Streck® Urine Preserve maintains cell-free RNA in urine

With the expanding opportunities for the use of urine in diagnostic applications, the experimental aim of this study was to determine whether urinary extracellular vesicle (uEV) messenger RNA (mRNA) concentration is maintained in Streck Urine Preserve (Streck UP).

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Methods

Second void urine was collected from three female donors, pooled, and aliquoted. Aliquots were left untreated or were preserved with Streck UP at a 1:9 ratio (Streck UP to donor urine). On day 0, after preservation, and after 3 and 7 days of ambient temperature bench top storage (20 °C to 26 °C), uEVs were isolated using miRCURY exosome cell/urine/CSF kit (Qiagen) according to the kit protocol. Isolated uEVs from 5 mL urine were resuspended in 250 µL resuspension buffer included in the kit and stored at -80 °C until use. Nanoparticle tracking analysis (NTA) was used to measure the number of isolated uEVs. The camera and analysis settings on NanoSight NS300 (Malvern Panalytical) were optimized and kept constant between samples. All samples were run in triplicate at a 1:100 dilution in PBS, yielding nanoparticle concentration per frame in the recommended range in accordance with the manufacturer's recommendations. Total RNA was extracted from 200 µL isolated uEVs using miRNeasy mini kit (Qiagen) following the kit protocol with adapted Proteinase K digestion step and Invitrogen TRIzol LS extraction process. In short, uEVs in 10% Proteinase K Solution (Qiagen) with 2% SDS, 30 mM Tris, 10 mM EDTA were incubated at 60 °C for 1 hour before RNA extraction. Three sample volumes of TRIzol LS Reagent were used in place of QIAzol Lysis Reagent. Reverse transcription (RT) was followed using Bio-Rad iScript RT reagents and protocol. Droplet Digital PCR (ddPCR) was used to quantify two housekeeping mRNA targets, beta-actin (*ACTB*) and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) according to Bio-Rad QX200 protocol. The sequences and concentrations for the primers and probes are summarized in Table 1. Duplicate reactions were run and data were plotted as mean copies/mL urine ± SD.

Name	Sequence	Conc. (nM)
GADPH Forward_584F	CAA CTT TGG TAT CGT GGA AGG	900
GAPDH Reverse_708R	GTA GAG GCA GGG ATG ATG TT	900
GAPDH Probe_598FAM	/56-FAM/CA TGA CCA C/ZEN/A GTC CAT GCC ATC AC/3IABkFQ/	250
ACTB Forward_788F	CCC TGG AGA AGA GCT ACG AG	900
ACTB Reverse_1087R	GCT CAG GAG GAG CAA TGA T	900
ACTB Probe_864FAM	/56-FAM/AC TCT TCC A/ZEN/G CCT TCC TTC CTG G/3IABkFQ/	250

Table 1: Summary of primers and probes used in the ddPCR analysis.

Results

The abundance and size of uEVs in urine samples preserved with Streck UP stored at ambient temperature was evaluated by NTA. As shown in Figure 1, compared with immediately processed Day 0 urine sample, preserved urine maintained both the count and mean size of uEVs up to 7 days at ambient temperature. No significant differences were noted between Day 0 and the preserved samples. The count and size of uEVs in unpreserved samples were not tracked due to interference by the bacteria outgrowth when stored at ambient temperature.

Given that uEV concentration does not change in urine samples preserved with Streck UP, we next used ddPCR to determine levels of mRNA targets thought to be encapsulated within uEVs. As shown in Figure 2, unpreserved urine showed a marked decrease in both *ACTB* and *GAPDH* uEV mRNA targets at Day 3 and 7 at ambient temperature when compared to Day 0 sample (processed immediately after collection) by ddPCR analysis. Streck UP maintains both targets to a significant extent. As urine is rich in ribonucleases, detectable cell-free RNA in urine is shielded from degradation by encapsulation within membrane-bound vesicles, such as exosomes. Unpreserved urine samples stored under ambient conditions result in marked bacterial outgrowth and simultaneous changes in urine pH and redox status, likely compromising both uEV compartmentalization and associated genetic content. Streck UP maintains the mRNA targets in uEVs by minimizing changes in the complex urinary environment.

Conclusion

Streck UP maintains uEV mRNA targets for an extended period of time at ambient temperature, providing researchers and clinical assay developers flexibility in urine sample shipping and processing.

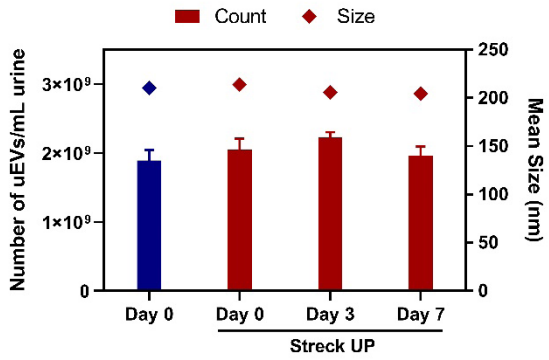

Figure 1: Concentration and mean size of uEVs in urine preserved with Streck UP (mean ± standard error)

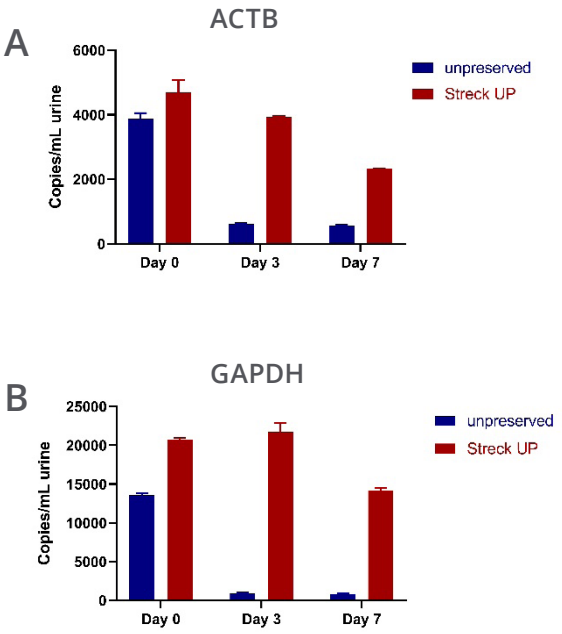

Figure 2: ddPCR analysis of (A) ACTB and (B) GAPDH levels in urine in uEVs.