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DNA quality and quantity from up to 16 years old post-mortem blood stored on FTA cards

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ABSTRACT

Blood samples preserved on FTA cards offer unique opportunities for genetic research. DNA recovered from these cards should be stable for long periods of time. However, it is not well established as how well the DNA stored on FTA card for substantial time periods meets the demands of forensic or genomic DNA analyses and especially so for from post-mortem (PM) samples in which the quality can vary upon initial collection. The aim of this study was to evaluate the time-dependent degradation on DNA quality and quantity extracted from up to 16 years old post-mortem bloodstained FTA cards.

Four random FTA samples from eight time points spanning 1998 to 2013 ($n = 32$) were collected and extracted in triplicate. The quantity and quality of the extracted DNA **s**amples were determined with Quantifiler® Human Plus (HP) Quantification kit. Internal sample and sample-to-sample variation were evaluated by comparing recovered DNA yields. The DNA from the triplicate samplings were subsequently combined and normalized for further analysis. The practical effect of degradation on DNA quality was evaluated from normalized samples both with forensic and pharmacogenetic target markers.

Our results suggest that (1) a PM change, e.g. blood clotting prior to sampling, affects the recovered DNA yield, creating both internal and sample-to-sample variation; (2) a negative correlation between the FTA card storage time and DNA quantity ($r = -0.836$ at the 0.01 level) was observed; (3) a positive correlation ($r = 0.738$ at the level 0.01) was found between FTA card storage time and degradation levels. However, no inhibition was observed with the method used. The effect of degradation was manifested clearly with functional applications. Although complete STR-profiles were obtained for all samples, there was evidence of degradation manifested as decreased peak heights in the larger-sized amplicons. Lower amplification success was notable with the large 5.1 kb *CYP2D6* gene fragment which strongly supports degradation of the stored samples.

According to our results, DNA stored on FTA cards is rather stable over a long time period. DNA extracted from this storage medium can be used as human identification purposes as the method used is sufficiently sensitive and amplicon sizes tend to be <400 bp. However, DNA integrity was affected during storage. This effect should be taken into account depending on the intended application especially if high quality DNA and long PCR amplicons are required.

1. Introduction

Dried blood spot technology has been used to preserve samples since the 1960s as part of newborn screenings [1]. Filter paper based storage methods have also gained popularity in other fields, such as microbiology [2], biological fieldwork [3], bio-banking [4,5], human genomics [6,7], epigenomics [8], pharmacogenetics [9,10], and forensic investigations [11–13]. Sample preservation on filter paper enables simple and flexible sample collection that allows for facile transport and archiving at room temperature. In the 1990's chemically treated filter papers became available.

Whatman™ Flinder's Technology Associates (FTA) Cards (GE Healthcare Bio-Sciences Corp., NJ, USA) are based on a

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chemically-treated matrix, which lyses cells from a variety of tissue sources (e.g. blood, saliva, plant tissue). Upon immediate cell lysis the released DNA is bound within the supporting material, e.g. a card. The matrix protects nucleic acids from damaging agents (e.g. nucleases, oxidative agents and bacterial growth) which serves to reduce degradation. According to the manufacturer, even 22-year-old blood samples can yield short tandem repeat (STR) profiles commonly generated in forensic genetics [14]. However, most of these studies evaluated fresh blood samples which are applied immediately on the FTA cards, although other sample types also have been reported [11,15–17]. Many of these studies have targeted forensically-relevant STR markers [18,19] or single nucleotide polymorphisms (SNPs) [20], and only few studies report the results from genome wide association studies [21]. DNA quality and quantity in PM samples with variable PM intervals (PMIs) stored on FTA paper have not tested to determine if such samples meet the demands of forensic or other genomic DNA analyses.

This study evaluates time-dependent DNA degradation and the ability to amplify STRs and a longer genomic target (i.e. the *CYP2D6* gene) in PM blood samples on FTA cards stored up to 16 years. First, the internal sample variation was evaluated by comparing DNA yields obtained from three different spots of the same bloodstain sample. Sample-to-sample variation was evaluated in a similar manner. Second, the quality and quantity of recovered DNA as well as the level of inhibition associated with the DNA extracts were assessed. Finally, the effective DNA yield was evaluated by determining the amplification success of short and relatively large amplicons using forensic and pharmacogenetic genotyping applications, respectively.

2. Materials and methods

Blood samples stored on the FTA Gene cards were obtained from forensic autopsies performed in 1998-2013. Certified standardized procedures were implemented in all autopsy procedures, including the collection of PM blood and its application onto the FTA cards. The sampled cadavers showed variation in the peri-mortem pathophysiological condition and in the extent of PM changes. To evaluate the time-dependent DNA degradation four samples were selected at random from each of the eight evenly separated time points covering the entire storage period (Fig. 1a).

2.1. DNA extraction and quantification

All reagents and instruments used were from Applied Biosystems (Foster City, CA, USA), Life Technology Corporation (Carlsbad, CA, USA), unless mentioned otherwise.

FTA cards were pierced with a Harris Micro-Punch™-puncher (Ted Pella, Inc., Redding, CA, USA). Each FTA bloodstain sample was extracted in triplicate, using four (2.0 mm diameter) punches as input material for each extraction (Fig. 1b). To avoid cross-contamination, the tip of the puncher was cleaned between samples by piercing clean filter paper multiple times.

The bloodstain discs were lysed according to the manufacturer's protocol using PrepFiler *Express*™ Forensic DNA Extraction Kit, and DNA was extracted with the semi-automated AutoMate *Express*™ Forensic DNA Extraction System. Recovered DNA was eluted to a 50 μl final volume with the elution buffer provided. The quality and quantity of extracted DNA were determined with Quantifiler® HP DNA Quantification Kit using 7500 Real-Time PCR system. Data analysis was performed with the HID Real-Time PCR Analysis Software v1.2.

After quantification of each extract, the triplicate samples were pooled and re-quantified with the Quantifiler® HP kit. Based on these results, the amount of PCR template DNA was normalized to 2 ng and 35 ng for STR analysis and *CYP2D6* whole gene amplification, respectively. For the normalization, quantification results of the small autosomal target locus of the Quantifiler® HP kit was used as recommended by the manufacturer [22].

DNA degradation was assessed by the degradation index (DI) defined by the Quantifiler® HP DNA Quantification kit user's manual [22]. DI measures the amplification success ratio of a small (80 bp) and a large (214 bp) autosomal target fragment. Values of $DI > 1$ indicate poorer amplification of the large fragment compared with that of the short fragment, which according to the Quantifiler® HP user's manual indicates sample degradation.

Assessment of inhibition was based on amplification success of the internal positive control (IPC) in the RT-PCR assay of the Quantifiler® HP kit. Inhibiting agents present in a sample cause lower amplification success of the IPC, shown as an upward shift in the cycle threshold (C_t) value. Under normal conditions (no inhibition), the IPC passes the threshold around cycle 27. C_t-values above 30 are considered an indication of inhibition [22].

2.2. Evaluation of the amplification success

To measure the practical effect of degradation, amplification success of two types of autosomal loci, differing in length, was analyzed. Amplification of short fragments was assessed with GlobalFiler®, a forensic STR multiplex assay (75-444 bp amplicons), and long fragments with amplification of a 5.1 kb genomic *CYP2D6* gene fragment.

2.3. STR analysis

GlobalFiler® multiplex assay, which amplifies 21 autosomal STR loci, two Y chromosomal markers (DYS391, Y indel) and one sex determining marker (Amelogenin), was performed according to the manufacturer's protocol, except that the reaction volume (7.5 μl) and 25 PCR cycles used. These amplification conditions have been validated in our laboratory and are part of the accredited method. Amplified DNA fragments were separated by capillary electrophoresis using a 24-capillary 3500xL Genetic Analyzer, polymer 4 (POP-4™) and HID36_POP4xl Run Module. Samples were run along with 500LIZ™ dye size standard. Data analysis was performed with GeneMapper® ID-X ver. 1.4 software. For allele calling, 400 relative fluorescence units (rfu) were used as a peak amplitude threshold, and the fragment sizes were called using the "Local Southern" sizing method.

The STR-data was analyzed by comparing allele peak heights observed in the FTA samples with those obtained for samples of good quality, i.e. the positive control. Peak heights of the two heterozygotic peaks were summed. For the analysis, the loci were assigned relatively evenly into four size categories based on the average amplicon size: <130 bp (D3S1358, D2S441, D19S433, D22S1045, D10S1248, D8S1179), 130-200 bp (D1S1656, vWA, TH01, D5S818), 200-300 bp (D16S539, D21S11, D13S317, D7S820, D12S391), and >300 bp (CSF1PO, TPOX, D18S51, FGA, SE33, D2S1338). For each sampling time point, the average of all peak heights observed within a size class was then compared with the average peak heights observed in two independent PCRs of the positive control (DNA Control 007).

2.4. CYP2D6 analysis

To evaluate the integrity of longer DNA fragments the pharmacogenetically interesting *CYP2D6* gene was chosen. *CYP2D6* gene is part of the large *CYP2D* cluster which is formed together with two pseudogenes, *CYP2D7* and *CYP2D8P* [23]. To exclude the highly homologous pseudogenes from the target of interest, the