

Urine Preservation and Stabilization and its Application in Microbial Urinalysis

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Introduction

Urine is an ideal sample input for many applications because of its easy and non-invasive collection procedure. However, nucleic acid detection in urine can lack sensitivity and be problematic due to nucleic acid instability and bacterial contamination in the sample. In addition, biased microbial lysis in urine hinders accurate sample analysis. To address these problems, we developed improved urine collection and purification procedures for molecular and microbial testing, from sample collection to sample analysis.

In this study, a stool sample was spiked into a urine sample to simulate stool contamination in urine. Next, **Urine Conditioning Buffer™ (UCB™)**, a uniquely formulated nucleic acid preservation and stabilization reagent, was added to the urine sample. To analyze the stability of the nucleic acids in the sample, urine was stored at room temperature at different time intervals: 0 days, 2 weeks, and 1 month. At each timepoint, DNA was extracted using the **ZymoBIOMICS® DNA Miniprep Kit**, which includes an efficient lysis system which enables unbiased lysis of microbes. The microbial composition of the DNA was then profiled via 16S rRNA gene targeted sequencing.

The results of the 16S rRNA gene targeted sequencing analysis showed that the bacterial profile composition of UCB™-preserved urine remained consistent throughout the different time intervals (0 days, 2 weeks, and 1 month), indicating that there was no bacterial blooms or nucleic acid degradation. In this analysis, we also showed that DNA from both gram-positive and gram-negative bacteria were successfully extracted using the ZymoBIOMICS® DNA Miniprep Kit.

These findings demonstrated that UCB™ can be reliably used as a preservative for urine samples collected for microbiome profiling because it inhibits microbial growth and preserves the DNA in the urine sample at room temperature over a month period. Furthermore, UCB™ was designed to concentrate the sample, making the large unwieldy urine volumes more manageable (e.g. 40 ml is concentrated to < 200 µl) without leading to microbial profiling bias. Finally, UCB™ uses precipitation to separate out the DNA fraction, which avoids relying entirely on centrifugation for separation and leads to the recovery of DNA that is typically lost in supernatant removal.

Here, we presented an improved urine collection procedure to stabilize nucleic acids in urine samples, coupled to an unbiased microbial urinalysis.

Urine Collection and Preservation

Standardized Urine Collection



Figure 1. Custom Filled UCB™ into any Collection Device.
The small volume requirement of UCB™ (70 µl UCB™ / 1 ml urine) makes it easy to custom filled UCB™ into any collection device.

UCB™ Preserves DNA in Urine at Ambient and Increased Temperatures

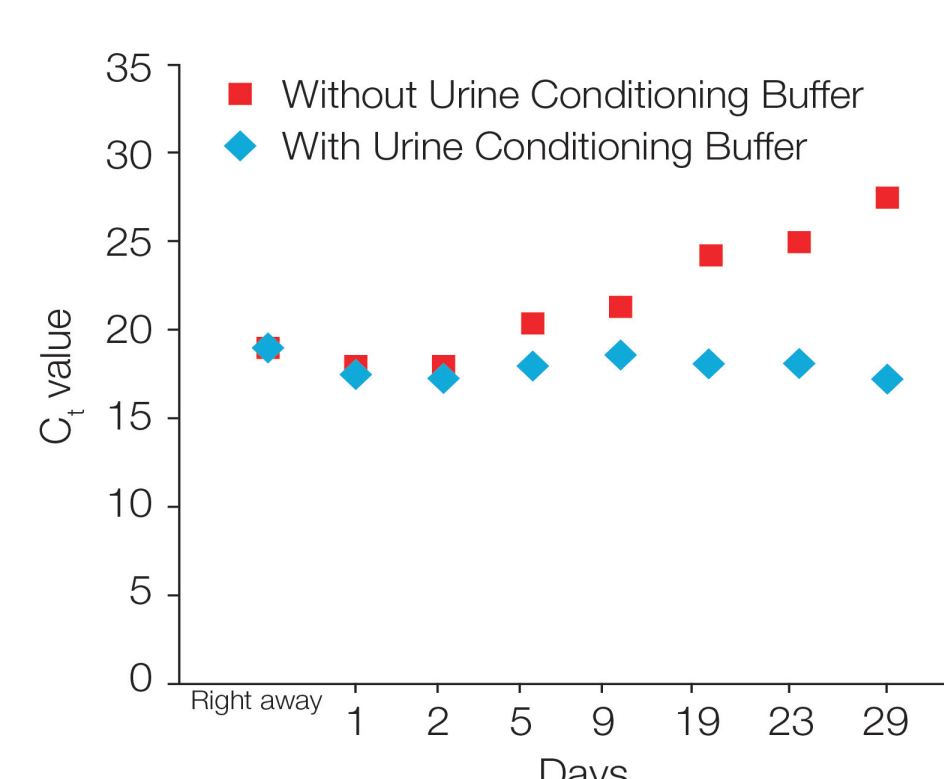


Figure 2. DNA in urine can be preserved for up to 1 month at ambient temperature.
Total (cellular and cell-free) urine DNA was incubated with and without UCB™ for 29 days. For each sample, 5 ml urine from a healthy female donor was processed using the Quick-DNA™ Urine Kit and the Ct values were determined by qPCR. Experiment was performed in technical duplicates.

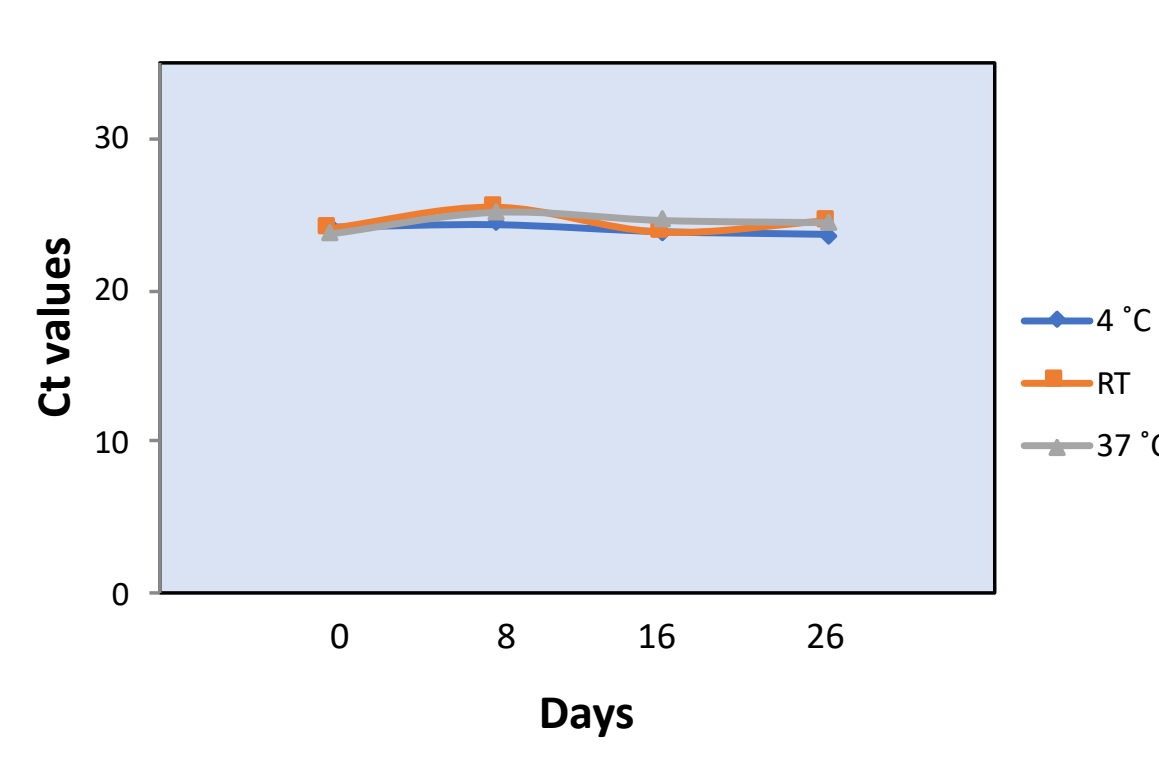


Figure 3. UCB™ preserves DNA in urine stored at different temperatures.
Urine added with UCB™ was stored at different temperatures (4 °C, Room Temperature (RT), and 37 °C) and analyzed over a period of 26 days. At each time point, total DNA was isolated from samples using the Quick-DNA™ Urine Kit. Corresponding Ct values were obtained from qPCR analysis. Experiment was performed in technical duplicates.

UCB™ Prevents Microbial Growth at Ambient Temperature

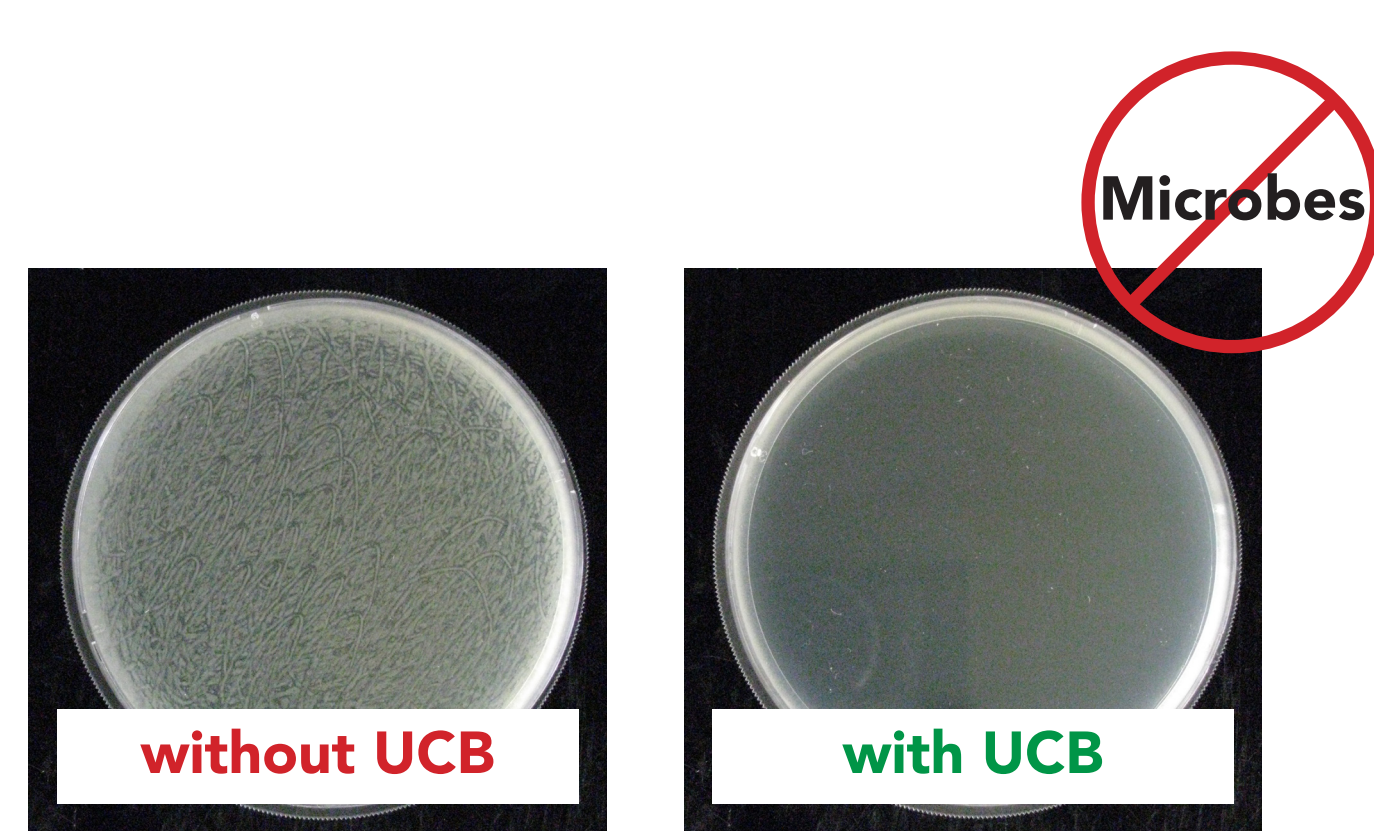
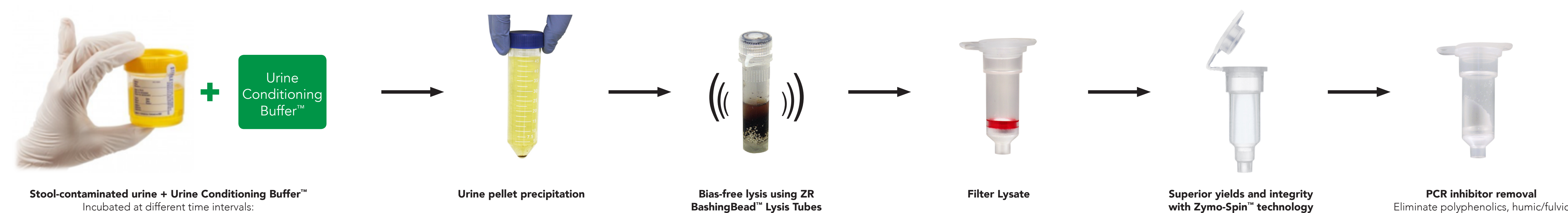


Figure 4. UCB™ prevents microbial growth at ambient temperature.
Urine was stored with and without UCB™ for 6 days. On day 6, microbial colony formation was determined by plating 500 µl urine on LB agar, followed by incubation at 37 °C overnight.

Unbiased Microbial DNA Testing

DNA extraction by ZymoBIOMICS® DNA Kit

Experimental design:



Ultra-Pure DNA from Inhibitor Rich Samples

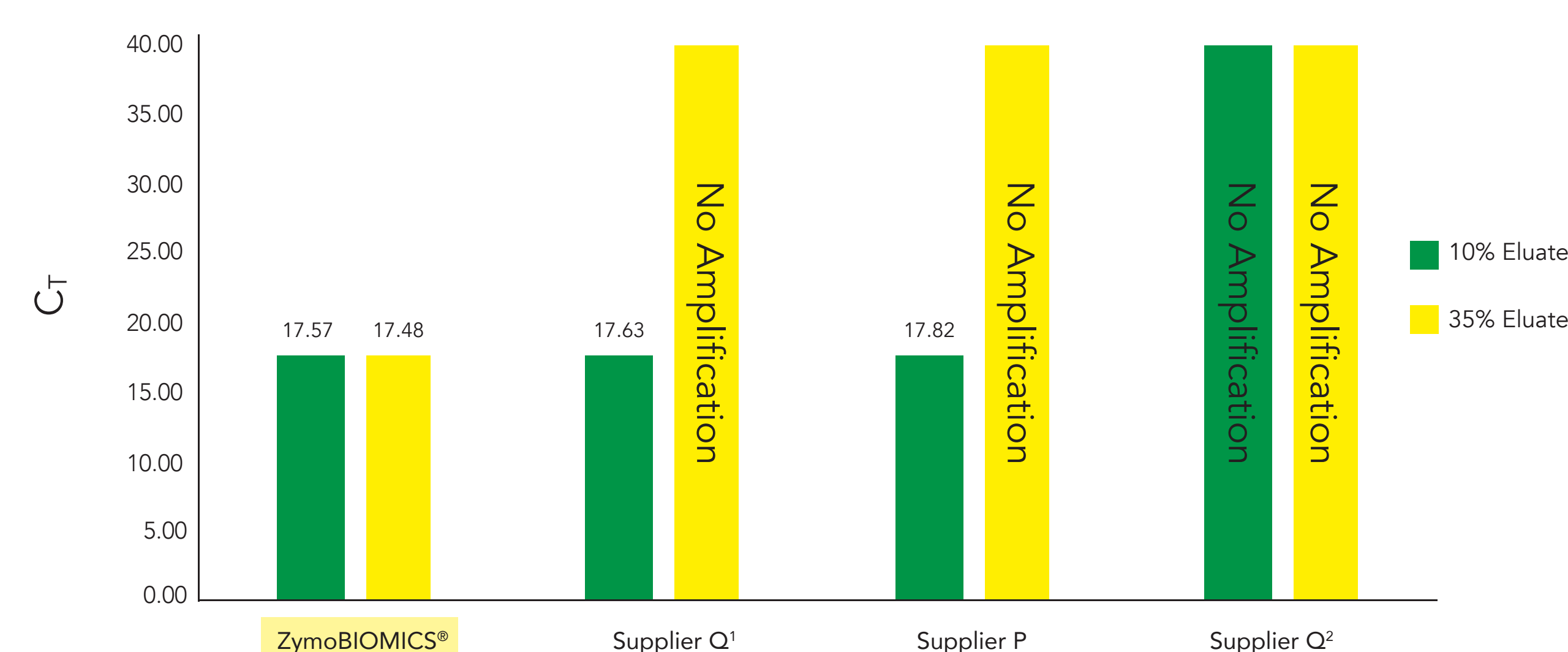


Figure 5. The ZymoBIOMICS® DNA Miniprep Kit provides inhibitor-free DNA even when challenged with extremely inhibitor rich samples.
Real-time PCR was used to evaluate eluates recovered using the ZymoBIOMICS® DNA Miniprep Kit, and kits from Supplier M, P, and Q. Reaction volume consisted of either 10% or 35% of the eluate from each kit to detect the presence of PCR inhibitors. Each reaction contained 25 ng of *Brettanomyces* DNA. Delayed and/or no amplification indicates PCR inhibition from inefficient inhibitor removal.

Bias-Free Microbial DNA Extraction using ZymoBIOMICS® DNA Miniprep Kit Validated with the ZymoBIOMICS® Microbial Community Standard

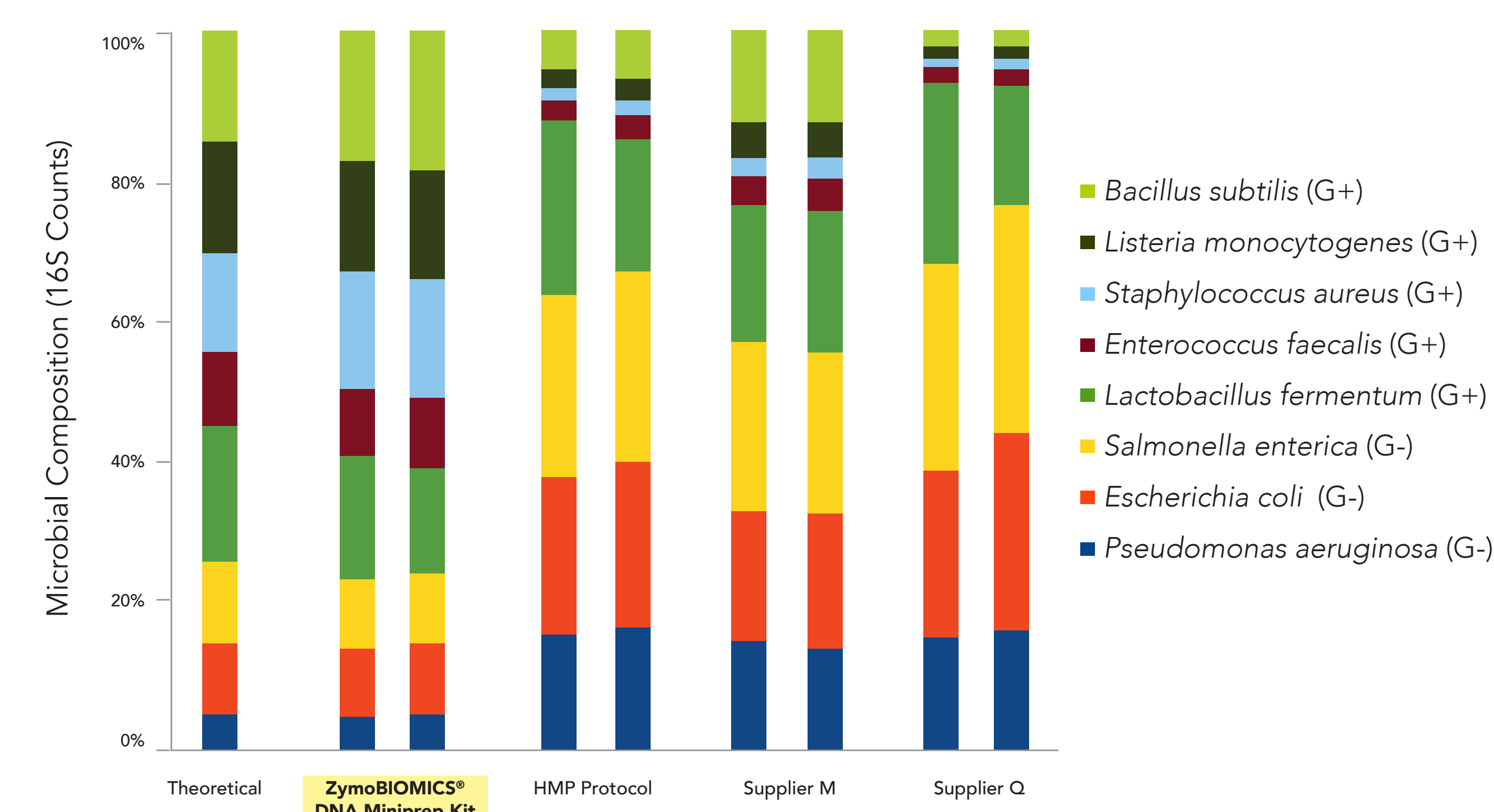


Figure 6. The ZymoBIOMICS® DNA Miniprep Kit provides unbiased representation of the organisms extracted from the ZymoBIOMICS® Microbial Community Standard.
DNA was extracted from ZymoBIOMICS® Microbial Community Standard using four different DNA extraction methods (ZymoBIOMICS® DNA Miniprep Kit, Human Microbiome Project (HMP) Protocol, Supplier M, and Supplier Q) and analyzed using 16S rRNA gene sequencing. 16S rRNA genes were amplified with primers targeting v3-4 region and the amplicons were sequenced on Illumina® MiSeq™ (2 x 250bp). Overlapping paired-end reads were assembled into complete amplicon sequences. The composition profile was determined based on sequence counts after mapping amplicon sequences to the known 16S rRNA of the eight different bacterial species.

Results

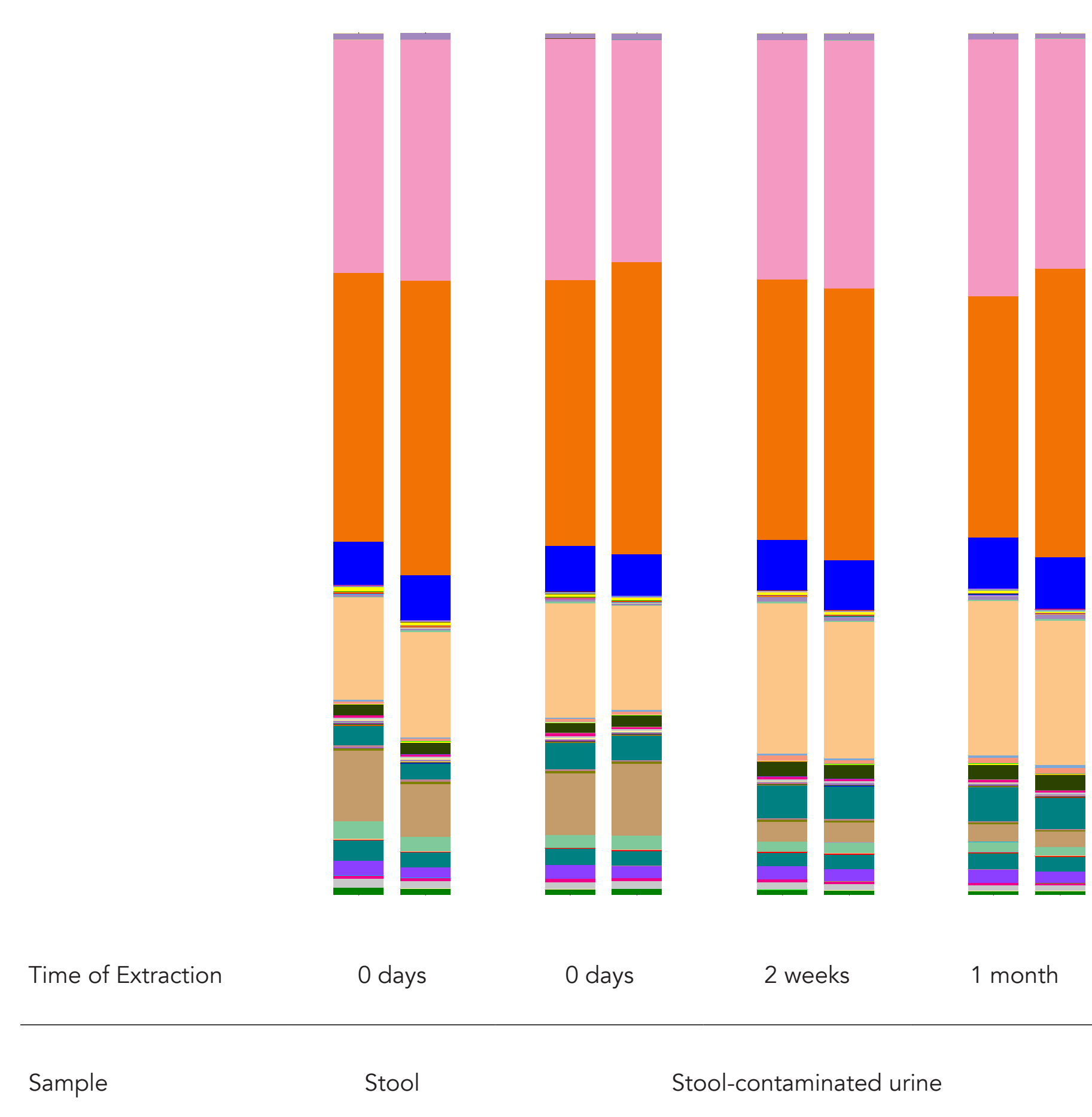


Figure 7. Genus composition of urine preserved in Urine Conditioning Buffer™ (UCB™), which preserves the microbial composition of urine with stimulated stool contamination for a month at room temperature.
Stool-contaminated urine was stored with UCB™ at room temperature and analyzed over a month (0 days, 2 weeks, and 1 month). All DNA was extracted using the ZymoBIOMICS® DNA Miniprep Kit. DNA from pure stool was also extracted as a control to show bias-free microbial lysis. The extracted DNA was then subjected to microbial composition profiling via 16S rRNA gene targeted sequencing. Experiment was performed in technical duplicates.

Conclusion

In this study, we present an improved urine collection procedure to stabilize urine samples coupled to an unbiased microbial urinalysis. This is achieved by using a uniquely formulated urine preservation and precipitation reagent, **Urine Conditioning Buffer™**, and an innovative lysis system that enables efficient and unbiased lysis and DNA extraction of microbes, **ZymoBIOMICS® DNA Miniprep Kit**.

Urine Conditioning Buffer™:

- ✓ Small volume requirement: Can be custom filled to any collection device (70 µl UCB™ / 1 ml urine).
- ✓ Effectively preserves DNA in urine at ambient temperature for up to 1 month.
- ✓ Inhibits microbial growth during long-term (cold-free) storage of urine samples.

ZymoBIOMICS® DNA Miniprep Kit:

- ✓ Ultra-pure, inhibitor-free DNA from many microbiome sample types (feces, soil, water, biofilms, swabs, body fluid, etc.) that is ideal for all downstream application including PCR, arrays, 16S rRNA gene sequencing, and shotgun sequencing.
- ✓ Innovative lysis system enables efficient and unbiased lysis of microbes including Gram-positive/negative bacteria, fungus, protozoans, and algae for accurate microbial community profiling.

Urine Conditioning Buffer™ and ZymoBIOMICS® DNA Miniprep kit provide a total solution for urine based microbial urinalysis including sample collection, stabilization, and bias-free microbial DNA lysis and inhibitor-free DNA purification.