Genomic DNA Isolation from blood collected on different anticoagulants: Citrate, EDTA & Heparin

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INTRODUCTION

Stool is an excellent sample source for diagnosing cases of viral and bacterial gastroenteritis[1,2] and is also a noninvasive source of human genomic DNA from exfoliated cells of the colon, which is often used in diagnosis of colorectal cancer[3]. The isolation of high quality DNA from stool is not without its problems however. The presence of phenolic compounds, metabolites and polysaccharides in stool make the isolation of quality nucleic acid samples that are free of PCR inhibitors very challenging[4]. Furthermore, the presence of RNases and DNases in stool poses a logistical problem in the form of nucleic acid degradation that occurs during sample collection and transport[5] . Current techniques which do not make use of preservative require that stool be collected into vials, transported on ice and then frozen at -20°C when received by the diagnostic testing facility. The addition of preservative to the collection vials eliminates the need to immediately process or freeze the stool samples and allows the samples to be shipped at ambient temperature.

Norgen Biotek Corp. has developed a Stool Nucleic Acid Preservative which allows for the long-term preservation of stool samples at ambient room temperature, making this buffer ideal for stool storage and shipping. This buffer is available as a product on its own, and preserved stool samples are compatible for use with our various stool isolation kits, including our Stool DNA Isolation Kit. The Stool Nucleic Acid Preservative is an aqueous storage buffer designed to prevent the growth of contaminating bacteria and virus while preserving the DNA and RNA of pathogenic microorganisms within the sample. The preservative also inactivates pathogenic viruses while stabilizing their DNA which allows for safer handling and transport of samples. This application note illustrates that DNA subsequently isolated from the preserved samples using Norgen's Stool DNA Isolation Kit is of a high quality and can be used directly in sensitive downstream diagnostic assays such as real-time PCR.

METHODS AND MATERIALS

DNA Isolation

A single stool sample was collected from a healthy donor using Norgen's Stool Nucleic Acid Collection and Transport Tubes (Cat. 45620, 45650) according to the accompanying protocol. Various volumes of preserved sample (0.1 mL, 0.2 mL, 0.4 mL and 0.6 mL) were then processed using Norgen's Stool DNA Isolation Kit (Cat. 27600). Preserved samples were compared against 200 mg frozen stool samples not mixed with preservative. All samples were spiked with adenovirus (1.25 x 10^4 pfu) and homogenized at 4.0 m/s for 20 seconds using the FastPrep®-24 homogenizer (MP Biomedicals). All samples were then processed following the recommended kit protocol. All samples were done in triplicate in two trials.

Gel electrophoresis

For visual analysis, 10 µL of DNA from the final DNA elution was loaded onto a 1% agarose TAE gel and run for 25 minutes at 150 V alongside Norgen's HighRanger 1 kb DNA ladder (Cat. 11900). The gel photo was taken using an AlphaImagerTM IS-2200 (Alpha Innotech).

PCR Amplification

The purified DNA was then used as the template in realtime PCR reactions. For the detection of adenoviral DNA, 2 µL of isolated DNA was added to 18 µL of real-time PCR reaction mixture (TaqMan) containing 0.25 µM serotype 5 hexon gene primer mix. For the detection of genomic DNA, 2 µL of isolated DNA was added to 18 µL of real-time PCR reaction mixture (TaqMan) containing 0.5 µM of GAPDH primer mix. TaqMan reactions were run using the real-time program; 95°C for 3 minutes for an initial denaturation, 50 cycles of 95°C for 15 seconds for denaturation and 60°C for 30 seconds for annealing and extension. For the detection of bacterial DNA, 2 µL of isolated DNA was added to 18 µL of real-time PCR reaction mixture (SYBR Green) containing 1 µM 16S primer mix. SYBR Green reactions were run using the real-time program; 95°C for 3 minutes for an initial denaturation, 45 cycles of 95°C for 15 seconds for denaturation, 60°C for 30 seconds for annealing and 72°C for 45 seconds for extension. Melting curve analysis was also performed on 16S-amplified samples. The reaction was run on an iCycler iQ real-time system (Bio-Rad).

RESULTS AND DISCUSSION

Norgen's Stool Nucleic Acid Preservative allows for the preservation of stool DNA at room temperature for more than 21 weeks prior to DNA isolation. This application note illustrates that DNA subsequently isolated from thepreserved samples using Norgen's Stool DNA Isolation Kit is of a high quality, and can be used directly in sensitive downstream diagnostic assays such as real-time PCR for human and bacterial gene detection. In addition, we also determined the ideal amount of preserved stool to be used when isolating DNA with Norgen's Stool DNA Isolation Kit. In determining the ideal amount of preserved stool to use, DNA quantity and DNA quality were compared by visualizing isolated DNA on a 1% TAE agarose gel and by spectrophotometric analysis. Real-time PCR analysis of GAPDH (human genomic DNA), serotype 5 hexon (adenoviral DNA) and 16S rDNA (prokaryotic DNA) genes was also used to determine the optimal tradeoff between DNA yield and quality.

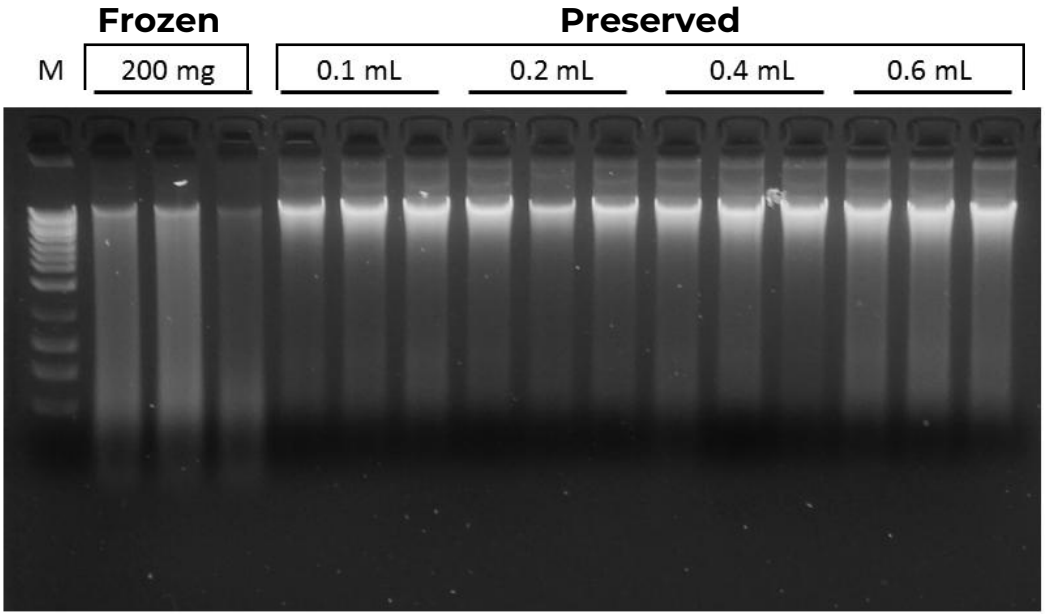

Figure 1. DNA isolated from various volumes of preserved stool samples compared to DNA isolated from 200 mg frozen stool (Trial 2). M = Norgen's HighRanger 1 kb DNA ladder.

DNA Quantity

DNA quantity was assessed using both gel electrophoresis **(Figure 1)** and spectrophotometry **(Figure 2C**). While spectrophotometry suggested that the DNA yield from frozen samples was comparable to the DNA yield from 0.4 mL of preserved samples **(Figure 2C)**, gel electrophoresis revealed that DNA isolated from all volumes of preserved samples (even as low as 0.1 mL) was far greater in yield than DNA isolated from frozen samples **(Figure 1)**. The high DNA yield of frozen samples, as generated from spectrophotometric analysis of sample concentration, was likely a result of smaller molecular weight DNA fragments from food which were not present in the preserved samples.

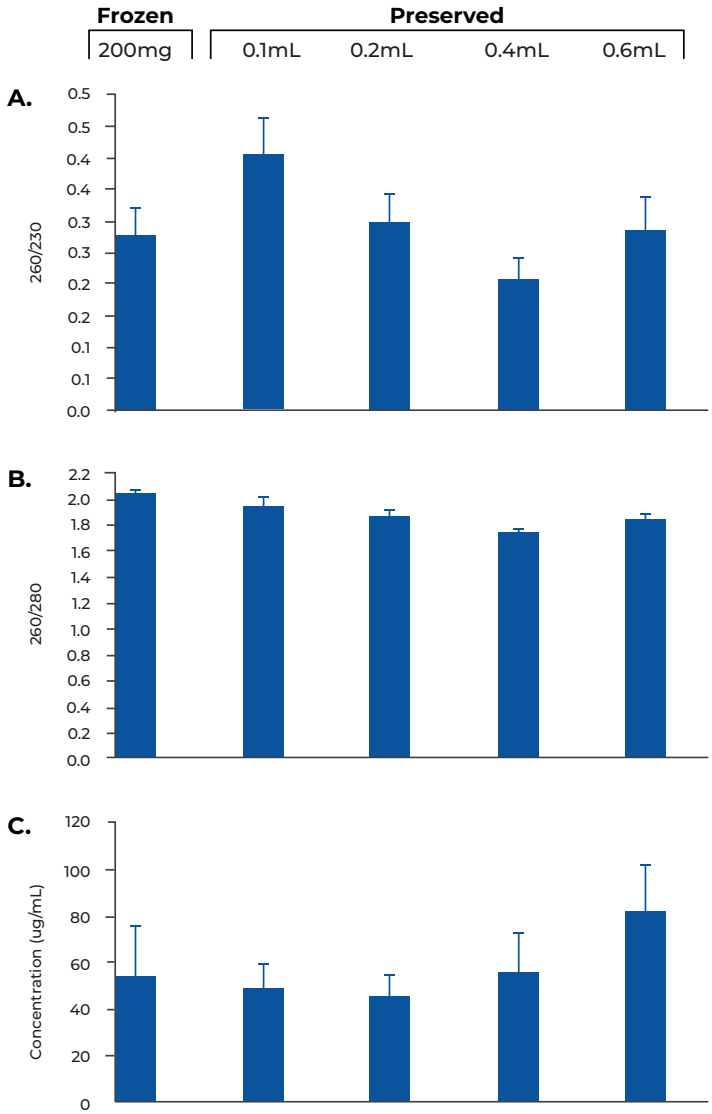

Figure 2. 260/230 ratios (A), 260/280 ratios (B) and concentrations (C) of DNA obtained by spectrophotometric analysis of 25x diluted DNA samples obtained from frozen and preserved stool samples of various amounts. Error bars represent standard error of the mean from two trials (n = 6). Measurements made using Fisher Scientific's Ultrospec 2100 Pro.

DNA Quality

DNA quality was assessed by both spectrophotometry **(Figure 2A and 2B)** and by real-time PCR **(Figure 3 and Table 1)**. When comparing the DNA quality ratios calculated by the spectrophotometer (260/230 and260/280), it is clear that the 0.1 mL preserved samples are of a similar quality, if not higher quality, than the frozen samples **(Figure 2A and 2B)**.

This is further re-enforced by the real-time PCR data for GAPDH **(Figure 3A)**, serotype 5 hexon (Figure 3B) and 16S rDNA **(Figure 3C)**. Amplification of human **(GAPDH; Figure 3A)**, adenoviral (**serotype 5 hexon; Figure 3B)** and bacterial (16S; Figure 3C) DNA reached cycle threshold (Ct) earliest in the 0.1 mL preserved samples (light green) than for any other volume of preserved sample **(Table 1)**. Absence of DNA amplification was common for 0.4 mL and 0.6 mL preserved samples likely due to higher concentration of PCR-inhibiting metabolites, and humic acids. Samples isolated from frozen stool amplified, albeit at a much higher Ct than the 0.1 mL preserved samples **(Table 1)**.

Stool	Ct											
	GAPDH				Adenovirus				16S rDNA			
	Trial 1	Trial 2	AVG	SE	Trial 1	Trial 2	AVG	SE	Trial 1	Trial2	AVG	SE
Frozen (Red)	36.9 35.7 36	34.1 34.4 34.5	35.3	0.5	19.7 19.7 19.2	18.6 18.7 20.3	19.4	0.3	23.7 22.9 23.9	19 18.9 21	21.6	0.9
0.1 mL preserved (light Green)	30.5 30.7 31.4	33.3 32.6 31.5	31.7	0.4	163.6 17.3 17.2	19.3 18.9 18.8	18.0	0.5	20.5 20.8 21.3	19.2 19.1 19	20.0	0.4
0.2 mL preserved (dark green)	32.7 32.3 36.7	32.7 34.9 33.8	33.9	0.7	17.9 17.4 22	19.8 20.5 19.9	19.6	0.7	21 21 20.8	18.6 22 19.3	20.5	0.5
0.4 mL preserved (blue)	34.6 N/A 36.6	33.6 N/A N/A	34.9	0.9	17.7 19.9 18.1	19.9 N/A N/A	18.9	0.6	21.9 29.4 22.4	19.3 N/A N/A	23.3	2.5
0.6 mL preserved (pink)	33.7 36 36.9	N/A N/A 36.5	35.8	0.7	18.1 18.4 18.3	20.8 N/A 19.9	19.1	0.5	21.9 21.9 21.4	19 N/A 19.7	20.8	0.7
NTC (Black)	N/A N/A	N/A N/A	N/A	N/A	N/A 38.5	N/A N/A	N/A	N/A	30.7 31.3	31.1 30.8	31.0	0.1

Table 1. Human genomic (GAPDH), adenoviral (serotype 5 hexon), and prokaryotic (16S rDNA) DNA detection in frozen and preserved stool samples by real-time PCR analysis following DNA isolation using Norgen's Stool DNA Isolation Kit

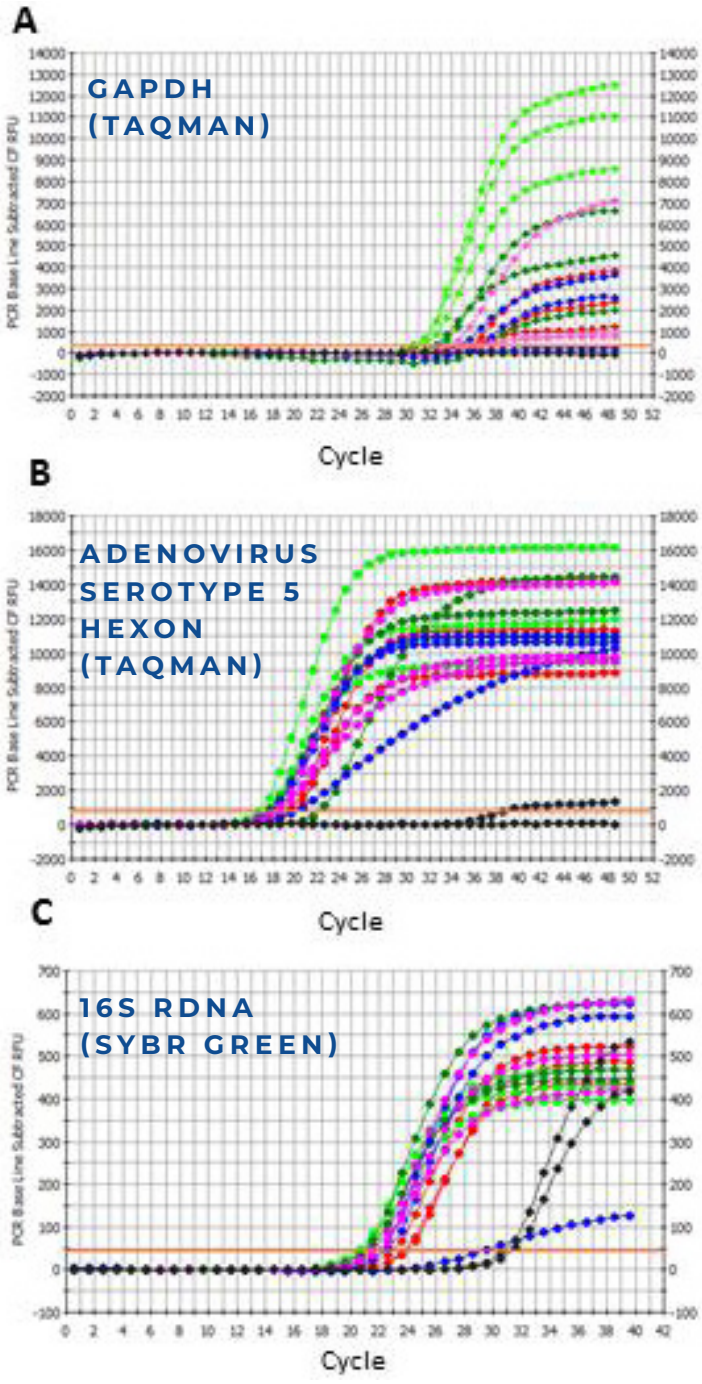

Figure 3. Human genomic (GAPDH; A), adenoviral (serotype 5 hexon; B), and prokaryotic (16S rDNA; C) DNA detection in frozen and preserved stool samples by real-time PCR analysis following DNA isolation using Norgen's Stool DNA Isolation Kit.

CONCLUSION

In evaluating DNA yield from various amounts of preserved stool sample in comparison to DNA yield from 200 mg of frozen stool, results indicate that even 0.1 mL of preserved sample yields a comparable or higher yield of DNA. Furthermore, the DNA isolated from the 0.1 mL preserved sample is of a higher quality than the frozen sample as indicated by 260/230 values and by gel electrophoresis.

Finally, when translated to real-time PCR detection of human, viral and bacterial DNA, the high quality of the DNA isolated from the 0.1 mL preserved sample results in earlier amplification, making this volume of preserved sample ideal for use with Norgen's Stool DNA Isolation Kit.

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