Technical Focus

Direct PCR Amplification of Blood Samples on Copan NUCLEIC-CARD™ Systems Using the AmpFℓSTR® Identifiler® Direct PCR Amplification Kit

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- Blood samples spotted on Copan NUCLEIC-CARD™* systems generate high-quality profiles when amplified with the AmpFℓSTR® Identifiler® Direct PCR Amplification kit
- Under the experimental conditions tested, Copan NUCLEIC-CARD™ systems perform comparably with other chemically-coated sample collection cards, exhibiting robust cell lysis and DNA stability over time
- Combining the Copan NUCLEIC-CARD™ with the Identifiler® Direct kit streamlines the laboratory workflow to help generate accurate results rapidly and reliably

Introduction

The number of single-source samples processed by laboratories have increased rapidly in recent years due to the success of national DNA databases. In an effort to avoid backlogs and meet demands for the delivery of rapid, high-quality results, laboratories are looking for ways to simplify and streamline the processing of these most straightforward of forensic samples.

Automation of the existing process is part of the solution, but even greater efficiency savings can be achieved by also removing rate-limiting steps from within the workflow where possible. Direct amplification technology has advanced dramatically in recent years, eliminating the need for time-consuming sample extraction and quantitation processes. At the same time, the range of substrates available for the collection of database and casework reference samples has also expanded, offering laboratories an unprecedented selection.

Copan, a market leader in the area of sample collection tools, has recently developed the NUCLEIC-CARD™, a chemically-treated sample collection matrix that lyses cells and preserves DNA spotted onto the paper substrate. Processing blood samples stored on the Copan NUCLEIC-CARD™ with a direct amplification chemistry enables laboratories to maximize sample processing speed. Choosing a direct amplification chemistry such as the AmpFℓSTR® Identifiler® Direct PCR Amplification kit, which has been designed specifically to deliver high first-pass success rates, further optimizes the workflow. A DNA analysis protocol that combines the innovative technology of the Identifiler® Direct PCR kit with the Copan NUCLEIC-CARD™ provides a rapid and cost-effective solution for laboratories that process a high volume of single-source samples.

*Note: The Copan NUCLEIC-CARD™ system is available in all countries except the United States.

Experiments and results

Materials and methods

To replicate the typical single-source sample processing work flow, experiments were conducted using blood on a variety of sample collection cards. Samples were prepared by spotting blood from 20 individuals onto Whatman® FTA® cards, untreated cards and three lots of Copan NUCLEIC-CARD™ 1.2 mm discs were punched from each card type and amplified in duplicate (with the exception of the sensitivity study) with the Identifiler® Direct kit using a PCR reaction volume of 25 µL. Amplification was performed on a Veriti® thermal cycler, with all reactions conducted directly on the sample disc. Capillary electrophoresis was performed on the Applied Biosystems® 3500 Genetic Analyzer, and the resulting data was analyzed using GeneMapper® *ID-X* v1.2 software. All analysis was conducted using two different thresholds of 150 and 450 RFU to simulate different levels of interpretation thresholds that may be applied under operational conditions (these thresholds are appropriate to the Applied Biosystems® 3500 Genetic Analyzer; lower thresholds would apply on Applied Biosystems® 31XX instruments). Unless stated otherwise, all data was evaluated according to the following metrics: First Pass Success Rate (defined as a full profile typed from one amplification and one CE injection), Mean Peak Height, Intracolor Balance, and Intralocus Balance. A comprehensive overview of the experiments conducted and data assessments performed as part of this study is shown in Table 1.

Results

Sensitivity study

When amplified for 25 cycles, all blood samples on FTA® and NUCLEIC-CARD™ produced full profiles at peak amplitude

Table 1

Study Type	Sample/Substrate	Data Assessment
Sensitivity	• 20 x blood on FTA® • 20 x blood on untreated card • 20 x blood on 1 x lot NUCLEIC-CARD™	Evaluation of replicate plates amplified for 25, 26, and 27 cycles to identify optimum cycle number for subsequent experiments Metrics: number of amplification failures (presence of a mixture, allelic dropout); number of off-scale alleles; first pass success rate at 150 and 450 RFU
Performance	• 20 x blood on FTA® • 20 x blood on 3 x lots NUCLEIC-CARD™	Evaluation of lot-to-lot consistency of NUCLEIC-CARD™ Performance comparison of NUCLEIC-CARD™ to FTA® card
Stability	• 20 x blood on FTA® • 20 x blood on 2 x lots NUCLEIC-CARD™ • All samples stored for 1 month at room temperature	Performance comparison of aged NUCLEIC-CARD™ lots Performance comparison of aged NUCLEIC-CARD™ and aged FTA® card Performance comparison of aged and fresh NUCLEIC-CARD™ and FTA® card
Inhibition	1 x blank 1.2 mm and 1 x blank 2.0 mm discs from FTA®, NUCLEIC-CARD™ and untreated card added to amplifications containing 4 ng of 9947A control DNA in duplicate	Evaluation of inhibition of PCR amplification as a result of cell lysis compound embedded in each type of sample collection card

Table 1. Experiments conducted in this study.

thresholds of 150 and 450 RFU and no off-scale alleles were observed (Table 2). No reproducible artifacts or peaks indicative of contamination were observed for any of the three card types. Punches from blood on untreated cards produced full profiles for only 9/20 samples at a threshold of 150 RFU and only 1/20 samples at a threshold of 450 RFU suggesting insufficient cell lysis. 25 cycles was selected as the optimum cycle number, as it produced an excellent first-pass success rate, while minimizing allelic drop-out and off-scale alleles. Significant levels of off-scale data were observed at higher cycle numbers (26 and 27). Accordingly, all samples in the subsequent experiments were amplified for 25 cycles.

Table 2

		Analysis at 150 RFU		Analysis at 450 RFU		Observations
Substrate	N	Number of Full Profiles	First Pass Success Rate	Number of Full Profiles	First pass Success Rate	Number of Homozygote Off-Scale Alleles
FTA® card	20	20/20	100%	20/20	100%	0
NUCLEIC-CARD™	20	20/20	100%	20/20	100%	0
Untreated card	20	9/20	45%	1/20	5%	0

Table 2. Summary of sensitivity study results.

Table 3

		Analysis at 150 RFU		Analysis at 450 RFU	
Substrate	N	Number of Full Profiles	First Pass Success Rate	Number of Full Profiles	First Pass Success Rate
FTA® Card	39[1]	39/39[1]	100%	39/39	100%
NUCLEIC-CARD™ Lot 1	40	40/40	100%	40/40	100%
NUCLEIC-CARD™ Lot 2	40	40/40	100%	39/40	97.5%
NUCLEIC-CARD™ Lot 3	40	40/40	100%	40/40	100%
Untreated card	40	15/40	37.5%	1/40	2.5%

Table 3. Summary of performance study first pass success rate results. Results for one sample were excluded due to injection issues[1].

Performance study

All samples on FTA® and NUCLEIC-CARD™ produced full profiles at a threshold of 150 RFU and all samples on FTA® cards, and all but one sample on NUCLEIC-CARD™ produced full profiles at a threshold of 450 RFU (Table 3). In contrast, samples on untreated cards produced 15/20 full profiles at a threshold of 150 RFU and only 1/40 full profiles at 450 RFU.

Mean Peak Height results for blood samples on all three lots of NUCLEIC-CARD™ were higher than for blood samples on FTA® cards, but also produced a wider range of peak heights (Figure 1). As a result, a total of 8 samples on NUCLEIC-CARD™ produced off-scale homozygous allele peaks, whereas no off-scale data was observed for FTA® cards. Refinement of the cycle number can be helpful in achieving a balance between first pass success rate and detection of off-scale alleles.

Intralocus balance results were comparable between FTA® cards and all three lots of NUCLEIC-CARD™, with the mean for all four sets of samples falling between 92% and 93% (Figure 2).

Intracolor balance results were also similar between the samples on FTA® cards and all three lots of NUCLEIC-CARD™. However, NUCLEIC-CARD™ consistently produced data with slightly higher intracolor balance than that of the FTA® cards (Figure 3).

Figure 1

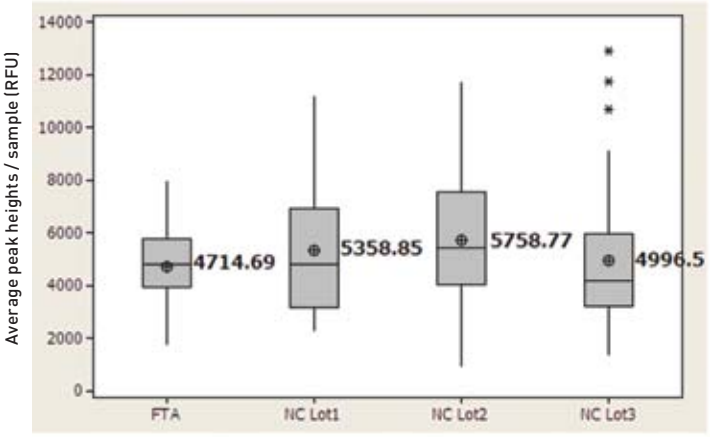

Figure 1. Mean peak heights (RFU) for samples amplified from FTA® card and NUCLEIC-CARD™ punches.

Figure 2

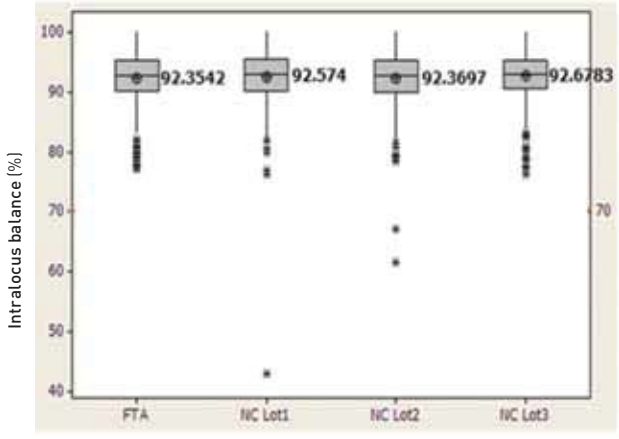

Figure 2. Intralocus balance (%) results for samples amplified from FTA® card and NUCLEIC-CARD™ system punches.

Figure 3

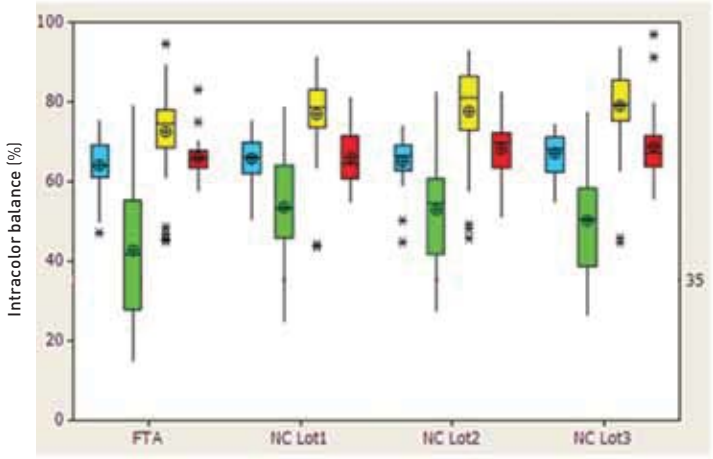

Figure 3. Intracolor balance expressed as a percentage and graphed by dye color for samples amplified from FTA® card and NUCLEIC-CARD™ system punches.

Stability study

First-pass success rates were 100% for all the samples on NUCLEIC-CARD™ and FTA® cards that had been aged for one month before sample processing. Mean peak heights were consistent between both lots of aged samples on NUCLEIC-CARD™, between aged samples on treated and FTA® cards and between aged and fresh samples on NUCLEIC-CARD™ and FTA® cards (Figure 4).

Balance between the two allele peaks in a heterozygote (intralocus balance) was comparable across all samples and substrates tested. Each set of cards displayed an intralocus balance ratio of 92% to 94% (Figure 5).

Intracolor balance was comparable between one month-old samples, if slightly higher for the blood samples on NUCLEIC-CARD™. It was also similar among both fresh and one-month-aged samples (Figure 6).

Figure 4

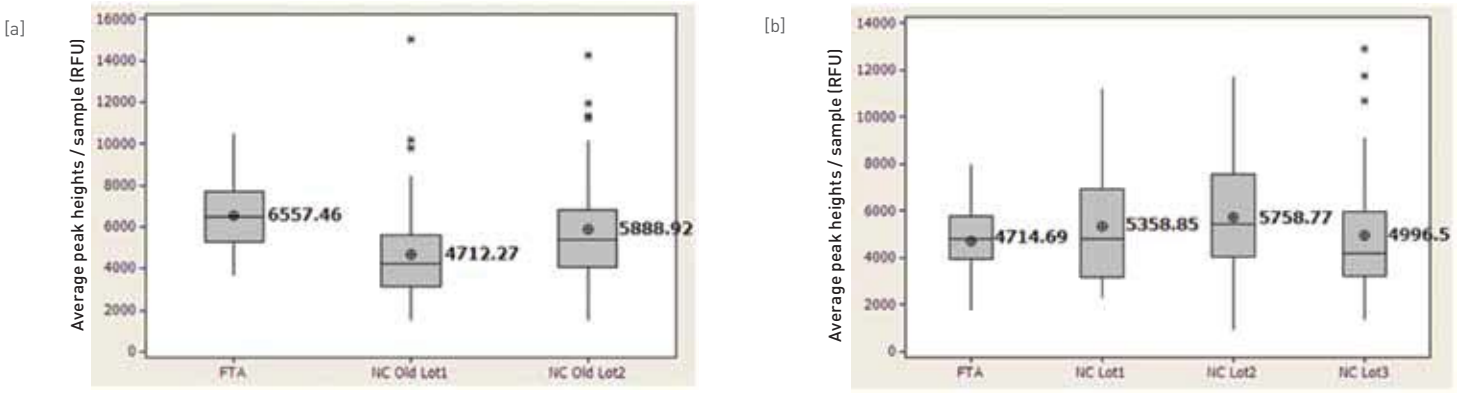

Figure 4. Comparison of mean peak height (RFU) for samples spotted onto FTA® cards and Copan NUCLEIC-CARD™ then aged for one month (a) versus samples freshly spotted onto FTA® cards and NUCLEIC-CARD™ (b).

Figure 5

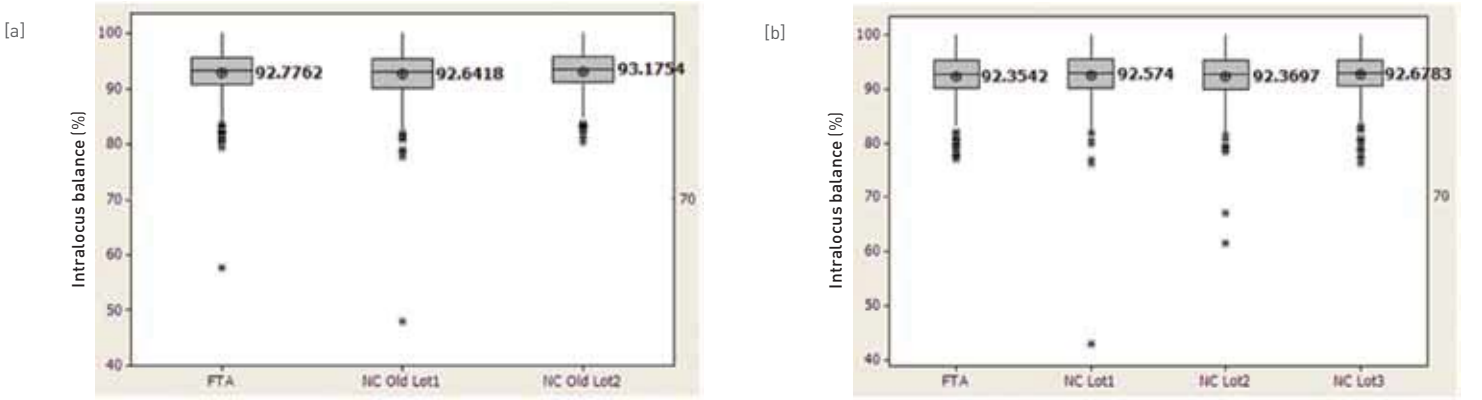

Figure 5. Comparison of mean intralocus balance (%) results for samples spotted onto FTA® cards and NUCLEIC-CARD™ then aged for one month (a) versus samples freshly spotted onto FTA® cards and NUCLEIC-CARD™ (b).

Figure 6

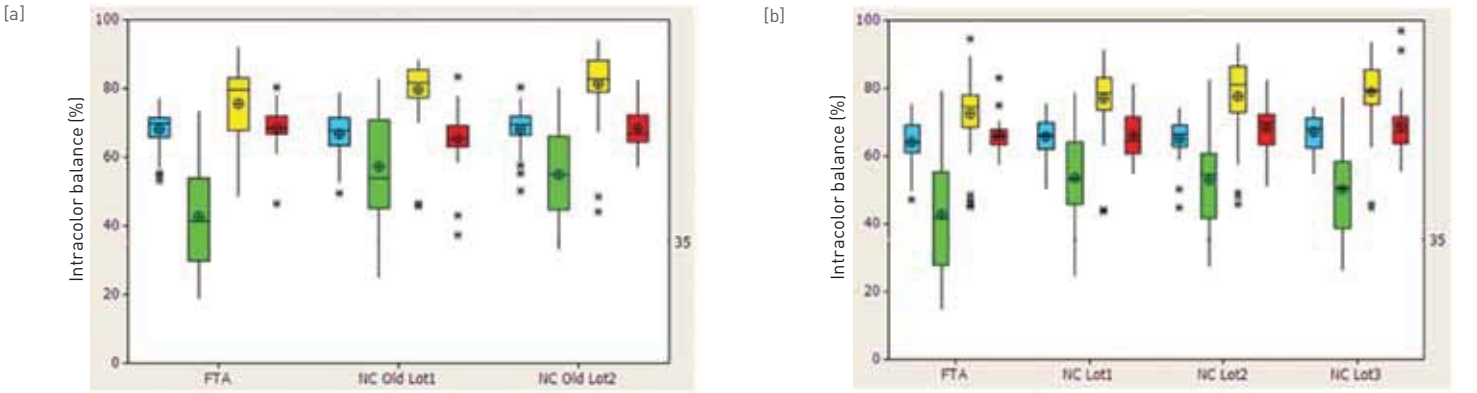

Figure 6. Comparison of intracolor balance (%) results graphed by dye color for samples spotted onto FTA® cards and NUCLEIC-CARD™ then aged for one month (a) versus samples freshly spotted onto FTA® cards and NUCLEIC-CARD™ (b).

The two lots of NUCLEIC-CARD™ used for the one-month-aged samples were distinct from the three lots used to prepare fresh samples, yet similar results were obtained for all five separate NUCLEIC-CARD™ lots. Therefore, NUCLEIC-CARD™ demonstrated both consistency from lot to lot and stability under commonly used storage conditions.

Inhibition study

Results indicated that the addition of blank 1.2 mm discs from both FTA® and NUCLEIC-CARD™ resulted in little inhibition of the reaction, but 2.0 mm discs punched from the same cards resulted in complete sample inhibition (Figures 7 and 8). As expected, no inhibition was observed in either 1.2 mm or 2.0 mm from untreated cards due to the lack of treatment reagents being introduced (Figure 9).

Conclusion

During the course of these experiments, blood samples spotted onto multiple lots of Copan NUCLEIC-CARD™ and amplified with the AmpFℓSTR® Identifiler® Direct PCR Amplification kit consistently generated high-quality profiles. The NUCLEIC-CARD™ did not introduce any contamination or inhibition into the workflow and show comparable performance to Whatman® FTA® cards. Given these results, the use of NUCLEIC-CARD™ in combination with the AmpFℓSTR® Identifiler® Direct PCR Amplification kit represents a valuable sample processing solution for forensic laboratories interested in implementing an efficient and high-quality workflow for single-source blood samples.

Figure 7

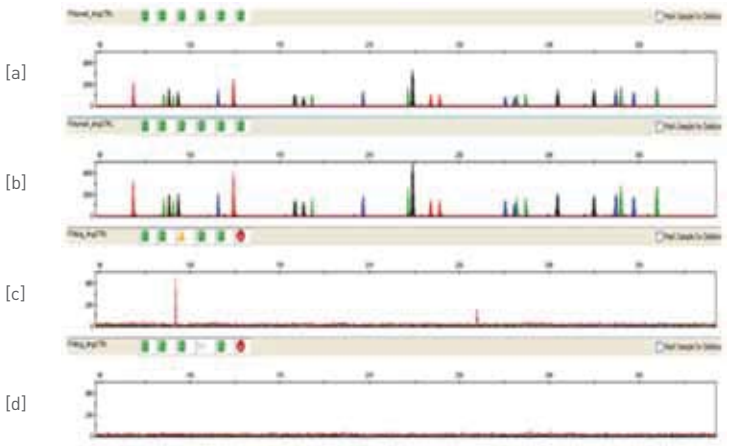

Figure 7. Comparison of data produced by 4 ng of 9947A control DNA amplified in the presence of blank 1.2 mm (a and b) and 2.0 mm (c and d) FTA® discs.

Figure 8

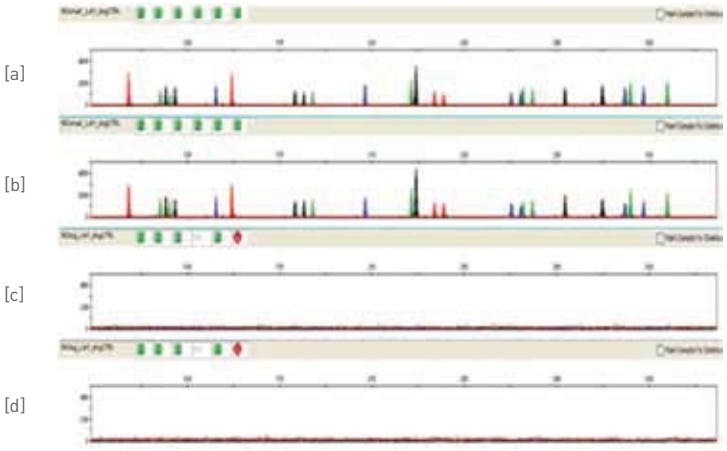

Figure 8. Comparison of data produced by 4 ng of 9947A control DNA amplified in the presence of blank 1.2 mm (a and b) and 2.0 mm (c and d) NUCLEIC-CARD™ discs.

Figure 9

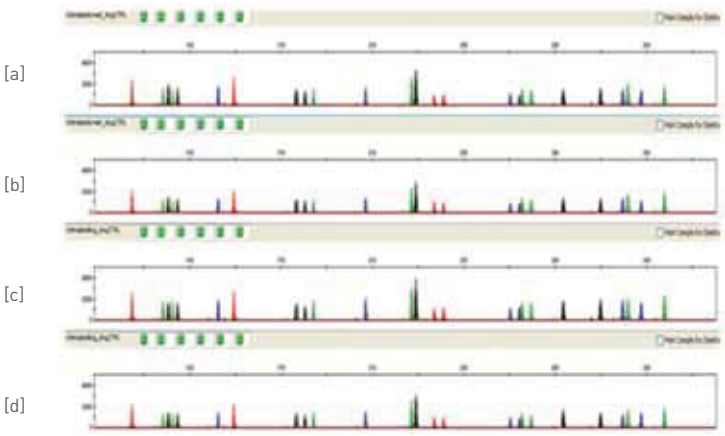

Figure 9. Comparison of data produced by 4 ng of 9947A control DNA amplified in the presence of blank 1.2 mm (a and b) and 2.0 mm (c and d) discs from untreated paper.

How to Cite This Article
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