Automated high-volume cfDNA extraction on Hamilton STAR achieves analytical equivalence to manual nRichDX Revolution® protocol while enabling 96-sample batch processing

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INTRODUCTION

Scaling liquid-biopsy studies requires workflows that preserve the sensitivity of high-volume cfDNA extraction without the labor and variability of manual processing. We implemented the nRichDX Revolution® kit on a Hamilton STAR liquid-handling platform and assessed analytical concordance, operational gains relative to the bench SOP, and cross-contamination.

MATERIALS & METHODS

Banked human plasma and urine (inputs 1–20 mL) were extracted in matched pairs either manually (nRichDX cfDNA Isolation Kit IFU) or on Hamilton STAR using identical chemistries and volumes; the only variable was automation of wash/elution steps. Replicates were run across ≥2 reagent lots and ≥3 days. Primary endpoints were (i)fragment-quality metrics from Agilent cfDNA ScreenTape (50–700 bp region; modal size, mono/di-nucleosome peak ratio, DNA Integrity Number/analog), and (ii) downstream assay performance by locus-specific qPCR/ddPCR (oncology targets/spike-in controls). Statistics included paired comparisons, Bland–Altman bias/limits of agreement, and variance components to estimate within/between-run CV.

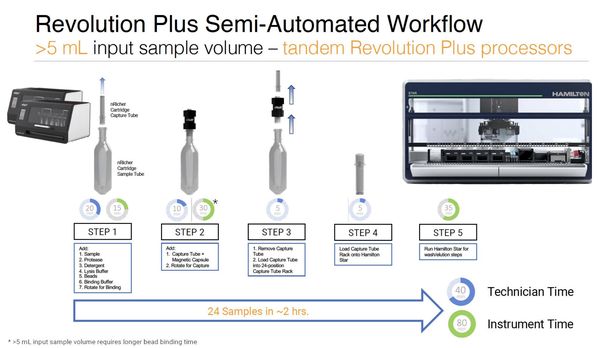
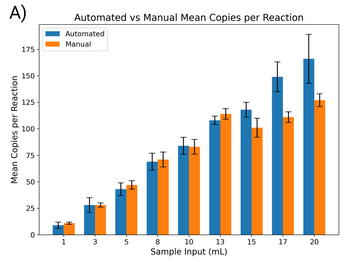


Figure 1. nRichDX Revolution cfDNA Max 20 Kit on Hamilton STAR workflow.

RESULTS



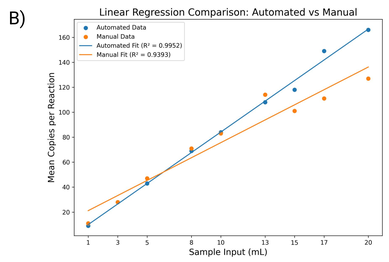


Figure 2. Automated vs Manual Copies per Reaction via PCR. **A)** Mean copies per reaction by sample input volume. **B)** Hamilton Automation vs Manual, Linear Regression. Both A and B show similar performance through 13 mL of sample input, once sample input goes beyond 13 mL, automation continues increasing recovered copies per reaction while manual has a slight decrease before increasing slightly at the 17 mL and 20 mL sample inputs.

	1	2	3	4	5	6
A	Spiked / Positive	Non-Spiked / Negative	Spiked / Positive	Non-Spiked / Negative	Spiked/ Positive	Non-Spiked / Negative
B	Non-Spiked / Negative	Spiked / Positive	Non-Spiked / Negative	Spiked / Positive	Non-Spiked / Negative	Spiked / Positive
C	Spiked / Positive	Non-Spiked / Negative	Spiked / Positive	Non-Spiked / Negative	Spiked / Positive	Non-Spiked / Negative
D	Non-Spiked / Negative	Spiked / Positive	Non-Spiked / Negative	Spiked / Positive	Non-Spiked / Negative	Spiked / Positive

Figure 3. Automation Checkerboard Plate map during the automated wash and elution steps on the Hamilton STAR liquid-handling platform. Two plates with this setup were run simultaneously (n = 48). Results for samples by position. Both sets of 24 samples had identical results with zero cross-contamination observed—all spiked samples were positive via PCR, while all non-spiked samples remained negative.

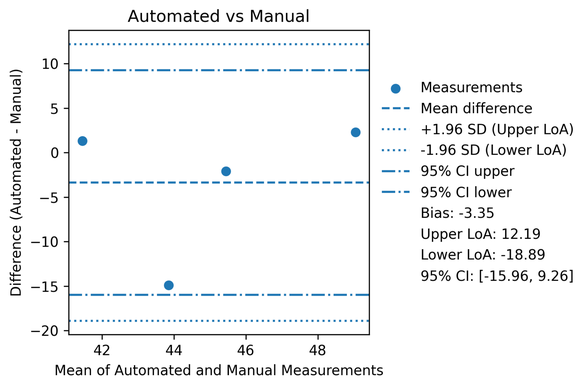


Figure 2. Automated vs Manual Bland-Altman Analysis. The mean difference (bias) between the two methods was −3.35. The 95% limits of agreement ranged from −18.89 to 12.19, indicating the interval within which 95% of differences between the two methods are expected to lie. The dashed line represents the mean difference, and the dotted lines represent the limits of agreement.

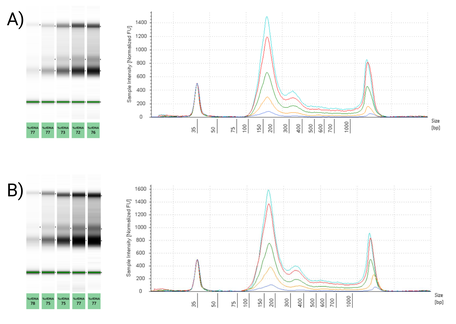


Figure 4. Automated vs Manual %cfDNA via Tape Station. For both A and B, sample volume inputs left to right: 1 mL (blue), 5 mL (orange), 10 mL (green), 15 mL (red), 20 mL (light blue). **A)** %cfDNA results for automated wash/elution steps samples. **B)** %cfDNA results for manually processed samples.

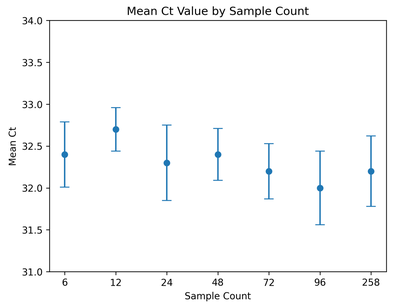


Figure 5. Variation At Each Sample Count, 5 mL sample inputs. Average Ct value from each individual extraction on the Hamilton by sample count. Sample counts 6, 12, 24, 48, 72, and 96 represent individual runs and their respective variation within the run; the sample count of 258 is all replicates across each of the runs (6, 12, 24, 48, 72, 96) which represents variation between runs.

CONCLUSION

Implementing the nRichDX Revolution cfDNA kit on Hamilton STAR preserves yield, fragment integrity, and variant-assay concordance relative to the manual IFU, while unlocking true 96-sample throughput and substantial reductions in hands-on time and overall turnaround. This data supports the adoption of automation to scale cfDNA programs for larger cohorts and more consistent day-to-day operations without compromising analytical performance.

AACR American Association for Cancer Research®