Colli-Pee® UAS™ Combined with nRichDX Revolution System™, a Promising Urinary Cell-free DNA Collection, Preservation and Extraction Workflow

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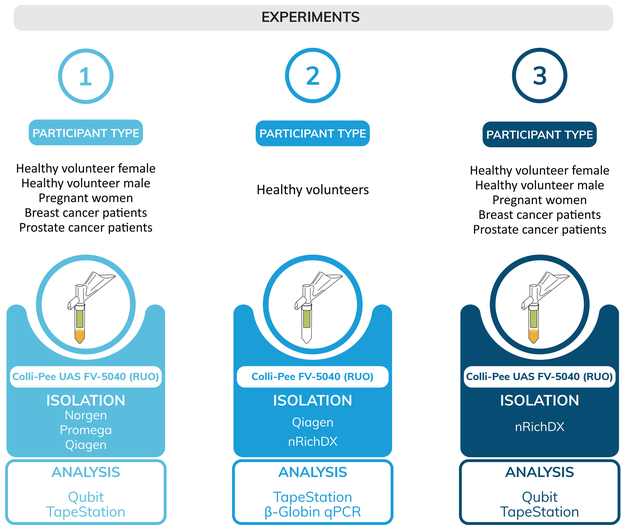
INTRODUCTION

Urine as liquid biopsy for non-invasive cancer diagnostics is gaining interest. Standardization of sample collection, storage, and processing are critical enablers driving the clinical utility of urine for cancer research, detection, screening, or treatment monitoring. Here, we investigated a first-void urine collection Colli-Pee® device and a urine preservative UAS™ from Novosanis, combined with a cell-free DNA (cfDNA) extraction platform, Revolution System™ from nRichDX, as a seamless workflow for urine sample collection, handling, and processing.

MATERIALS AND METHODS

First, three commercial cfDNA extraction kits: QIAamp Circulating Nucleic Acid kit (Qiagen), Urine Cell-Free Circulating DNA Purification Maxi kit (Norgen), and Custom Maxwell RSC ccfDNA kit (Promega), input volumes ranging from 4 mL for Promega and Qiagen to 12 mL for Norgen, were compared using samples collected with the Colli-Pee® UAS™ FV-5040 from healthy volunteers (n= 27) and cancer patients (n=10). Secondly, the Qiagen kit (4 mL) and the Revolution System™ (20 mL) were compared by using unpreserved and UAS™ preserved urine samples held at room temperature (RT) for 7 days. Both unpreserved and preserved urine samples were investigated under freeze-thaw cycling conditions to evaluate batch processing compatibility with the Revolution System™. Finally, the Revolution System™ was used to extract cfDNA from samples of healthy volunteers (n=13) and cancer patients (n=7), input volume of 20 mL. The performance was measured using Qubit dsDNA HS assay (ThermoFisher), cfDNA ScreenTape (Agilent), HS D5000 ScreenTape (Agilent), and β-globin qPCR assay. See schematic overview below.



RESULTS

Comparison of Norgen, Promega and, Qiagen extraction kits on urine samples from a clinical setting

All three extraction kits performed comparable on urine samples from a clinical setting. However, the Qiagen kit showed a higher DNA concentration than the Norgen and Promega kits.

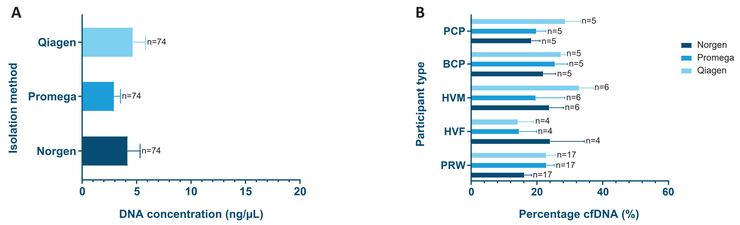


Figure 1: Comparison of Norgen, Promega and, Qiagen extraction kits on urine samples from clinical setting.
Qubit DNA analysis (1A) and TapeStation cell-free DNA (cfDNA) analysis (1B) of samples from different participant types after extraction using the Norgen, Promega and, Qiagen extraction kits. All results are depicted as mean ± SEM. Abbreviations: BCP, breast cancer patients; cfDNA, cell-free DNA; HVF, healthy female volunteers; HVM, healthy male volunteers; n, sample size; PCP, prostate cancer patients; PRW, pregnant women.

Scientific comparison Revolution System™ with Qiagen on urine samples from healthy volunteers

Unpreserved samples showed degradation of cfDNA and loss of cfDNA profiles *(data not shown)* while no significant difference was observed in UAS™ preserved urine samples under both RT storage and freeze-thaw cycling *(data not shown)* conditions. Additionally, a slight difference in Ct values is demonstrated between Qiagen and nRichDX, showing that both methods performed almost equivalent. The Revolution System™ showed high cfDNA yield and sensitivity.

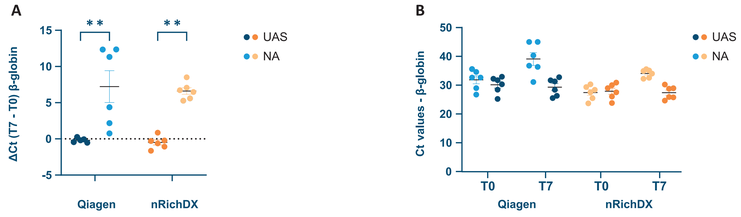


Figure 2: Comparison of Revolution System™ and Qiagen extraction kit on urine samples from healthy volunteers.
Comparison of the Revolution System™ and Qiagen extraction kit for UAS™-preserved and unpreserved urine samples after 7 days at room temperature (20-26°C) using β-globin qPCR analysis (2A & 2B). The results are depicted as ΔCt values (2A) and Ct values (2B). The experiment was performed with n=6 and the results are depicted as mean ± SEM. Abbreviations: n, sample size; NA, no chemistry addition; T0, baseline; T7, 7 days; UAS, addition of UAS™ preservative.

Suitability of Revolution System™ on urine samples from clinical setting

The nRichDX Revolution System™ showed an average DNA concentration (Figure 3A) and cfDNA percentage (Figure 3B) of 13.30 ng/µL and 23.22%, respectively, across all samples of different participant types, with the highest percentage for healthy female volunteers (39.90%) and lowest percentage for pregnant women (8.52%). Both Qubit analysis and TapeStation analysis showed the clear suitability of the Revolution System™ on urine samples from clinical setting.

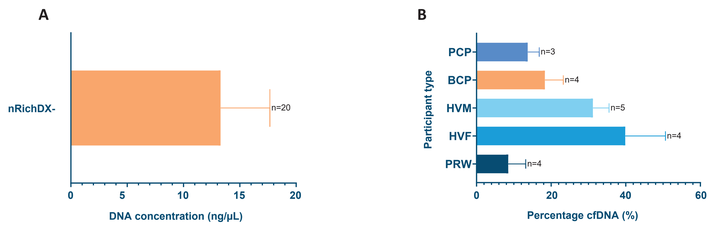


Figure 3: Suitability of Revolution System™ on urine samples from clinical setting.
Qubit DNA analysis (3A) and TapeStation cell-free DNA analysis (3B) of samples from different participant types after extraction using the Revolution System™ (nRichDX). All results are depicted as mean ± SEM. Abbreviations: BCP, breast cancer patients; cfDNA, cell-free DNA; HVF, healthy female volunteers; HVM, healthy male volunteers; n, sample size; PCP, prostate cancer patients; PRW, pregnant women.

Qualitative comparison of the extraction kits

Each extraction workflow requires different steps whereby Qiagen incurs the most steps and transfers, followed by the Promega kit. The Revolution System™ and Norgen kit comprised of the lowest number of steps. The Revolution System™ was also least prone to manual error.

Table 1: Qualitative comparison of the extraction kits.
All four extraction methods are compared based on possible input volume in milimeters (mL), number of protocol steps for the extraction of a 20 mL urine sample, run time for the extraction of a 20 mL urine sample with the incubation times between brackets.

Aim	cfDNA extraction from 1 sample of 20 mL urine			
Parameter	nRichDX	Norgen	Promega	Qiagen
Urine volume possible	20 mL	20 mL	5x4 mL	5x4 mL
Protocol steps	12	12	5x6 = 30	5x17 = 85
Total run time (min)	180	60	80	120
Hands-on time (min)	30	25	30	55

CONCLUSION

The nRichDX Revolution extraction platform enables processing of large urine volumes leading to an increased cfDNA yield and sensitivity. Additionally, UAS™ preserved samples showed compatibility with all available tested cfDNA extraction kits or platforms, demonstrating the agnostic nature of the UAS™ chemistry. Overall, the innovative Colli-Pee® UAS™ FV-5040 (RUO), urine collection and analyte preservation device, combined with the nRichDX Revolution System™, offers an improved workflow for investigating urinary biomarkers.

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