APPLICATION NOTE #57

Comparative Study of DNA Isolated from Stool Using Norgen's Stool Nucleic Acid Isolation Kit and Norgen's Stool DNA Isolation Midi Kit (Slurry Format) Versus Competitor's DNA Stool Mini Kit

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INTRODUCTION

The use of stool as a biological sample for nucleic acid isolation and subsequent biomarker or viral nucleic acid detection has only recently been recognized. Despite the invasive nature and questionable sensitivity of colonoscopy, it is still used as the gold standard for colorectal carcinomas (CRC)[1]. Stool DNA, on the other hand, has shown remarkable sensitivity rates considering its noninvasiveness, even detecting lesions that have been missed using colonoscopy[1].

One issue with the use of stool as a diagnostic sample is that the majority of DNA found in stool arises from bacterial or pathogenic sources, as opposed to human cells. One way to overcome this issue is to process a larger amount of stool, thus increasing the likelihood of detecting the gene of interest using qPCR. Unfortunately, many commercially available kits rely on a spin-column format for processing stool samples, and due to column overloading issues, sample sizes are often restricted to under 250 mg.

Norgen Biotek Corp. has recently developed a stool DNA isolation kit designed to increase the starting amount of stool used for isolation. It utilizes a silicon carbide slurry matrix, designed to lyse stool samples and overcome column overloading issues. Using this method, customers can extract DNA from up to 1 gram of stool, significantly increasing the overall concentration and yield of DNA obtained from one stool DNA isolation.

The purpose of this study is to compare Norgen's Stool DNA Isolation Midi Kit (Slurry Format) (Cat# 51100) to Norgen's Stool Nucleic Acid Isolation Kit (Cat# 45600) and to Competitor's DNA Stool Mini Kit. Comparisons will be based on DNA yield, quality and qPCR performance.

METHODS AND MATERIALS

Stool DNA Extraction

DNA was extracted from one stool sample using Norgen's Stool DNA Isolation Midi Kit (Slurry Format; Cat# 51100), Norgen's Stool Nucleic Acid Isolation Kit (Cat# 45600) and Competitor's DNA Stool Mini Kit, as per the manufacturer's instruction.

Norgen's Stool DNA Isolation Midi Kit (Slurry Format)

Norgen's Stool DNA Isolation Midi kit involved lysing the 1 gram of stool, vortexing, and spinning samples down at 3000rpm for 5 min. Once 2 mL of supernatant was transferred to a new tube, RNase was added, along with binding buffer. Samples were then incubated on ice for 5 minutes, and 2 mL of supernatant was again transferred to a new tube. Slurry was then added to the supernatant, along with 96-100% ethanol. Samples were then vortexed, and spun down for 1 minute. The resin pellet was then washed twice, and transferred to a filter spin column. Samples were then eluted from the column, and re-bound to a silicon carbide spin column. Samples were then washed and eluted.

Norgen's Stool Nucleic Acid Isolation Kit

For Norgen's Stool Nucleic Acid Isolation Kit, 200 mg of stool was used as the input. Lysis solution and a lysis additive was added, and samples were vortexed and spun down. The supernatant was then transferred to a new tube, binding solution was added, and the samples were incubated on ice for 10 minutes. Samples were then spun down, supernatants added to a new tube, and 70% ethanol was added. Samples were then bound to a column, washed and eluted.

Competitor's DNA Stool Mini Kit

Once again, for the DNA Stool Mini Kit, 200 mg of stool was used. Buffer ASL was added, and samples were vortexed, then spun down. Supernatants were then transferred to a new tube, and an InhibitEX tablet was suspended into the sample. Samples were then spun down, and supernatants were transferred to a new tube. Samples were then spun down, and supernatants were again transferred to a new tube, along with proteinase K, and Buffer AL. Samples were then incubated for 10 minutes at 70°C. Ethanol was then added, and samples were transferred to a spin column. Samples were then washed and eluted.

Spectrophotometry and Gel Electrophoresis

Stool DNA quantity and quality was measured using the NanoVue Plus Spectrophotometer (GE Healthcare). Ten microliters of each sample was also run on a 1.2% agarose gel.

Real-Time PCR

The purified DNA was then used as the template in a realtime PCR reaction. Briefly, 2 µL of isolated DNA was added to 20 µL of a real-time PCR reaction mixture containing 10 µL of Norgen's 2X PCR Mastermix (Cat# 28007) spiked with SYBR® Green dye, 5 mM 18S or 16S primer pair, and nuclease-free water. GAPDH was also detected using the TaqMan® probe system, with the same components as the previously mentioned PCR, however SYBR green was not required. All PCR samples were amplified under the realtime program; 95°C for 3 minutes for an initial denaturation, 50 cycles of 95°C for 15 seconds for denaturation, 60°C for annealing and extension. The reaction was run on an iCycler iQ Realtime System (Bio-Rad).

RESULTS AND DISCUSSION

The use of stool as a diagnostic medium is steadily increasing, as collection is easy and non-invasive, and previous tests have found high sensitivity and specificity rates from using stool DNA to diagnose lesions, as opposed to colonoscopy. As a result, the need for a reliable stool DNA isolation method is increasingly becoming apparent. Also, with more and more researchers looking to isolate DNA from larger amounts of stool, a robust stool DNA isolation method is required to process up to 1g of stool.

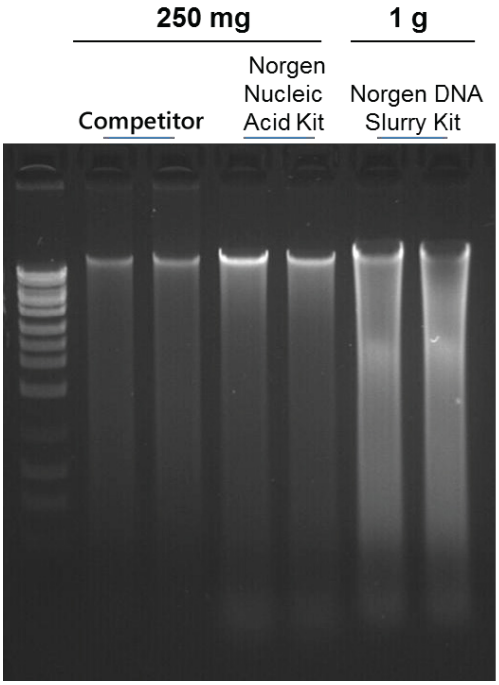

Figure 1. Purified saliva DNA isolated from swabs collected by two donors (A and B), run on a 1X TAE 1.0% agarose gel. High quality saliva DNA was isolated from both samples, with donor A showing a higher yield of DNA than donor B.

In this study, DNA was isolated from one stool sample using the three aforementioned methods. All samples were then run on a 1.2%, 1X TAE agarose gel **(Figure 1)**. Both of Norgen's kits and Competitor's kit successfully isolated DNA from stool, with a higher yield found from Norgen's Stool DNA Isolation Midi kit (Slurry format) samples.

Next, in order to determine the quality of the DNA isolated, a qPCR was run to detect three genes: bacteria 16S, human 18S and human GAPDH. Both bacteria and host DNA are often used in diagnostics, as they can be used to detect not only pathogens in stool, but also to detect cancer biomarkers (specifically colorectal cancer biomarkers).

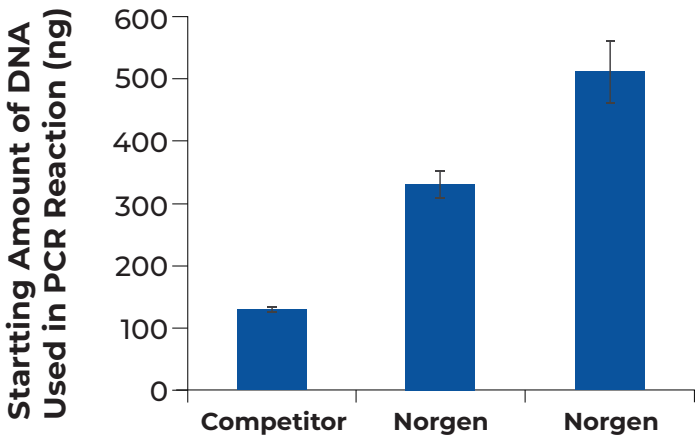

Figure 2. The starting amount of stool DNA used in each PCR reaction, from three different methods. As Norgen's 1 gram slurry samples were found to have the highest overall concentration and yield, the amount of DNA used in the PCR reaction was also significantly higher than the Norgen 250 mg method, and Competitor's samples.

The use of larger volumes of stool being used in DNA isolation processes often poses as an issue, as PCR inhibitors are notoriously found in stool samples. These inhibitors are often co-eluted with stool DNA, making downstream applications a challenge. In this experiment, we isolated DNA from 250 mg of stool using two different kits, and from 1 gram of stool using Norgen's slurry method. Norgen's Stool DNA Isolation Midi Kit (Slurry Format) is the only known stool DNA isolation kit that is able to process up to 1 gram of stool.

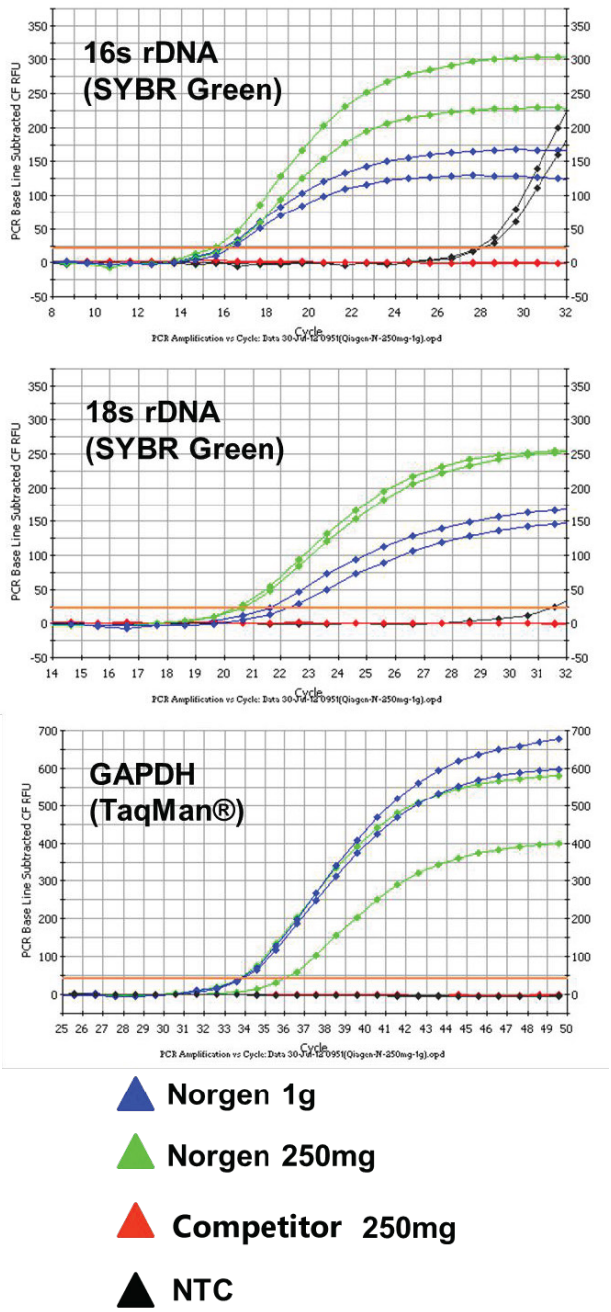

Figure 3. Detection of pathogenic and host genes using qPCR. Two Norgen methods and one Competitor method were used to isolate DNA from either 250 mg or 1 gram of stool. The elutions were then used in a qPCR reaction to detect human 18S and GAPDH as well as bacterial 16S. Norgen's slurry method was used to isolate DNA from 1 gram of stool (blue), while the other Norgen method and the Competitor method were used to isolate DNA from 250 mg of stool (green and red, respectively.)

As each aforementioned stool DNA isolation method has a unique elution volume, the resultant concentration of stool DNA was intrinsically variable. Without adjusting sample elution volume, a uniform volume of 2 µL was used from each sample in order to determine how well each method performs in downstream applications independently, without altering the provided protocol. **Figure 2** graphically depicts the difference in starting amount of stool DNA used for each PCR reaction.

Stool Nucleic Acid Isolation Kit Cat.45600

A higher amount of starting DNA concentration in a PCR reaction also means a higher concentration of co-eluted PCR inhibitors. Norgen's 1 gram slurry method samples were found to have the highest starting DNA concentration **(Figure 2)**, and Competitor was found to have the lowest.

The results of the PCR can be found in **Figure 3**. While Norgen's samples amplified for all three genes using both kits, Competitor's samples were completely inhibited. Ct values generated from the two different Norgen kits were similar, possibly indicating saturation of the PCR signal.

Stool DNA Isolation Kit Cat.27600

It is important to note the difference in starting DNA concentration in the PCR reactions when interpreting results. Despite the significantly higher amount of DNA used for Norgen's samples (thus higher amounts of PCR inhibitors), these samples amplified consistently. On the other hand Competitor's samples (which were more diluted) were completely inhibited.

CONCLUSIONS

From the data presented in this report, the following can be concluded:

1. Norgen's Stool DNA Isolation Midi Kit (Slurry Format) Can Be Used to Successfully Isolate DNA from 1 gram of Stool. In this report, we demonstrated the ability to isolate DNA from 1 gram of stool using a slurry method. This drastically increased the amount of DNA in the elution, when compared to isolating DNA from 250 mg of stool.
2. Norgen's Stool DNA Isolation Midi Kit (Slurry Format) Isolates DNA Ready for Sensitive Downstream Applications, such as qPCR. We have demonstrated that despite isolating DNA from 1 gram of stool, Norgen's slurry method was able to outperform Competitor in keeping PCR inhibition to a minimum. PCR inhibitor concentrations were so high in the competitor kit that no amplification was found.

REFERENCES

1. Lucas AL, Hwang C, and Frucht H. 2012. Strengthening the case for stool DNA tests as first-line colorectal cancer screening: Are we there yet? Clin. Gastroenterol. H. 10(3): 214-215.

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