Comparative Study of DNA Isolated from Increasing Volumes of Blood Using Norgen's Blood Genomic DNA Isolation Kit Versus Qiagen's QIAamp DNA Blood Mini Kit

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INTRODUCTION

Blood is an excellent sample for diagnostic purposes. It is rich in protein and nucleic acid-based biomarkers that can be used to detect a panel of diseases long before any physical symptoms become apparent. Studies have shown that cancer biomarkers, such as differentially methylated target genes, are detectable in blood samples and have proven to be extremely sensitive and specific for given cancer types[1]. These biomarkers can be cell associated, such as those found in leukocytes[2], or cell-free, such as those found in plasma or serum samples. For this reason, blood is often the sample of choice for biomarker or diagnostic research.

Investigators utilizing blood in their research have unique needs, based on their downstream applications and sample volume. For some studies, less blood may be processed in order to preserve precious samples. On the other hand, some studies require a high yield of DNA from their samples, and thus require higher volumes of sample to be processed. The method used for blood DNA isolation can heavily influence the results of research-based or diagnostic tests associated with blood. Investigators must ensure that their blood DNA isolation method is flexible, i.e. a linear increase in blood volume being processed leads to a linear increase in DNA concentration and yield. A robust blood DNA isolation kit eliminates sample processing biases, and increases data reproducibility.

The purpose of this study is to compare two commercially available blood DNA isolation methods: Norgen's Blood Genomic DNA Isolation Kit (Cat# 46300) and Qiagen's QIAamp DNA Blood Mini Kit (Cat# 51104) for their ability to isolate high quality and high quantities of genomic DNA from increasing volumes of blood.

MATERIALS AND METHODS

Sample collection

Ten milliliters of blood was collected in Ethylenediaminetetraacetic acid (EDTA) tubes, from one healthy individual, by a trained professional. The sample was frozen at -70ºC until processed.

Blood DNA extraction

DNA was extracted from the thawed blood sample using Norgen's Blood Genomic DNA Isolation Kit (Cat# 46300) and Qiagen's QIAamp DNA Blood Mini Kit (Cat# 51104), as per the manufacturer's instruction.

Norgen's Blood Genomic DNA Isolation Kit

Briefly, Proteinase K was added to a microcentrifuge tube, followed by 20, 50, 100 or 200 µL of blood. Lysis Solution was then added, and samples were vortexed and incubated at 55ºC for 10 minutes. Next, ethanol was added to each sample, and samples were bound, washed and eluted as per the manufacturer's protocol.

Qiagen's QIAamp DNA Blood Mini Kit

Briefly, Qiagen Protease was added to the bottom of a microcentrifuge tube, followed by 20, 50, 100 or 200 µL of blood. Buffer AL was then added, and samples were vortexed and then incubated at 56ºC for 10 minutes. Ethanol was added to each sample following incubation, and samples were bound, washed and eluted as per the manufacturer's instruction.

Spectrophotometry

Blood DNA quantity was measured using the UltraSpec 2100 Pro (Fisher Scientific). Fifty microliters of each DNA elution was diluted with 450 µL of nuclease-free water, and OD measurements were taken using the cuvette-based spectrophotometry method.

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Real-Time PCR

The purified DNA was then used as the template in a real-time TaqMan® PCR reaction. Briefly, 9 µL of isolated DNA was added to 20 µL of real-time PCR reaction mixture containing 10 µL of Norgen's 2X PCR Mastermix (Cat# 28007), 0.4 µL from a 5mM GAPDH primer pair mix, 0.2 µL of the TaqMan® probe, and nuclease-free water. The PCR samples were amplified under the real-time program; 95°C for 3 minutes for an initial denaturation, 40 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

Blood is an excellent resource for research and diagnostic purposes. Systemic diseases can be detected through a simple blood test, and changes in DNA (both cellular and cell-free) can be detected from blood, giving rise to its high potential for screening for a panel of diseases.

The key to the success of any study utilizing blood is a reliable blood DNA isolation method that performs optimally from a range of sample volumes. One can determine the flexibility of a kit by increasing the sample volume in order to see the linearity of the increase in DNA yield. In this study, DNA was isolated from 20, 50, 100 and 200 µL of blood using Norgen's kit, and Qiagen's kit. Fifteen microliters of each 200 µL elution was then run on a 1X TAE 1.0% agarose gel to visually inspect the isolated gDNA (**Figure 1**). Both Norgen and Qiagen were able to isolate DNA from each sample volume, with similar yields and quality observed for both kits.

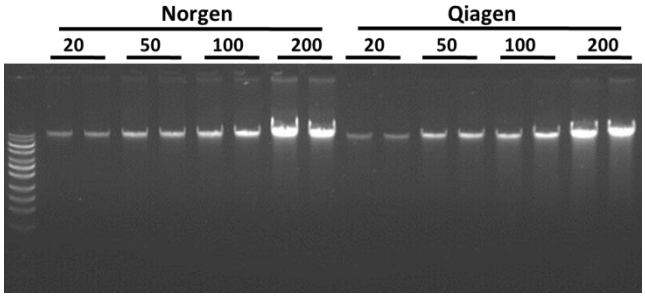

Figure 1. Resolution of DNA collected in EDTA tubes and isolated using Norgen's Blood Genomic DNA Isolation Kit and Qiagen's QIAamp DNA Blood Mini Kit. Fifteen microliters of 200 µL elutions were run on 1X TAE 1.0% agarose gel. Marker= Norgen's UltraRanger DNA Ladder.

In order to determine the yield of DNA isolated from each volume of blood using both blood DNA isolation kits, samples were measured using a cuvette-based spectrophotometry method (UltraSpec 2100 Pro; Fisher Scientific). Each sample was eluted twice, in two different microcentrifuge tubes, and DNA yields were determined for both elutions (**Figure 2**). Norgen samples were found to have higher yields from the first elution of the lowest blood volume samples (20, 50 and 100 µL), and with the inclusion of the second elution, Norgen samples recovered an overall higher amount of DNA than Qiagen samples, from the same blood volume processed.

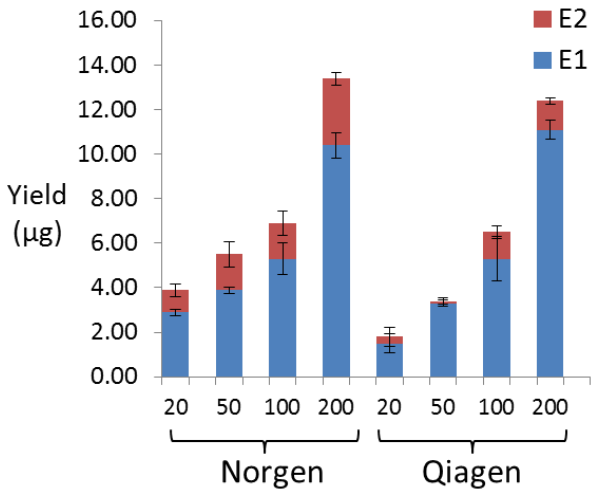

Figure 2. The difference in yield isolated from different volumes of blood using Norgen's vs. Qiagen's kit. Fifty microliters of each sample was diluted in 450 µL of nuclease-free water, and DNA concentrations were measured using the UltraSpec 2100 Pro (Fisher Scientific). Two elutions were performed for all samples.

DNA quality was determined through the use of a TaqMan® Real-Time PCR method. In order to assess sample inhibition, 9 µL of each sample was used in the reaction. The Ct values were then graphed (**Figure 3)**.

For all volumes of blood processed, Norgen consistently displayed a lower Ct value when compared to Qiagen's samples (with the only exception being the similar performance between the two kits when processing 100 µL of blood).

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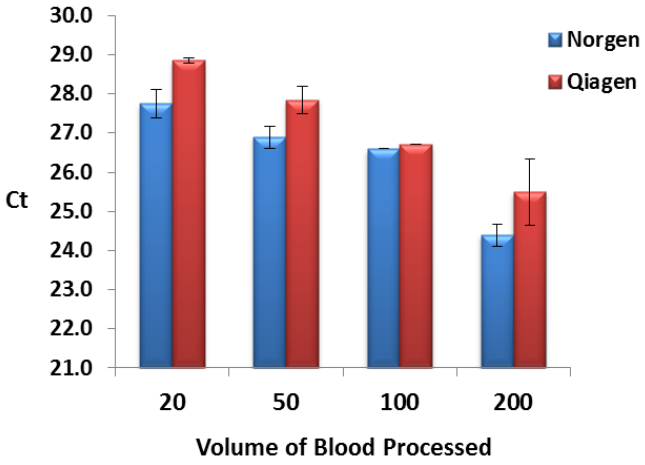

Figure 3. The difference in Ct values between Norgen and Qiagen blood samples, using a Taqman® qPCR reaction. Nine microliters of each elution was used in a 20µl qPCR reaction involving GAPDH primers.

CONCLUSIONS

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

1. Norgen's Blood Genomic DNA Isolation Kit isolates the highest yields of DNA from all volumes of blood processed, when compared to a leading competitor kit.
2. While the majority of DNA on Norgen's columns is eluted in the first 200 µL elution, yields can be increased by 10-20% by performing a second elution.
3. Norgen's Blood Genomic DNA Isolation Kit outperforms competitor kits in sensitive downstream applications, such as qPCR.
4. Norgen samples are free from inhibitors, as 9 µL of elution was used in each PCR reaction, with no delay in Ct values when compared to 2 µL used in the PCR reaction (data not shown).

REFERENCES

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