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

M. Simkin[1], M. Abdalla[1], Y. Haj-Ahmad, Ph.D[1,2]
[1]Norgen Biotek Corporation, Thorold, Ontario, Canada
[2]Centre for Biotechnology. Brock University, St. Catharines, Ontario, Canada

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 Competitor's 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 Competitor's 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 Competitor'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.

3430 Schmon Parkway
Thorold, ON Canada L2V 4Y6
Phone: 905-227-8848 • Fax: 905-227-1061
Toll-Free (North America): 1-866-667-4362
www.norgenbiotek.com

(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 Competitor's 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 Competitor'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 Competitor's 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.

REFERENCES

1. Lo YMD, et al. 1998. Quantitative analysis of fetal DNA in maternal plasma and serum: Implications for noninvasive prenatal diagnosis. Am J Hum Genet, 62: 768–775.
2. Chiua RWK, et al. 2008. Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma. P Natl Acad Sci USA, 105 (51): 20458-20463.
3. Beersma MFC, et al. 2010. Quantification of viral DNA and liver enzymes in plasma improves early diagnosis and management of herpes simplex virus hepatitis. J Vir Hepat, 18 (4): e160–e166.
4. Jahr S, et al. 2001. DNA fragments in the blood plasma of cancer patients: Quantitations and evidence for their origin from apoptotic and necrotic cells. Cancer res, 61: 1659-1665.

3430 Schmon Parkway
Thorold, ON Canada L2V 4Y6

Phone: 905-227-8848 • Fax: 905-227-1061
Toll-Free (North America): 1-866-667-4362
www.norgenbiotek.com