Viability Of Microorganisms in Saliva & Stool Collection and Transportation Preservatives

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Booth #662

Two preservatives were introduced several years ago from Norgen Biotek Corp. to stabilize nucleic acids (NA) in saliva samples (Saliva DNA Collection and Preservation Devices) and stool samples (Stool Nucleic Acid Collection and Transport Tubes) for transportation at ambient temperature. Prior studies have shown that the preservatives could decrease the variability of microbiota authenticity during transportation and improve the NA quality and quantity. The objective of this experiment was to determine whether microorganisms remain viable in these preservatives and for how long. In this study, the viability of three microorganisms (*Escherichia coli*, *Listeria monocytogenes* and *Saccharomyces cerevisiae*) were monitored over time (0, 4, 8 and 24 hours) with or without preservatives. At each time point, samples were spotted on specific media and were then incubated at 37°C for *E. coli* and 30°C for *L. Monocytogenes and S. cerevisiae,* then analyzed for growth at 24-48 hr after plating. Total DNA was also isolated at each time point to see the effect of the preservative on cell mortality and cell lysis using qPCR. Our results showed the organisms tested became non-viable within 8hrs in stool preservative while their growth is zero after an hour. These results demonstrate that the saliva and stool NA Collection and Transport Tubes/Devices are effective at disinfecting microorganisms. DNA analysis also showed evidence that the preservatives effectively lysed microorganisms in 24 hours. Overall, Norgen's preservatives (stool and saliva) were not only able to preserve NA but also at kill microorganisms in a short period of time in clinically important human specimens. This may be very beneficial and a convenient method to handle potentially infectious human specimens at core facilities.

Introduction

Norgen Biotek's preservatives have proven the fact that they can effectively stabilize nucleic acids from a variety of human specimens including stool, saliva, urine, and blood during temperature-variable transportation and handling conditions. Recently, the importance of sample handling at labs or core facilities has risen due to the prevalence of secondary infection from human specimens, which must be treated as infectious materials.

In this study, we evaluated the viability of various microorganisms in both stool and saliva preservatives to elucidate the effectiveness of Norgen's preservatives as static agents, capable of instantly inactivating microorganism viability.

Methods & Materials

Table 1. Summary of plating methods and contents.

Specimens		Positive control (culture)	PBS mixed		Preservative mixed	
			Saliva (1:1)	Stool (1:2)	Saliva (1:1)	Stool (1:2)
No spiking		N/A	1	1	2	2
Spiking	*E.coli* (as a G-)	10^8 cfu	3	3	3	3
	L. monocytogenes (as a G+)	10^8 cfu	3	3	3	3
	S. cerevisiae (as a yeast)	10^8 cfu	3	3	3	3

- With the remaining culture, DNA was isolated using Norgen's Saliva DNA Isolation Kit (Cat # RU45400) and Stool DNA Isolation Kit (Cat # 27600), and subsequently analyzed using agarose gel electrophoresis
- Three µL of each DNA sample was used in a 20µL PCR reaction, with an in-house qPCR Master Mix, and specific primers and probes for GADPH and 16S rDNA analysis. The qPCR amplification cycle was 95 °C for 15 seconds, 60 °C for 30 seconds, and 72 °C for 45 seconds x 40 cycles.

Results & Discussion

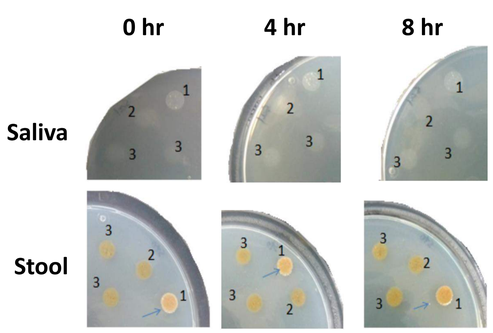

Figure 1. Viability of the microorganism *L. monocytogenes* spiked in both the saliva preservative and the stool preservative. As well as saliva and stool mixed with only preservative or only 1X PBS.

Table 2. Cell viability results. Cell viability rate: ~10^8 cfu = ++++ > +++ > ++ > + > 0 cfu = No growth

Time Incubated with the Preservative at RT		Saliva Preservative				Stool Preservative			
		0 h	4 h	8 h	24 h	0 h	4 h	8 h	24 h
In 1X PBS		+++	+++	+++	+++	+++	+++	+++	+++
In Preservative	*E.coli* (as a G-)	0	0	0	0	0	0	0	0
	L. monocytogenes (as a G+)	0	0	0	0	0	0	0	0
	S. cerevisiae (as a yeast)	0	0	0	0	0	0	0	0

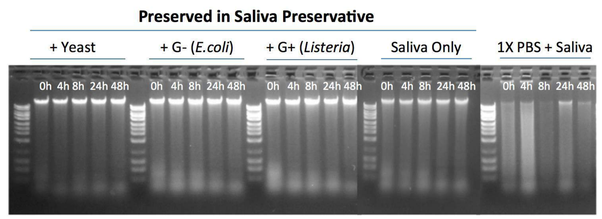

Figure 2. Resolution of an agarose gel containing saliva preservative spiked with the three different microorganisms, as well as saliva containing only 1X PBS

- Regardless of the spiked microorganisms, DNA preserved in Saliva Preservative shows maintained DNA integrity and quantity compared to 1X PBS
- Preservative ensures DNA profile is the same at time of collection vs. analysis

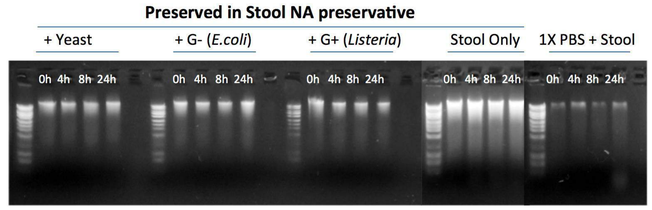

Figure 3. Resolution of an agarose gel containing stool preservative spiked with the three different microorganisms, as well as stool containing only 1X PBS

- Regardless of the spiked microorganisms, DNA preserved in Stool Preservative shows maintained DNA integrity and quantity compared to 1X PBS
- Greater DNA yield in the stool sample only, due to the instant cell lysis of microorganisms by the preservative

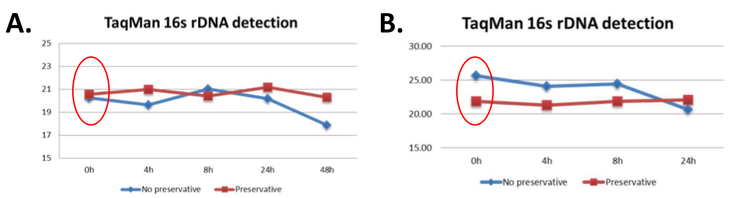

Figure 4. Real Time PCR of 16s rDNA results indicating the Ct value over a period of 48 hours for the (A) Saliva sample and (B) stool sample.

- The circles indicate two different modes of actions: A) Saliva preservative - Inactivation of cell viability, B) Stool preservative - Instant cell lysis
- No Preservative (PBS) affected the population variance with time, resulting in alteration of the original microbiome data
- DNA profile at time of collection is maintained by preservative

Conclusions

1. Norgen's preservatives (Saliva and Stool) were able to inactivate instantly the microorganisms' viability at 0h (*E. coli, L. monocytogenes,* and *S. cerevisiae*)
2. The mode of action of Norgen's stool preservative was slightly different from the saliva preservative in that it could lyse cells immediately, at the point of the mixing (0 h), resulting in a higher DNA yield (Figure 3) and earlier Ct from 16s rDNA detection (Figure 4). In contrast, the saliva preservative could inhibit the growth of microorganisms without lysis, whilst effectively stabilizing the DNA.
3. The benefit of this study may be found in the transportation and handling of potentially infectious human specimens for a core lab facility where high amounts of samples are to be stored and handled.

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