FTA cards: high-quality media for storage and transport of DNA

Whatman™ FTA™ provides a wide variety of products to meet the requirements of sample collection, transportation, and storage of DNA. FTA cards provide a simple solution to collect, preserve, and purify biological samples at room temperature for downstream DNA analysis. GE addresses the needs of its databasing and forensic customers with its Whatman FTA technology.

FTA technology
As global databases are expanded, forensic laboratories are faced with increasing numbers of biological samples that require processing. Although laboratory automation and new direct STR amplification chemistries are alleviating some of the challenges experienced in databasing processes, forensic laboratories still require standardized collection procedures, consistent DNA collection media, and stabilized nucleic acids that can be analyzed years after collection.

GE manufactures high-quality FTA sample collection cards and kits. Additionally, we offer a wide range of off-the-shelf and custom FTA products for the collection of human blood, buccal cells, and saliva.

The FTA family of products facilitates collection, transport, purification, and long-term, room temperature storage of DNA on a single sample collection card. We can confirm that these simple cards are well-suited for databasing and forensic applications.

FTA cards provide flexibility; one sample applied to an FTA card can be used with multiple downstream technologies. FTA may be applied to a number of common forensic workflows, including:

- DNA extraction using organic solvents or commercially available kits
- DNA quantitation using real-time PCR
- Short tandem repeat (STR) analysis using direct or standard (conventional) chemistries
- Mitochondrial DNA sequencing
- Single nucleotide polymorphism (SNP) analysis

FTA encompasses a high-quality paper coating chemistry that will lyse eukaryotic cells on contact, denature proteins, and protect DNA from degradation. FTA contains chemical denaturants and a free radical scavenger; the DNA remains tightly bound when proteins and inhibitors are washed from the matrix.

FTA card types and DNA analysis options
Two types of FTA cards are available to accommodate different sample types. Indicating cards have been designed for use with clear samples such as buccal cells and saliva. The cards include a pink indicating dye that turns white when a clear sample is applied. Non-indicating cards are used with blood and other samples that are not clear.

GE manufactures, chemically coats, and cuts all non-indicating and Indicating FTA paper to exacting standards (see Manufacturing).

There are three options for DNA analysis with FTA cards: direct amplification; standard amplification with rinsing ("punch-in method"); and extraction of DNA from the card.

Long-term DNA stability
FTA cards are ideal for long-term DNA preservation. They have been shown to be more efficient than untreated matrices for both long-term preservation and storage of DNA (see Comparison of FTA and untreated paper).

FTA cards lyse eukaryotic cells on contact. Following capture, DNA is protected from environmental and enzymatic damage by a proprietary chemical composition impregnated on the card.

DNA has been shown to remain intact on FTA cards at ambient temperatures for years, so there is no need for freezers to archive the samples. For example, DNA from 12-year-old buccal samples and 22-year-old blood on FTA cards has generated STR data with good signal strength in both direct (Fig 1) and standard (Fig 2) amplifications.
Several countries have introduced regulations requiring long-term archiving and retesting of samples years after collection. For example, in the US, several states require post-conviction testing, which may occur years after samples are stored. The chemical matrix on FTA protects nucleic acids from ultraviolet (UV) radiation (see UV protection of DNA) and other environmental agents. The protective qualities of FTA chemistry enable the long-term archiving of samples.

We have successfully amplified DNA from samples stored on FTA cards for over a decade in multi-barrier pouches (with desiccant) at ambient temperatures (see Direct STR amplification).

**Direct STR amplification**

Direct STR profiling of DNA from samples on FTA cards is simple: remove the card (with applied sample) from the pouch; take a punch from the card; and analyze the punch. The remainder of the card may be re-archived in a multi-barrier pouch for future use. Direct amplification chemistries streamline sample processing, because these methods eliminate purification and washing. DNA from buccal and blood samples, stored on FTA at ambient temperature for 12 and 22 years respectively, was amplified using an AmpFlSTR Identifier Direct PCR Amplification Kit (Applied Biosystems). Both sample types exhibited strong signals and good quality peaks (Fig 1A and 1B).

**Conventional (standard) STR amplification**

DNA from 12-year-old buccal and 22-year-old blood samples was amplified using the AmpFlSTR Profiler Plus™ or AmpFlSTR COflier™ PCR Amplification Kits (Applied Biosystems). STR data from both the buccal and blood samples showed 100% concordance with data from samples collected at t = 0 (data not shown) and produced high-quality results that were above minimum thresholds. Figure 2 shows Profiler Plus data for a 12-year-old buccal sample (Fig 2A) and a 22-year-old blood sample (Fig 2B). Similar results were obtained with the COflier kit (data not shown).

![Fig 1](image1.png)  
**Fig 1.** Direct STR profiles of (A) a 12-year-old buccal sample collected on Indicating FTA paper; and (B) a 22-year-old blood sample collected on FTA paper. Both samples were processed using an AmpFlSTR Identifier Direct PCR Amplification Kit (Applied Biosystems) and the manufacturer’s standard protocol. Samples were run on an Applied Biosystems™ 3130xl Capillary Electrophoresis Genetic Analyzer using standard conditions. The samples were analyzed using GeneMapper™ ID 3.2 (Applied Biosystems).

![Fig 2](image2.png)  
**Fig 2.** STR profiles of (A) a 12-year-old buccal sample collected on Indicating FTA paper and (B) a 22-year-old blood sample collected on FTA paper. DNA was amplified using AmpFlSTR Profiler Plus PCR Amplification Kit and a modified procedure. The samples were run on an Applied Biosystems 3130xl Capillary Electrophoresis Genetic Analyzer using standard conditions. Results were analyzed using GeneMapper ID 3.2.
UV protection of DNA

One of the characteristics of FTA paper that enables long-term archiving of DNA is its protection against UV radiation. Exposure to UV can cause degradation of DNA in samples, compromising the long-term stability of archived samples. FTA chemistry protects DNA from the harsh impact of UV exposure to increase the stability of archived samples.

Purified DNA was spotted onto FTA (treated) and untreated cotton fiber-based Whatman 903™ paper. DNA was exposed to 9.9 × 10⁵ µjoules of UV radiation and then quantitated using real-time PCR. These results were compared to those from samples stored in the dark. Samples stored on FTA and exposed to UV radiation showed an average Cₜ (threshold cycle) shift of 1.42 cycles, which represents a 2.7-fold decrease in DNA integrity. UV exposure of samples stored on an untreated matrix resulted in an average Cₜ shift of 9.75 cycles, representing an 862-fold decrease in DNA integrity (data not shown). The minimal Cₜ shift with FTA reflects the high proportion of intact template DNA and the enhanced level of protection offered by FTA. These data demonstrate that greater UV protection for DNA is provided by FTA paper than by untreated paper.

Ease of use

With the availability of direct amplification chemistries, sample processing and forensic workflows are streamlined. Preparation of samples for use with direct amplification chemistry requires only the addition of the punch to the STR reagent, eliminating the need for purification and wash steps.

Comparison of FTA and untreated paper

Previously we compared FTA and untreated cards, with purified DNA applied, in real-time PCR following UV irradiation (see UV protection of DNA). Here we compared direct STR profiling results between FTA and untreated cards. Thirty-nine buccal samples each were collected on Whatman 903 paper (untreated) and FTA indicating Micro Cards. A 1.2 mm punch was taken from each paper for amplification using the AmpFLSTR Identifier Direct PCR Amplification Kit (Applied Biosystems) according to the manufacturer’s instructions. The number of complete STR profiles, success rate, and total average peak height for each paper were assessed (Table 1).

A substantial performance difference was seen between 903 and FTA papers processed with the Identifier Direct chemistry. A success rate of 97% was observed with the FTA (treated) collection product compared with a 41% success rate using the 903 (untreated) paper (Table 1 and Fig 3). The untreated paper also exhibited PCR inhibition/inefficient amplification in many samples, as evidenced by the low overall average peak height (Table 1 and Fig 4).
Punching tools for FTA cards

Manual tools
GE offers the Uni-Core™ punch tool in several sizes for use with FTA cards. This tool is designed with a very sharp, stainless steel cutting edge. Harris Uni-Core is a disposable tool that provides up to 500 punches. It is available with 1.2, 2.0, and 3.0 mm diameter tips. There is no sample carryover when recommended procedures are followed[1]. The cutting mat ensures clean sample cuts and extends the life of the cutting tip.

Automated punch system
Hamilton easyPunch™ is an automation solution co-developed by GE and Hamilton Robotics and is intended for the forensic laboratory. The system is designed for the integration of FTA sample collection cards with the easyPunch robot, facilitating a fully automated system for forensic workflows. The key design features of this technology include card and sample location imaging to obtain the optimal sample punch, full sample and card traceability, automated punching, and liquid handling. easyPunch has been designed to deliver substantial simplification to high-throughput punching of FTA cards and subsequent sample processing.

Manufacturing
DNA-free manufacturing standards
GE’s manufacturing processes minimize the risk of human DNA contamination for its FTA products used to collect and analyze biological material for forensics purposes.

Clean room environment
GE manufactures FTA cards in a clean room environment in which airborne particles are controlled.

The clean room is constructed and used in a manner that minimizes the introduction, generation, and retention of particles, and in which other relevant parameters (e.g., temperature, humidity, and pressure) are controlled as necessary.

Quality
In order to ensure consistent product quality, all FTA cards are quality control tested and certified to guarantee reliable DNA reference sample analysis. GE has a zero QC failure rate (i.e., no failures on batches) for generating STR profiles on FTA cards.

All FTA products are manufactured in a controlled environment (see Clean room environment), with additional controls in place to minimize DNA contamination. All FTA products are subject to documented quality processes.

Enhanced certification level available
An enhanced certification level is available for customers who must comply with PAS377, which requires certification that FTA is free from human amplifiable DNA. A certificate is provided with each batch in order to confirm the testing results. This standard is offered as an additional service and is used by many of our customers worldwide.

In order to assess enhanced DNA-free status, Indicating FTA cards are tested for DNA-free status using Direct STR profiling and a strict card sampling regimen. Positive controls, consisting of a 2800M Control DNA Standard (Promega), are amplified at a concentration of 20 pg/µl in order to demonstrate the detection capabilities of the testing system. A single disc from an FTA card is removed using a 1.2 mm Harris punch. The STR profile is generated using the Promega PowerPlex™ 18D Kit according to the manufacturer’s instructions. Peaks generated from blank cards are required to conform to specific acceptance/defect criteria. In addition, peaks that cannot be explained as either biology- or technology-related artifacts are assumed to be real[2]. GE confirms that it has reviewed PAS377:2012 (Specification for consumables used in the collection, preservation and process of material for forensic analysis) for requirements relating to DNA-free product manufacture and that it complies with aspects applicable to FTA manufacture.

Draft standard ISO 18385
Standard ISO 18385 ‘Minimizing the risk of DNA contamination in products used to collect and analyze biological material for forensic purposes’ is currently in draft and is available from national standards organizations for commentary purposes. GE is actively engaged in this feedback process and is preparing product processes for readiness of the launch of this ISO standard. This process includes both creation of a DNA Elimination Database and assessment and validation of appropriate technologies to ensure a DNA-free product every time. However, GE does not currently collect DNA profiles for contamination detection reference purposes.
**Product development**
GE has invested in rapid FTA card development and prototyping, which allows a means to provide quick and efficient, low cost, low volume sample card profiles for developmental validation purposes.

**Quality management**
GE has achieved the status of a Registered Firm of Assessed Capability and holds Certificate No. F597874 dated 30 August 2013, to produce goods or services in respect of, and in accordance with, ISO 9001:2008. The GE International Quality Systems are audited by the British Standards Institute annually against these standards.

**Operational improvements**
GE has driven efficiencies in labor utilization and has developed enhanced mechanisms to meet FTA demand profiles while ensuring a consistent workforce in order to retain product knowledge and expertise. A full training and development program is in place to ensure knowledge sharing and succession planning within the FTA manufacturing team.

**Corporate experience and excellence**
GE is committed to a forward program of Continuous Improvement. This strategic directive reinforces our approach to understanding and exceeding our customer expectations in all aspects of our supply chain and operations management.

The Continuous Improvement program is guided by both a number of frameworks (Lean, Six Sigma, Product Quality, Certified Line, and GE professional maintenance) and a 3- to 5-year strategic vision. This approach is supported by an extensive workplace training and educational program where all employees are actively encouraged to challenge and drive improvement projects and strategic cultural change.

These improvements will allow GE to produce FTA technology and other products more efficiently and predictably, and they will support our target for customer satisfaction and growth.

**Summary and conclusions**
GE provides a range of high-quality sample collection products based on Whatman FTA technology and offers reliable solutions for transportation and long-term preservation of DNA. These benefits are essential in a wide range of applications including forensic, pharmaceutical, biotechnological, research, and screening applications. DNA-containing samples that can be collected include blood, buccal cells, saliva, and tissue. DNA and other biomolecules collected with these products may, after simple purification steps, be used for many types of downstream analyses.

GE can customize kits that include any of our FTA products and accessories.

**References**
1. Application note: Cross-contamination study: carryover does not occur during punching and processing of FTA or Clonesaver™ cards. GE, 51621 (2003).