

Streck® Urine Preserve maintains the integrity of exfoliated cells in urine

With the expanding opportunities for the use of urine in diagnostic research applications, the experimental aim of this study was to explore the effect of Streck Urine Preserve (Streck UP) on maintaining the integrity of exfoliated cells in urine when stored at ambient temperature for up to 7 days. Human breast cancer cells stably expressing green fluorescent protein (mimicking cells shed into urine) were spiked in fresh human urine to model the preservation activity of Streck UP.

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Methods

T47D/GFP cells (Cell Biolabs, Inc.) were cultured in Dulbecco's Modified Eagle Medium (DMEM, high glucose) supplemented with 10% fetal bovine serum, 0.1 mM MEM non-Essential Amino Acids, 2 mM L-glutamine, and 1% Pen-Strep. On the day of spike-in, second void urine was collected from one female donor and one male donor, pooled, and aliquoted. Pooled urine aliquots were then preserved with or without Streck UP at a 1:9 ratio (Streck UP to donor urine). Trypsin-dissociated T47D/GFP cells were resuspended in PBS, spiked into the urine at a concentration of 0.5×10^6 cells/mL, and subsequently stored at ambient temperature (20 °C to 26 °C) for up to 7 days. The spiked urine samples were protected from light during storage to minimize quenching of the fluorescence. On Day 0 (day of spike), 4 and 7, samples were mixed and 100 μ L aliquots were immobilized onto coated glass microscope slides using Cytospin 3 (Thermo Shandon) at 500 rpm for 7 minutes. One set of slides was stained with ProLong antifade mountant with DAPI (Invitrogen) and imaged using a Zeiss Axio Observer inverted fluorescent microscope. A second set was stained with hematology stain (HARLECO Hemacolor Stain Set) and imaged using the same microscope in brightfield mode.

Results

The effect of Streck UP on maintaining the integrity of exfoliated cells was evaluated visually using fresh urine spiked with metastatic breast cancer cells as an experimental model. As shown in Figure 1, cells spiked into unpreserved urine samples demonstrated significant degradation by Day 4 and complete loss of cell structure on Day 7 due to prototypical cell death (membrane blebbing and nuclear condensation) and significant particulate matter from bacterial outgrowth. Preserved urine

exhibited minimal visual increases in cell debris and no noticeable sign of bacterial contamination. The preservative also maintained the appearance of the spiked "exfoliated" cancer cells. Similarly, structurally intact GFP-expressing cells with normally appearing nuclei (DAPI staining) were observed in preserved samples, as demonstrated in Figure 2. Strong GFP staining was observed out to 7 days. In contrast, the unpreserved sample demonstrated dim GFP signal from the cell debris and highly condensed and blebbing nuclei indicative of cell death at day 7.

Conclusion

Streck UP maintains the visual integrity of urine cellular components for up to 7 days when held at ambient temperature. Streck UP provides researchers and clinical assay developers flexibility in handling, shipping, and processing of urine specimens.

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