

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 non-invasiveness, even detecting lesions that have been missed using colonoscopy¹.

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.

MATERIALS AND METHODS

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

depicts the difference in starting amount of stool DNA used for each PCR reaction.

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.

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 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.

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.

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.)

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.

