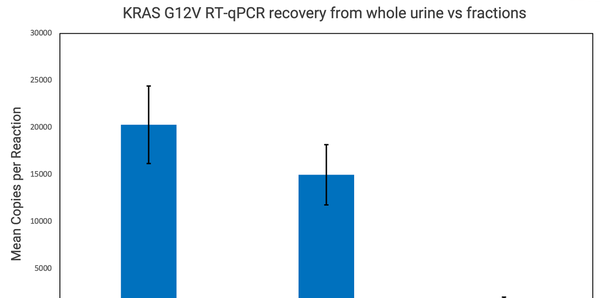
Run	Whole urine (single)	Pellet + Supernatant (sum)	Pellet only	Supernatant only	Whole vs sum
042325-JPS	1108.8 ± 46.6	886.2 ± 110.0	666.2 ± 116.0	220.0 ± 9.5	25.1%
080525-DC	435.7 ± 55.5	404.5 ± 66.4	249.3 ± 30.1	155.2 ± 36.7	7.7%
080525-JPS	670.0 ± 52.9	565.5 ± 40.8	415.2 ± 45.7	150.3 ± 6.1	18.5%

Figure 2. Whole-urine single extractions increases total DNA yield vs. fractionated workflow. Qubit fluorometric quantification of total DNA from a whole-urine single extraction versus fractionated pellet and supernatant extractions across three independent runs. The paired-sum bar represents elementwise pellet + supernatant sums from matched replicate sets.



Condition	Total RNA yield (ng)	Replicates
Whole urine (single extraction)	147.8 ± 3.8	4
Supernatant (cell-free fraction)	131.0 ± 6.5	4
Cell pellet (cell fraction)	Below detection (RNA Qubit)	4

Figure 3. Whole-urine workflow preserves RNA yield relative to supernatant-only extraction. Qubit fluorometric quantification of total RNA from a whole-urine single extraction versus supernatant-only and cell pellet samples. Pellet-only RNA was below Qubit detection under these conditions.



Condition	KRAS Ct	Mean Copies per Reaction	Replicates
Whole urine (single extraction)	26.92 ± 0.27	20,304 ± 4,114	4
Supernatant (cell-free fraction)	27.40 ± 0.33	14,985 ± 3,205	4
Cell pellet (cell fraction)	30.65 ± 0.39	1,582 ± 397	4

Figure 4. Improved KRAS G12V analytical recovery from whole urine (cell + supernatant). RT-qPCR of a spiked-in KRAS G12V standard showed improved analytical performance when extracting from whole urine (cell + supernatant) compared to either fraction alone.

CONCLUSION

The nRichDX Revolution Sample Prep System enables a streamlined whole urine, single-extraction workflow that captures nucleic acids from both the cellular and cell-free fractions without fractionation, reducing hands-on steps, transfers, and opportunities for loss or variability. Across multiple runs, whole-urine single extractions produced 7.7% – 25.1% higher mean DNA yield than the paired sum of pellet and supernatant extractions. Whole urine also preserved RNA yield and improved KRAS G12V RT-qPCR recovery relative to fraction-only workflows. Beyond performance, the consolidated workflow improves operational consistency (fewer manual decision points), supports scalable processing across input volumes, and aligns with high-throughput liquid biopsy operations by simplifying sample processing while maintaining analytical sensitivity.

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