Comparative Study of Plasma DNA Isolated Using Norgen's Plasma/Serum Circulating DNA Purification Kits Versus Qiagen's QIAamp DNA Blood Kits

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INTRODUCTION

Free circulating DNA found in plasma and serum samples is an excellent source for biomarker discovery. Collection is minimally invasive, and DNA isolated from plasma can be used for genetic and epigenetic testing, as well as for diagnostic purposes.

Fetal DNA has been found to be readily detected in maternal plasma and serum (1), which can be used not only for pregnancy confirmation and gender determination, but also for prenatal diagnostics, such as chromosomal aneuploidies (2). Viral nucleic acids can also be detected in plasma/serum samples, which is often used to screen for HIV (3). From a biomarker standpoint, studies have found that plasma DNA levels are significantly increased in cancer states, and an important portion of this DNA originates from the tumour itself (4). Therefore, plasma DNA has endless potential for a variety of research, diagnostic and screening purposes.

PCR inhibition is a common issue for researchers utilizing plasma as a source for biomarkers. This is due to the presence of proteins, nucleases, and impurities that can compromise the plasma DNA elution, leading to downstream application issues. In this study, we compared two sets of commercially available plasma DNA isolation methods: Norgen's Plasma/Serum Circulating DNA Purification Mini, Midi and Maxi Kits (Slurry Format) and Qiagen's QIAamp DNA Blood Mini, Midi and Maxi Kits. Each competitor method covers a range of 200 µL to 10mL of plasma across three kits.

MATERIALS AND METHODS

Plasma Preparation and DNA Extraction

Blood was collected into sodium citrate tubes, and plasma was prepared according to standard procedures. Briefly, whole blood samples (isolated directly into their specific anticoagulant) were centrifuged twice for 15 minutes at 2000 x g each to obtain cell-free plasma. Plasma was then aliquoted into 50mL aliquots (to avoid multiple freeze thaw cycles), and immediately frozen at -70°C until used.

DNA was extracted from 0.2 mL – 10 mL of plasma using a total of 5 kits from two different lines of products: Norgen's Plasma/Serum Circulating DNA Purification Mini, Midi and Maxi Kits, and Qiagen's QIAamp DNA Blood Midi and Maxi Kits. For Norgen's kits, the mini kit covers a range of 0.2 mL-0.4 mL of plasma, the midi kit covers a range of 0.4 mL-2 mL, and the maxi kit covers 2 mL-10 mL of plasma. For Qiagen's kits, the midi kit covers 0.3 mL-2mL, and the maxi kit covers 3mL to 10mL. Qiagen also has a mini kit, which covers plasma volumes up to 0.2 mL. This kit was not used in our study.

Real-Time PCR

The purified DNA was then used as the template in a real-time PCR reaction. Usually, 3 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 5S or 15S primer pair, and nuclease-free water. In some experiments, increasing volumes of template were used (3, 6 and 9 µL). All PCR samples were amplified under the real-time 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 72°C for extension. The reaction was run on an iCycler iQ Realtime System (Bio-Rad).

Real-time PCR is the best method to determine the performance of a plasma DNA isolation kit, as the amount of DNA in a plasma sample is often too low and too variable in length to be detected on an agarose gel. Also, plasma samples contain short, fragmented DNA that cannot be measured accurately using spectrophotometry.

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RESULTS AND DISCUSSION

The use of plasma as a diagnostic medium is quite common, as collection is minimally invasive, it can be used to screen or test for a variety of diseases, and it can be used for genetic studies. The key to the success of using plasma for research or diagnostic purposes is a reliable, robust plasma DNA isolation method.

In this study we isolated DNA from various plasma volumes, ranging from 200 µL to 10 mL, to compare Norgen's and Qiagen's plasma DNA kits. The first comparison made between Norgen and Qiagen was DNA isolated from 0.2 mL and 0.4 mL of plasma using Norgen's Plasma/Serum DNA Purification Mini Kit, and Qiagen's QIAmp DNA Blood Midi Kit. Elutions were run in a qPCR (iCycler) using 3 µL of elution per reaction, with primers to detect the human 5S gene (**Figure 1**). Norgen samples were found to amplify at the same rate as Qiagen, or better, for this input volume. Moreover, Qiagen samples showed both gDNA and RNA contamination whereas Norgen's kit showed neither gDNA contamination nor co-purified RNA along with the plasma circulating DNA (data not shown).

Next, plasma DNA was isolated from 0.5 mL and 2 mL of plasma using Norgen's Plasma/Serum DNA Purification Midi kit, and Qiagen's QIAmp DNA Blood Midi Kit. The purified DNA was then used in a qPCR reaction to detect the human 5S gene (**Figure 2**). For these two input volumes, Norgen samples clearly amplify sooner than Qiagen samples, indicating that the DNA isolated from 0.5 mL and 2 mL using Norgen's kit is superior in quality and/or quantity compared to Qiagen's kit.

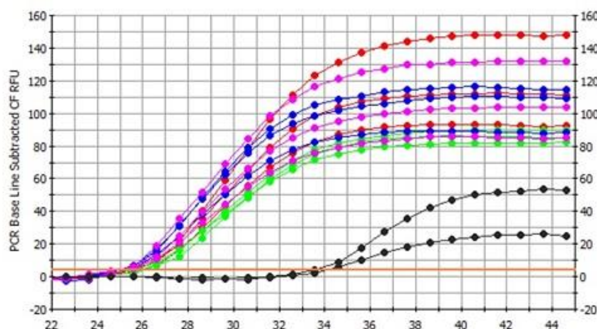
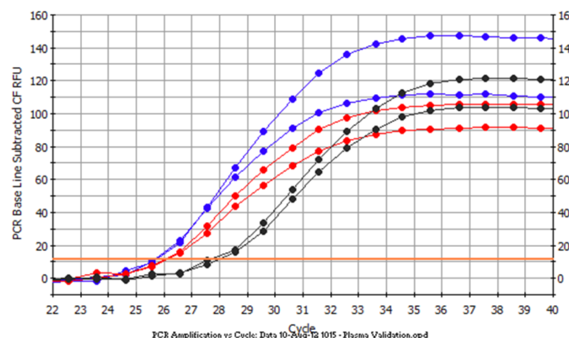
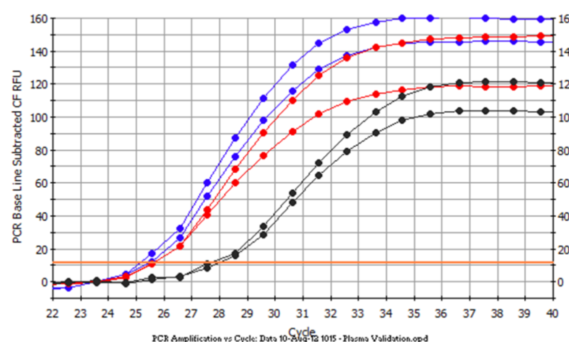


Figure 1. Isolation and Detection of Circulating DNA Isolated from Different Plasma Volumes. Norgen's Plasma/Serum DNA Purification Mini Kit (Slurry Format) and Qiagen's QIAamp DNA Blood Midi kit were used to isolate circulating DNA from 0.2 mL and 0.4 mL plasma. Three microlitres of the purified DNA was used as the template in a qPCR reaction to detect the human 5S gene. The red and blue lines correspond to DNA isolated from 0.2 mL and 0.4 mL plasma using Norgen's Kit, whereas the green and pink lines correspond to DNA isolated from 0.2 mL and 0.4 mL of plasma using Qiagen's kit.

0.5mL Plasma



2mL Plasma



Norgen Qiagen NTC

Figure 2. Detection of Human 5S Gene from 0.5mL and 2mL of Plasma. Norgen's Plasma/Serum Circulating DNA Purification Midi Kit was compared to Qiagen's QIAamp DNA Midi Kit using 0.5 mL (top image) and 2 mL (bottom image) of plasma. Norgen's samples=blue, Qiagen's samples=red. Three microliters of each elution was used in a 20 µL qPCR reaction to detect the human 5S gene.

A common issue with processing high volumes of plasma is that inhibitors naturally present in plasma samples are often co-eluted, and with higher volumes of plasma comes higher concentrations of these inhibitors.

Using the same samples isolated for Figure 2, increasing volumes of elution were used in a qPCR reaction in order to determine the relative amount of PCR inhibitors present in each sample. For this experiment, two human genes were detected: the 5S gene (**Figure 3a**) and the S15 gene (**Figure 3b**). It was found that an increase in elution volume used in

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the PCR did not greatly affect the Ct value generated from Norgen samples. Qiagen's samples, on the other hand, were found to show a higher degree of PCR inhibition, as 9 µL of elution led to a drastic increase in Ct value. This trend was apparent for both 0.5 mL (top image) and 2 mL (bottom image) of plasma, as well as for 5S (Figure 3a) and S15 primers (Figure 3b).

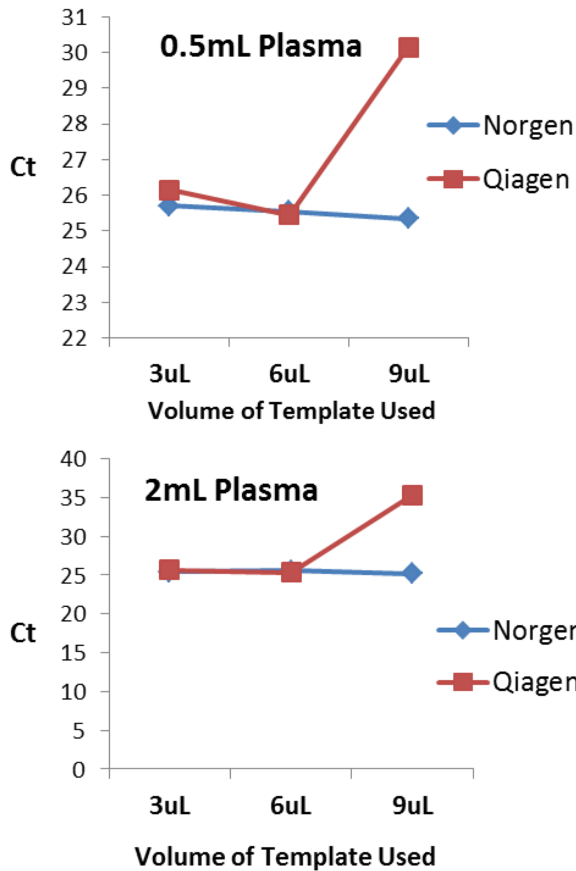

Figure 3a. Determination of the Amount of Inhibition Present in Plasma DNA Samples when Detecting the Human 5S Gene. DNA was isolated from 0.5 mL of plasma (top image) and 2 mL of plasma (bottom image) using Norgen's Plasma/Serum Circulating DNA Purification Midi Kit and Qiagen's QIAmp DNA Blood Midi Kit. Increasing volumes of the elution (3, 6 and 9 µL) were used in a 20 µL qPCR reaction to observe any increase in Ct value. An increase in Ct value with increasing amount of template would be a clear indication of PCR inhibitors present in the sample. The primers used flank the human 5S gene. An increase in elution volume used in the PCR did not greatly affect the Ct value generated from Norgen samples, however inhibiton was observed when 9 µL of Qiagen's elution was used as the template.

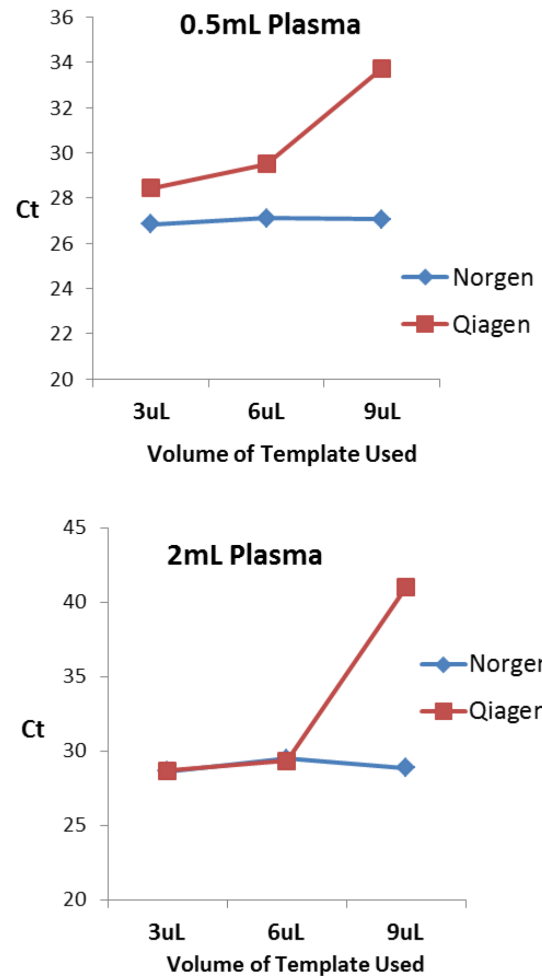

Figure 3b. Determination of the Amount of Inhibition Present in Plasma DNA Samples when Detecting the Human S15 Gene. DNA was isolated from 0.5 (top image) and 2 mL (bottom image) of plasma using Norgen's Plasma/Serum Circulating DNA Purification Midi Kit and Qiagen's QIAmp DNA Blood Midi Kit. Increasing volumes of elution (3, 6 and 9 µL) were used in a 20 µL qPCR reaction to observe any increase in Ct value. An increase in Ct value with increasing amount of template would be a clear indication of PCR inhibitors present in the sample. The primers used flank the human S15 gene.

Lastly, DNA was isolated from 5 mL of plasma using Norgen's Plasma/Serum Circulating DNA Purification Maxi Kit, and Qiagen QIAmp Blood DNA Maxi Kit. For this volume, 1 x 10^6 Adenoviral (AdV) particles were spiked into each plasma sample. Similar to the lower volume samples, 5S and S15 were used to detect the human genes present in the samples, and the AdV DNA Binding Protein (DBP) gene was chosen to detect the exogenous AdV DNA. The

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results are summarized into **Figure 4**. At this plasma volume, the difference between Norgen's and Qiagen's DNA isolation technology is the most evident. For all three genes (5S, S15, and DBP [Adenovirus spike in], Norgen samples amplified more than 2 Cts sooner.

For S15 in particular, Norgen samples amplified at ~22 Ct, while Qiagen samples were closer to 30 Ct. Norgen also recovered a significantly higher amount of viral DNA, as demonstrated by the DBP gene used to detect the spiked-in AdV DNA. Norgen's samples amplified ~4 Cts sooner than Qiagen's for this gene.

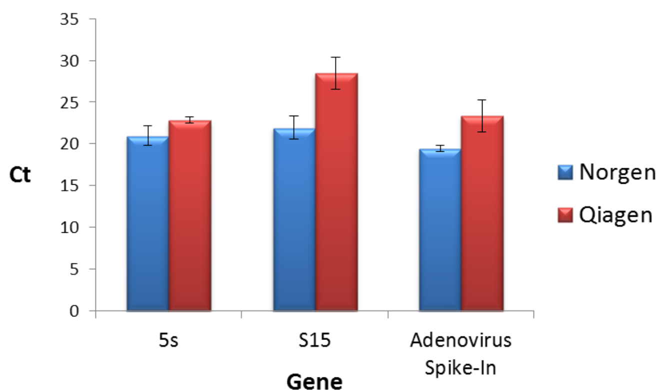

Figure 4. Detection of Human and Viral DNA from 5 mL of Plasma. Norgen's Plasma/Serum Circulating DNA Purification Maxi Kit was compared to Qiagen's QIAamp DNA Maxi Kit using 5 mL of plasma. Three microliters of each elution was used in a 20 µL qPCR reaction to detect the human 5S and S15 genes, as well as the AdV DBP gene.

CONCLUSIONS

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

1. Norgen's Plasma/Serum Circulating DNA Purification Kits (Mini, Midi and Maxi) outperform the leading competitor kits at all volumes tested (from 0.2 mL to 5 mL).
2. Norgen's plasma kits recover a higher amount of spiked-in viral particles, making them more useful for pathogen detection from plasma.
3. Norgen's plasma kits co-purify less PCR inhibitors, which was made evident through increasing the amount of template used in a qPCR reaction detecting two different genes.

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