

Abstract

There is a need for RNA preservation buffers for microbiome work, specifically buffers applicable to both bacterial and viral samples that do not inhibit later downstream molecular genetic procedures. The goal of this study was to evaluate the ability of two commercially available buffers to preserve microbial and viral RNA for later genetic analysis following storage under typical field or clinical conditions. In this study, we used an automated nucleic acid electrophoresis system to assess the quality of RNA. In addition the viral RNA was assessed for use directly in reverse transcriptase and quantitative PCR protocols.

Escherichia coli cells stored for 1, 2, 7 and 14 days at both room temperature (RT) and 4°C in Puritan PurSafe® DNA/RNA Preservative. Total RNA yields from PurSafe® were not significantly different from original starting concentrations at all time points and storage temperatures. Gel analysis indicated that neither storage temperature nor storage time had a significant effect on the RNA quality.

Influenza A virus (H1N1 – ATCC VR-1469) was propagated on Madin-Darby Canine Kidney Cells. Lysates were clarified by low-speed centrifugation, filtration (0.45µm), ultracentrifugation, and resuspension in Eagle's Minimum Essential Medium without fetal calf serum. Serial dilutions from 1.5×10⁷ to 1.5×10³ TCID₅₀/ml were made. Sterile swabs were immersed aliquots of each dilution and allowed to absorb for 15-20 seconds then placed in vials containing Puritan Opti-Swab® medium and stored for 1, 2 and 7 days at either RT or 4°C. Quantitative real-time PCR (Qiagen Influenza A RT-PCR Assay) was used to quantify Influenza A concentrations. QPCR detected a linear positive relationship between the initial sample concentration and detected relative copy number across all concentrations. Neither storage time nor temperature had a significant effect on viral RNA quality or the ability to quantify viral concentration.

Methods

E. coli RNA:

Top10 *E. coli* cells were grown up in LB (50 ml) culture overnight in 250 ml flask with shaking at 37° C. Turbid cultures were aliquoted into 34 1.5 ml centrifuge tubes, ~1.5 ml each. Tubes were spun down to pellet cells and remove LB media. To each centrifuge tube 1 ml of Puritan PurSafe® was added. Cells were not resuspended. Two tubes (without either buffer) were immediately stored at -80° C as the time zero samples. Remaining centrifuge tubes with buffer were stored at either 4°C or room temperature (RT) for 1, 2, 7 and 14 days. There were two replicates for each storage temperature and time point. At the designated time point samples were removed from 4°C or RT and placed at -80°C.

RNA isolation was performed as per manufacturer's protocol (Promega SV Total RNA Isolation System, Madison WI.). RNA was resuspended in 50 µl nuclease-free water. RNA concentrations were determined with a NanoVue spectrophotometer. RNA quality and gel analysis was conducted with an Experion Automated Electrophoresis System (BioRad, Hercules, CA) as per manufacturer's protocol. Samples were diluted 3-fold to be within the Experion Electrophoresis System's quantitative and qualitative range of 25-100 ng/µl and 5-500 ng/µl respectively. Differences in RNA yields with storage temperature and time were analyzed using ANOVA.



Viral RNA Methods (continued)

Influenza A virus ATCC VR-1469 was propagated on monolayers of Madin-Darby Canine Kidney Cells (MDCK). Resulting lysates were then clarified by low-speed centrifugation (5000xg/20min/4°C), followed by filtration (0.45µm), pelleted by ultracentrifugation (100,000xg/1hr/4°C) and resuspended in 3 mL Eagle's Minimum Essential Medium (EMEM) without fetal calf serum. Virus stocks were enumerated using the Reed-Muench method and reported as 50% tissue culture infectious dose per milliliter (TCID₅₀/mL). Serial ten-fold dilutions of virus stocks were made ranging from 1.5×10⁷ to 1.5×10³ TCID₅₀/mL.

Aliquots (100µL) of each dilution were transferred into a 96-well microtiter plate and sterile swabs were immersed in the corresponding wells and allowed to absorb for 15-20 seconds. Following absorption, the swabs were placed in vials containing Puritan Opti-Swab® medium and were held in the medium for 24, 48, and 168 hours at either RT or 4°C. In addition the 1.5×10⁵ TCID₅₀/mL samples were held at RT for 1 month. RNA isolations were performed using standard kits as per the manufacturer's protocol.

Influenza A (Qiagen virotype Influenza A RT-PCR assay) concentrations using a BioRad CFX96™ PCR Detection System. Copy numbers of pathogenic targets were quantified based on threshold cycle (Ct, the cycle at which the baseline-corrected normalized fluorescence is significantly above background signal). Mean differences in copy number were compared to initial concentrations to assess degradation with storage time and temperature using multivariate ANOVA.



Results

E. coli RNA

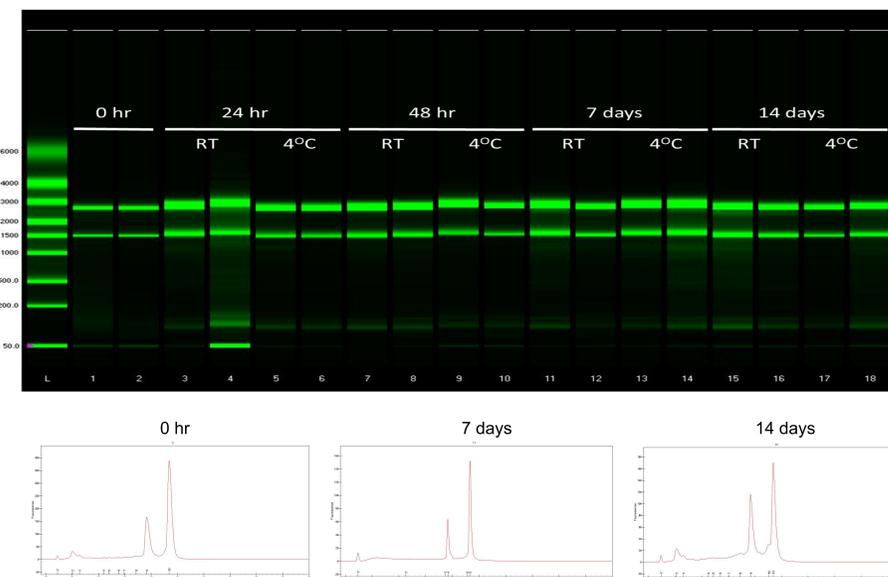
Table 1: Total RNA concentrations at different time intervals based on temperature and preservation media

Time	Mean RNA Conc (ng/µl)	
	Room Temp	4°C
0 hr	891.1	
24 hr	738.3	1,219.9
48 hr	2,564.2	754.7
7 days	1,801.7	1,760.8

Table 2: Statistical analysis showing significant effect of media on total RNA yields. No difference seen with time or temperature.

Effect	df	Mean RNA Conc (ng/µl)			
		SS	MS	F	P
Time (hr)	4	2625476	656369	1.41676	0.312
Temp	1	1787	1787	0.00386	0.952
Time (hr)*Temp	4	4245414	1061353	2.29092	0.148
Error	8	3706303	463288		
Total	17	10705761			

Figure 2. Gel electrophoresis of Prokaryote samples. Note presence of distinct ribosomal bands indicating high quality RNA



Viral RNA Results (continued)

Viral RNA

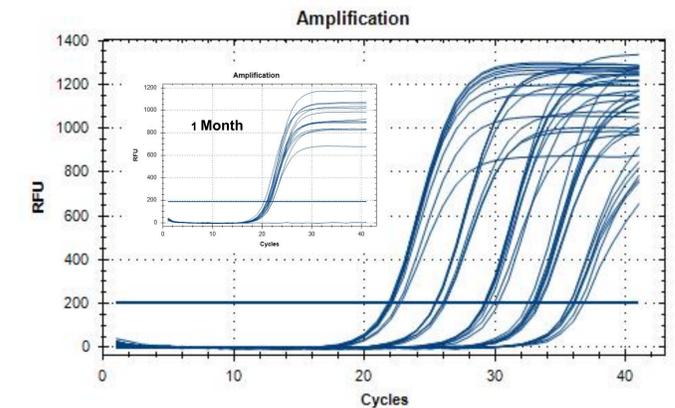
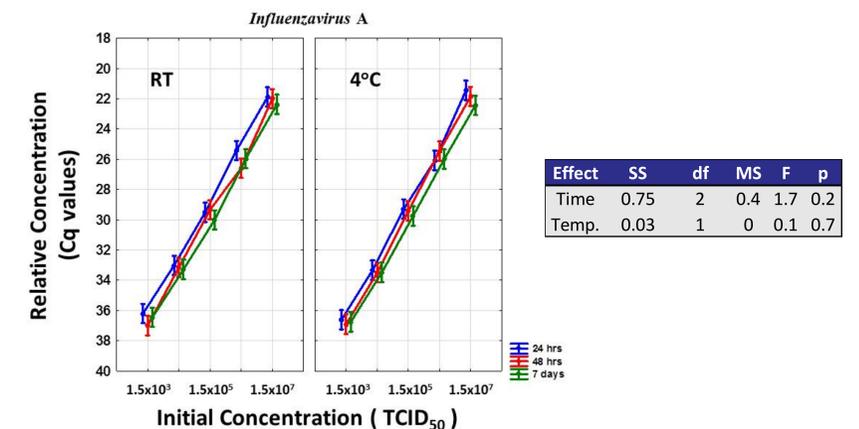


Figure 2. Quantitative RT-PCR detection of Influenza A virus RNA. Fluorescence readings were measured by BioRad CFX96™ PCR Detection System. The threshold for determining Cq value for each sample is indicated by the horizontal line. (RFU= relative fluorescence unit). Curves show samples for dilutions ranging from 1.5×10⁷ to 1.5×10³ TCID₅₀/mL. Inset shows samples (1.5×10⁵ TCID₅₀/mL) held for 1 month.



Conclusions

- Puritan Medical Product's PurSafe® and Opti-Swab® is a viable collection and transport media for the storage and preservation of RNA, allowing for the qualification and quantification of RNA suitable for genetic analysis.
- PROKARYOTE: Yields of RNA from Puritan PurSafe® were significantly greater than a comparable commercial product (p < 0.001) and were not significantly different than time zero samples. Storage temperature (4°C or room temperature) did not have a significant effect (P=0.56) on the Puritan PurSafe®'s ability to preserve RNA for genetic analysis.
- VIRAL: Storage time (1, 2, 7, 30 days) also did not have a significant effect on the Puritan Opti-Swab®'s ability to preserve viral RNA for genetic analysis. Q-RT-PCR did not detect differences in relative concentrations of samples stored for extended periods at 4°C or room temperature.

These results indicate that there was little or no degradation in RNA regardless of storage temperature or storage time. Performance of Puritan DNA/RNA® Safe in preserving RNA samples was equivalent to or better than the comparable commercial product based on total RNA yields and gel electrophoresis.