Application note 28-9822-24 AA

Comparative analysis of FTA™ and NucleoSave™ cards

In this study, Whatman™ FTA cards were compared with NucleoSave cards in the following analyses: yield and functionality (in real-time PCR and short tandem repeat genotyping) of extracted DNA after room temperature storage; long-term stability of stored DNA using UV irradiation as a model of accelerated aging; antimicrobial activity of the matrices against representative bacterial and fungal species; and suitability of the matrices for direct "paper-in" DNA amplification. Whatman FTA cards provided superior results to NucleoSave cards in all analyses.

Introduction

Whatman FTA cards provide a simple solution for the room temperature collection, storage, transport, and isolation of nucleic acid samples for analysis. FTA cards contain chemicals that lyse cells, denature proteins, and protect nucleic acids from damage caused by nucleases, reactive oxygen species, and UV light. When crude biological samples, such as whole blood, cells, or plant or animal tissue, are applied directly to an FTA card, cell membranes and organelles are lysed and nucleic acids become entrapped in the fibers of the matrix. The nucleic acids remain immobilized and stabilized for immediate processing, transport, or long-term room temperature storage.

In the current study we describe the use of FTA and NucleoSave cards for the storage of blood samples, and present a comparative analysis of the yield, quality, and performance of the purified genomic DNA (gDNA) in subsequent downstream applications such as real-time PCR and short tandem repeat (STR) genotyping. In addition, we compare the long-term stability of DNA stored on both card types using UV irradiation as a model of accelerated aging, and assess the antimicrobial activity of each card type against a range of bacterial and fungal species.

Materials and Methods

Quantitation and STR analysis of DNA purified from FTA and NucleoSave cards

Sample preparation

Whole human blood (65 µl) was applied to FTA cards (GE Healthcare) or NucleoSave cards (Macherey-Nagel). Cards were dried at room temperature for ≥ 20 h, then stored in a Secador™ auto-desiccator cabinet (Bel-Art Products) at room temperature for 4 wk. Cards that had not been stored were used as controls. Discs (3.0 mm diameter) were punched from each matrix. DNA was extracted from FTA and NucleoSave discs using illustra™ tissue & cells genomicPrep Mini Spin Kit (GE Healthcare) and NucleoSpin™ Tissue Kit (Macherey-Nagel), respectively, according to each manufacturer's protocol.

DNA quantitation

Real-time PCR amplification was performed in 96 well plates using Quantifiler™ Human DNA Quantification Kit (Applied Biosystems) according to the manufacturer's instructions. Amplifications were performed on a 7900HT Fast Real-Time PCR System (Applied Biosystems) using 2 µl of purified gDNA.

STR genotyping

STR analysis is a common technique used to establish the identity of an individual from samples collected at an earlier date. Sample storage and preparation techniques must produce DNA of sufficient quality, purity, and quantity to provide a complete STR profile.

STR genotyping was used to assess the quality and purity of DNA purified from blood stored on FTA and NucleoSave cards for 4 wk at room temperature. DNA was extracted as described above. DNA concentration was determined using the Quantifiler Human DNA Quantification Kit.

Purified gDNA (1 ng) was used for PCR amplification of fifteen STRs and amelogenin using the AmpFLSTR™ Identifiler PCR Amplification Kit according to the manufacturer's protocol. Amplifications were performed on the Gold-plated 96-Well GeneAmp™ PCR System 9700. Capillary electrophoresis was performed on a 3130xl Genetic Analyzer, followed by GeneMapper™ ID v3.2 software analysis. All amplification reagents, equipment, and software were purchased from Applied Biosystems.

Analysis of DNA integrity

To compare the long-term stability of DNA stored on FTA and NucleoSave cards, exposure to UV irradiation was used as a model of accelerated aging. DNA yield (used as an indication of DNA integrity) was measured using endpoint PCR analysis.

Sample preparation

Plasmid DNA (1 ng) was applied to FTA and NucleoSave cards. Samples were dried at room temperature for ≥ 20 hrs. The following day, half of the samples were exposed to 0.99 Joules of UV irradiation. Discs (3.0 mm) were punched from each matrix.

DNA was purified from FTA and NucleoSave discs using illustra cells & tissue genomicPrep Mini Spin Kit (GE Healthcare) according to the manufacturer's protocol. (Note that only one purification kit was used to isolate DNA from both card types to ensure that an equivalent quantity of DNA was purified from each.)

Endpoint PCR analysis

Endpoint PCR was used to assess the amount of plasmid DNA purified from FTA and NucleoSave cards that was suitable for amplification following UV exposure.

PCR master mix (95 µl) containing 1 U rTaq2 DNA polymerase, 200 µM dNTPs (both GE Healthcare) and 200 nM primers for tumor protein p53 ORF (Sigma-Genosys) was added to 5 µl of purified gDNA. PCR amplification was performed using a PTC-200 thermal cycler (MJ Research). PCR cycling conditions were: 94°C for 3 min, followed by 30 cycles of 94°C for 30 sec; 55°C for 1 min; 72°C for 2 min, and a final incubation at 72°C for 10 min.

Analysis of antimicrobial activity

Microbial strains

Six microbial strains were used for testing the antimicrobial activity of FTA and NucleoSave cards: *Escherichia coli*, ATCC28922; *Staphylococcus aureus*, ATCC25923; *Bacillus subtilis*, ATCC10774; *Candida albicans*, ATCC10231; *Pseudomonas aeruginosa*, ATCC10145; and an environmental isolate of *Aspergillus niger*. Bacterial strains were obtained from American Type Culture Collection.

Sample preparation and analysis

Bactericidal and fungicidal activity was assessed on TSA and Sabouraud 4% glucose media (both Fluka), respectively. FTA or NucleoSave cards (6.0 mm discs) were placed aseptically onto a pre-inoculated microbial lawn (15 cm plate; ~ 2×10^5 colony-forming units/ml test organism; 1, 2) and incubated overnight. Antimicrobial activity was assessed by measuring the diameter of zones of clearing around the disc.

Evaluation of suitability for direct "paper-in" DNA amplification

Sample preparation

Two options exist for DNA preparation from FTA cards: DNA can be prepared directly for amplification using a "paper-in" process, or by purification using illustra tissue & cells genomicPrep Mini Spin Kit (GE Healthcare). Macherey-Nagel recommends purifying DNA from NucleoSave using NucleoSpin Tissue Kit prior to downstream analysis. Blood spotted onto FTA and NucleoSave cards was processed and used for "paper-in" PCR to compare the suitability of each card type for direct PCR analysis.

Whole human blood (65 µl) was applied to FTA or NucleoSave cards. Cards were dried at room temperature for ≥ 20 h, then stored in a Secador auto-desiccator cabinet at room temperature for less than 1 wk. Discs (1.2 mm diameter) were punched from each matrix. Discs were washed with FTA Purification Reagent (GE Healthcare) and rinsed with TE^{-1} (10 mM Tris-HCl, 0.1 mM EDTA, pH 8) buffer.

Endpoint PCR amplification

Following disc processing and drying, PCR master mix (50 µl) containing 1 U FideliTaq DNA polymerase, 200 µM dNTPs (both GE Healthcare) and 200 nM primers for β-globin (Sigma-Genosys) was added to each well in a 96-well PCR plate. PCR amplification was performed using a PTC-200 thermal cycler (MJ Research). PCR cycling conditions were: 94°C for 3 min, followed by 35 cycles of 94°C for 30 sec; 55°C for 1 min; 72°C for 4 min, and a final incubation at 72°C for 5 min.

Results and discussion

Quantitation and STR analysis of DNA purified from FTA and NucleoSave cards

DNA quantitation

Statistical analysis using a one-way ANOVA showed that FTA cards produced a significantly higher yield ($p < 0.001$) than NucleoSave cards (Fig 1A). The average yield obtained from FTA cards was 122.38 ng (± 15.69), compared with 31.24 ng (± 6.78) for NucleoSave cards. (Note that the yield obtained from NucleoSave cards in this experiment is consistent with that published in Macherey Nagel's Application Note for NucleoSave cards [i.e., 5–30 ng; 3]).

FTA cards also produced a significantly higher yield ($p < 0.001$) than NucleoSave cards following 4 wk storage at room temperature (Fig 1B). The average yield obtained from FTA cards was 54.67 ng (± 14.81) compared with 7.74 ng (± 4.22) from NucleoSave cards. When compared with samples that had not been stored (Fig 1A), the yield obtained from FTA cards decreased by 52% after 4 wk, and the yield from NucleoSave cards fell by 73%. These data demonstrate that FTA cards provide a more stable matrix for the long-term, room temperature storage of blood samples.

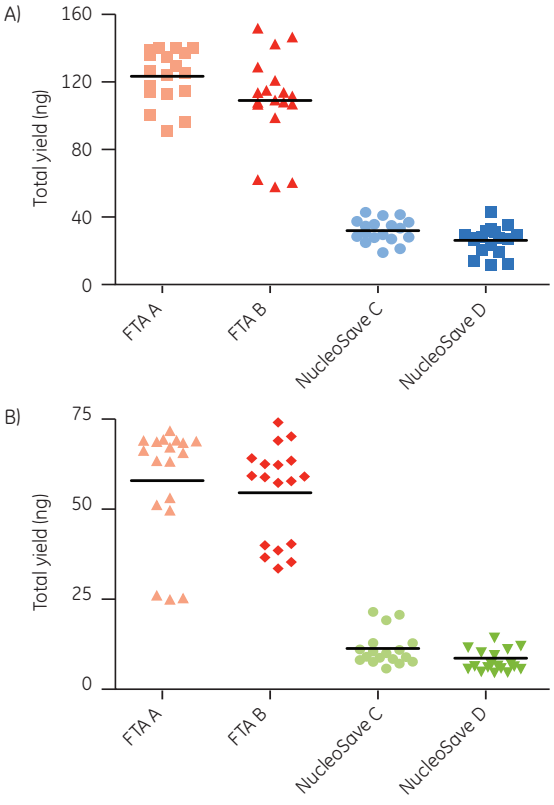

Fig 1. Total yield (ng) of DNA purified from discs taken from the center of two batches of FTA (A and B) and NucleoSave (C and D) cards that had been spotted with 65 µl of whole human blood. DNA yield was quantitated by real-time PCR. Each point on the graph represents the recovery of DNA from a single disc (n = 18). (A) Total yield from cards that were not stored. (B) Total yield after 4 wk room temperature storage in an auto-desiccator.

Table 1. Percent accuracy of allele calls for 16 loci determined by STR profiling of DNA purified from discs taken from the center of FTA and NucleoSave cards spotted with 65 µl of whole human blood.

locus	FTA			NucleoSave		
	Allele 1	Allele 2	Summary	Allele 1	Allele 2	Summary
D8S1179	100%	100%	6 correct calls out of 6	66.6%	33.3%	3 correct calls out of 6
D21S11	100%	100%	6 correct calls out of 6	66.6%	66.6%	4 correct calls out of 6
D7S820	100%	100%	6 correct calls out of 6	33.3%	0%	1 correct call out of 6
CSF1PO	100%	N/A	3 correct calls out of 3	66.6%	N/A	2 correct calls out of 3
D3S1358	100%	100%	6 correct calls out of 6	66.6%	66.6%	4 correct calls out of 6
TH01	100%	100%	6 correct calls out of 6	66.6%	33.3%	3 correct calls out of 6
D13S317	100%	100%	6 correct calls out of 6	100%	100%	6 correct calls out of 6
D16S539	100%	100%	6 correct calls out of 6	66.6%	66.6%	4 correct calls out of 6
D2S1338	100%	100%	6 correct calls out of 6	66.6%	66.6%	4 correct calls out of 6
D19S433	100%	100%	6 correct calls out of 6	66.6%	66.6%	4 correct calls out of 6
vWA	100%	100%	6 correct calls out of 6	66.6%	66.6%	4 correct calls out of 6
TPOX	100%	N/A	3 correct calls out of 3	100%	N/A	3 correct calls out of 3
D18S51	100%	100%	6 correct calls out of 6	0%	0%	0 correct calls out of 6
AMEL	100%	N/A	3 correct calls out of 3	100%	N/A	3 correct calls out of 3
D5S818	100%	100%	6 correct calls out of 6	66.6%	66.6%	4 correct calls out of 6
FGA	100%	100%	6 correct calls out of 6	66.6%	66.6%	4 correct calls out of 6
Total number of samples			3			3
Total possible allele calls			87			87
Total correct allele calls			87			53
% Accuracy			**100%**			**60.9%**

N/A = not applicable

STR genotyping

Statistical analysis using a Mann-Whitney non-parametric t-test showed that FTA cards produced a significantly higher accuracy ($p < 0.001$) than NucleoSave cards based on the percentage of correct allele calls (Table 1). For DNA purified from FTA cards, 87 correct allele calls were made out of a possible 87 (100% accuracy). In comparison, DNA purified from NucleoSave cards gave 53 correct allele calls out of a possible 87 (60.9% accuracy). As shown in Figure 2, all gDNA samples purified from FTA cards provided a full profile of 16 loci, in contrast to the partial profiles obtained for all gDNA samples purified from NucleoSave cards. These results demonstrate the superior quality of gDNA purified from FTA cards.

A)

B)

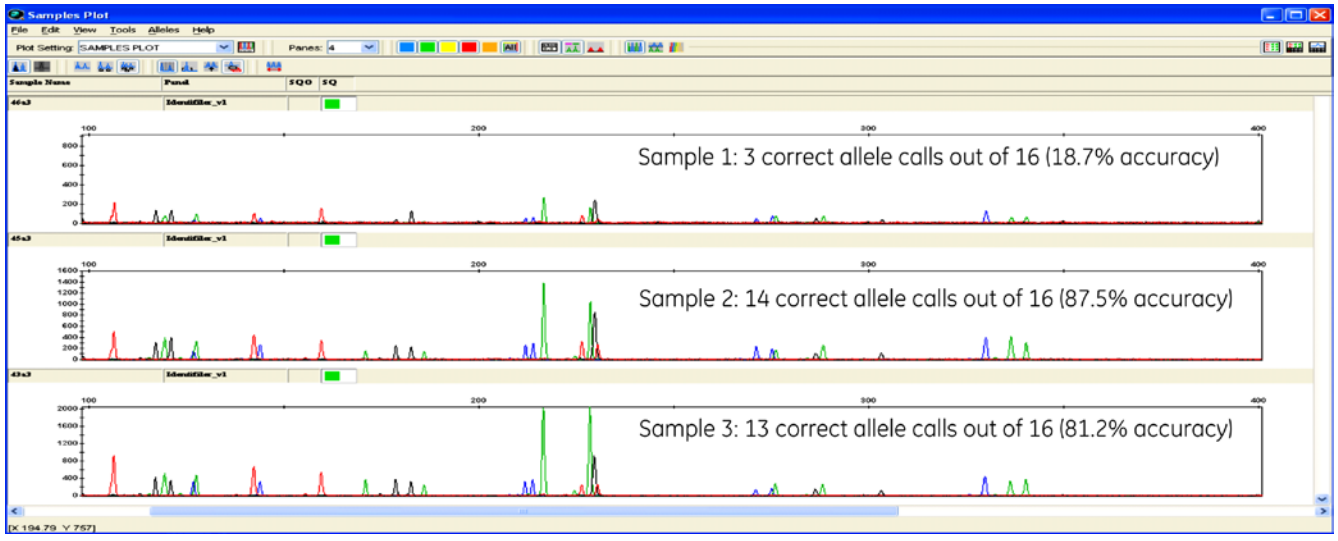

Fig 2. AmpFLSTR Identifiler STR electropherograms from DNA purified from discs taken from the center of (A) FTA and (B) NucleoSave cards spotted with 65 µl of whole human blood.

Analysis of DNA integrity

Endpoint PCR analysis

When PCR products were visualized on an agarose gel there was no noticeable reduction in band intensity (yield) following UV irradiation for DNA purified from FTA cards (Fig 3). However, amplification of DNA purified from NucleoSave cards showed a visible reduction in band intensity following UV exposure (Fig 3). These results demonstrate that FTA cards possess greater protective properties against UV irradiation than do NucleoSave cards.

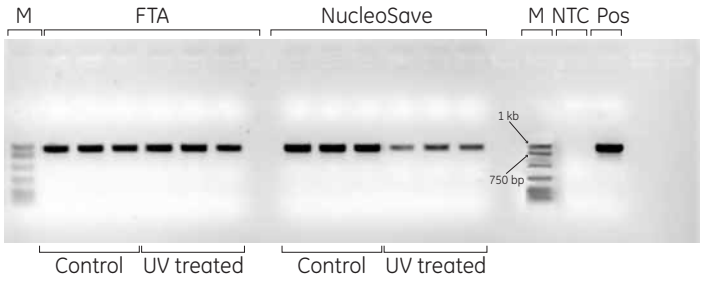

Fig 3. Agarose gel analysis (1X TAE, 1% agarose) of PCR products amplified from plasmid DNA purified from 3.0 mm discs taken from the center of FTA and NucleoSave cards (spotted with 1 ng of plasmid DNA). Half of each card type had previously been exposed to 0.99 Joules of UV irradiation.
M, PCR Markers (Promega); NTC = no template control; Pos = positive control (400 pg of plasmid DNA).

Analysis of antimicrobial activity

Growth of all six microorganisms was inhibited in the presence of discs removed from FTA cards but not from NucleoSave cards (Fig 4). For example, FTA discs inhibited the growth of *A. niger,* producing clearance zones measuring up to 17.5 mm in diameter (Fig 5). In contrast, growth inhibition was not observed for NucleoSave discs. (Note that there are no claims made in Macherey-Nagel's literature for microbial inactivation by NucleoSave cards).

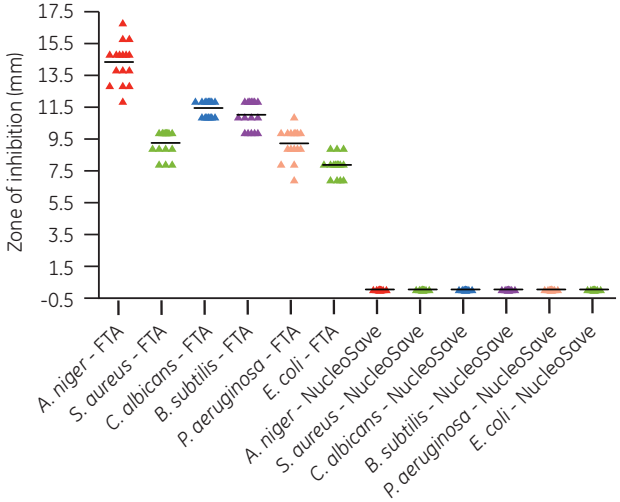

Fig 4. Zone of growth inhibition (mm) for six microbial strains [at ~ 1 to 2.5 × 10^5 colony-forming units (CFU)/ml)] grown in the presence of FTA and NucleoSave discs. FTA conferred antimicrobial activity against the full range of bacterial and fungal species tested. NucleoSave cards showed no inhibitory action against any of the organisms tested (Note that there are no claims made in Macherey-Nagel's literature for microbial inactivation by NucleoSave cards).

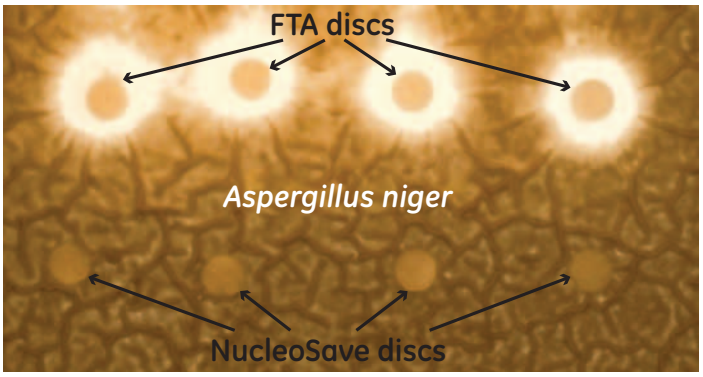

Fig 5. Zones of growth inhibition for *A. niger* (seeded on Sabouraud 4% dextrose agar at 2.5 × 10^5 CFU/ml) grown in the presence of FTA and NucleoSave discs.

Evaluation of suitability for direct "paper-in" DNA amplification

Endpoint PCR amplification

PCR products (3.6 kb) were amplified from FTA discs, while no PCR products were amplified from NucleoSave (NucSave) discs (Fig 6). These results demonstrate that FTA cards are better suited to rapid "paper-in" processing methods than are NucleoSave cards. In addition, blood samples stored on FTA can be prepared for PCR and subsequent forensic analysis (data not shown) without the need for extensive DNA purification and extraction processes.

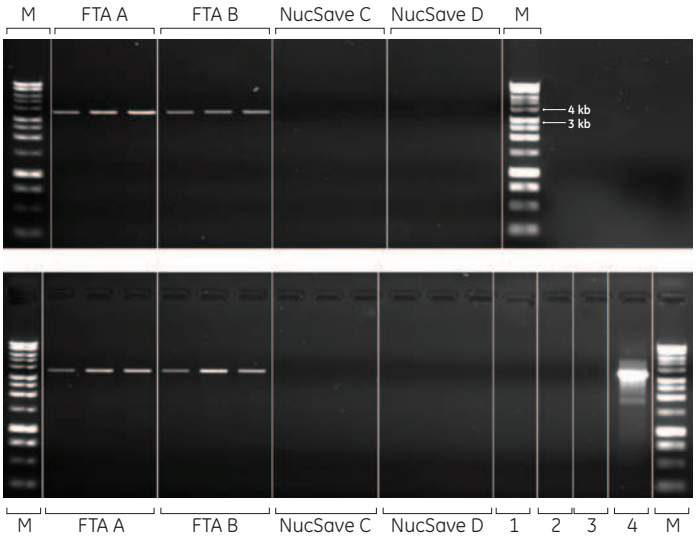

Fig 6. Agarose gel analysis (1X TAE, 1% agarose) of 3.6 kb PCR products (β-globin, single copy gene) amplified directly (using "paper-in" PCR process) from 1.2 mm discs taken from the center of two batches of FTA (A and B) and NucleoSave (C and D) cards spotted with 65 µl of whole human blood.
M, 1kb DNA Ladder (Promega); Lane 1, no template control (NTC) for blank FTA disc; Lane 2, NTC for blank NucleoSave disc; Lane 3, negative control (no disc); Lane 4, positive control (50 ng of gDNA).

Conclusions

DNA purified from discs taken from FTA and NucleoSave cards spotted with whole human blood was suitable for real-time PCR and STR genotyping. These data showed that the yield, purity, and quality of DNA purified from FTA were superior to that of DNA purified from NucleoSave cards.

The long-term stability of DNA stored on FTA and NucleoSave cards was compared using UV irradiation as a model of accelerated aging. Endpoint PCR results demonstrate that FTA cards posess superior protective properties against UV irradiation-induced damage to stored DNA over NucleoSave cards.

FTA and NucleoSave cards were assessed for antimicrobial activity against a range of bacterial and fungal species. Results showed that FTA conferred antimicrobial activity against the full range of bacterial and fungal species tested, but NucleoSave cards showed no inhibitory action against any of the organisms. (Note that there are no claims made in Macherey-Nagel's literature for microbial inactivation by NucleoSave cards).

Blood spotted onto FTA and NucleoSave cards was processed and used for "paper-in" PCR to compare the suitability of each card type for direct PCR analysis. Results showed that PCR products (3.6 kb) were amplified from FTA discs, while no PCR products were amplified from NucleoSave discs. These data demonstrate that FTA cards are better suited to rapid "paper-in" processing methods than are NucleoSave cards.

The advantages and benefits of FTA cards are discussed below.

- Simple collection: Direct room temperature sample application and immediate protection of nucleic acids from degradation enables convenient sample collection in the laboratory or in the field.
- Room temperature storage: Nucleic acids are stabilized, ready for shipping or long-term room temperature storage without the need for refrigeration.
- Pathogen inactivation: FTA confers inherent antimicrobial activity against a range of viral, bacterial, and fungal species reducing exposure of laboratory personnel to potentially hazardous microbes during shipping and handling.
- Fast purification: Nucleic acids can be purified on the FTA card in three simple steps, all in a single tube at room temperature. DNA remains immobilized on the matrix and is ready for "paper-in" PCR or other amplification techniques. Alternatively, nucleic acids can be isolated from the FTA card using organic extraction, Chelex™ (Bio-Rad Laboratories) purification, or a standard gDNA purification kit such as illustra tissue & cells genomicPrep Mini Spin Kit from GE Healthcare (4), demonstrating the versatility of FTA sample processing methods.
- Preservation of DNA integrity: Superior protective properties against UV irradiation serve to maintain the integrity of DNA samples archived on FTA cards.
- Amenable to automation: Automation speeds the handling of multiple FTA punches and standardizes DNA purification. Punches can be washed easily and prepared for PCR on a variety of liquid handling instruments.

References

1. McFarland, J. The nephelometer: an instrument for estimating the numbers of bacteria in suspensions used for calculating the opsonic index and for vaccines. *JAMA* **49**, 1176–1178 (1907).
2. Forbes, B. A. *et al.* Bailey and Scott's diagnostic microbiology, 10th ed. Mosby, Inc., Missouri (1998).
3. Convenient storage of blood samples at room temperature. Macherey-Nagel. KATEN300045 NuSave Cards en1/10/0/6.2008 PD.
4. Application note: Extraction of DNA from Whatman FTA cards. GE Healthcare, 28-9822-22. Edition AA (2010).

Ordering Information

FTA Cards and Indicating FTA Cards

Product	Quantity (cards/pk)	Sample areas/card	Maximum volume/ sample area (µl)	Maximum total volume/card (µl)	Code number
FTA Classic Card	100	4	125	500	WB120205
Indicating FTA Classic Card[1]	100	4	125	500	WB120206
FTA Mini Card	100	2	125	250	WB120055
Indicating FTA Mini Card[1]	100	2	125	250	WB120056
FTA Micro Card	100	1	125	125	WB120210
Indicating FTA Micro Card[1]	100	1	125	125	WB120211
FTA Gene Card[2]	100	3	75	225	WB120208
PlantSaver™ Card[3]	100	4	25	100	WB120065
CloneSaver™ Card	5	96	5	480	WB120028
EasiCollect™	50	1	125	125	WB120462
FTA Elute Micro Card	100	4	30	120	WB120410
FTA Elute Micro Card	25	4	30	120	WB120401

[1] Indicating FTA Cards change color from pink to white when sample is applied. They are recommended for use with clear samples
[2] FTA Gene Cards are compatible for use with automated liquid sampling systems when used with FTA Gene Card Trays (see below)
[3] Plant homogenate; 10 mg/50 µl PBS

FTA Reagents, Kits, and Accessories

Product	Quantity	Code number
illustra tissue & cells genomicPrep Mini Spin Kit	50 reactions	28-9042-75
	250 reactions	28-9042-76
FTA Purification Reagent	500 ml	WB120204
FTA Gene Card Trays	20	WB100030
FTA Kit	1	WB120067
FTA Plant Kit	1	WB120068
Sterile Foam Tipped Applicator Swabs	100	WB100032
Desiccant (1 g)	1000	WB100003
Multi-Barrier Pouch, Small (for Mini, Micro, and Gene Cards)	100	WB100036
Multi-Barrier Pouch, Large (for Classic Cards)	100	WB100037
CloneSaver Resealable Multi-Barrier Pouch	50	WB100024
Harris Micro Punch 1.2 mm (with Mat)	1	WB100005
Harris Uni-Core Disposable 1.25 mm Punches (with Mat)	4	WB100028
Harris Micro Punch 2.0 mm (with Mat)	1	WB100007
Harris Uni-Core Disposable 2.0 mm Punches (with Mat)	4	WB100029
Replacement Cutting Mat	1	WB100020
Replacement Tip 1.2 mm	1	WB100006
Replacement Tip 2.0 mm	1	WB100008
Harris Uni-Core Punch 3.0 mm (with Mat)	4	WB100039
Harris Micro Punch 3.0 mm (with Mat)	1	WB100038
Replacement Tip 3.0 mm	1	WB100042

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