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A note on the use of FTA™ technology for storage of blood samples for DNA analysis and removal of PCR inhibitors

M.P. Mullen[1,2]†, D.J. Howard[2], R. Powell[1Φ] and J.P. Hanrahan[2]
[1]*Department of Microbiology, National University of Ireland, Galway, Ireland*
[2]*Teagasc, Animal Production Research Centre, Athenry, Co. Galway, Ireland*

FTA™ technology is widely used across many molecular disciplines for sample capture, storage and analysis. The use of this technology for the long-term storage of blood samples for DNA analysis was examined as well as its potential to remove inhibitors from DNA samples previously extracted from blood with PCR inhibitors remaining. It was found that blood spots stored on FTA™ cards for 8 years at room temperature gave successful PCR products and that FTA™ cards are a useful tool for removing substances in samples which interfere with or inhibit, the PCR reaction.

Keywords: blood; DNA storage; FTA™; PCR inhibitor

Introduction

The generation of numerous DNA samples from biological specimens is now an element in many research projects. Preparing and archiving a large collection of samples for DNA analysis can present logistical problems. One solution is the blood storage medium FTA™ Classic Cards. An FTA™ card safely stores genomic DNA, in the form of dried spots of whole blood, or can be used with almost any sample type, including cultured cells, buccal cells, plant material, bacteria, plasmids, solid tissue, viral particles and M13 plaques (www.whatmann.com). Upon sample application onto the cards, cell membranes and organelles are lysed and the released nucleic acids are

†Corresponding author: michael.mullen@teagasc.ie
ΦDr. Richard Powell, a valued colleague, died unexpectedly during the course of the work described herein.

entrapped in the matrix. The nucleic acids remain immobilized and are stabilized for transport, immediate processing or long-term storage at room temperature. Stored at room temperature, genomic DNA on FTA™ is reported by the manufacturers to be stable for at least 17 years (www.whatmann.com). Prior to analysis of the bound DNA, the samples are purified by washing with FTA™ Purification Reagent and removing the stabilising chemicals and cellular inhibitors of enzymatic reactions. The FTA™ purification method is very mild and similar to detergent based protocols (Montgomery and Sise, 1990) as compared to traditional phenol based purification methods (Maniatis, Fritsch and Sambrook, 1989). Since the DNA remains bound to the paper, the manipulations to purify the DNA are simplified and amenable to automation. DNA samples on FTA™ cards offer a very compact archival system compared to glass vials or plastic tubes in freezers. FTA™ technology has also been shown to have practically identical limits of detection in PCR to those using traditionally extracted DNA (Alhassan *et al.*, 2007). Successful PCR is dependant on the sample being free of polymerase inhibitors such as haemoglobin (Abu Al-Soud and Rådström, 2001), lactoferrin (Abu Al-Soud and Rådström, 2001) and IgG (Abu Al-Soud, Jönsson and Rådström, 2000) commonly found in blood. As little as 0.004% v/v of blood in the PCR reaction is sufficient for complete inhibition to occur (Rådström *et al.*, 2003). The occurrence of PCR inhibitors in samples post DNA extraction has been described for sources such as stools (Stauffer *et al.*, 2008) and blood (Akane *et al.*, 1994). This present study assessed the suitability of FTA™ technology for inhibitor removal and long-term storage (8 years) of DNA at room temperature.

Materials and Methods

Reagents
FTA™ reagents were obtained from Whatmann and PCR reagents from Invitrogen, unless otherwise noted.

Samples
Four samples of dried sheep blood spotted on FTA™ cards in 2000 were tested (in 2008) to confirm the long term stability of DNA. Four samples of sheep DNA that had been extracted using a traditional detergent method but contained inhibitors were used for the inhibition removal analysis. An individual sample of DNA of known quality and quantity was used as both an internal amplification control (IAC) and an external amplification control (EAC). The IAC was used to confirm the presence of inhibitors in the pre extracted DNA samples, and the EAC was used to confirm the correct operation of the equipment and reagents used in the PCR.

Preparation of samples from an FTA™ card
DNA samples, extracted previously using a traditional detergent method, were spotted onto FTA™ cards and allowed to dry overnight at room temperature. Using a Harris Micro-Punch™ apparatus with mat (Whatmann), a 1.2 mm diameter punch was taken from dried spots from both the freshly spotted extracted samples and the samples spotted in the year 2000. The micro-punch was cleansed with alcohol between punches to prevent cross-contamination. The punches were processed according to the manufacturer's instructions. Briefly, the punches were washed three times with FTA™ purification reagent for 5 min at room temperature, followed by two washes with TE (10 mM Tris pH 8.0, 0.1 mM EDTA). The washed punches were dried for 30 min

at 60 °C. Processed FTA™ Card punches were assayed immediately.

Amplification

The processed FTA™ punches (1.2 mm punches were equivalent to ~20 ng genomic DNA) were placed in PCR tubes, with the following components added and the final volume adjusted to 50 µL with sterile double distilled H_2O: 200 nM each primer, 800 µM DNTPs, 1.5 mM $MgCl_2$ & 2.5 U *Taq* polymerase. Using an Eppendorf Mastercycler the samples were heated to 95 °C for 3 min followed by 30 cycles of 95 °C for 30 s, 63 °C for 30 s, 72 °C for 30 s, with a final extension step of 72 °C for 5 min. The reaction products were held at 4 °C until use, typically within 24 h.

Two primer sets were used in the amplification, both amplifying segments of the BMP15 gene located on the ovine X chromosome; one produced 191 bp DNA fragment and the second produced 141 bp DNA fragment (Table 1).

The PCR products were visualised using a 2.5% agarose gel with ethidium bromide staining. A DNA marker of known DNA fragment sizes (100 bp ladder) was run along side the samples to aid in identification of the products.

Results and Discussion

In this study, it was found that the FTA™ cards enabled successful PCR with DNA samples extracted from blood that have previously failed to yield PCR products. The presence of inhibitors was confirmed by the addition of an internal amplification control to the PCR reaction. The absence of the PCR product from both of the DNA templates confirmed the presence of inhibitors in the test sample. This contaminated DNA upon treatment with the FTA™ system gave a high quantity of quality PCR products (Figures 1 and 2). The EAC was successful in all cases and produced the desired PCR product, confirming that the parameters and components in the PCR were correct (Figure 1).

Figure 2 shows the amplification of 191 bp PCR products of an additional four extracted DNA samples which were previously known to contain inhibitors.

The DNA that was spotted onto the FTA™ cards in year 2000 gave successful amplification of the target 191 bp product after 8 years stored on FTA™ cards at room temperature (Figure 2).

Samples which have repeatedly failed to yield PCR products due to inhibitors can be rescued for testing using the FTA™ system. Although this testing only encompassed removing inhibitors from blood, it is most likely that PCR inhibitors found for other sample sources such as food or feed, and well established problematic sources such as urine and faeces, would also be removed from the DNA.

FTA™ usefulness for the preservation of biological samples for subsequent DNA analysis was also examined. It was found that DNA from blood samples stored on FTA™ classic cards is stable for at least 8

Table 1. Nucleotide sequences of the primer sets used

Primer name	Strand	Nucleotide sequence	PCR product size (bp)
BMP15F2	Forward	5'-CACTGTCTTCTTGTTACTGTATTTCAATGAGAC-3'	141
BMP15R4	Reverse	5'-GATGCAATACTGCCTGCTTG-3'	
BMP15HPYF	Forward	5'-GCAGCCAAGAGGTAGTGAGG-3'	191
BMP15HPYR	Reverse	5'-CAATACTGCCTGCTTGACGA-3'	

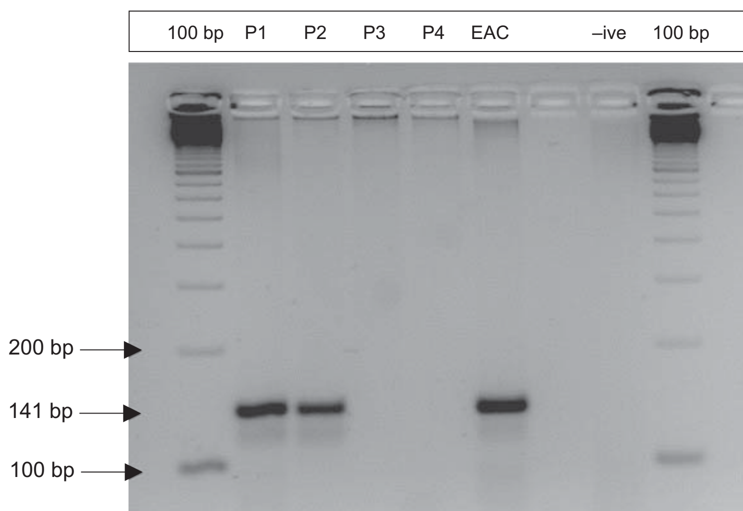

Figure 1. PCR amplification of DNA samples pre- and post-FTA™ purification. 100 bp: Molecular size marker (100, 200, 300, 400 bp, etc.). EAC: External amplification control (DNA of known quality and quantity was used as a positive control for the PCR). The same DNA was used for the internal amplification control (IAC). P1–P2: 141 bp PCR product from the DNA samples post-FTA™ purification. P3–P4: Absence of PCR product from both the IAC and the sample DNA prior to FTA™ treatment. –ive ctl: Negative control (no DNA added).

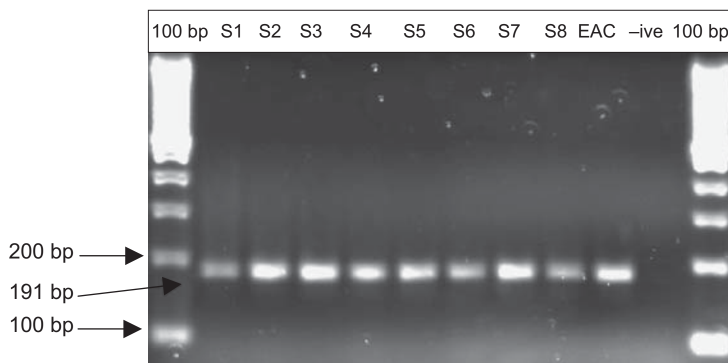

Figure 2. PCR amplification of 8-year old blood spots and FTA™ treated DNA samples which contained inhibitors. 100 bp: Molecular size marker (100, 200, 300, 400 bp, etc.). EAC: External amplification control (DNA of known quality and quantity was used as a positive control for the PCR). S1–S4: PCR products from 8-year old blood spots, stored using the FTA™ system. S5–S8: 191 bp PCR products from existing DNA samples extracted with inhibitors present and purified with the FTA™ system. –ive ctl: Negative control (no DNA added).

years and provided ample PCR products for downstream analysis such as sequencing or genotyping. However, this system does have some drawbacks such as limited return of DNA from each dried blood spot (with approximately 20 ng per 1.2 mm diameter punch), and the requirement for carrier free genomic DNA and accurate DNA concentrations for certain commercial analysis, e.g., SNP (single nucleotide polymorphism) chips and genotyping platforms. Therefore, it is still prudent to use traditional extraction procedures from whole blood as a primary method for DNA extraction, where practicable. Carrier bound DNA from systems such as FTA™ may still be used for DNA demanding applications but will require whole genome amplification (WGA) to provide sufficient DNA. WGA has been shown to have very high concordance (>99.8%) with intact genomic DNA (Barker *et al.,* 2004) when analysed across a 2320 SNP panel but has not been proven on all commercial platforms and may introduce bias to the genotyping results (Dean *et al.,* 2002). The best use of the FTA™ system may lie in a secondary role for processing of samples, e.g., for routine screening of established SNPs of importance or candidate gene SNP discovery, without the need to repeatedly return to the stock solutions. It is evident, from considarations such us ease of use, space saving, long term stability and storage at room temperature, that the FTA™ card technology is a valuable tool for molecular biology.

References

Abu Al-Soud, W., Jönsson, L.J. and Rådström, P. 2000. Identification and characterization of immunoglobulin G in blood as a major inhibitor of diagnostic PCR. *Journal of Clinical Microbiology* **38:** 345–350.

Abu Al-Soud, W. and Rådström, P. 2001. Purification and characterization of PCR-inhibitory components in blood cells. *Journal of Clinical Microbiology* **39:** 485–493.

Akane, A., Matsubara, K., Nakamura, H., Takahashi, S. and Kimura, K. 1994. Identification of the heme compound copurified with deoxyribonucleic acid (DNA) from bloodstains, a major inhibitor of polymerase chain reaction (PCR) amplification. *Journal of Forensic Science* **39:** 362–372.

Alhassan, A., Iseki, H., Kim, C., Yokoyama, N. and Igarashi, I. 2007. Comparison of polymerase chain reaction methods for the detection of *Theileria equi* infection using whole blood compared with pre-extracted DNA samples as PCR templates. *Tropical Animal Health and Production* **39:** 369–374.

Http://www.whatman.com/FTANucleicAcidCollectionStorageandPurification.aspx [Accessed October 2008].

Maniatis, T., Fritsch, E.F. and Sambrook, J. 1989. "Molecular Cloning: A Laboratory Manual", Second Edition. Cold Spring Harbor Press, Cold Spring Harbor, New York, 2344 pages.

Montgomery, G.W. and Sise, J.A. 1990. Extraction of DNA from sheep white blood cells. *New Zealand Journal of Agricultural Research* **33:** 437–441.

Stauffer, S.H., Birkenheur, A.J., Levy, M.G. and Marr, H. 2008. Evaluation of four DNA extraction methods for the detection of *Tritrichomonas foetus* in feline stool specimens by polymerase chain reaction*. Journal of Veterinary Diagnostic Investigations* **20:** 639–641.

Rådström, P., Knutsson, R., Wolffs, P., Dahlenborg, M., Löfström, C. 2003. PCR detection of microbial pathogens. *Methods in Molecular Biology* **216:** 31–50.

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