Isolation of Genomic DNA from Dried Blood Collected on Different Materials: Blood Cards, Cloth and Solid Surface using Norgen's Dried Blood Spot Genomic DNA Isolation 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.

Blood DNA is used in a variety of lab procedures, from diagnostics to forensic applications. Dried blood spots can be found on almost any material, and investigators have unique needs based on the type of material that the dried blood spot has been recovered on. The type of material can affect the results of blood DNA isolation kits, ultimately influencing the results of research-based, diagnostic and identification tests associated with blood. Another factor that must be considered is the age of the dried blood. The amount of time that blood has been on a material can affect the quality and quantity of the DNA it contains. Investigators must ensure that their blood DNA isolation method is flexible, i.e. it can work efficiently on isolating blood from a range of materials.

The purpose of this study is to isolate blood genomic DNA from dried blood collected on blood cards, cloth and solid surfaces using Norgen's Dried Blood Spot Genomic DNA Isolation Kit (Cat# 36000) after one day and one week post-collection.

MATERIALS AND METHODS

Sample collection

Fresh blood was collected from 3 donors on blood cards, cloth, and as drops on a solid surface (petri dishes). The blood was then allowed to dry at room temperature.

Blood DNA extraction

DNA was extracted from the dried blood spot samples using Norgen's Dried Blood Spot Genomic DNA Isolation Kit, as per the manufacturer's instruction. Blood cards as well as cloth were excised as punches of 3x3mm diameter and placed into clean tubes, then 100 µL of digestion buffer was added to the tubes and vortexed for 10 seconds. Blood clots on solid surface were collected by adding 50 µL of the kit's digestion buffer on the blood spot to dissolve then the liquid was collected by a swab and the swab tip with the liquid was placed into a clean tube containing another 50 µL of the digestion buffer, then the tube was vortexed for 10 seconds. All tubes were then incubated at 85ºC for 10 minutes and spun briefly in a centrifuge. Next, Proteinase K was added to the microcentrifuge tube, plus 300 µL of the Lysis Solution was added, and samples were vortexed and incubated at 55ºC for 10 minutes. Next, 250 µL ethanol was added to each sample, and samples were bound, washed and eluted as per the manufacturer's protocol, with elution of the DNA in a 50 µL volume.

Real-Time PCR

The purified DNA was then used as the template in a real-time TaqMan® PCR reaction. Briefly, 2 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 of a 25 µM GAPDH primer pair mix and 0.2 µL of the TaqMan® probe. The volume was brought up to 20 µL using 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).

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RESULTS AND DISCUSSION

Blood is an excellent resource for diagnostic procedures, forensic applications and identification purposes. It is beneficial for studies utilizing dried blood spots to use a blood DNA isolation method that performs optimally for blood found on any material, as the samples available may be of different types. In this study, DNA was isolated from dried blood spots on cards, cloth and solid surfaces from three different donors using Norgen's Dried Blood Spot Genomic DNA Isolation Kit. Fifteen microliters of each 50 µL elution was then run on a 1X TAE 1.0% agarose gel to visually inspect the isolated gDNA from isolations done after one day and one week post-collection **(Figure 1 a,b)**. All the samples showed good DNA integrity with different yields, as the samples were collected from different donors. High integrity DNA was obtained from samples after one day as well as one week post-collection.

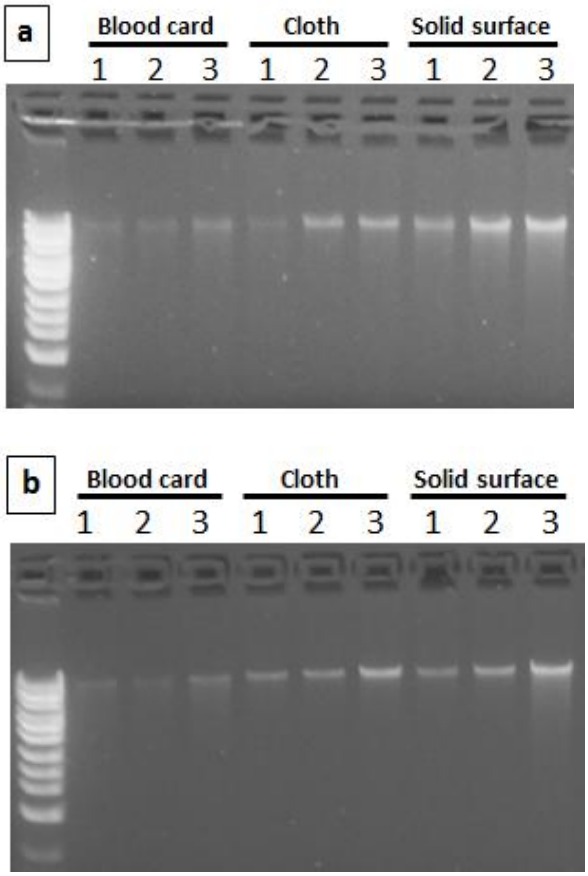


Figure 1. Resolution of DNA isolated from different materials using Norgen's Dried Blood Spot Genomic DNA Isolation Kit. Fifteen microliters of 50 µL elutions were run on 1X TAE 1.0% agarose gel. Panel (a) shows DNA isolated one day post-collection and Panel (b) shows DNA isolated after 1 week post-collection. Marker = Norgen's UltraRanger DNA Ladder. DNA quality was determined through the use of a TaqMan® Real-Time PCR method. Two µL of each sample was used in the reaction. The Ct values were then graphed from each donor from both the one day and one week post-collection isolations **(Figure 2 a,b)**. The average Ct value for blood isolated from each material is illustrated in **Figure 3 (a,b)**. Positive amplification was obtained from all samples regardless of the collection method or time post-collection (1 day or 1 week). The overall Ct average for each collection method (average Ct from the three donors per each collection method) shows non-significant differences between samples isolated after one day post collection and those isolated after one week post-collection.

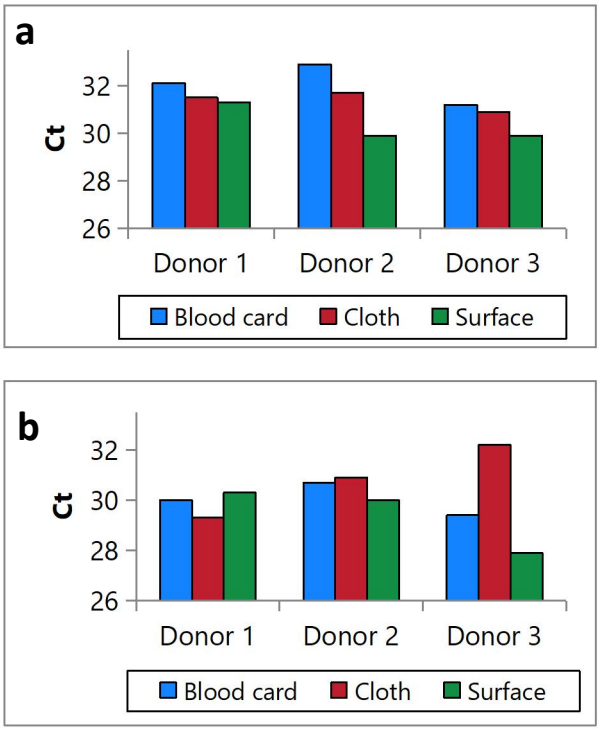
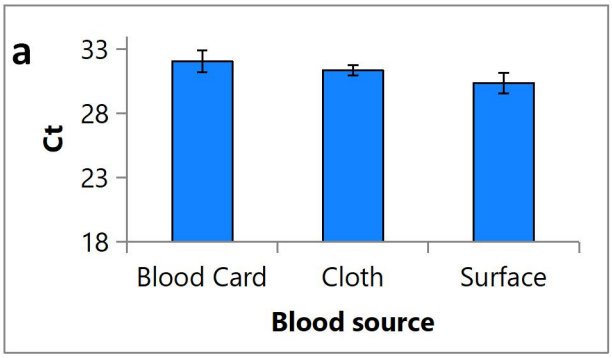


Figure 2. The difference in Ct values obtained from a Taqman® qPCR reaction performed on DNA isolated from different materials using Norgen's Dry Blood Spot Genomic DNA Isolation Kit. Two microliters of each elution were used in a 20 µl qPCR reaction involving GAPDH primers. Panel (a) corresponds to isolation done one day post-collection and Panel (b) corresponds to isolation done 1 week post-collection.



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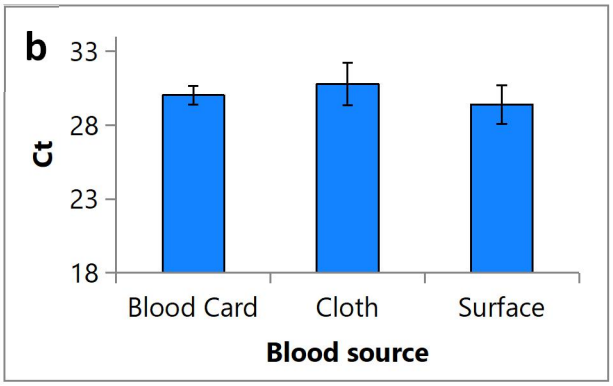

Figure 3. The average Ct value between DNA isolated from the different materials using a Taqman® qPCR reaction performed on DNA isolated from different materials using Norgen's Dry Blood Spot Genomic DNA Isolation Kit. Two microliters of each elution were used in a 20 µL qPCR reaction involving GAPDH primers. Panel (a) corresponds to solation done one day post-collection and Panel (b) corresponds to isolation done 1 week post-collection.

CONCLUSIONS

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

1. Norgen's Dried Blood Spot Genomic DNA Isolation Kit can isolate good integrity DNA from cloth as well as surface after one day or one week post-collection drying at room temperature.
2. All of the isolated DNA have high quality and free of PCR inhibitors.

REFERENCES

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