Technical Focus

Direct PCR Amplification of Buccal Samples on the NUCLEIC-CARD™ COLOR With the AmpFℓSTR® Identifiler® Direct PCR Amplification Kit

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- The Copan NUCLEIC-CARD™ COLOR system is designed for collection and preservation of single-source buccal samples used in forensics.
- NUCLEIC-CARD™ COLOR is available as a stand-alone collection card that can be used with 4N6FLOQSwabs™, or in a convenient Collection Device with the 4N6FLOQSwabs™ Lollipop Swab prepackaged with the card.
- The use of Copan NUCLEIC-CARD™ COLOR with 4N6FLOQSwabs™ for buccal sample collection generates high-quality genotypes when amplified with the AmpFℓSTR® Identifiler® Direct PCR Amplification kit.

Introduction

In forensic investigations, buccal swab samples are some of the most challenging samples to process in terms of reproducibility and success rate when DNA is amplified for STR profiling in direct mode (i.e., without sample extraction and quantification). High-throughput laboratories are seeking a solution to help reduce the cost and increase the first pass success rate of buccal samples, in order to lower the backlog and make the reference profiling of these samples more reliable and efficient.

Copan NUCLEIC-CARD™ COLOR is an indicating sample collection system that has been specifically designed to facilitate processing of colorless samples while maintaining the standard features of NUCLEIC-CARD™ in terms of cell lysing capability, long term DNA integrity, and preservation. NUCLEIC-CARD™ COLOR can be used in conjunction with the 4N6FLOQSwabs™ Regular size tip (Figure 1) or is combined with a 4N6FLOQSwabs™ Lollipop Swab and packaged as the NUCLEIC-CARD™ Collection Device (Figure 2). The NUCLEIC-CARD™ COLOR changes from a pink background to white when buccal samples are deposited onto the card. The change in background allows visualization of where the sample was deposited making it easier for manual and automated punching and increasing the probability of getting full STR profiles. NUCLEIC-CARD™ COLOR combined with the AmpFℓSTR® Identifiler® Direct PCR Amplification kit or other Life Technologies direct amplification kits represents a reliable and cost-effective solution to process single-source buccal samples for forensic applications.

Figure 1 & 2

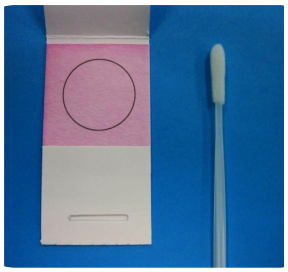


Figure 1. NUCLEIC-CARD™ COLOR and 4N6FLOQSwabs™ Regular size tip.

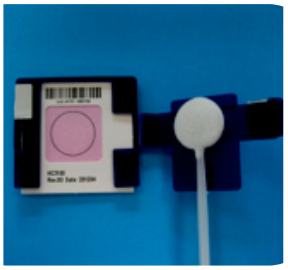


Figure 2. NUCLEIC-CARD™ Collection Device.

Experiments and results

Materials and methods

Studies were designed to compare the NUCLEIC-CARD™ Collection Device, the NUCLEIC-CARD™ COLOR, and the EasiCollect™ device (Whatman) with foam swab and Indicating FTA® card (Whatman). Buccal samples were collected based on each of the manufacturer's recommendation and air dried for one hour prior to storing at room temperature. With the exception of the samples used in the stability study, samples were stored for three days prior to processing. 1.2 mm punches were collected and amplified with the AmpFℓSTR® Identifiler® Direct PCR Amplification Kit using the recommended protocol. PCR was performed on the Veriti® PCR Thermal Cycler, capillary electrophoresis was performed on an Applied Biosystems® 3130 Genetic Analyzer, and the analysis was performed with GeneMapper® *ID-X* v1.3. Analysis was conducted using thresholds of 50 and 150 RFU, and data were evaluated for First Pass Success Rate (full profile obtained from one amplification and one CE injection), Mean Peak Height, Intracolor Balance, and Intralocus Balance. A detailed description of the experiments performed and the purpose of the data assessment for that experiment is outlined in Table 1.

Table 1

Study Type	Sample Type/Substrate	Data Assessment
Sensitivity	30 saliva donors on Indicating FTA® with EasiCollect™ 30 saliva donors on NUCLEIC-CARD™ COLOR	26 and 27 cycles tested to evaluate optimum cycle number for subsequent experiments
Performance	38 saliva donors on Indicating FTA® with EasiCollect™ 30 saliva donors x3 on NUCLEIC-CARD™ COLOR with 4N6FLOQSwabs™ Regular 30 saliva donors x2 on NUCLEIC-CARD™ Collection Device	Performance comparison of Indicating FTA® and the NUCLEIC-CARD™ COLOR with both types of 4N6FLOQSwabs™
Inhibition	4x Indicating FTA® with PBS + 4 ng 9947 Control DNA 4x NUCLEIC-CARD™ COLOR with PBS + 4 ng 9947 Control DNA	Comparison of possible inhibition due to indicating dye and other reagents by punching various locations on the Indicating FTA® and the NUCLEIC-CARD™ COLOR
Stability	2 lots NUCLEIC-CARD™ COLOR x 3 saliva donors x 4 temperatures (4°C, 22°C, 30°C, 55°C) x 5 time points (0, 2, and 6 months and simulated 24 and 72 months) 2 lots NUCLEIC-CARD™ COLOR with donor sample deposited after each x 4 temperatures (4°C, 22°C, 30°C, 55°C) x 5 time points (0, 2, and 6 months and simulated 24 and 72 months)	Evaluation of performance of samples deposited onto NUCLEIC-CARD™ COLOR and then aged

Results

Sensitivity study

When amplified for 27 cycles and analyzed with a 50 RFU threshold, the 4N6FLOQSwabs™ samples used with NUCLEIC-CARD™ COLOR produced full profiles with 3 observations of off-scale alleles, while 29/30 buccal swab samples on Indicating FTA® CARDS produced full profiles with 11 off-scale alleles seen. When analyzed at a peak amplitude threshold of 150 RFU, both NUCLEIC-CARD™ COLOR and Indicating FTA® CARDS had 29/30 samples produce full profiles. No reproducible artifacts or peaks indicative of contamination were observed for any of the two card types tested. 27 cycles was selected as the optimum cycle number, as it produced an excellent first pass success rate while minimizing null or partial profiles when compared to 26 cycle protocol. Accordingly, all samples in the subsequent experiments were amplified for 27 cycles. Results are summarized in Table 2.

Performance study

A total of 188 buccal samples were tested: 60 samples collected with the NUCLEIC-CARD™ Collection Device, 90 samples obtained with NUCLEIC-CARD™ COLOR and 4N6FLOQSwabs™ Regular size tip, and 38 buccal samples spotted in FTA® CARDS with the EasiCollect™ device. 149/150 samples on NUCLEIC-CARD™ COLOR and 37/38 samples on Indicating FTA® CARDS produced full profiles at a threshold of 50 RFU, while 145/150 samples on

Table 2

			Analysis at 50 RFU		Analysis at 150 RFU		Observations
Substrate:	PCR cycles	N samples	Number of Full Profile	First Pass Success Rate	Number of Full Profiles	First Pass Success Rate	Number of Homozygote Off-Scale Alleles
Indicating FTA® CARDS	26	30	29/30	96.7%	28/30	93.3%	4
NUCLEIC-CARD™ COLOR	26	30	28/30	93.3%	26/30	86.7%	1
Indicating FTA® CARDS	27	30	29/30	96.7%	29/30	96.7%	11
NUCLEIC-CARD™ COLOR	27	30	30/30	100.0%	29/30	96.7%	3

Table 3

Substrate:	N samples	Analysis at 50 RFU		Analysis at 150 RFU	
		Number of Full Profile	First Pass Success Rate	Number of Full Profiles	First Pass Success Rate
Indicating FTA® CARDS	38	37/38	97.4%	36/38	94.7%
NUCLEIC-CARD™ COLOR with 4N6FLOQSwabs™ Regular	90	90/90	100.0%	87/90	96.7%
NUCLEIC-CARD™ Collection Device	60	59/60	98.3%	58/60	96.7%

Figure 3

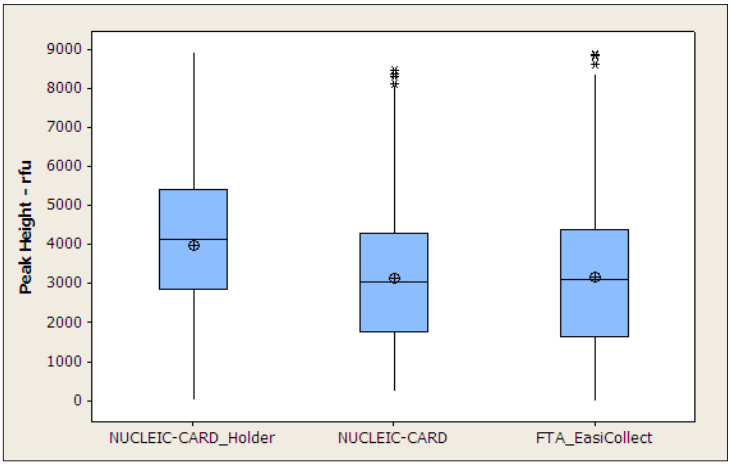


Figure 3. Peak height comparison of the NUCLEIC-CARD™ Collection Device, NUCLEIC-CARD™ COLOR and Indicating FTA® Cards generated in the Performance evaluation.

Figure 4

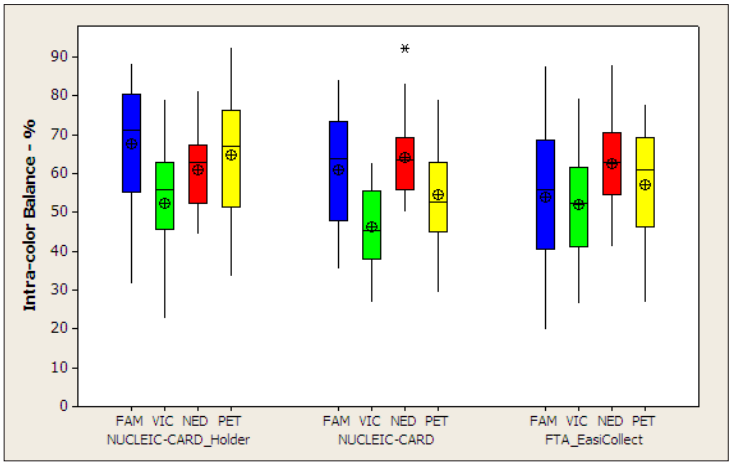


Figure 4. Intra-color balance of the NUCLEIC-CARD™ Collection Device, NUCLEIC-CARD™ COLOR and Indicating FTA® Cards obtained in the Performance evaluation.

NUCLEIC-CARD™ COLOR and 36/38 samples on Indicating FTA® CARDS produced full profiles at a threshold of 150 RFU (Table 3) Average peak heights were higher for the NUCLEIC-CARD™ Collection Device as compared to the NUCLEIC-CARD™ COLOR and 4N6FLOQSwabs™ Regular size tip and the EasiCollect™ device with Indicating FTA® paper. No differences were observed when the samples were collected with the NUCLEIC-CARD™ COLOR and 4N6FLOQSwabs™ Regular size tip and the EasiCollect™ device (Figure 3). Intra-color balance results were comparable between the three sample collection methods (Figure 4). No differences were observed in the intra-locus alleles (NUCLEIC-CARD™ COLOR Collection Device PHR = 0.912±0.085; NUCLEIC-CARD™ COLOR, 0.905±0.087; FTA® EasiCollect™ 0.907±0.070).

Inhibition study

40 µL of PBS was spotted onto 4 different NUCLEIC-CARD™ COLOR and 4 different Indicating FTA® cards. 1.2 mm punches were taken in different areas of the card and amplified with 4 ng of 9947A to assess the possibility of inhibition from the indicating dye (Figure 5). In the area where the strongest concentration of dye is seen (Area 2), some inhibition was seen with both card types, while more allele dropout was seen with the Indicating FTA® cards. No differences were observed between the NUCLEIC-CARD™ COLOR and the Indicating FTA® cards in Areas 1 and 3.

Figure 5

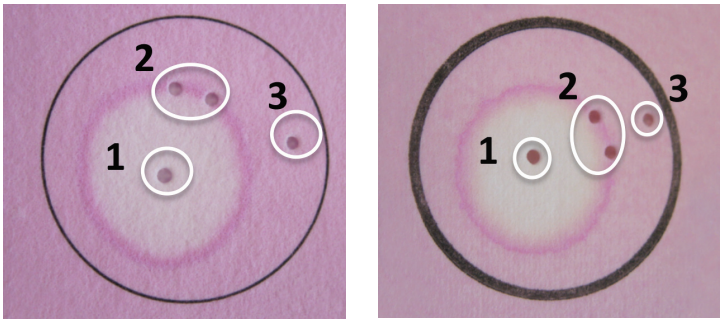
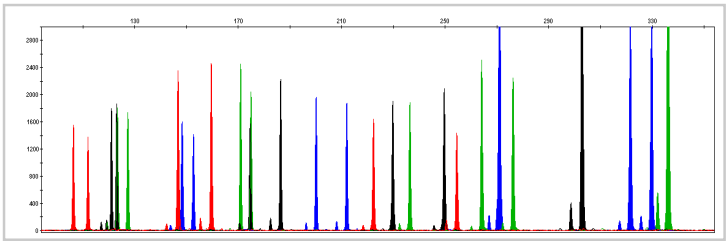
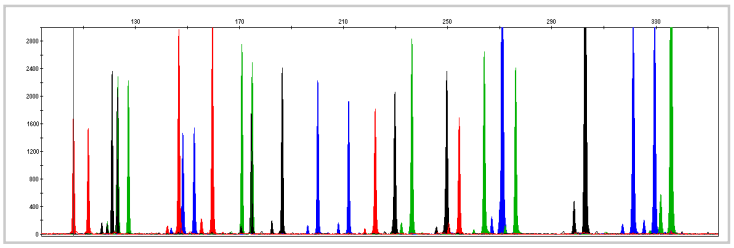


Figure 5. Area 1, 2, and 3 after spotting PBS on a NUCLEIC-CARD™ COLOR (left) and on indicating FTA® card (right).
Peak height comparison between NUCLEIC CARD™ COLOR and indicating FTA® cards after amplification with AmpFℓSTR® Identifiler® Direct.

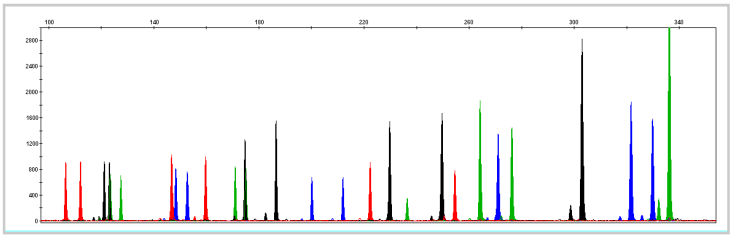
Area 1, NUCLEIC-CARD™ COLOR



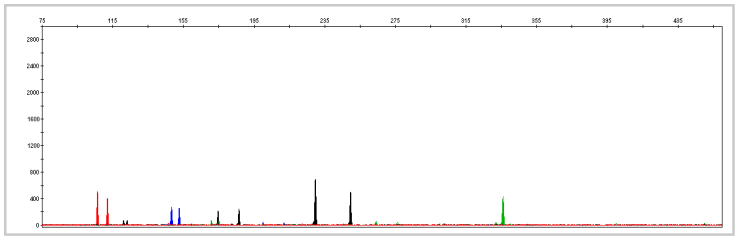
Area 1, from Indicating FTA® card



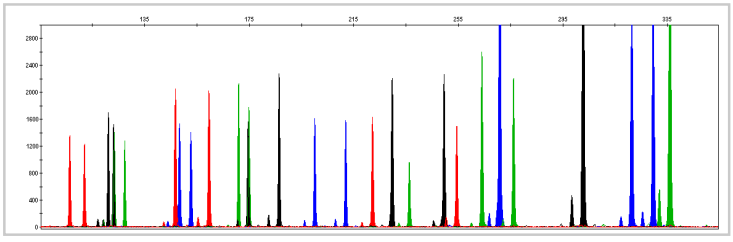
Area 2, NUCLEIC-CARD™ COLOR



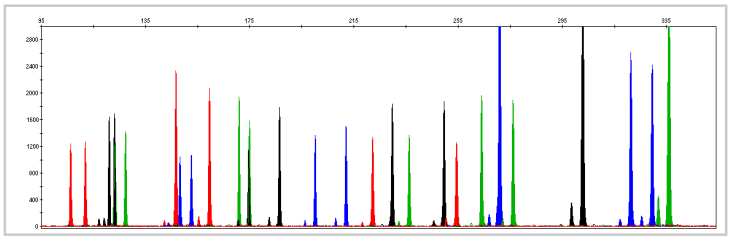
Area 2, from Indicating FTA® card



Area 3, NUCLEIC-CARD™ COLOR



Area 3, from Indicating FTA® card



Stability study

Samples from 3 different donors were deposited onto 2 different lots of NUCLEIC-CARD™ COLOR and stored for 2 to 6 months away from direct light exposure. Based on broadly-accepted accelerated stability protocols, incubation for 2 and 6 months at 55°C correspond to a real aging of approximately 24 and 72 months.

Differences were seen with the average peak heights between samples and the different length of time in storage that was assumed to be normal variation seen in buccal samples for different donors (Figure 6). Even after simulated storage at 72 months, full profiles with no artifacts were generated for all cards processed. No differences were observed in terms of ICB in all the stability scenarios (Figure 7). Real time stability studies are ongoing to confirm the results obtained with the accelerated aging conditions.

Figure 6

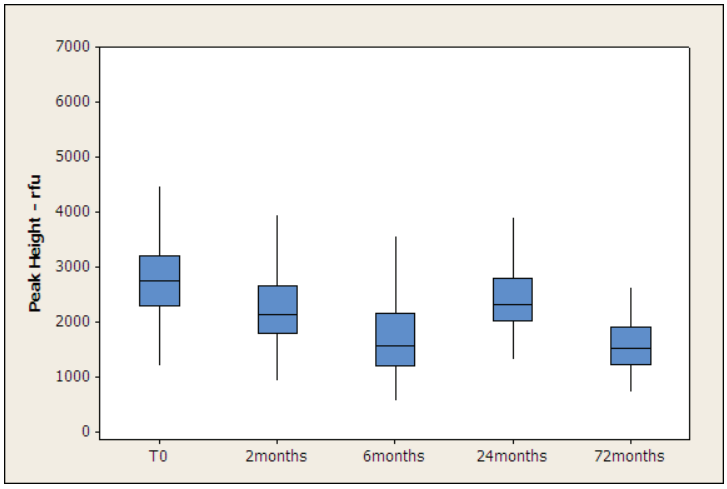


Figure 6. Peak height after long term storage of the NUCLEIC-CARD™ COLOR.

Figure 7

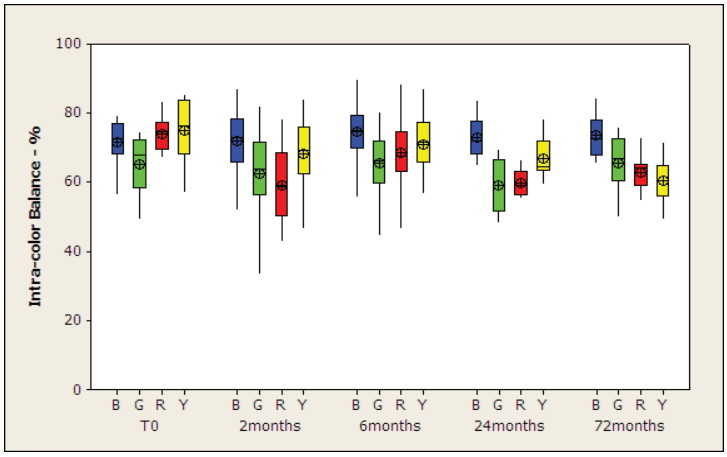


Figure 7. Intra-color balance after long term storage of the NUCLEIC-CARD™ COLOR.

Conclusions

In this comparative study, buccal samples collected from different donors, using the NUCLEIC-CARD™ Collection Device and the NUCLEIC-CARD™ COLOR with the 4N6FLOQSwabs™ Regular size tip and amplified with the AmpFℓSTR® Identifiler® Direct PCR Amplification kit generated full and high-quality profiles. The NUCLEIC-CARD™ COLOR products consistently produced peak heights and intra-color balance that were comparable or sometimes higher than those generated from samples collected with the EasiCollect™ device using Indicating FTA® cards. Stability tests demonstrated the robustness of the NUCLEIC-CARD™ COLOR showing lysing and good profile generating capabilities across time and temperatures. Together with Copan 4N6FLOQSwabs™, the NUCLEIC-CARD™ COLOR and the AmpFℓSTR® Identifiler® Direct PCR Amplification kit demonstrated to be a consistent and successful processing tool for direct profiling of buccal cell samples.

References

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