DNA Extraction from Blood Stored on FTA Cards

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Abstract
Informed consent was obtained from 238 unrelated Libyan individuals (Benghazi region). DNA was extracted from blood stains collected on FTA cards About 1.2 mm FTA1 disc and 1 ng of DNA purified from buccal swabs was used products were separated and detected using the ABI Prism 310xl Genetic Analyzer (Applied Biosystems) according to the manufacturer’s recommended protocol. The data were analysed using GeneMapper ID v3.2 (Applied Biosystems). Quality control - The laboratory has participated in the Y-STR Haplotyping Quality Assurance Exercise (Certified at 2010-5-20). We conclude that amplification of DNA stored on FTA cards can be used safely to analyse DNA profiles.

Introduction
DNA-based genetic analysis has a long-standing and important role in research, medical diagnostics, and DNA profiling of individuals. Characterization or typing of blood, serum, and other body fluid and tissues has been used for forensic and clinical purposes for more than 50 years [1]. In the 25 years, methods have become available for DNA typing showing differences in the genetic material itself. Development in genetic and modern molecular biology has accumulated knowledge about the human genome [2], especially with the significant progress of the Human Genome Project [3].

Blood and Buccal Swabs Collection
Approximately 200 µl of venous blood samples were collected in tubes containing EDTA to prevent blood coagulation. The collected blood samples were stored on FTA cards (Figure 1). Epithelial nucleated cells were collected using swabs, by asking the volunteer to rub the swab on the inside surface of his/her cheek and subsequently drying the swabs by holding them at room temperature exposed to air for approximately 5 minutes. The DNA samples (swabs and FTA cards) were placed into respective containers and stored at room temperature. It was confirmed that refrigeration or freezing was not neccessary and that good results were obtained from most samples stored at room temperature. Figure 1 shows one swab sample collected from an individual in Benghazi stored under the same conditions as a sample collected on an FTA card.

Figure 1: FTA Cards Used to Store Blood Collected from Benghazi Subjects (Whatman)
FTA paper is absorbent cellulose–based paper that contains chemical substances to protect DNA molecules from nuclease degradation and prevent bacterial growth as a result, DNA on FTA paper is stable at room temperature over a period of several years. A major advantage of FTA paper is that consistent results may be obtained without quantification (used directly without having to quantify the ideal amount of DNA).

Figure 2: Atypical Electropherogram Showing 17 Y Filer Loci Results of DNA Extracted from Swab Collected in Benghazi and Brought to UK without Refrigeration or Freezing [4]

DNA was extracted from blood stains collected on FTA® cards (Whatman, Kent, UK) using FTA® Purification Reagent to wash the cards (Whatman) following the manufacturer’s protocol. A drop of 150 µl of fresh whole blood was spotted onto FTA® card and dried at room temperature. A circle 1.2 mm in diameter of card containing dried blood was punched out of the FTA® card using a Harris micro punch and placed in an Eppendorf PCR tube (alcohol was used to sterlise punches between samples). The FTA® card was washed by adding 200 µl Whatman FTA® purification reagent and allowed to stand for 5 min at room temperature, following the manufacturer’s protocol. This wash step was repeated three times.
Subsequently, the disc was washed twice with distilled water. The washed disc, now white, was then incubated at room temperature for 1 hour and subsequently ready for being used for the PCR protocol. A novel method was developed to wash discs by washing them twice, the first one by adding 200 μl of the solution and keeping it overnight, and then washing again for 5 minutes with the same amount of solution. Generally good results were obtained with this method.

**Buccal Swab Spin Protocol (QIAamp®DNA Blood Mini Kit)**

For samples obtained with buccal swabs from volunteers, QIAamp®DNA Blood Mini Kit (QIAGEN, Hilden, Germany) was used and DNA was quantified using a StepOnePlus™ Real-Time PCR System (Applied Biosystems, Foster City, USA).

Buccal swabs were placed in a 2 ml microcentrifuge tube and 400 μl (cotton and DACRON swab) or 600 μl (Omni Swab) PBS (Phosphate Buffered Saline) added to the sample. A volume of 20 μl of a QIAGEN Protease stock solution (100 mg/ml; 7000 units/ml) (an endolytic protease that cleaves peptide bonds at the carboxylic sides of aliphatic, aromatic, or hydrophobic amino acids) to the sample. Proteinase K in the extraction buffer inactivates nucleases and aids in the lysis of epithelial and white blood cells to free nuclear DNA. Subsequently, 400 μl of Buffer AL were added and the sample mixed immediately by vortexing for 15 s. Following incubation at 56°C for 10 min., the tube was briefly centrifuged to remove drops from inside the lid. A volume of 400 μl of ethanol (96–100%) was added and the sample mixed again by vortexing, then centrifuged to remove drops from inside the lid. Carefully 700 μl of the mixture was applied to the QIAamp spin column (in a 2 ml collection tube) without wetting the rim, the cap was closed, and centrifuged at 6000 x g (8000 rpm) for 1 min. The QIAamp spin column was placed in a clean 2 ml Collection tube, and the tube containing the filtrate discarded. This step was repeated by applying up to 700 μl of the remaining mixture to the spin column. The QIAamp spin column was opened carefully and 500 μl Buffer AW1 was added without wetting the rim. The cap was closed and the tube centrifuged at 6000 x g (8000 rpm) for 1 min. The QIAamp spin column was placed in a clean 2 ml collection tube, and the collection tube containing the filtrate discarded. The QIAamp spin column was carefully opened and 500 μl Buffer AW2 added without wetting the rim. The tube was centrifuged at full speed (20,000 x g; 14,000 rpm) for 3 min. The QIAamp spin column was placed in a clean 1.5 ml microcentrifuge tube, and the collection tube containing the filtrate discarded. The QIAamp spin column was opened carefully and 150 μl Buffer AE or distilled water added. After incubation at room temperature for 1 min, the tube was centrifuged at 6000 x g (8000 rpm) for 1 min and then either used directly or stored at 4°C (less than 2 wks) or <20°C (>2wks).

**Alternative Method for Extracting Liquid DNA from FTA Cards**

This protocol describes purification of total (genomic) DNA from whole blood stained FTA paper (to extract DNA in liquid form and to be quantified rather than to use cards directly without quantification), using the QIagen spin protocol DNA Purification from blood or body fluids procedure. 20 μl of QIAGEN protease (100 mg/ml; 7000 units/ml) was added into the bottom of a 1.5 ml microcentrifuge tube; discs were punched from FTA paper with blood sample stains (6 discs of 1.2 mm) and placed in a microcentrifuge tube; 400 μl AL buffer was added to the sample and mixed by pulse-vortexing for 15s; The tube was then incubated at 56°C for 10-20 min followed by briefly centrifuging to remove drops from the inside of the lid; 200 μl ethanol (96–100%) were added to the sample, and mixed again by pulse-vortexing for 15 s. After mixing the microcentrifuge tube was briefly centrifuged to remove drops from the inside of the lid; the mixture from step 5 was carefully applied to the QIAamp Mini spin column (in a 2 ml collection tube) without transferring the FTA paper. The tube centrifuged at 6000 x g (8000 rpm) for 1 min. The QIAamp Mini spin column was placed in a clean 2 ml collection tube and the tube containing the filtrate was discarded. The QIAamp Mini spin column was opened carefully and 500 μl Buffer AW1 was added without wetting the rim. The cap was closed and the...
tube centrifuged at 6000 x g (8000 rpm) for 1 min. The QIAamp Mini spin column was placed in a clean 2 ml collection tube and the collection tube containing the filtrate was discarded. The QIAamp Mini spin column was opened carefully and 500 μl Buffer AW2 added without wetting the rim. The tube centrifuged at full speed (20,000 x g; 14,000 rpm) for 3 min. The QIAamp Mini spin column was placed in a new 2 ml collection tube and the old collection tube was discarded with the filtrate. The collection tube was centrifuged at full speed for 1 min. The QIAamp Mini spin column was placed in a clean 1.5 ml microcentrifuge tube, and the collection tube containing the filtrate was discarded. The QIAamp Mini spin column was carefully opened and 100-200 μl Buffer AE or distilled water was added. The tube was incubated at room temperature (15–25°C) for 1 min, and then centrifuged at 6000 x g (8000 rpm) for 1 min. Pure liquid DNA in solution instead of on FTA paper was now ready to be used in different experiments. This method for extracting DNA in liquid form resulted in amplifiable DNA, as can be seen in Figure 3.

Figure 4: Electropherogram Showing the Profile of 15 Autosomal Loci Using the Identifiler Kit for One of the Benghazi Samples [5]

Amplification Using Bloodstained FTA Cards
FTA™-treated DNA collection cards can be useful for the collection, storage, and processing of biological samples. A small punched out piece of the bloodstained card placed directly into an amplification tube, purified, and amplified. Previous studies have indicated that a
1.2-mm bloodstained punched disc contains approximately 5-20 ng DNA. Accordingly, an appropriate cycle number for this high quantity of DNA is 27 cycles (yhrd.org). A 1.2 mm diameter FTA disc and 1 ng purified DNA from a buccal swab were directly used to amplify 17 STR loci using the AmpFISTR Yfiler PCR kit and AmpFI STR Identifier kit for fifteen autosomal loci in accordance with the manufacturer's instructions. PCR reagent was: 9.2 µl, 5 µl of primers, and 0.8 µl Taq Polymerase were added to 1.2 mm diameter FTA discs or 1ng genomic DNA from buccal swabs inside PCR tubes (total volume 15 µl). PCR was carried out in a Thermocycler 9700 (Applied Biosystems) (see 2.7). PCR conditions were as follows: starting temperature was 95°C for 11 min, (denaturing temperature 95°C for 1min, annealing 59°C for 1min, extension 60°C for 1min, 27 cycles), then 60°C for 80 minutes for Y-filer and 60 minutes to allow the completion of replications for Identifier and holding at 4°C, according to the manufacturer's protocol (AmpFISTR Yfiler and Identifier Amplification KitsUser's Manual).

Conclusion
Worldwide genetic databases are useful tools in forensic genetics, given that any individual profile must be compared against a population dataset.
In this study we used FTA cards to translate the blood samples from Libya to UK and we amplify them by cutting the samples from the cards directly without using any method of DNA extraction and we got same results of liquid DNA. That is mean we can use them in cases from abroad.

References