Streck® Urine Preserve maintains viral RNA in urine

With the expansion of opportunities for the use of urine in diagnostic research, Streck researchers sought to determine if Streck Urine Preserve (Streck UP) functioned to maintain viral RNA. To demonstrate the viral RNA preservation attributes of Streck UP, heat-inactivated Zika virus (ZIKV) was added in fresh human urine upon collection and stored at ambient temperature for up to 7 days.

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Methods

Second void urine was collected from one female donor and one male donor, pooled, and aliquoted with or without the addition of Streck UP at a 1:9 ratio (Streck UP to donor urine). Heat-inactivated ZIKV (BEI Resources, PRVABC59 cat# NR-50369) was spiked in at three different titers: 10^6, 10^5, 10^4 RNA copies per mL urine. Spiked urine samples were then stored at ambient temperature (20 °C to 26 °C) up to 7 days. On Day 0, 3 and 7, urine samples were centrifuged at 1500 ×g for 10 minutes, and the supernatant was further aliquoted and stored at -80 °C before RNA extraction. Viral RNA was extracted using the QIAamp Viral RNA Mini kit (Qiagen). The protocol was modified with the addition of a Proteinase K digestion step. In short, 78 µL Proteinase K solution (Qiagen) was added to 700 µL viral lysate containing 140 µL of urine sample and 560 µL Buffer AVL (+carrier RNA).

The lysate mixture was incubated at 56 °C on a shaking heat block for 1 hour. Adjusted volume of ethanol (560 µL + 78 µL equal volume of Proteinase K solution) was then added to the lysate mixture before proceeding through the extraction process as written. RealStar® Zika Virus RT-PCR Kit (Altona Diagnostics) was used to detect ZIKV specific RNA. An internal control included in the kit was added during urine extraction and used as a sample preparation normalizer. A 30 µL reaction (5 µL Master Mix A, 15 µL Master Mix B, 10 µL RNA template) was run on Applied Biosystems® 7500 FAST Real-Time PCR System. Duplicate reactions were run and normalized fold change was calculated using the ΔΔCt method and reported as mean ± SD.

Results

The effect of Streck UP on the maintenance of ZIKV RNA in urine at ambient temperature over an extended period of time was evaluated. As shown in Figure 1, spiked urine samples preserved with Streck UP at Day 3 and Day 7 showed ZIKV RNA levels comparable to immediate processing on Day 0. A marked decrease of 10-fold or more was observed in unpreserved urine on both Day 3 and 7 at all three viral titers. At the 10^5 copies/mL urine titer, ZIKV RNA in unpreserved urine cannot be detected at Day 7. At the lowest titer (10^4 copies/mL urine) tested, neither the Day 3 nor Day 7 unpreserved sample contained detectible ZIKV RNA, suggesting ZIKV RNA degradation and thereby loss in unpreserved urine samples.

In addition, as higher ZIKV RNA in preserved sample over unpreserved sample at Day 0 was noted, the stability of ZIKV RNA in urine after immediate spike-in was further tested. As shown in Figure 2, unpreserved urine exhibited an observable loss in the detected ZIKV RNA when compared with the same ZIKV titer in Tris-EDTA (TE) buffer. Streck UP preserves the ZIKV RNA to a significant extent and the preservation effect is more pronounced in lower titers.

Conclusion

Streck UP can effectively stabilize viral RNA as demonstrated with the ZIKV RNA model in fresh urine specimens at different viral titers for up to 7 days of ambient temperature storage. According to CDC guidelines, when testing urine specimens for viral content like ZIKV RNA, samples are required to be kept cold (2 °C to 8 °C) or frozen (≤ -20 °C) for storage and shipping. Streck UP provides researchers and clinical assay developers flexibility with the handling, shipping and processing of urine specimens.

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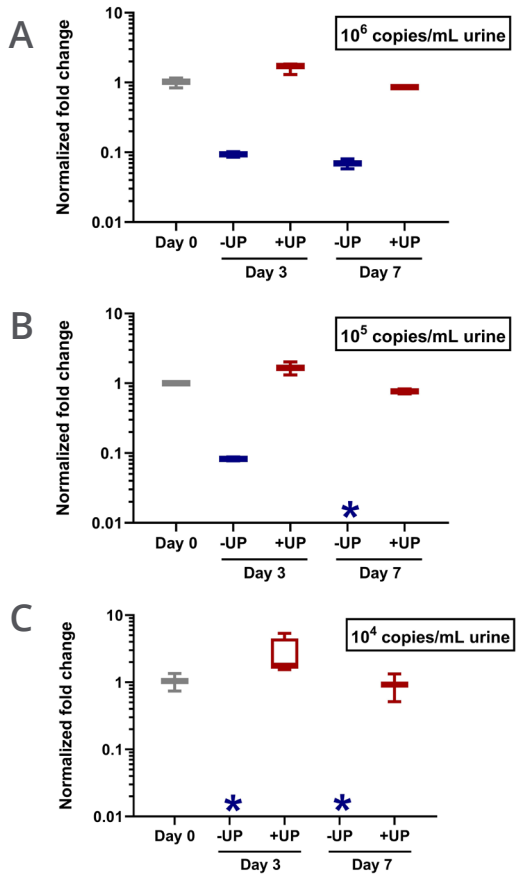

*Figure 1: Normalized fold change in detected ZIKV RNA in preserved or unpreserved urine at Day 3 and Day 7 after storage at ambient temperature compared with the same ZIKV titer in urine at Day 0. ZIKV RNA were spiked in at (A) 10^6, (B) 10^5 and (C) 10^4 RNA copies/mL urine. * ZIKV specific RNA is under the detection limit.*

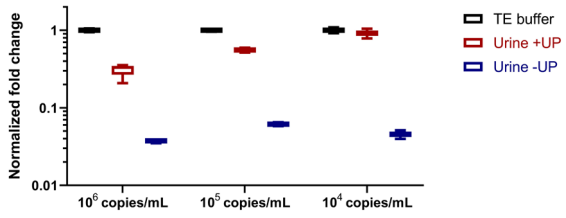

Figure 2: Normalized fold change in detected ZIKV RNA in preserved or unpreserved urine compared with the same ZIKV titer in TE buffer.