

Long Term Stability of RNA Stored in Norgen's Urine Preservation Buffer

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INTRODUCTION

In recent years attention has been turning to the use of non-invasive samples for genetic and diagnostic analysis, including the use of urine. The isolation of high quality RNA from urine is not without its problems however. The number of RNA-containing cells found in urine can vary significantly from individual to individual. Adequate amounts of urine must therefore be collected to ensure that RNA can be extracted in an amount sufficient for testing. As enzymes that degrade RNA are found in urine, methods must also be employed to protect the RNA in the sample. For currently available collection devices which do not employ preservative this requires that the urine be collected and held then frozen at -20°C as soon as possible. The addition of preservative to the collected urine eliminates the need to immediately process or freeze the urine samples and allows the samples to be shipped at ambient temperature.

Norgen Biotek Corp. has developed a Urine Preservative which allows for the long-term preservation of urine samples at ambient room temperature, making this reagent ideal for urine storage and shipping. This liquid preservative is available as a product on its own, and is also included with our different urine concentration, preservation, storage and purification devices and kits. The Urine Preservative is an aqueous storage buffer that stabilizes the RNA for long-term storage at ambient temperature. Since the buffer prevents the growth of microorganisms and inactivates viruses it also allows the samples to be handled and shipped safely. The RNA subsequently isolated from the preserved samples is of a high quality and can be used directly in sensitive downstream diagnostic assays such as Quantitative PCR.

In this application note, the long-term stability of Urine RNA in Norgen's Urine Preservative is analyzed. Preserved urine samples were stored at -20°C, 4°C, room temperature

and 55°C for up to 6 months, and the RNA was subsequently isolated and analyzed.

MATERIALS AND METHODS

RNA Isolation

Urine samples were collected from numerous donors and mixed, and then aliquoted into 20 mL urine samples. The aliquoted urine samples were concentrated using Norgen's Urine Concentration Tube. Norgen's Urine Preservative was then mixed with the concentrated urine and was stored at -20°C, 4°C, room temperature and 55°C for up to 6 months. Urine RNA was subsequently isolated at time 0, 1 month, 2 months, 4 months and 6 months from the concentrated urine/preservative sample using Norgen's Urine RNA Concentration, Preservation and Isolation Kit.

PCR Amplification

The purified RNA was then used as the template in a real-time PCR reaction. Briefly, 2 µL of isolated RNA was added in to 20 µL of reverse transcription PCR reaction mixture containing 2.5 mM 5S Reverse primer pair. Following the RT-PCR reaction, 5 µL of RT-PCR reaction was added in to 20 µL real-time PCR reaction mixture (SYBR Green) containing 2.5 mM 5S primer pair. The PCR samples were amplified under the real-time program; 95°C for 5 min for an initial denaturation, 40 cycles of 95°C for 15 second for denaturation and 60°C for annealing and extension. The reaction was run on an iCycler iQ real-time system (Bio-Rad).

RESULTS AND DISCUSSION

Urine samples often need to be stored for a period of time prior to RNA isolation and analysis. Traditionally urine samples are frozen at -20°C as soon as possible, however such storage leads to increased costs and is not always convenient, especially in resource-limited settings. The ability to safely store urine samples at ambient temperatures without any degradation of the RNA is ideal. Here, urine samples are concentrated and mixed with Norgen's Urine Preservative and stored at -20°C, 4°C, room temperature and 55°C for up to 6 months. Urine RNA was isolated from the concentrated and preserved urine

samples stored at the 4 different temperatures in triplicate at time 0, 1 month, 2 months, 4 months and 6 months.

These RNA samples were then used as the template in a real-time PCR reaction to detect the 5S gene. RNA samples must be of a high quality in order to be used successfully in downstream amplification reactions. As it can be seen in Figure 1, the RNA isolated from the concentrated and preserved urine samples stored at -20°C, 4°C, room temperature and 55°C for all the time points could all be successfully amplified using real time PCR. Furthermore, the quality of the urine RNA stored at all 4 temperatures did not change from 0 months to 6 months, as indicated by the fact that the Ct value remained consistent.

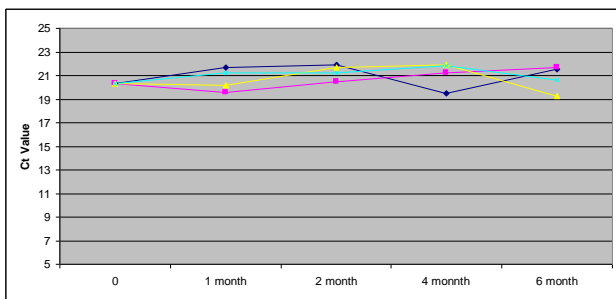


Figure 1. Quantitative PCR detecting 5S gene from urine RNA isolated from different temperatures (-20°C, 4°C room temperature and 55°C) at different time points (time 0, 1 month, 2 months, 4 months and 6 months). The quality of the urine RNA stored at all 4 temperatures did not change from 0 month to 6 months.

The urine samples stored at 55°C can allow us to extrapolate results based on accelerated-aging. According to the Arrhenius equation, the higher the temperature the faster a given chemical reaction will proceed, including RNA degradation. Accordingly, the rate of a chemical reaction typically decreases by half for every 10°C decrease in temperature. Therefore the rate of chemical degradation of RNA at room temperature can be expected to be 6-fold slower than the rate of degradation at 55°C. Based on this calculation the urine preserved at 55°C for 6 months indicates equal stability to urine preserved for 2.5 years at room temperature (24°C). Therefore, based on the successful PCR results for samples stored at 55°C shown above, urine samples stored in Norgen’s Urine Preservative are stable at room temperature for up to 2.5 years.

CONCLUSION

High quality urine RNA can be isolated and successfully amplified from urine samples stored in Norgen’s Urine Preservative at -20°C, 4°C room temperature and 55°C for 6 months. Based on accelerated-aging, urine samples stored at 55°C for 6 months in Norgen’s Urine Preservative are equivalent to samples stored for 2.5 years at room temperature.