Short Research Article

RHD gene amplification on reused FTA card

ABSTRACT

Aims: *RHD* gene analysis performed directly from repeatedly extracted DNA on FTA card disc has not been studied. Here we demonstrate a direct real-time PCR of *RHD* gene exon 10 by reusing FTA card.

Study design: This is an experimental study to observe the *RHD* exon 10 amplification on repeatedly extracted DNA on FTA card.

Methodology: Five samples from dry blood spot on FTA cards were subjected to wash and lysis four times without additional extraction process. The lysates on each round was demonstrated for signs of amplification by melting curve analysis and agarose gel electrophoresis.

Results: successful amplification of *RHD* gene exon 10 was observed by melting curve analysis (melting temperature 84.85℃) and reconfirmed by 2% agarose gel electrophoresis (393 bp fragment).

Conclusion: Repeated amplification of DNA from direct FTA card with dried blood sample without the need of time consuming extraction process was possible.

Keywords: FTA card, *RHD* gene, Real-time PCR

ABBREVIATIONS

K2EDTA: K2 Ethylenediaminetetraacetic acid; PCR: Polymerase chain reaction; TAT: Turnaround time

1. INTRODUCTION

Deoxyribonucleic acid (DNA) can be obtained from different biological samples stored on FTA cards (1-3). DNA on FTA card had been amplified directly and post extraction using conventional polymerase chain reaction (PCR). A study by Choi and colleagues showed that DNA extraction method used for dried blood spot on FTA card is suitable for diagnostic screening in drug discovery (4). Pak Yang and Chas André from Thermo Fisher Scientific were able to amplify different genomic DNA on dried blood from direct FTA card (1 mm disc direct into PCR reaction) without the need of extraction process (5). However once processed, sample disc cannot be reused as DNA template by this direct method. Repeated extraction of DNA from processed FTA card has been possible and successful (6), but no studies demonstrated that with direct FTA card processed disc. Here, we modify previously described method and demonstrate repeated DNA yield from reusing FTA card by analysing *RHD* exon 10 using direct real time PCR melting curve indicator.

2. MATERIALS & METHODS

Sample preparation

A total of 40 µl K2EDTA blood sample per well was applied on Whatman FTA card (GE Healthcare) and dried at 80oC for 30 minutes. The samples were stored at room temperature until used. For sample preparation; 3 mm disc was punched out of the sample in FTA card and placed in 1.5 microcentrifuge tube. Quick washing and lysing process were followed as described previously (5). The samples were washed with 20 µl of ddH2O at 50oC for 3 minutes. Tris-EDTA buffer pH 8.0 (Qiagen, UK) was added after water removal and the samples were incubated for 5 minutes at 98oC in a heating block. Lysate was transferred to 0.5 ml tube and the FTA card was subjected to new washing and lysing round. The process repeated for total of four times with each sample.

Real time PCR

A qualitative study was performed using real time PCR using melting curve analysis indicator. Each sample with 4 lystaes round sub-tubes was run in duplicates. The real time PCR was used to demonstrate q1 the presence of *RHD* gene exon 10. *RHD* positive and negative samples were used as controls. The primer pair used was as follows: Forward- 5'-TAGGCTGTTTCAAGAGATCAAGC-3'; Reverse- 5'-AGCTTACTGGATGACCACCA-3' (7) which amplifies product of 393 bp.

Real time PCR was performed in a total volume of 22 µl consisting of 1x qPCRBIO SyGreen master mix, 400nM of each primer and 4 µl of lysate (DNA template). The reaction was carried out in Rotor-Gene Q thermocycler (Qiagen). Thermocycler was run for 40 cycles under the following condition: initial denaturation at 95oC for 3 min, denaturation at 95oC for 5 sec, annealing and extension at 60oC for 20 sec.

3. RESULTS

We observed, *RHD* gene exon 10 amplification in all 4 tubes for both samples with average melting curve temperature of 84.85oC (Figure 1). This was validated and reconfirmed by 2% agarose gel electrophoresis that gave a single band of expected 393 bp DNA product (Figure 2).

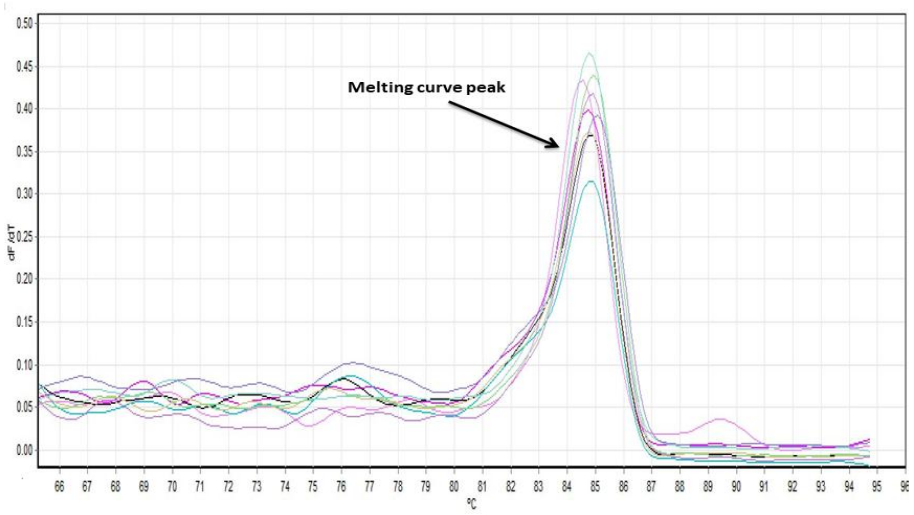
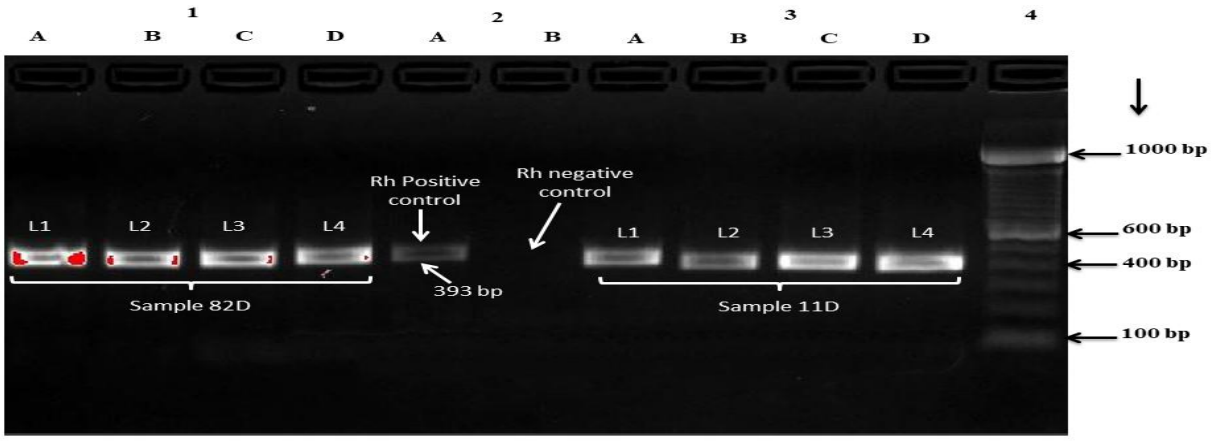


Fig. 1. *RHD* gene exon 10 melting curve analysis of representative samples from washing 1 through 4 rounds. All lysates showed melting curve peak between 84.60 and 84.85℃ with



median temperature of 84.85℃.

Fig. 2. Representative samples for *RHD* exon 10 amplification on 2% gel electrophoresis. Lanes 1A, 1B, 1C and 1D: sample 82D with amplification of *RHD* exon 10 in each lysate L1 through L4 (393 bp fragment). Lane 2A: Amplification exon 10 of Rh(D) positive control (393 bp fragment), Lane 2B: D negative control with no amplification of *RHD* exon 10. Lanes 3A, 3B, 3C and 3D: sample 11D with amplification of *RHD* exon 10 in each lysate L1 through L4 (393 bp fragment).Lane 4: 100 bp DNA ladder.

4. DISCUSSION

In this study, a total of five dry blood spot samples from FTA card subjected to four rounds of washing and lysis were tested to amplify *RHD* gene exon 10. We successfully amplified 393 bp product with this modified method. These data suggest using of direct FTA disc containing dry blood spot may be stored for a prolonged period and reused for another round. Minimal storage space and room temperature storage are two advantages of FTA cards use (8). As we demonstrated successful amplification of one of the red cell genotyping, these cards may be used in blood banks in future for donors typing without the demand of large freezer spaces to store DNA for years.

Fast turnaround time (TAT) is one of the quality signs of laboratory service (9). Thus the use of direct FTA card without extraction improves TAT ensuring better quality service. Wanger and colleagues study on red cell genotyping direct PCR using plasma or serum demonstrated fast TAT with analysis being completed in 40 minutes only (10). We also demonstrated improved TAT as samples were amplified without need of extraction saving two to three hours.

5. CONCLUSION

Repeated amplification of DNA from direct FTA card with dried blood sample without extraction process was possible. This eliminates extraction process reducing TAT for better laboratory quality service and limits the demand of freezer space for DNA storage. In future, we would further explore the present study to include more samples for direct FTA card testing which will include red blood cell *RHD* genotyping for the presence of exon 1 through 10.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

Ethical Approval:

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

CONSENT FOR PUBLICATION

Participant's written consent is obtained for publication.

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