NORGEN BIOTEK CORP.

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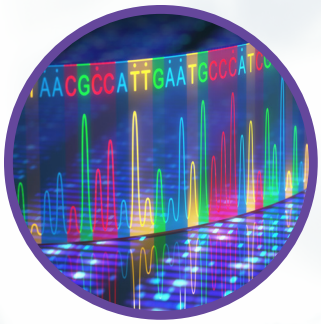
First Issue!

Microbial Digest



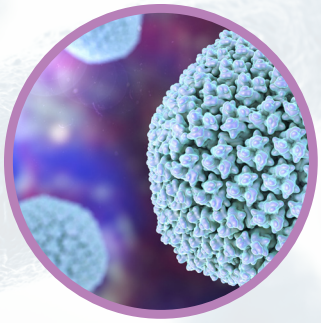
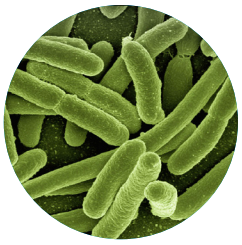


Table of Contents

Demographics



Pathogen Detection

Pediatrics

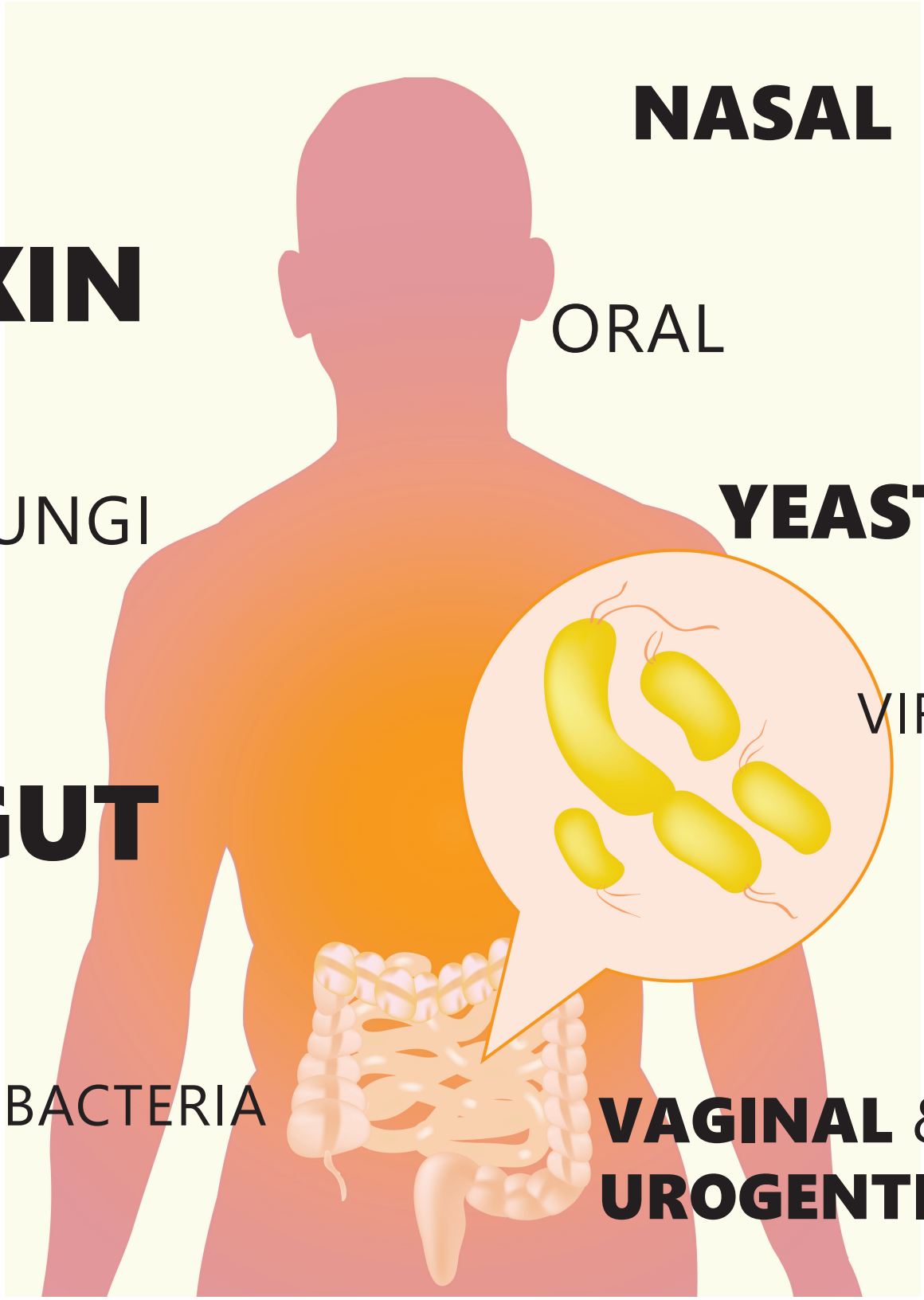
Pharmacology

Therapeutics

Nutrigenomics/ Probiotics

Cancer Screening

The Human Microbiome



What is a microbiome ?

The NIH Human Microbiome Project defines the microbiome as the collective genomes of the microbes (composed of bacteria, bacteriophage, fungi, protozoa and viruses) that reside inside living beings.[1] The human microbiota consists of the 10-100 trillion symbiotic microbial cells, primarily contained within the gut, but also in the mouth, the skin, the nose, the vagina, etc. The human microbiome consists of the genes these cells harbour.[2]

Various terminologies can create confusion: for example, "microbiota" (the microbial taxa associated with humans) and "microbiome" (the catalog of these microbes and their genes) are often interchanged. In addition, "metagenomics" originally referred to shotgun characterization of total DNA, although now it is increasingly being applied to studies of marker genes such as the 16S rRNA gene."[3]

Why is it so important ?

Bacterial Cells > Human Cells: We contain about 10 times as many microbial cells as human cells.[1] The human gut microbiota consists of many different types of bacteria - over 1000 bacterial species have been identified. However, the presence and prevalence of these bacteria changes person to person, with only approximately 160 species per person per fecal sample. This variance is based on many factors: bacterial infections, antibiotic treatment, lifestyle, surgeries, dietary changes or other health changes.[4]

Impact on Health: An analysis of the full gene content and composition of the microbiomes living in the human body (i.e. the metagenome) predicts that there may be more than 8 million unique microbial genes associated with the microbiomes across the human body of healthy adults. When compared to the total number of human genes, this suggests that the genetic contribution of the microbiome to the human supraorganism may be many hundreds of times greater than the genetic contribution from the human genome.[1] This means that the human gut microbiome can have a profound effect on the types of diseases we encounter and how we heal/recover.

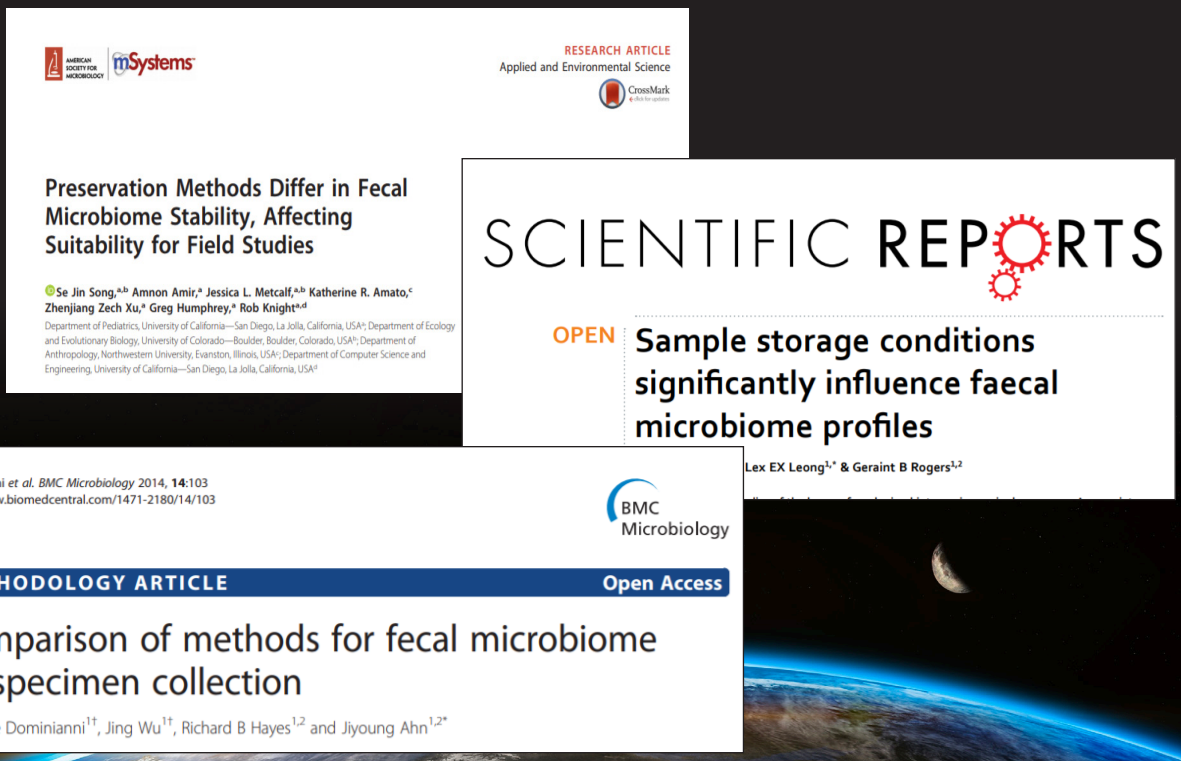
References

[1] Yang J. The Human Microbiome Project: Extending the definition of what constitutes a human . NIH Human Genome Project. 2012.
[2] Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The human microbiome project. Nature. 2007;449:804–810.
[3] Ursell LK, Metcalf JL, Parfrey LW, Knight R. Defining the human microbiome. Nutrition reviews. 2012;70 Suppl 1:S38-44.
[4] Rodríguez JM et al. The composition of the gut microbiota throughout life, with an emphasis on early life. Microbial ecology in health and disease. 2015;2;26:26050.

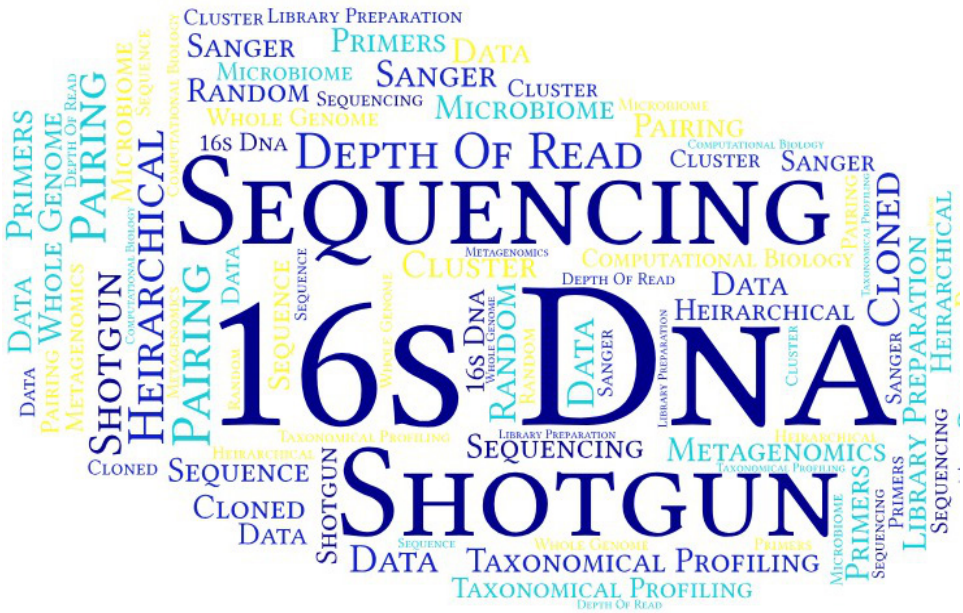
The Importance of Sample Collection & Stabilization

Maintaining the integrity of samples collected is a major challenge in microbiome research. From the point of collection, the microbial composition of a sample can begin to change. For this reason, freezing is often considered the gold standard. However, this is not always an option when collections are done off-site or in remote locations. In addition, cold chain transport and storage can be costly and therefore prohibit the number of samples you can collect/analyze. Thus, use of a chemical preservation system is becoming widely accepted by researchers as they try to circumvent bias in data analysis.

Emerging microbiome applications call for better sample collection methods



STABLE SAMPLES



REPRODUCIBLE RESULTS

Is Chemical Stabilization the new GOLD STANDARD?

Key Advantages

- **Stabilize microbial profiles from point of collection:** Stable samples are the key to reproducible results
- **Bacteriostatic:** Stop microbial growth from point of collection
- **Preserve nucleic acids at ambient temperature:** No need for costly and inconvenient cold chain shipping & storage
- **Renders samples safe & non-infectious:** Inactivate viruses, ship with peace of mind
- **Non-invasive home-based collection:** Enables donors to provide samples from the comfort of their home
- **Ensure sample homogeniety:** Minimize bias in data analysis

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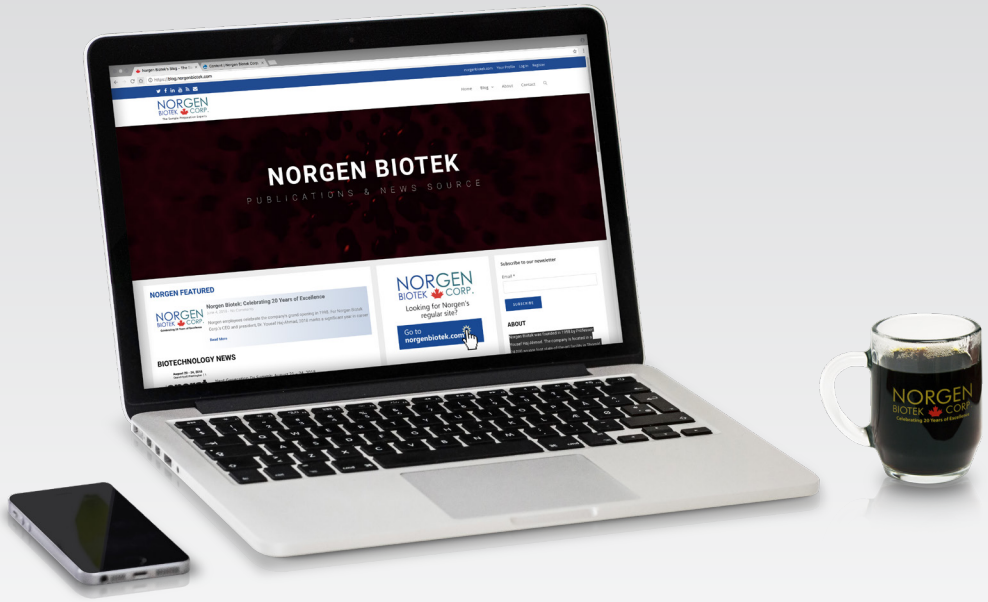
Publications & News Source



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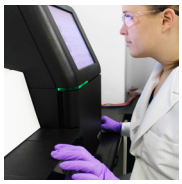
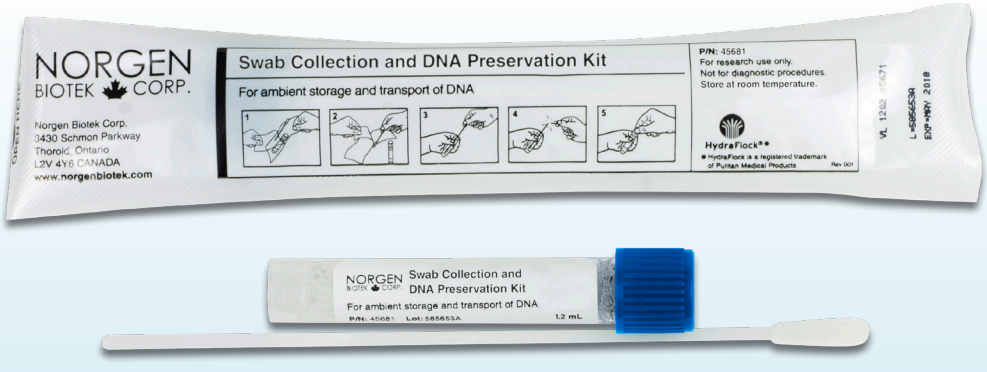


NORGEN BIOTEK CORP.

Stabilize **Microbiota Profiles** From Point of Collection

- Collect from nasal, buccal, saliva, fecal, skin, surfaces, and more
- DNA preservation at room temperature over 2 years
- Compatible with Norgen's **Microbiome DNA Isolation Kit** (Cat. 64100)

Swab Collection and DNA Preservation Kit (Cat. 45690)



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SPOTLIGHT Microbial Diversity

Comparison of Stool DNA Preservation Methods and their Impact on Gut Microbiome Diversity

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INTRODUCTION

Appropriate preservation and storage of stool samples is crucial in maintaining DNA fidelity and microbial community composition for downstream applications and analysis, including next-generation sequencing (NGS) and microbiome characterization. Post-collection bias may be introduced in cases where sample material is inadequately preserved, highlighting the importance of preservation in study design. Currently, rapid freezing to −80°C is considered best-practice for preserving stool samples; however, this modality may be unfeasible when sample collection takes place in non-laboratory settings, such as in participants' homes.

This application note investigates differences in microbiota authenticity, DNA concentration, and DNA fidelity when stool is preserved by rapid freezing to −80°C, using Norgen Biotek's Stool Preservative, or using TE buffer. Following storage for 1, 3, and 6 days, stool DNA was isolated from two samples for each preservative condition and several parameters were assessed, including DNA concentration, 260/230 ratio, and 260/280 ratio. Next-generation sequencing of the fecal microbiome was also performed. Norgen Biotek's Stool Preservative was associated with the greatest DNA concentration, and the least divergence in microbiota diversity and composition throughout the study. Similarly, rapid freezing was associated with minimal divergence in microbiota diversity and composition. However, samples preserved using TE buffer showed substantial divergence in microbiota diversity.

Therefore, this application note illustrates the utility of Norgen Biotek's Stool Preservative for accurate, stable characterization of the gut microbiome, and shows that this preservative can be used in cases where freezing and coldchain transport is unavailable.

MATERIALS AND METHODS

Stool sample collection and processing

Stool samples were collected from two healthy donors and three 200 mg aliquots from each donor were immediately applied to each different storage condition; ambient temperature, -80˚C, TE buffer (pH 8.0) and Norgen's Stool Nucleic Acid Collection and Preservation Tubes (Cat. 45630, 45660) containing Norgen's Stool Preservative. The aliquots were used in order to minimize the potential of inter-subject variation.

Stool DNA extraction and quantification

DNA extraction was performed using Norgen's Stool DNA Isolation Kit (Cat. 27600). Inputs of 200 µL were used from either the TE buffer/stool mixture or Norgen's Stool Preservative/stool mixture and 200 mg from fresh or frozen stool samples for Day 0, Day 1, Day 3 & Day 6. Stool samples were bead homogenized after adding 800 µL of Lysis Buffer L for preserved samples or 1 mL for fresh and frozen samples. Next, the samples were centrifuged and 100 µL of Binding Buffer I was added to the clean supernatant and incubated on ice for 10 minutes. Equal amounts of 70% Ethanol were then added to the clean supernatant from Binding Buffer I lysate after centrifugation. The protocol was then followed as written to complete the DNA isolation. For visual inspection of genomic DNA, 10 µL of the elution was loaded onto a 1.2 % agarose TAE gel and run for 30 minutes at 150 V alongside Norgen's HighRanger 1 kb DNA ladder (Cat. 11900). Gel photos were taken using an AlphaImager™ IS-2200 (Alpha Innotech). The purified DNA from each of the four conditions was quantified with the A260, A280 A260/A230 and A260/A280 ratios using the NanoDrop™ 2000 Spectrophotometer.

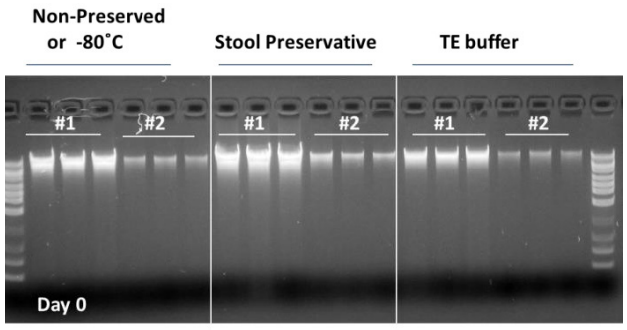
16S rRNA gene sequencing

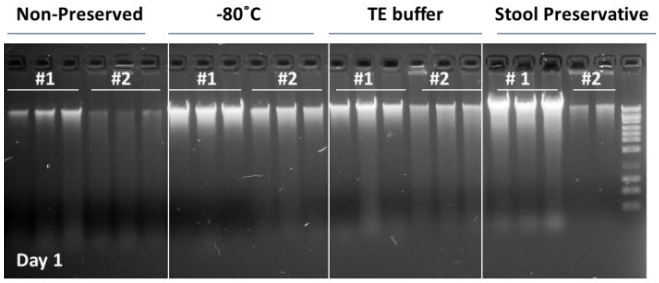
The V3-V4 hypervariable region of the bacterial 16S rRNA gene was amplified from 12.5 ng of stool DNA quantified by PicoGreen. The amplicons were then cleaned, Dualindexed with the Nextera XT Index Kit (Illumina Inc., San Diego, CA, USA) according to the

manufacturer's instructions and sequenced according to the Illumina MiSeq 16S Metagenomic Sequencing Library Preparation protocol). The final library was paired-end sequenced at 2 × 300 bp using a MiSeq Reagent Kit v3 on the Illumina MiSeq platform. Sequencing was performed at Norgen Biotek. For Bioinformatic analysis, the sequencing data was analyzed using the Illumina 16S metagenomics app.

RESULTS AND DISCUSSION

Stool samples were collected from two healthy donors, yet the DNA yield was notably different, thus indicating the diversity of microorganisms between individuals. In general, Donor Sample #1 demonstrated higher DNA yields than Donor Sample #2 regardless of the preservation method used. The DNA yield was substantially reduced within 24 hours from non-preserved samples after storage at room temperature. In contrast, samples preserved in Norgen's Stool Preservative or stored at -80˚C showed a consistency in DNA yield up to 6 days (Figure 1, 2). Interestingly, the DNA yield from samples stored in TE buffer seemed consistent on the gel over time (Figure 1), but Figure 2 showed more detailed changes with DNA yield decreasing in Sample #1 and increasing in Sample #2 during the storage period. This fluctuation of DNA yield may have been influenced by the change of microorganisms' growing condition (aerobic or anaerobic) during the storage (Figure 2).





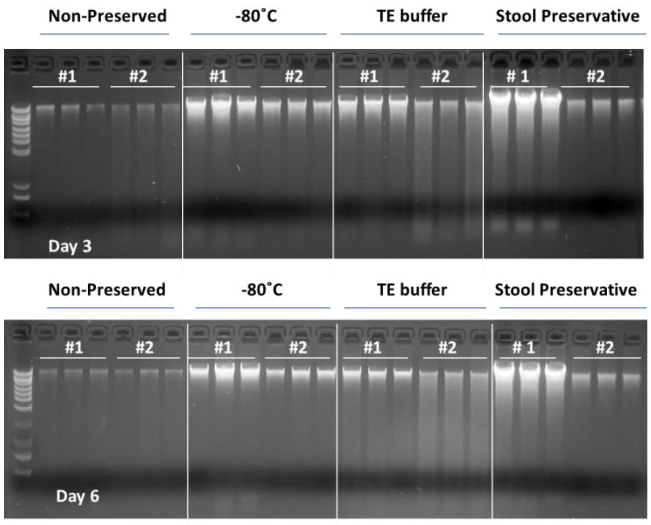


Figure 1. Isolation of DNA from Stool Preserved Using Different Preservative Methods for Day 0, 1, 3 and 6. DNA was isolated using Norgen's Stool DNA Isolation kit (Cat. 27600) and 10 µL of elution was loaded on 1.2% 1x TAE Agarose gel.

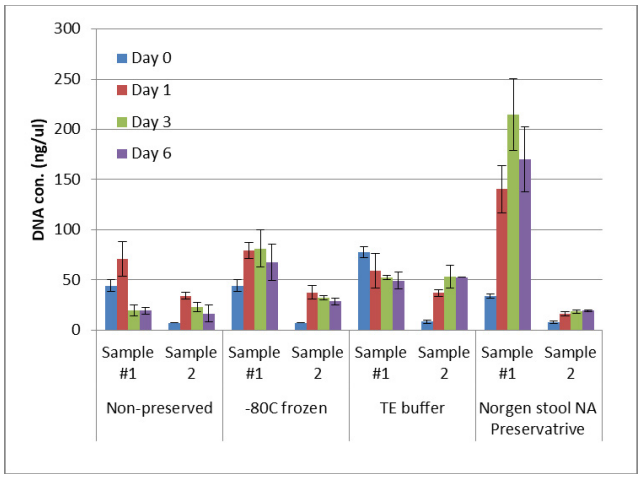


Figure 2. DNA Concentration of Samples Isolated from Stool Preserved Using Different Preservative Methods Over Six Days.

In terms of DNA quality (260/230 and 260/280), the nonpreserved fresh sample had a decrease in the ratio over time from both Sample #1 and #2 (Figure 3 and 4). The change of 260/230 ratio was quite noticable in the nonpreserved samples, as well as the -80°C storage condition, while samples preserved in Norgen's Stool Preservative showed minimal variation in 260/280 and 260/230 ratio over the 6 days (Figure 3 and 4).

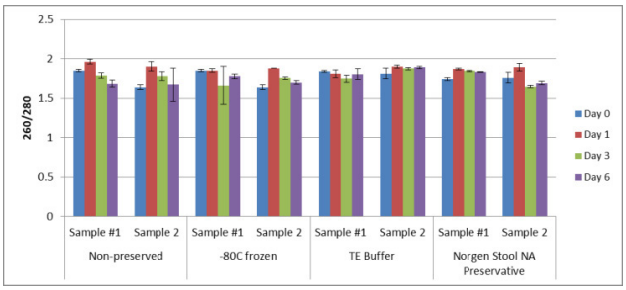


Figure 3. 260/280 Ratio of Samples Isolated from Stool Using Different Preservative Methods Over Six Days. The 260/280 ratio of the -80˚C frozen samples and the samples preserved in Norgen's Preservative demonstrated values closest to 1.8 over the six day period. The non-preserved samples were associated with a continual decline in the 260/280 ratio, indicative of lesser DNA quality.

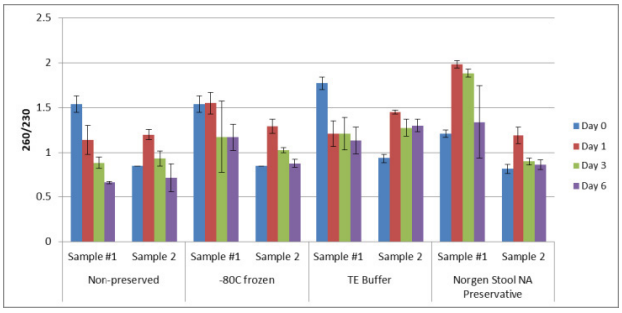


Figure 4. 260/230 Ratio of Samples Isolated from Stool Using Different Preservative Methods Over Six Days. The non-preserved samples were associated with a continual decline in the 260/230 ratio, indicative of contamination and lesser DNA quality. A similar decline was observed for the TE-preserved samples. The Norgenpreserved and rapid freezing samples were associated with 260/230 ratios closest to 1.8 over the six day period, indicative of minimal salt contamination.

The effect of storage conditions on faecal microbiota was assessed based on the relative abundance of bacterial phyla.The results indicated a substantial divergence in the non preserved sample by Day 1, with an increase in Thermi, a decrease in Tenericutes, and a decrease in Nitrospirae. Gradual divergence was also observed in TE-preserved samples, with a decrease in Tenericutes and an increase in Thermi.

Norgen's Stool Preservative and rapid freezing at -80˚C were associated with the least divergence in microbiota diversity and composition throughout the study, with the abundance of Thermi and Tenericutes varying minimally over time (Figure 5). Therefore, Norgen's Stool Preservative and rapid freezing provided the most accurate, representative measurements of bacterial phyla abundance

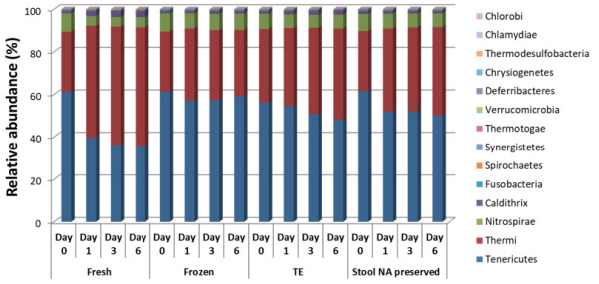


Figure 5. Relative Abundance of Bacterial Phyla in Stool DNA Preserved Using Different Methods. Day 0 is reflective of the microbial community composition at the time of collection; measurements thereafter reflect divergence from this baseline composition.

CONCLUSIONS

1. **Norgen's Stool Preservative is recommended for optimal DNA fidelity and concentration** — Quality and quantity of DNA rapidly decrease unless frozen at -80°C or preserved in Norgen Biotek's Stool Preservative.

2. **Norgen's Stool Preservative is associated with the least divergence in microbiome** — Norgen's Stool Preservative and rapid freezing were associated with the least divergence in microbiota diversity and composition throughout the study.

SPOTLIGHT DNA Fragment Size

Determination of the DNA Molecular Weight (MW) from different Norgen Columns and Isolation Methods

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INTRODUCTION

The isolation of large molecule weight DNA is required for certain sequencing applications such as paired-end sequencing of long-range DNA fragments for de novo assembly of mammalian genomes. It has been a belief that DNA isolated using a spin column is fragmented, and that the maximum size isolated is approximately 25 kb. Here, Norgen Biotek investigated the molecular weight (MW) of DNA purified using spin column chromatography based on five different column types (Micro Spin Column, Mini Spin Column, Midi Spin Column, Maxi Spin Column, 96-Well plate) as well as purification based on magnetic beads and alcohol precipitation.

MATERIALS AND METHODS

DNA Sample preparation

Fifty million HeLa cells were pelleted, and 15 mL of Buffer SK was added to lyse the cells (~3.3 M cells/mL). Different lysate volumes and centrifuge speeds were then applied according to the various column types and isolation methods as indicated in Table 1.

Table 1. Specifications of the type of column and method used for DNA isolation.

	Method used for DNA isolation	Lysis input volume	Centrifuge speed (RPM)
1	Micro Spin Column	0.5 ml	6000 rpm
2		0.5 ml	6000 rpm
3	Mini Spin Column	0.5 ml	6000 rpm
4		0.5 ml	6000 rpm
5	Midi Spin Column	1.5 ml	3000 rpm
6		1.5 ml	3000 rpm
7	Maxi Spin Column	3 ml	3000 rpm
8		4 ml	3000 rpm
9	96-Well Plate	0.5 mL	3000 rpm
10		0.5 mL	3000 rpm
11	Magnetic bead	1.5 mL	N/A
12	Alcohol ppt	1.5 mL	N/A

* 50 M HeLa cells in 15 mL of Buffer SK

Gel electrophoresis

For visual analysis, 10 µL of DNA from the elution was loaded onto a 1.2% 1x TAE agarose gel and run for 25 minutes at 150 V. The gel photo was taken using an AlphaImagerTM IS-2200 (Alpha Innotech).

Pulsed-Field Gel Electrophoresis (PFGE)

The purified DNA was used for Pulsed-Field Gel Electrophoresis (PFGE) for better resolution of the MW range. One µg of DNA was loaded on the PFGE gel and run at 4 V for 16 hours followed by 6 V for 6 hours.

RESULTS AND DISCUSSION

The profile of DNA isolated using Norgen's different spin columns and isolation methods was compared between 1.2% 1x TAE gel (Fig. 1) and PFGE (Fig. 2).

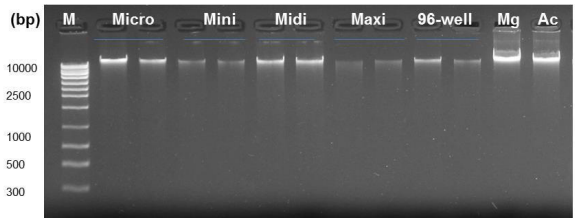


Figure 1. DNA resolution on 1x TAE 1.2% agarose gel. DNA was isolated using five column types (Micro Spin Column, Mini Spin Column, Midi Spin Column, Maxi Spin Column, 96-Well Plate) by spin column chromatography as well as two other isolation methods (Mg: Magnetic bead system, Ac: Alcohol precipitation). Lane M is Norgen's HighRanger 1 kb DNA Ladder (Cat# 11900).

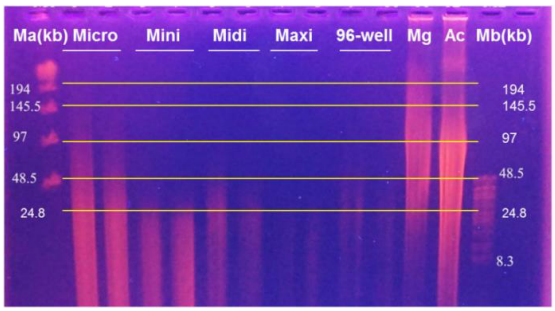


Figure 2. PFGE profile showing the range of the different DNA MW isolated using five column types (Micro Spin Column, Mini Spin Column, Midi Spin Column, Maxi Spin Column, 96-Well Plate) by spin column chromatography as well as two other isolation methods (Mg: Magnetic bead system, Ac: Alcohol precipitation). Lane Ma: NEB Lambda PFG ladder Cat # N0341. Lane Mb: Bio-Rad Chef DNA size standards: 8-48kb, Cat# 170-3707.

CONCLUSIONS

1. PFGE reveals that the molecular weight (MW) of the DNA isolated using Norgen's columns and methods is larger than 25 kb in general.

2. The centrifuge speed applied to the different size of columns, including the 96-well plate, did not affect the MW of the purified DNA.

3. Isolation using magnetic beads or alcohol precipitation isolated higher MW DNA. 4

4. A detailed summary of the variation in DNA MW is presented in Table 2.

Table 2. Overview of PFGE results showing variable DNA MW from different column types and isolation methods.

Method used for DNA isolatio	% of DNA molecular weight (MW)			
	>100 kb	50-100 kb	25-50 kb	<25 kb
Micro Spin Column	3l	35	35	27
	3	35	35	27
Mini Spin Column	0	5	45	55
	0	5	45	55
Midi Spin Column	0	10	50	40
	0	10	50	40
Maxi Spin Column	0	10	50	40
	0	10	50	40
96-Well Plate	0	10	50	40
	0	5	45	55
Magnetic bead	10	50	30	10
Alcohol ppt	5	40	40	15

Table 3. Examples of Norgen's kits that utilize the different types of columns and methods for DNA isolation from various sample types.

	Method used for DNA isolation	Example of Norgen	Cat No.
1	Micro Spin Column	Cells and Tissue DNA Isolation Micro Kit	57300
2		Urine DNA Isolation Micro Kit	18100
3	Mini Spin Column	Saliva DNA Isolation Kit	RU45400
4		Stool DNA Isolation Kit	27600
5	Midi Spin Column	Blood DNA Isolation Midi Kit	51400
6		Urine Cell-Free Circulating DNA Purification Midi Kit	56700
7	Maxi Spin Column	Soil DNA Isolation Maxi Kit	62000
8		Urine Cell-Free Circulating DNA Purification Maxi Kit	56800
9	96-Well Plate	Soil DNA Isolation 96-Well Kit	26560
10		Fungi/Yeast Genomic DNA Isolation 96-Well Kit	27350
11	Magnetic bead	Stool DNA Isolation Kit (Magnetic Bead System)	55700
12	Alcohol ppt	Saliva DNA Collection, Preservation and Isolation Kit	RU35700

SPOTLIGHT Sample Transport

Effect of Stool Transportation Conditions on Microbiota Diversity

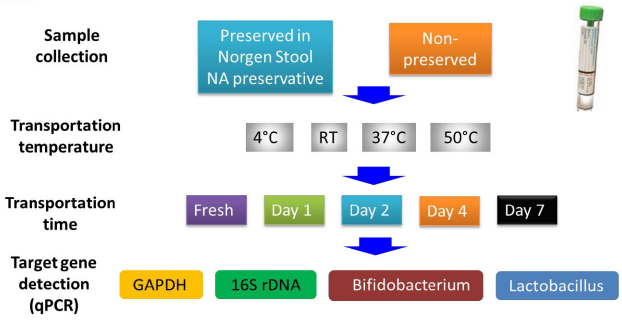
Abstract

Nucleic acid (NA) based applications are a powerful tool driving research in the area of human cancer marker discovery, pathogen diagnosis and genetic tests. These nucleic acids are isolated from many different human specimens, and it is known that the method of sample transportation prior to nucleic acid purification can influence the sample's stability, greatly affecting the NA content and therefore resulting in data variability. Despite this knowledge, however, the importance of sample preservation and transportation has not been extensively studied before. Therefore, this study focuses on the effect of transportation conditions, particularly temperature fluctuation, on microbiota diversity in human stool. A number of different microbial targets were monitored by qPCR from stool samples exposed to 5 different temperatures, with and without the addition of a stool NA preservative (Norgen Biotek Corp.). The results indicate an intriguing observation in that the population of a certain microorganism was affected by the temperature and the incubation time, resulting in variability of the microbial population analysis as demonstrated by qPCR.

W.-S. Kim, PhD[1], Nezar Rghei[1] and Yousef Haj-Ahmad, PhD[1, 2]

[1] Norgen Biotek Corporation, Thorold, Ontario, Canada
[2] Brock University, St. Catharines, Ontario, Canada

MATERIALS AND METHODS



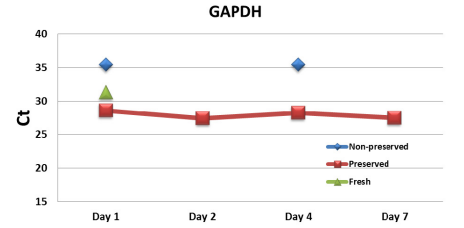
Outline of the experiment set up . Samples were collected from healthy donors and split into the 4 collection tubes with or without Norgen stool nucleic acid (NA) preservative. Each tube was incubated at a different temperature to simulate the various transportation conditions. Total DNA then was isolated at different time points, and the DNA was directly used for real-time PCR (qPCR) for the target gene monitoring.

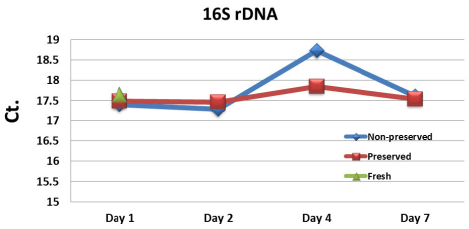
RESULTS

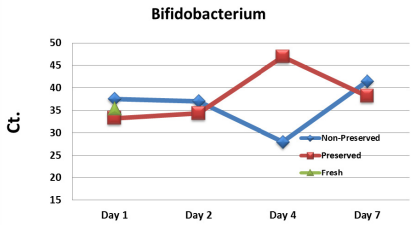
- Preservativenaffects stool DNA quality (A260/280 and A230/280) and quantity (concentration) for the variable sample transportation conditions.

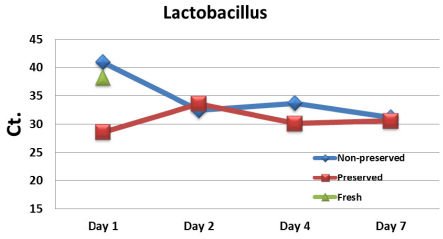
- **Preservative was able to provide more reliable gene stability, as indicated by real-time PCR (SYBR Green).** Human (GAPDH), Gram negative (16S rDNA) and two gastrointestinal Gram positive bacteria were monitored from the stool samples stored at ambient or 50°C temperature with/without Norgen's stool nucleic acid (NA) preservative.

AMBIENT TEMPERATURE

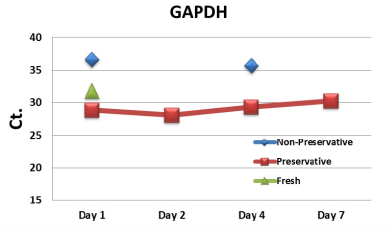


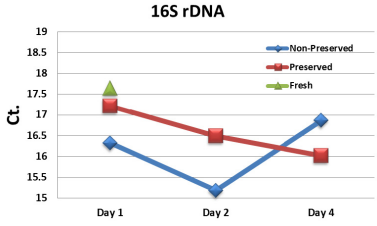


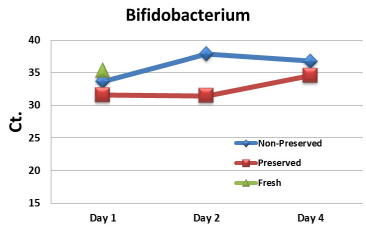


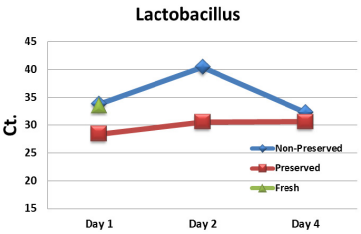


50°C





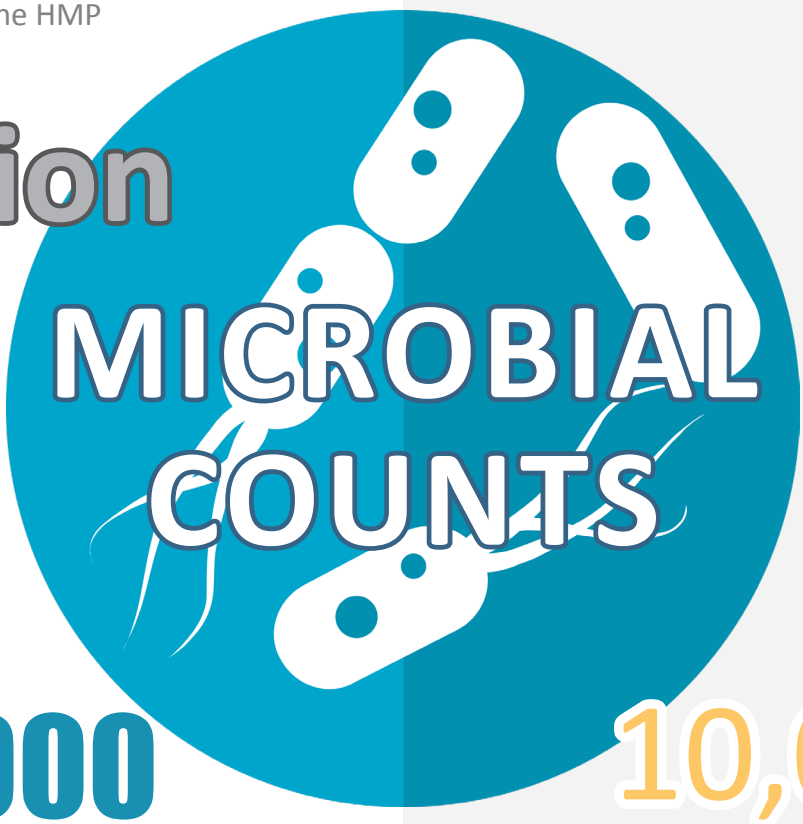




SUMMARY

- Preservation of biological information at the site of sample collection, without altering the original nature of the specimens, is critical.
- The human gene GAPDH was well stabilized in preserved stool samples, even with a temperature shift (RT to 50°C)
- Stool preservative improves the DNA quality (A260/230) and yield from the samples being transported at RT, warm (37°C) or hot (50°C) temperature conditions.
- The population of Gram negative bacteria (indicated by 16s DNA gene) was found to change dramatically without the preservative during the simulated transportation period.

MICROBIAL COUNTS



10:1

Reported ratio of microbial cells to human cells, prior to the start of the HMP

$170 million

Funding received by the Human Microbiome Project (HMP) by the NIH Common Fund[6]

2 million

Number of people per year in the US infected with bacteria that is resistant to antibiotics[7]

1-2kg

Weight of the microbes living inside your gut (roughly the weight of your brain)

23,000

Number of deaths per year due to antibiotic resistant infections[7]

10,000

Number of different species occupying the human body[8]

364x

Number of genes the microbiome provides that contribute to human survival vs. the human genome (8 million vs. 22,000)[8]

References

[6] NIH Common Fund: https://commonfund.nih.gov/hmp
[7] https://www.cdc.gov/drugresistance/index.html
[8] https://newsinhealth.nih.gov/2012/11/your-microbes-you

SPOTLIGHT Microbial Inactivation

Inhibitory Effect of Norgen's Saliva DNA Preservative on the Growth of Bacteria and Yeast

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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 saliva. In contrast to blood samples, saliva can be self-collected, is less costly to ship and easier to store and process. Human genomic DNA extracted from buccal epithelial cells and white blood cells found in saliva can be used in various applications including diagnostic assays, epidemiological studies and surveys.

Norgen Biotek Corp. has developed a Saliva DNA Preservative which allows for the long-term preservation of saliva samples at ambient temperature, making this buffer ideal for saliva storage and shipping. This buffer is available as a product on its own, and is also included with our different saliva DNA collection, preservation, shipping, storage and purification devices and kits. The Saliva DNA Preservative is an aqueous storage buffer designed for rapid cellular lysis and subsequent preservation of saliva DNA from fresh specimens. This buffer stabilizes the DNA for long-term storage at ambient temperature. Here, we show that the buffer also prevents the growth of microorganisms, thereby resulting in a non-infectious sample that can be handled and shipped safely, as safety is a primary concern when working with any human samples including saliva.

MATERIALS AND METHODS

Bacteria and Yeast

Two microorganisms were used in this study. The first is the bacteria *Escherichia coli* DH5α and the second is the yeast *Saccharomyces cerevisiae*.

Incubation and plating

Microorganisms were grown overnight in LB (for bacteria) and YPD (for yeast). The next day, the copy number of each culture was counted and 1 million cells of each microorganism were pelleted and resuspended in 500 µL of fresh saliva preserved in Norgen's Saliva DNA Preservative. From each condition five 100 µL aliquots were plated on agar-LB plates (for bacteria) and agar-YPD (for yeast). Controls from both microorganisms were pelleted and resuspended in 500 µL of LB or YPD with no Preservative, Saliva/Medium mixture and Medium / Preservative mixture (Table 1). All treatments were plated as mentioned earlier. Plates were incubated overnight at 37°C (bacteria) or 30°C (yeast). The next day, colonies from each plate were counted.

RESULTS AND DISCUSSION

Determination of microorganism growth rate in the saliva/preservative mixture is important to estimate the related risk in preserving saliva samples that contain potential infectious microorganisms. In the present study, no growth was detected from microorganisms mixed with the preservative and saliva, or preservative and growth medium. However, other conditions that did not contain the preservative showed positive growth (Table 1). Therefore Norgen's preservative is inhibiting the growth of bacteria and yeast, thereby resulting in non-infectious samples that can be handled and shipped safely.

Table 1. Spiking Mixtures and Composition

Mixture	Diluent	Saliva DNA Preservation Buffer (2X)	Growth Status
Saliva/Preservative	Saliva	Yes	-
Mini Spin Column	Saliva	No	+
Midi Spin Column	LB or YPD	Yes	-
Maxi Spin Column	LB or YPD	Yes	-

CONCLUSION

Norgen's Saliva DNA Preservative inhibits the growth of bacteria and yeast, thereby resulting in non-infectious samples that can be handled and shipped safely.

Viability Of Microorganisms in Saliva & Stool Collection and Transportation Preservatives

Abstract

Two sample collection and preservation devices 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. These devices contain Norgen's Saliva DNA Preservative and Norgen's Stool Preservative, respectively. Prior studies have shown that the preservatives could minimize changes in the microbiota diversity 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 hours 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 immediately became non-viable in Norgen's Stool Preservative and Norgen's Saliva DNA Preservative. These results demonstrate that the Saliva and Stool NA Collection and Transport Tubes/Devices are effective at killing 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 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.

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[2] Brock University, St. Catharines, Ontario, Canada

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.

MATERIALS AND METHODS

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

- Stool samples were collected and were either mixed with PBS (1:2 ratio) or mixed with Norgen's Stool Preservative (1:2 ratio). The samples were then spiked with 108 *E.coli*, 108 L. monocytogenes or 108 *S.cerevisiae*. Unspiked samples mixed with PBS and preservative were used as a control. At each time point (0 hours, 4 hours, 8 hours, 24 hours) 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 hours after plating.

- Saliva samples were collected and were either mixed with PBS (1:1 ratio) or mixed with Norgen's Saliva DNA Preservative (1:1 ratio). The samples were then spiked with 108 *E.coli*, 108 *L. monocytogenes* or 108 *S.cerevisiae*. Unspiked samples mixed with PBS and preservative were used as a control. At each time point (0 hours, 4 hours, 8 hours, 24 hours) 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 hours after plating.

- With the remaining culture that was not used for plating at each time point, 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

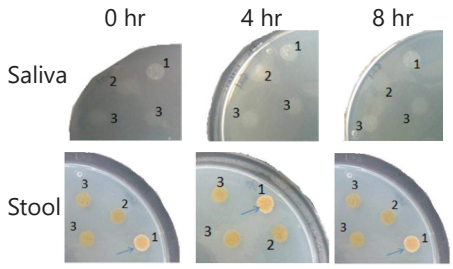


Figure 1. Viability of the microorganism *L. monocytogenes* spiked in both the saliva preservative and the stool preservative (labelled as 3), as well as saliva and stool mixed with only preservative (labelled as 2) or only 1X PBS (labelled as 1). Numbers correspond to the numbering in Table 1.

Table 2. Cell viability results. Cell viability rate: ~108 cfu = ++++ > +++ > ++ > + > 0 cfu = No growth

Time Incubated with the Preservative at RT		Saliva Preservative				Stool Preservative			
		0h	4h	8h	24h	0h	4h	8h	24h
In 1X PBS		+++	+++	+++	+++	+++	+++	+++	+++
Spiking	*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

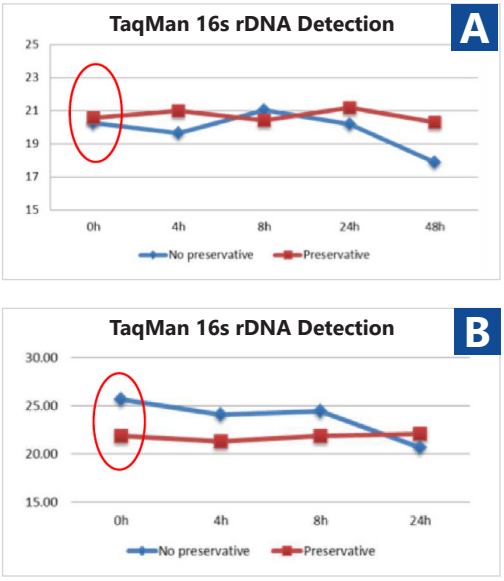


Figure 2. Real Time PCR of 16s rDNA results indicating the Ct value over a period of 48 hours for the (A) Saliva sample and (B) stool sample.

- he 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
- The DNA profile at the time of collection is maintained by using Norgen's preservatives, as indicated by the consistent Ct values seen in both A) and B)

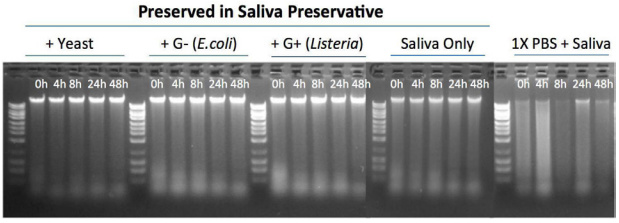


Figure 3. Resolution of an agarose gel containing Norgen's Saliva DNA Preservative spiked with the three different microorganisms, as well as saliva containing only 1X PBS

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

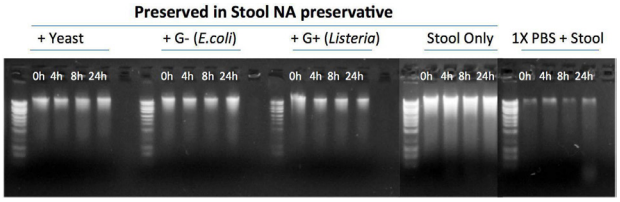


Figure 4. Resolution of an agarose gel containing Norgen's stool preservative spiked with the three different microorganisms, as well as stool containing only 1X PBS

- Regardless of the spiked microorganisms, DNA preserved in Norgen's Stool Preservative shows that DNA integrity and quantity is maintained compared to 1X PBS
- Greater DNA yield can be seen in the stool sample only (stool preserved with Norgen's Stool Preservative), due to the instant cell lysis of microorganisms by the preservative

CONCLUSIONS

1. Norgen's preservatives (Saliva and Stool) were able to instantly inactivate the microorganisms' viability at 0 h (*E. coli, L. monocytogenes*, and *S. cerevisiae*) as seen in Table 2.

2. The mode of action of Norgen's Stool Preservative was slightly different from Norgen's Saliva DNA Preservative in that it could lyse cells immediately, at the point of mixing (0 h), resulting in a higher DNA yield (Figure 3) and earlier Ct from 16s rDNA detection (Figure 4). In contrast, the Saliva DNA 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.

STABLE SAMPLES:
REPRODUCIBLE RESULTS

1 USER-FRIENDLY COLLECTION:
no mess & odour-free

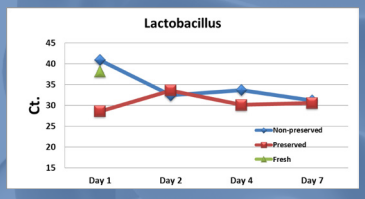
SCOOP DESIGN
no mess!

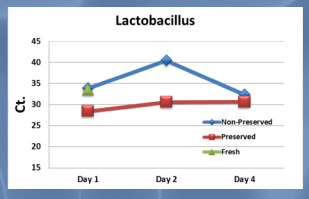
NO ODOUR
no distraction!

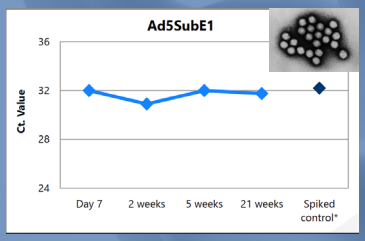
2 NO NEED TO FREEZE:
eliminate cost of cold chain transport/ storage

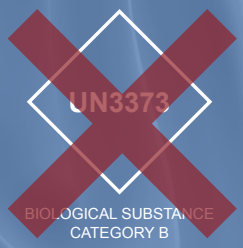
DRY ICE

3 RENDER SAMPLES NONINFECTIOUS:
stop microbial growth & inactivate viruses

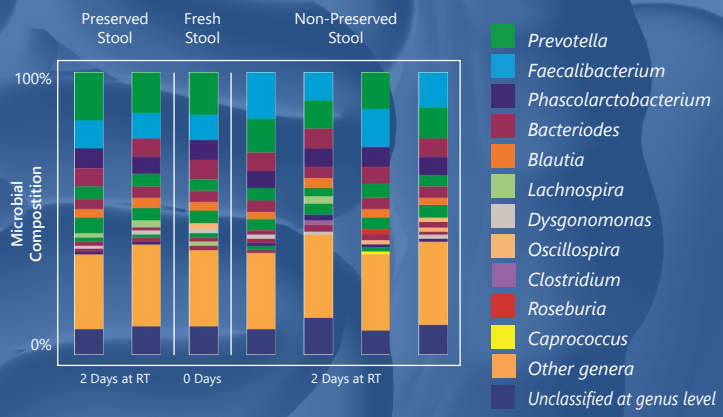








4 BACTERIOSTATIC & HOMOGENOUS:
minimize bias in data analysis



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