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Short communication

Comparison of performance of genetics 4N6 FLOQSwabs™ with or without surfactant to rayon swabs

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ABSTRACT

The collection of traces is the first step in the process of forensic genetics analysis. Currently, several different techniques are used (eg. gauze). Nevertheless, swabbing appears to be the most common of these. In a second step, the sampling devices should allow the use of preliminary tests in combination with an immunological confirmatory test (e.g. Hexagon Obti or Hemdirect). Our previous study shows that sampling with Genetics 4N6FLOQswabs™ coated with surfactant reduces by a factor of at least 100 the detection threshold of blood using two immunological tests. The aim of this work was to compare the ability to recover blood trace and the compatibility with immunological confirmatory test of various Genetics 4N6FLOQswabs™ nylon flocked swabs with or without surfactant.

The results obtain in this study show that Genetics 4N6FLOQswabs™ not coated with surfactant and Human DNA free FLOQswabs™ were suitable for the used in combination with immunological blood detection tests. Nevertheless, the Genetics 4N6FLOQswabs™ not surfactant coated give a better blood trace recovery.

1. Introduction

In forensic labs, the recovery of trace is done using several methodologies like swabbing or wet gauze. Single or double swabbing method to collect biological materials is the most common.

Generally the swabbing is performed using cotton swabs, but several works suggest that nylon flocked swabs (Genetics 4N6FLOQSwabs™, Copan) could improve the DNA recovery[1] or the DNA typing[2] although this benefit seems to be dependent of the extraction methods used.[3]

A recent work shows that use of polyester swab wetted with 0.01% SDS as a swabbing medium improved DNA collection.[4,5]

In our previous validation study, we observed that sampling with Genetics 4N6FLOQSwabs™ reduces by a factor of at least 100 the detection threshold of blood using the Hexagon Obti and Hemdirect immunological test. Using infrared spectral analyses, we identified the presence of sulfosuccinate ester on the genetic Genetics 4N6FLOQSwabs™.[6] This surfactant is responsible to the inhibition of the blood immunological test.

In order to allow the use of Genetics 4N6FLOQSwabs™ also with immunological assays besides molecular testing, we have been working in close collaboration with Copan to validate a new production of Genetics 4N6FLOQSwabs™. We compared the Genetics 4N6FLOQSwabs™ that contain surfactant (OLD 4N6), the new production of Genetics 4N6FLOQSwabs™ without surfactant (NEW 4N6) and a Copan line of a Human DNA free FLOQswabs™ marked as Medical Devices used for diagnostic testing. Rayon swabs were used as reference (RAYON). Using various dilutions of blood, we tested the recovery of DNA and the compatibility between these two new flocked swabs with two immunological blood detection strips.

2. Materials and methods

2.1. Hexagon Obti test

Four human blood samples from different volunteers were collected in EDTA tubes. Blood samples were diluted with phosphate buffer saline to obtain dilutions ranging from 10X to $1.6 \cdot 10^5$X. Fifty µl of each dilution were deposited on rayon swabs (Copan, Italy), Genetics 4N6FLOQSwabs™ (Copan, Italy), a new production of Genetics 4N6FLOQSwabs™ (Copan, Italy) and a line of a Human DNA free FLOQswabs™ (Copan, Italy), marked as Medical Devices used for diagnostic testing.

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Thereafter, each sampling support was incubated in 200 μl of the Hexagon Obti buffer overnight at 4 °C. This gave final dilutions between 50 to $8 \cdot 10^5$ times. Subsequently, 80 μl of this mixture were deposited on the Hexagon Obti strip (Human GmbH, Germany) and the results were recorded 10 min and 1 h after deposition of the sample. As control, each diluted blood sample was incubated in 200 μl of the Hexagon Obti buffer overnight at 4 °C without support. As above, 80 μl of this mixture were deposited on the Hexagon Obti strip (Human GmbH, Germany) and results were recorded following the same timing.

2.2. HemDirect test

For this test, our four human blood samples were diluted in MilliQ water to get dilutions ranging from 20X to $1{\cdot}6\ 10^5$X. Fifty μl of each dilution were placed on rayon swabs (Copan, Italy), Genetics 4N6FLOQSwabs™ (Copan, Italy), a new production of Genetics 4N6FLOQSwabs™ (Copan, Italy) and a line of a Human DNA free FLOQswabs™ (Copan, Italy). Then, each sampling support was incubated in 200 μl of the HemDirect buffer overnight at 4 °C. This gave final dilutions between 100 to $8 \cdot 10^5$ times. Subsequently, swabs were submitted to a quick spin in a spin basket and 120 μl of the obtained liquid were deposited on the HemDirect strip (Seratec, Germany). Results were recorded 10 min and 1 h after deposition of the sample. As control, diluted blood samples (n = 5) were incubated in 200 μl of the HemDirect buffer overnight at 4 °C without support. 120 μl of this mixture were placed on the HemDirect strip (Seratec, Germany) and results red after the same incubation times.

2.3. DNA extraction

Swabs were incubated in 240 μl of incubation buffer (DC920B-C, Promega, USA) added to 10 μl of protease K at 18 mg/ml (Promega, USA) at 56 °C overnight. Thereafter, the swabs were centrifuged using a spin basket. The elution was recovered, 500 μl of the lysis buffer of the DNA IQ™ Casework Sample Kit for Maxwell®16 (AS1210, Promega, USA) and 5 μl of DTT 1 M (V3151, Promega, USA) was added. The mix was submitted to DNA purification using Maxwell®16 robot (Promega, USA) and the kit DNA IQ™ Casework Sample for Maxwell®16 (AS1210,Promega, USA) following manufacturer instruction (Technical Bulletin – Tissue and Hair Extraction Kit (for use with DNA IQ™) Protocol – Instructions for use of product DC6740). The samples were eluted in 80 μl of elution buffer of the kit.

2.4. DNA quantification

The nuclear DNA in the extracts was quantified using the Quantifiler Trio system (Applied Biosystems, USA) and an Applied Biosystems 7500 Real-Time thermal cycler, according to the manufacturer's specifications. Each quantification was performed in duplicate. The average concentration was considered for the experiments.

3. Results

3.1. Hexagon Obti test

Table 1 presents the results obtained with the various dilutions tested in this study. The highest dilution enabling positive signal detection using OLD 4N6 was 100X. In comparison, the highest dilution allowing blood detection after 10 min was $8 \cdot 10^5$ for liquid and $1 \cdot 10^4$ for rayon swabs confirming our previous results showing that OLD 4N6 inhibited the detection of blood with the Hexagon Obti immunological strip.

Concerning the Human DNA free FLOQswabs™ and the NEW 4N6, the results are similar to the results obtained with the liquid control blood. In comparison to the rayon swabs, the performance of these two different flocked swabs regarding the immunological detection of blood is higher.

We also observed that for the lowest dilutions (50X and 100X) the signal of liquid, Human DNA free FLOQswabs™, the NEW 4N6 and RAYON decreased sharply probably due to the 'hook' effect.[7]

Detection after longer time (1 h) had weak or no influence.

3.2. HemDirect test

To confirm that both the NEW 4N6 and the Human DNA free FLOQswabs™ provided by Copan could be used with immunological detection tests, we performed the same analyses using HemDirect blood detection strips. For this test, we used dilutions ranging from 100X to $8 \cdot 10^5$ X.

The highest dilution enabling positive signal detection was 100X (Table 2) for OLD 4N6. The highest dilution allowing blood detection after 10 min was $1 \cdot 10^4$ for rayon swab as already observed. For the Human DNA free FLOQswabs™ and the NEW 4N6, the sensitivity reached the liquid control values proving the highest sensibility obtain with these new flocked swabs.

As observed using Hexagon Obti, detection after longer time (1 h) had little or no influence. We also observed a 'Hook' effect for the lowest dilution using liquid control, rayon swabs, Human DNA free FLOQswabs™ and the NEW 4N6.

3.3. DNA recovery

The most important quality of a swab is the recovery of trace and release of the DNA during the extraction process. To investigate these collection devices, blood was diluted and 5 or 10 μl of these dilution was deposed in a microscopic glass slices and dried overnight or, as reference, in a microtube and frozen immediately. The

Table 1
Number of positive tests using Hexagon Obti strips with the various dilutions deposited, or not, on different sampling supports. Liquid samples served as positive controls and standards. n = 5. *: test signal much weaker than control signal on the strip.

Final dilution	50X		100X		500X		2000X		10000X		$2 \cdot 10^5$X		$8 \cdot 10^5$X	
	10′	1 h	10′	1 h	10′	1 h	10′	1 h	10′	1 h	10′	1 h	10′	1 h
Liquid control	4*	4*	4*	4*	4	4	4	4	4	4	4	4	3*	4*
Human DNA free FLOQswabs™	4*	4*	4*	4*	4	4	4	4	4	4	3*	3*	1*	3*
											1	1		
OLD 4N6	4*	1	4*	2*	0	0	0	0	0	0	0	0	0	0
		3*		2										
NEW 4N6	4*	4*	4*	4*	4	4	4	4	4	4	2	2	2*	3*
											2*	2*		
RAYON	4*	4*	4*	4*	4	4	4	4	4	4	0	0	0	0