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Optimizing extraction of microbial DNA from urine: Advancing urinary microbiome research in bladder cancer

Chuang-Ming Zheng[1], Ho Won Kang[2,3], Seongmin Moon[3,4], Young Joon Byun[2], Won Tae Kim[2,3], Yung Hyun Choi[5], Sung-Kwon Moon[6], Xuan-Mei Piao[2], Seok Joong Yun[2,3]

[1]Key Laboratory of Cellular Function and Pharmacology of Jilin Province, Yanbian University, Yanji, China, [2]Department of Urology, Chungbuk National University College of Medicine, Cheongju, [3]Department of Urology, Chungbuk National University Hospital, Cheongju, [4]Department of Convergence of Medical Science, Chungbuk National University College of Medicine, Cheongju, [5]Department of Biochemistry, Dong-Eui University College of Oriental Medicine, Busan, [6]Department of Food Science and Technology, Chung-Ang University, Anseong, Korea

Purpose: This study aimed to evaluate and optimize microbial DNA extraction methods from urine, a non-invasive sample source, to enhance DNA quality, purity, and reliability for urinary microbiome research and biomarker discovery in bladder cancer.

Materials and Methods: A total of 302 individuals (258 with genitourinary cancers and 44 with benign urologic diseases) participated in this study. Urine samples were collected via sterile catheterization, resulting in 445 vials for microbial analysis. DNA extraction was performed using three protocols: the standard protocol (SP), water dilution protocol (WDP), and chelation-assisted protocol (CAP). DNA quality (concentration, purity, and contamination levels) was assessed using NanoDrop spectrophotometry. Microbial analysis was conducted on 138 samples (108 cancerous and 30 benign) using 16S rRNA sequencing. Prior to sequencing on the Illumina MiSeq platform, Victor 3 fluorometry was used for validation.

Results: WDP outperformed other methods, achieving significantly higher 260/280 and 260/230 ratios, indicating superior DNA purity and reduced contamination, while maintaining reliable DNA yields. CAP was excluded due to poor performance across all metrics. Microbial abundance was significantly higher in WDP-extracted samples ($p<0.0001$), whereas SP demonstrated higher alpha diversity indices ($p<0.01$), likely due to improved detection of low-abundance taxa. Beta diversity analysis showed no significant compositional differences between SP and WDP ($p=1.0$), supporting the reliability of WDP for microbiome research.

Conclusions: WDP is a highly effective and reliable method for microbial DNA extraction from urine, ensuring high-quality and reproducible results. Future research should address sample variability and crystal precipitation to further refine microbiome-based diagnostics and therapeutics.

Keywords: Metagenome; Methodological study; Urinary bladder neoplasms; Urine

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Corresponding Author: Seok Joong Yun https://orcid.org/0000-0001-7737-4746
Department of Urology, Chungbuk National University Hospital, Chungbuk National University College of Medicine, 776 1sunhwan-ro, Seowon-gu, Cheongju 28644, Korea
TEL: +82-43-269-6371, E-mail: sjyun@chungbuk.ac.kr
Xuan-Mei Piao https://orcid.org/0000-0001-6847-7743
Department of Urology, Chungbuk National University College of Medicine, 1 Chungdae-ro, Seowon-gu, Cheongju 28644, Korea
Tel: +82-43-269-6143, E-mail: phm1013@hotmail.com

INTRODUCTION

The microbiome comprises the collective genomes of microbial communities residing in specific environments [1]. Recent technological advancements have highlighted the impact of the human microbiome on health and disease [2-4]. Since the initiation of the Human Microbiome Project, numerous studies have demonstrated the microbiome's role in tumorigenesis and cancer progression [3-7]. Bladder cancer (BCa) is a significant public health concern, imposing considerable socio-economic burdens. Despite extensive research, its etiology remains poorly understood [8,9]. Because urine is in continuous contact with bladder tissue, it contains exfoliated cells, DNA, RNA, and proteins, making it an invaluable source for non-invasive biomarker discovery [10]. Compared to invasive techniques like cystoscopy, urine-based diagnostics offer a convenient, patient-friendly alternative with potential applications in disease monitoring and early detection [11].

Historically, urine was considered sterile [12,13]; however, modern sequencing technologies have identified bacterial communities within it [9,14,15]. The growing recognition of the urinary microbiome's role in health and disease [16-18] suggests that it may serve as a non-invasive biomarker for BCa detection and progression monitoring [19].

However, urinary microbiome research faces challenges due to inconsistencies in DNA extraction protocols [20,21]. The typically low bacterial concentration in urine, coupled with the presence of polymerase chain reaction (PCR) inhibitors such as urea, β-human chorionic gonadotropin, and various urinary crystals, hinders bacterial DNA recovery [22]. These inhibitors, influenced by factors such as pH and temperature, affect DNA yield and quality [23-29]. Saetun et al. [29] reported that calcium oxalate and larger amorphous crystals frequently form in frozen urine samples and that the addition of ethylenediaminetetraacetic acid (EDTA) can reduce crystal size by 25%. Comparative studies on crystal dissolution in EDTA, saline, and Dulbecco's phosphate-buffered saline have identified EDTA as the most effective agent [28]. Pre-treating urine samples with EDTA before DNA extraction raises the pH and reduces the concentration of inhibitory substances, providing a cost-effective, high-throughput approach for enhancing bacterial DNA recovery in diagnostic and molecular applications. However, this method does not fully resolve the challenges of low DNA yield and PCR inhibition in all samples [28].

Thus, optimizing DNA extraction from urine is crucial for improving microbiome-based research outcomes. This study compared traditional extraction methods with water dilution and Tris-EDTA-based approaches to identify an optimized strategy for obtaining high-quality microbial DNA suitable for BCa-related microbiome studies.

MATERIALS AND METHODS

1. Evaluation of DNA extraction approaches

DNA extraction was performed using the Quick-DNA™ Urine Kit (Zymo Research). To optimize the extraction process for microbiome analysis, three distinct protocols were tested, each using 6 mL of urine:

1) Standard protocol (SP)

Urine samples were processed directly according to the manufacturer's guidelines. This protocol involved conditioning the urine with Urine Conditioning Buffer to prevent DNA degradation and bacterial overgrowth at room temperature. The conditioned samples were then precipitated and centrifuged to collect the DNA pellet, which was resuspended in Genomic Lysis Buffer, treated with proteinase K, and incubated to digest proteins and enhance DNA purity. The resulting lysate was transferred to a spin column, where the DNA bound to the column matrix. After washing with specific buffers to remove contaminants, the purified DNA was eluted.

2) Water dilution protocol (WDP)

Urine samples were pre-diluted with 4 mL of Ultra-Pure™ Distilled Water (Thermo Scientific) prior to addition of Urine Conditioning Buffer. This protocol aims to increase DNA recovery by increasing the overall sample volume.

3) Chelation-assisted protocol (CAP)

Urine samples were pre-treated with 4 mL of Tris-EDTA Buffer, pH 9.0 (Biosesang) to dissolve urinary crystals and improve DNA yield. Following EDTA treatment, Urine Conditioning Buffer was added according to the manufacturer's instructions.

To ensure consistency in sample handling, DNA was extracted from all samples using these protocols under standardized conditions. After extraction, the DNA was stored at -80°C, and its concentration and purity were assessed using a NanoDrop One spectrophotometer (Thermo Scientific). Importantly, the thawing time for all frozen urine samples was standardized across the study to minimize variability and to ensure uniform conditions for each specimen. This approach helped to reduce potential bias associated with sample processing.

2. Statistical analysis and software tools

Continuous variables are expressed as mean±standard deviation. Data normality was assessed using the one-sample Kolmogorov–Smirnov test. The Mann–Whitney U test was used to compare DNA quantity and quality indices across different extraction protocols. The analyzed metrics included DNA concentration (ng/μL), 260/280 ratio, and 260/230 ratio, which were visualized using Python v3.11.8 (Python Software) with the Pandas, Matplotlib, and Seaborn libraries, as well as GraphPad Prism 8 (GraphPad Software).

All statistical analyses for 16S rRNA sequencing data were conducted using Python (scipy, statsmodels, seaborn) and R (vegan, DESeq2, ggplot2). Group comparisons were performed using the Mann–Whitney U test, followed by the Shapiro–Wilk normality test.

For alpha diversity analysis, the Shannon index, Simpson index, and Chao1 index were calculated to evaluate microbial richness, dominance, and evenness. Beta diversity analysis was conducted to assess microbial compositional differences between SP and WDP. Principal component analysis was used to visualize microbial community variations, while Bray-Curtis multidimensional scaling was applied to assess compositional dissimilarities.

To determine statistically significant differences in microbial community composition, PERMANOVA (permutational multivariate analysis of variance) was performed using the Adonis test. Results were considered statistically significant at $p<0.05$, with significance thresholds reported as follows: *$p<0.05$, **$p<0.01$, ***$p<0.001$, and ****$p<0.0001$.

Further details on materials and methods can be found in the online-only supplementary files (Supplementary Materials and Methods, Supplementary Fig. 1).

3. Ethics statement

The Ethics Committee of Chungbuk National University Hospital approved the protocol, and written informed consent was obtained from each subject. The collection and analysis of all samples were approved by the Institutional Review Board (IRB) of Chungbuk National University Hospital (IRB approval number: 2020-04-011-003), and informed consent was obtained from each subject.

RESULTS

1. Evaluation of the three different protocols used for microbial DNA extraction

The effectiveness of SP, WDP, and CAP was evaluated by measuring DNA concentration, purity (260/280 ratio), and contamination levels (260/230 ratio) (Table 1). To ensure robust comparison, 24 urine samples from eight individuals were processed using all three protocols. WDP demonstrated consistent performance, with a mean DNA concentration of 78.34±173.95 ng/μL, which was lower than SP (mean, 175.73±331.75 ng/μL), but with significantly less variability. CAP yielded the lowest concentration (mean, 62.89±145.85 ng/μL). Regarding DNA purity, WDP had the highest 260/280 ratio (1.53±0.32), indicating minimal protein or phenol contamination. In contrast, SP (1.28±0.54) and CAP (1.37±0.53) produced DNA of lower purity. Contamination levels, as indicated by the 260/230 ratio, were also lowest with WDP (1.87±1.57), outperforming both SP (1.36±0.64) and CAP (1.16±0.93). Due to its poor performance, CAP was excluded

Table 1. Quantity and quality of microbial DNA extracted from urine specimen using the three different methods

Sample	Standard protocol			Water dilution protocol			Chelation-assisted protocol		
	Conc. (ng/μL)	260/280	260/230	Conc. (ng/μL)	260/280	260/230	Conc. (ng/μL)	260/280	260/230
1	479.6	1.89	2.4	505.4	1.89	2.35	422.4	1.86	1.81
2	887.1	0.34	0.18	2.6	1.01	0.75	39.6	0.35	0.34
3	2	0.89	1.09	2.1	1.2	5.5	4.2	1.23	0.65
4	3.4	1.33	1.24	16.9	1.83	1.9	4.9	1.15	0.06
5	9.3	1.35	1.15	9.9	1.41	1.21	3	1.06	0.58
6	3.9	1.66	1.61	16.4	1.67	1.22	5.8	1.9	1.47
7	1.5	0.89	1.35	4.2	1.4	0.75	1.7	1.66	2.89
8	19	1.87	1.85	69.2	1.82	1.25	21.5	1.73	1.49
Minimum	1.50	0.34	0.18	2.10	1.01	0.75	1.70	0.35	0.06
Maximum	887.10	1.89	2.40	505.40	1.89	5.50	422.40	1.90	2.89
Mean	175.73	1.28	1.36	78.34	1.53	1.87	62.89	1.37	1.16
SD	331.75	0.54	0.64	173.95	0.32	1.57	145.85	0.53	0.93

Conc., concentration; SD, standard deviation.

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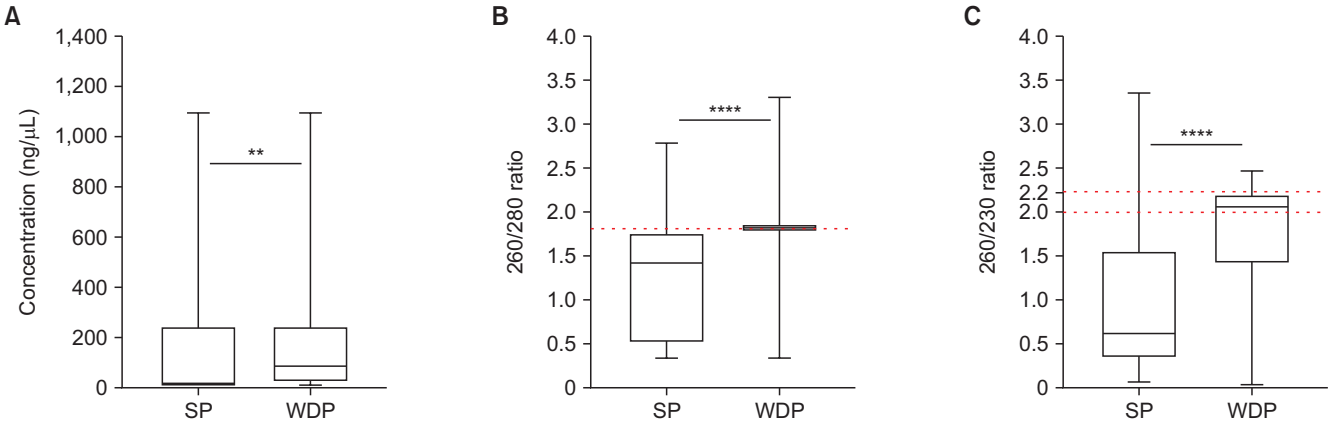

Fig. 1. Comparison of DNA concentration, 260/280 ratios, and 260/230 ratios after performing the standard protocol (SP) and water dilution protocol (WDP). (A) There is marked variability in the concentration of DNA (ng/μL) after the SP, whereas the concentration obtained using the WDP is more stable. (B) The 260/280 ratios indicate that the DNA purity yielded by the WDP is greater than that yielded by the SP. (C) The 260/230 ratios show that contamination levels after the WDP are lower than after the SP. **p<0.01, ****p<0.0001.

from further analysis. While SP produced higher DNA concentrations, WDP provided better quality and purity with more consistent results. To validate these findings, a larger sample set (n=197) was subsequently analyzed.

2. Comparison of the WDP and SP using a larger sample set

Among 197 urine specimens, 96 were processed using the SP and 101 using the WDP (Supplementary Table 1). As shown in Fig. 1, WDP consistently outperformed SP in terms of DNA purity and contamination levels. Although WDP yielded a slightly lower mean DNA concentration, it was significantly more consistent and reliable (p<0.01). Regarding DNA purity, WDP achieved significantly higher 260/280 ratios (p<0.0001), consistently close to the ideal value (~1.8), indicating minimal protein contamination. In contrast, SP exhibited a wider range of 260/280 ratios, with many samples falling below the acceptable threshold, suggesting notable protein impurities. WDP also excelled in reducing contamination, as evidenced by 260/230 ratios within the optimal range (2.0–2.2). Meanwhile, SP had significantly lower values (p<0.0001), indicating higher contamination levels from substances such as buffer residues or phenol. These results highlight WDP's superior performance, making it the preferred method for microbial DNA extraction due to its consistent quality and lower contamination levels compared to SP.

3. Comparative analysis of the WDP and SP in matched specimens

To directly compare WDP and SP, 50 matched urine samples from 25 individuals were analyzed. WDP demonstrated greater consistency across samples than SP (Fig. 2). While SP yielded a higher average DNA concentration (157.5±270.3 ng/μL) than WDP (75.7±146.4 ng/μL), its results were highly variable due to extreme outliers. In contrast, WDP produced more uniform DNA concentrations, ensuring reliable recovery. DNA purity, measured by the 260/280 ratio, was significantly higher in WDP (1.74±0.32) compared to SP (1.37±0.48), indicating fewer protein contaminants. Contamination levels, assessed via the 260/230 ratio, also favored WDP (1.53±1.06) over SP (1.28±0.73), with SP frequently falling below the acceptable range (2.0–2.2), suggesting the presence of organic contaminants. Overall, while SP sometimes produced higher DNA concentrations, its inconsistency and lower purity make it less reliable. WDP provides more consistent, high-quality DNA, making it the superior choice for microbial DNA extraction from urine samples.

4. Variability in DNA yield and reproducibility of extraction methods

To assess DNA extraction consistency, we analyzed 27 urine samples from 9 BCa patients using SP and WDP. Repeated extractions with SP (SP-1, SP-2, SP-3) showed stable DNA concentration, 260/280, and 260/230 ratios, indicating no major protocol-induced variability (Supplementary Fig. 2, Supplementary Table 2). However, DNA purity (260/280 ratios) was consistently higher with WDP, while SP showed greater variability, with many samples falling below the acceptable range (~1.8), suggesting protein contamination (Supplementary Fig. 2, middle panel). Contamination levels (260/230 ratios) were significantly lower for SP than WDP, with many SP samples failing to meet the ideal range (2.0–2.2), indicating residual contaminants (Supplementary Fig. 2,

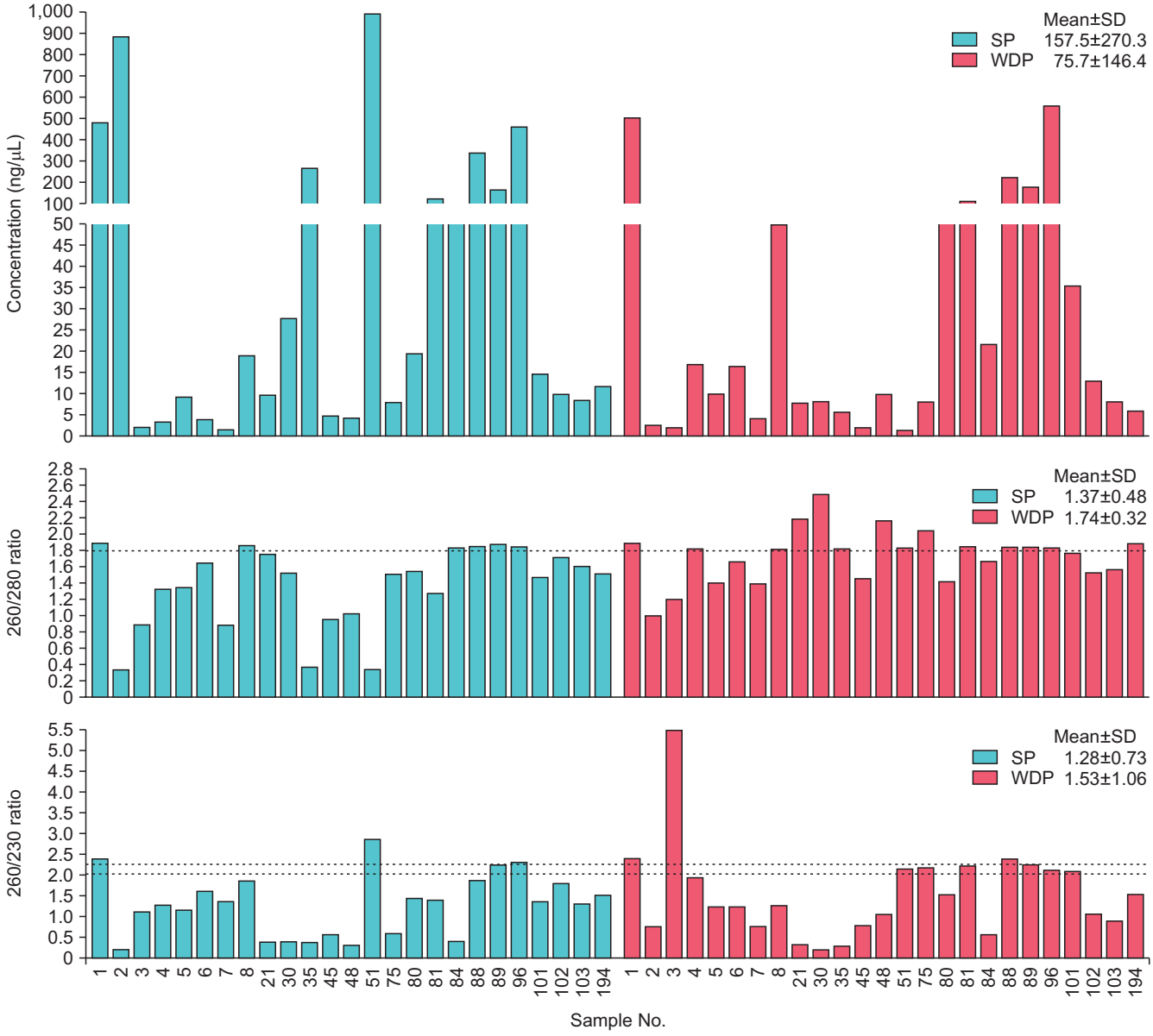

Fig. 2. Comparison of DNA extraction efficiency between standard protocol (SP) and water dilution protocol (WDP) in matched urine specimens. Bar graphs represent DNA concentration (ng/μL) (top), 260/280 purity ratio (middle), and 260/230 contamination ratio (bottom) for urine samples extracted using SP (blue) and WDP (red). DNA concentration: SP exhibited higher mean DNA yield (157.5±270.3 ng/μL) compared to WDP (75.7±146.4 ng/μL), but with substantial variability and extreme outliers. 260/280 ratio: WDP achieved significantly higher mean purity (1.74±0.32) compared to SP (1.37±0.48), with WDP values consistently closer to the optimal range (~1.8), indicating reduced protein contamination. 260/230 ratio: WDP showed lower contamination levels (1.53±1.06) compared to SP (1.28±0.73), with more samples approaching the acceptable range of 2.0–2.2, suggesting reduced buffer and organic compound interference. SD, standard deviation.

bottom panel). Additionally, repeated SP extractions showed high variation in contamination levels, raising concerns about reproducibility. In contrast, repeated WDP extractions (WDP-1, WDP-2, WDP-3) demonstrated strong consistency in concentration and purity, with values remaining within optimal thresholds (Supplementary Fig. 3, Supplementary Table 3). Overall, these results confirm that WDP is the more reliable method, offering consistent and high-quality DNA extractions, making it the preferred choice for microbial DNA analysis in urine samples.

5. Microbial community variability and DNA extraction efficiency

Quality control assessments before 16S rRNA sequencing showed that DNA concentration was significantly higher in WDP than SP ($p<0.0001$) (Fig. 3A), indicating superior extraction efficiency. DNA concentration remained consistently higher in WDP across both control and BCa samples (all

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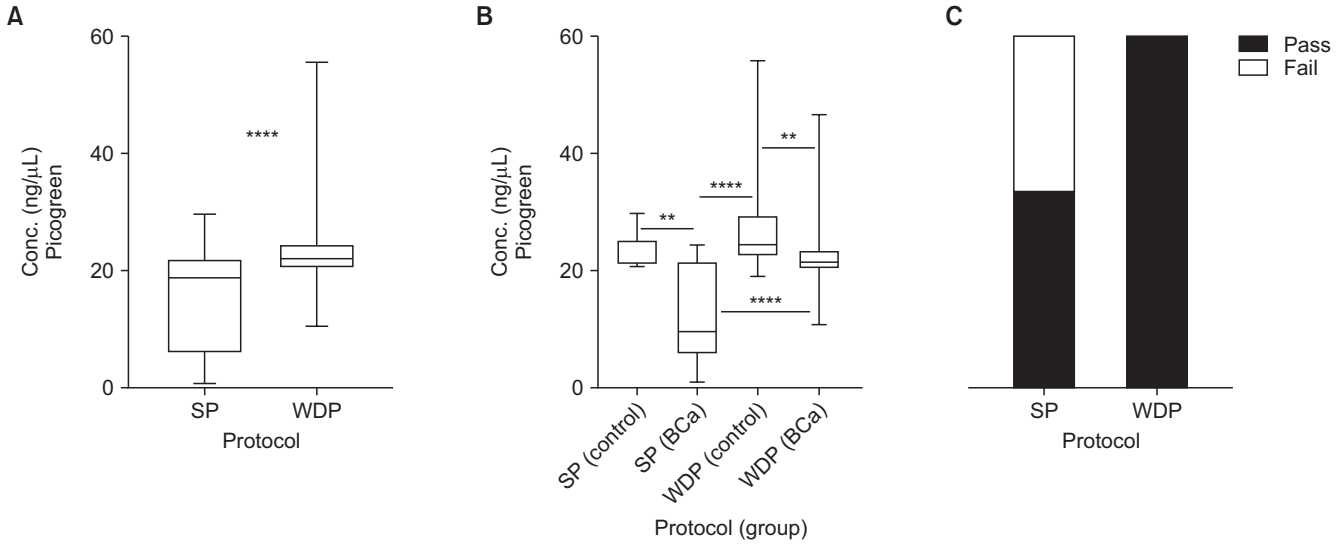

Fig. 3. Quality control assessment before 16S rRNA sequencing. (A) DNA concentration (ng/μL) comparison between standard protocol (SP) and water dilution protocol (WDP). WDP yielded significantly higher Picogreen DNA concentrations than SP, indicating superior extraction efficiency. (B) Subgroup analysis of DNA concentration in control and bladder cancer (BCa) samples. WDP consistently produced higher DNA concentrations than SP in both control and BCa groups, suggesting that extraction efficiency was primarily method-dependent rather than sample-type dependent. (C) Pass rate based on the 10 ng/μL threshold for sequencing. WDP exhibited a higher pass rate than SP, demonstrating greater reproducibility and reliability for microbial DNA extraction. **p<0.01, ****p<0.0001.

p<0.01) (Fig. 3B), confirming that extraction method, rather than sample type, was the key factor. Additionally, WDP had a higher pass rate based on the 10 ng/μL threshold (Fig. 3C), reinforcing its reproducibility. Microbial analysis revealed that total microbial abundance and $\log_2$ fold change were significantly higher with WDP than SP (p<0.0001) (Fig. 4A), indicating that WDP extracts more microbial DNA overall. While SP shows more consistent $\log_2$ fold change values, it may fail to capture key bacterial groups. Alpha diversity indices, including the Shannon index (richness/evenness), Simpson index (species dominance), and Chao1 index (species richness), were all higher in SP (p<0.01 respectively) (Fig. 4B). However, beta diversity analysis showed no significant differences in microbial composition between SP and WDP (p=1.0) (Fig. 4C). These results suggest that while WDP enhances DNA yield and microbial abundance, it does not significantly alter overall microbial diversity.

DISCUSSION

This study conducted a comprehensive evaluation of three microbial DNA extraction protocols for urine specimens, demonstrating that WDP consistently outperforms SP and CAP in terms of DNA purity, reproducibility, and reduced contamination levels. CAP, which employs Tris-EDTA buffer to enhance DNA recovery, was eliminated early due to its suboptimal performance (Table 1), yielding lower DNA concentrations, poor purity (260/280 ratio), and higher contamination levels (260/230 ratio) compared to the other methods. These findings suggest that chelation reagents may interfere with DNA extraction or introduce contaminants, making CAP unsuitable for high-quality DNA recovery. Since our study primarily aimed to evaluate extraction efficiency rather than investigate the specific biochemical impact of EDTA, further analysis of its effects was beyond our scope. Nonetheless, future research could explore EDTA's role in DNA recovery optimization.

When comparing SP and WDP across all samples, WDP consistently produced DNA of higher purity with lower contamination levels, although DNA concentrations were slightly lower than those obtained using SP (Fig. 1). WDP achieved 260/280 and 260/230 ratios that closely aligned with ideal ranges, indicating minimal contamination from proteins and organic compounds. In contrast, SP often yielded variable results, with many samples failing to meet acceptable quality thresholds, indicating compromised DNA integrity. While SP occasionally produced higher DNA concentrations, its variability and contamination levels make it less reliable for applications requiring DNA samples of consistently high quality.

Matched-sample analysis further confirmed WDP's advantages (Fig. 2). Direct comparisons showed that WDP consistently provided higher DNA purity (260/280 ratios) and lower contamination levels (260/230 ratios) than SP. By contrast, SP exhibited greater variability in DNA concentration and lower quality. Additional assessments across multiple

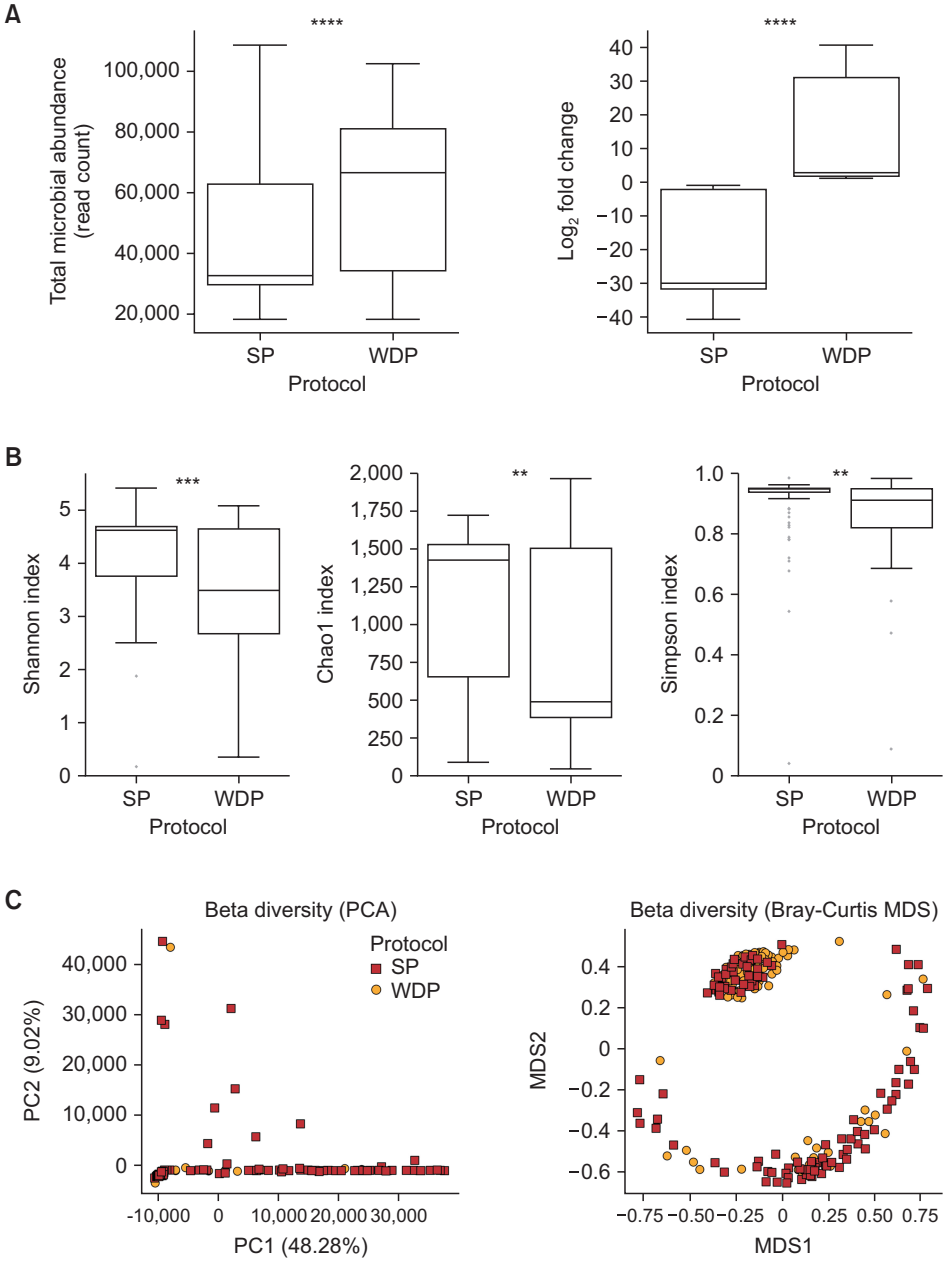

Fig. 4. Microbial community variability and diversity between standard protocol (SP) and water dilution protocol (WDP). (A) Total microbial abundance and log_2fold change. WDP-extracted samples exhibited significantly higher microbial abundance (left panel) and log_2fold change (right panel) compared to SP, indicating improved microbial DNA recovery. (B) Alpha diversity indices. SP showed significantly higher Shannon index, Chao1 index, and Simpson index than WDP, suggesting greater species richness and evenness. (C) Beta diversity analysis. Principal component analysis (PCA) (left panel) and Bray-Curtis multidimensional scaling (MDS) (right panel) show no distinct clustering between SP and WDP samples, indicating that the microbial composition remains largely comparable between the two extraction methods. **p<0.01, ***p<0.001, ****p<0.0001.

urine specimens reinforced this trend. Repeated extractions using SP (SP-1, SP-2, SP-3) resulted in lower DNA yield and quality, whereas WDP extractions (WDP-1, WDP-2, WDP-3) demonstrated strong reproducibility across samples (Supplementary Figs. 2, 3, Supplementary Tables 2, 3). These results highlight WDP's superior consistency, purity, and reliability for microbial DNA extraction from urine specimens. The consistently higher pass rate for WDP (based on the 10 ng/μL threshold) further supports its reliability and reproducibility in microbial DNA extraction for 16S rRNA sequencing.

Metagenomic analysis revealed that microbial abundance was significantly higher in WDP than in SP (p<0.0001) (Fig. 4A), suggesting that WDP is more effective at capturing a broader range of microbial DNA, thereby reducing the risk of missing key bacterial taxa. However, alpha diversity

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indices were notably higher in SP than in WDP ($p<0.01$) (Fig. 4B), likely due to increased detection of low-abundance taxa. The lower microbial DNA yield from SP may have reduced the dominance of highly abundant species, increasing species evenness and inflating diversity indices. Despite these differences in microbial abundance and diversity, beta diversity analysis showed no significant clustering differences between WDP and SP ($p=1.0$) (Fig. 4C), indicating that both protocols capture a largely comparable microbial community composition. This suggests that while WDP enhances DNA recovery and microbial abundance, it does not introduce significant bias in microbial community structure. The ability of WDP to extract higher-quality DNA while maintaining overall microbial diversity makes it the preferred method for applications requiring robust and reproducible microbiome analysis.

This comparative study emphasizes that diluting urine samples with DW at room temperature is critical to enhance DNA extraction efficiency. Stirring at room temperature or solvent dissolution alone had only a limited effect on the crystalline composition of samples [28,29]. The observed variability among samples likely stems from differences in their crystalline structures and compositions [24-27]. In our study, a subset of samples failed to yield DNA using any method. These cases were attributed to patient-related factors (e.g., underlying medical conditions or medication use) or urine-related factors (e.g., pH, ion concentration, or crystal composition). Further research is needed to elucidate the mechanisms underlying these biases and to develop strategies to mitigate their effects.

Overall, WDP emerges as the most robust and effective protocol for extracting microbial DNA from urine specimens. Its ability to consistently yield high-quality DNA with minimal contamination makes it the preferred choice for routine analyses and research requiring reproducible results. While SP may be suitable for applications prioritizing DNA yield, WDP offers a more balanced and reliable solution, particularly when purity and consistency are critical. These findings support the broader adoption of WDP in microbial DNA extraction workflows, particularly in clinical and research settings that require high-quality DNA.

CONCLUSIONS

WDP emerged as the most effective method for extracting microbial DNA from urine, offering an optimal balance of DNA purity, reproducibility, and minimal contamination. These findings support the broader application of WDP in both clinical diagnostics and research, particularly in studies requiring consistently high-quality DNA for downstream analyses. Future research should focus on validating WDP's performance across larger and more diverse populations while addressing challenges related to urine crystal composition and sample variability. This study represents a significant advancement in refining DNA extraction techniques, paving the way for more accurate and reliable investigations of the urinary microbiome.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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AUTHORS' CONTRIBUTIONS

Research conception and design: Xuan-Mei Piao and Seok Joong Yun. Data acquisition: Chuang-Ming Zheng, Seongmin Moon, Young Joon Byun, Ho Won Kang, and Xuan-Mei Piao. Statistical analysis: Chuang-Ming Zheng, Seongmin Moon, Young Joon Byun, Ho Won Kang, and Xuan-Mei Piao. Data analysis and interpretation: Won Tae Kim, Xuan-Mei Piao, and Seok Joong Yun. Drafting of the manuscript: Chuang-Ming Zheng, Ho Won Kang, and Xuan-Mei Piao. Critical revision of the manuscript: Won Tae Kim, Sung-Kwon Moon, Yung Hyun Choi, and Seok Joong Yun. Obtaining funding: Young Joon Byun, Xuan-Mei Piao, and Seok Joong Yun. Administrative, technical, or material support: Ho Won Kang, Won Tae Kim, and Seok Joong Yun. Supervision: Ho Won Kang, Sung-Kwon Moon, and Yung Hyun Choi. Approval of the final manuscript: all authors.

SUPPLEMENTARY MATERIALS

Supplementary materials can be found via https://doi.org/10.4111/icu.20240454.

REFERENCES

1. Cho I, Blaser MJ. The human microbiome: at the interface of health and disease. Nat Rev Genet 2012;13:260-70.
2. Proctor L. Priorities for the next 10 years of human microbiome research. Nature 2019;569:623-5.
3. Kau AL, Ahern PP, Griffin NW, Goodman AL, Gordon JI. Human nutrition, the gut microbiome and the immune system. Nature 2011;474:327-36.
4. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature 2012;486:207-14.
5. Schwabe RF, Jobin C. The microbiome and cancer. Nat Rev Cancer 2013;13:800-12.
6. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 2006;444:1027-31.
7. Smith MI, Yatsunenko T, Manary MJ, Trehan I, Mkakosya R, Cheng J, et al. Gut microbiomes of Malawian twin pairs discordant for kwashiorkor. Science 2013;339:548-54.
8. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71:209-49.
9. Yacouba A, Tidjani Alou M, Lagier JC, Dubourg G, Raoult D. Urinary microbiota and bladder cancer: a systematic review and a focus on uropathogens. Semin Cancer Biol 2022;86(Pt 3):875-84.
10. Maas M, Todenhöfer T, Black PC. Urine biomarkers in bladder cancer - current status and future perspectives. Nat Rev Urol 2023;20:597-614.
11. Piao XM, Kang H, Kim WJ, Yun SJ. Prominence of urinary biomarkers for bladder cancer in the COVID-19 era: from the commercially available to new prospective candidates. Investig Clin Urol 2021;62:500-19.
12. Wolfe AJ, Brubaker L. "Sterile urine" and the presence of bacteria. Eur Urol 2015;68:173-4.
13. Wolfe AJ, Toh E, Shibata N, Rong R, Kenton K, Fitzgerald M, et al. Evidence of uncultivated bacteria in the adult female bladder. J Clin Microbiol 2012;50:1376-83.
14. Lewis DA, Brown R, Williams J, White P, Jacobson SK, Marchesi JR, et al. The human urinary microbiome; bacterial DNA in voided urine of asymptomatic adults. Front Cell Infect Microbiol 2013;3:41.
15. Chorbińska J, Krajewski W, Nowak Ł, Małkiewicz B, Del Giudice F, Szydełko T. Urinary microbiome in bladder diseases-review. Biomedicines 2023;11:2816.
16. Fouts DE, Pieper R, Szpakowski S, Pohl H, Knoblach S, Suh MJ, et al. Integrated next-generation sequencing of 16S rDNA and metaproteomics differentiate the healthy urine microbiome from asymptomatic bacteriuria in neuropathic bladder associated with spinal cord injury. J Transl Med 2012;10:174.
17. Modena BD, Milam R, Harrison F, Cheeseman JA, Abecassis MM, Friedewald JJ, et al. Changes in urinary microbiome populations correlate in kidney transplants with interstitial fibrosis and tubular atrophy documented in early surveillance biopsies. Am J Transplant 2017;17:712-23.
18. Gottschick C, Deng ZL, Vital M, Masur C, Abels C, Pieper DH, et al. The urinary microbiota of men and women and its changes in women during bacterial vaginosis and antibiotic treatment. Microbiome 2017;5:99.
19. Min K, Kim HT, Lee EH, Park H, Ha YS. Bacteria for treatment: microbiome in bladder cancer. Biomedicines 2022;10:1783.
20. Sinha R, Abu-Ali G, Vogtmann E, Fodor AA, Ren B, Amir A, et al. Assessment of variation in microbial community amplicon sequencing by the Microbiome Quality Control (MBQC) project consortium. Nat Biotechnol 2017;35:1077-86.
21. Costea PI, Zeller G, Sunagawa S, Pelletier E, Alberti A, Levenez F, et al. Towards standards for human fecal sample processing in metagenomic studies. Nat Biotechnol 2017;35:1069-76.
22. Mahony J, Chong S, Jang D, Luinstra K, Faught M, Dalby D, et al. Urine specimens from pregnant and nonpregnant women inhibitory to amplification of Chlamydia trachomatis nucleic acid by PCR, ligase chain reaction, and transcription-mediated amplification: identification of urinary substances associated with inhibition and removal of inhibitory activity. J Clin Microbiol 1998;36:3122-6.
23. Ackerman AL, Anger JT, Khalique MU, Ackerman JE, Tang J, Kim J, et al.; NIH Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP). Optimization of DNA extraction from human urinary samples for mycobiome community profiling. PLoS One 2019;14:e0210306.
24. Fogazzi GB. Crystalluria: a neglected aspect of urinary sediment analysis. Nephrol Dial Transplant 1996;11:379-87.
25. Daudon M, Frochot V. Crystalluria. Clin Chem Lab Med 2015;53 Suppl 2:s1479-87.
26. Simerville JA, Maxted WC, Pahira JJ. Urinalysis: a comprehensive review. Am Fam Physician 2005;71:1153-62.
27. Brunzel NA. Fundamentals of urine and body fluid analysis. 5th ed. Elsevier Health Sciences; 2022.
28. Munch MM, Chambers LC, Manhart LE, Domogala D, Lopez A, Fredricks DN, et al. Optimizing bacterial DNA extraction in urine. PLoS One 2019;14:e0222962.
29. Saetun P, Semangoen T, Thongboonkerd V. Characterizations of urinary sediments precipitated after freezing and their effects on urinary protein and chemical analyses. Am J Physiol Renal Physiol 2009;296:F1346-54.

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