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Short Communication

Fast nuclear staining of head hair roots as a screening method for successful STR analysis in forensics

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ABSTRACT

The success rate of STR profiling of hairs found at a crime scene is quite low and negative results of hair analysis are frequently reported. To increase the success rate of DNA analysis of hairs in forensics, nuclei in hair roots can be counted after staining the hair root with DAPI. Two staining methods were tested: a longer method with two 1 h incubations in respectively a DAPI- and a wash-solution, and a fast, direct staining of the hair root on microscope slides.

The two staining methods were not significantly different. The results of the STR analysis for both procedures showed that 20 nuclei are necessary to obtain at least partial STR profiles. When more than 50 nuclei were counted, full STR profiles were always obtained. In 96% of the cases where no nuclei were detected, no STR profile could be obtained. However, 4% of the DAPI-negative hair roots resulted in at least partial STR profiles. Therefore, each forensic case has to be evaluated separately in function of the importance of the evidential value of the found hair. The fast staining method was applied in 36 forensic cases on 279 hairs in total.

A fast screening method using DAPI can be used to increase the success rate of hair analysis in forensics.

1. Introduction

Humans shed about 100 head hairs daily, mostly during hair grooming. A struggle involving hair pulling, however, can greatly accelerate hair loss. Therefore, head hairs from the victim or from the putative offender are frequently found at crime scenes, especially crimes of violence [1–3]. Short Tandem Repeat (STR) analysis of the hair root can identify the donor of the hair. In many forensic cases however, no reportable STR profiles are obtained from hairs collected at crime scenes [4,5], which can be explained by the growth phase of the hair. Hairs with intact root in the mitotically active anagen growing phase consistently yield reportable STR profiles while the inactive, naturally shed hairs in the telogen phase rarely yield informative STR profiles [5–9]. Unfortunately, 95% of the hairs found at a crime scene are telogen hairs [8,9].

The aim of this study was to optimize and validate a fast, non-destructive, easy to perform and inexpensive screening method to select those hair roots useful for STR analysis. Nuclei in hair roots can be stained overnight with 4′,6-diamidino-2-phenylindole or DAPI, a non-destructive and fluorescent dye that binds strongly to A–T rich regions in DNA [8,10]. The aim of this study was to validate a shorter staining protocol with DAPI and to evaluate the impact of the staining on subsequent STR profiling. Furthermore, the influence of forensic adhesive tapes, used to collect hairs at a crime scene, was investigated.

2. Materials and methods

2.1. Staining of hair roots with 1 h incubation in DAPI (part I)

58 head hairs (plucked or spontaneously shed hairs of various types and colors) were collected from 9 Caucasian volunteers. Hair roots were isolated by cutting the hairs approximately 1 cm above the hair root and were individually put into sterile 1.5 ml microcentrifuge eppendorfs. 10 μl of a DAPI/DABCO-solution (1.6 mg DAPI (Sigma); 2.24 g DABCO (1,4-diazabicyclo (2,2,2) octane) (Sigma), 10 ml Tris–HCl 0.2 M; pH 7.4) and 90 μl glycerol (Sigma) was added to the hair root. After 1 h incubation at room temperature in the dark, the hair root was removed from this solution and transferred to another microcentrifuge eppendorf. 10 μl of a wash-solution (2.24 g DABCO; 10 ml Tris–HCl 0.2 M pH

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7.4) and 90 μl glycerol was subsequently added to the hair root. After 1 h incubation, hair roots were removed from this wash-solution and put on UV-sterilized microscope slides cleaned with bleach and 70% ethanol. 10 μl of the wash-solution was added to the hair root and a coverslip glass was applied.

2.2. DAPI-staining of hair roots directly on microscope slides (part II)

In order to reduce the incubation time even further, 23 head hair roots (plucked or spontaneously shed hairs of various types and colors), collected from 7 Caucasian volunteers, were put directly on microscope slides after isolation, upon which 20 μl DAPI/DABCO-solution was added to the hair root. A coverslip glass was applied and hair roots were immediately visualized under the fluorescence microscope.

To compare both staining methods, hair roots of 54 naturally shed hairs from 5 Caucasian donors were stained directly on microscope slides (part II) upon which images were acquired. In a next step, hair roots were removed from the microscope slide and were stained again using the method described in part I. Images were again acquired. Both images of the same hair root were compared to each other.

2.3. Hairs on adhesive tapes

To investigate the influence of possible loss of nuclei due to the adhesive tape, 10 hairs plucked from 1 Caucasian donor were collected using adhesive tapes from the tape lifting kit (distributed by National Institution for Criminalistics and Criminology, Belgium) [11]. These hairs were removed from the adhesive tape and were stained directly with DAPI on microscope slides (part II).

2.4. Forensic cases

The screening method (part II) was applied on 279 hairs, collected in 36 forensic cases. Hairs were mainly collected from clothes and some from tape lifting kits applied on car seats.

2.5. Microscopic evaluation of the staining

Image acquisition was carried out with an AxioVert 200 M inverted fluorescence microscope (Carl Zeiss), equipped with the AxioVision multichannel fluorescence module and an AxioCam MRm camera (Carl Zeiss). Cell nuclei were visualized using Zeiss filter set no. 49 (G 365 nm, FT 395, BP 445/50). Slides were screened at 10× or 20× magnification using a Carl Zeiss short distance Plan-Apochromat® objective [12]. Nuclei present in the hair root were examined across several focal planes by performing a Z-stack multidimensional acquisition. A software module from Zeiss (extended focus, computation from Z-stack) was applied on the multidimensional acquired image, which results in a single image with a great depth of field, showing every nucleus present in the hair root. DAPI fluorescent blue spots showing the shape and size of the human follicular cells (~3–6 μm) were counted.

2.6. DNA extraction, amplification and detection

After microscopic evaluation, hair roots were removed from the microscope slide and transferred in a 1.5 ml microcentrifuge tube. 200 μl 5% Chelex®100 (Bio-Rad) was added to the hair root [13]. After vortexing for 10 s, samples were incubated overnight at 56 °C in a Thermomixer (Eppendorf). The following day, samples were incubated at 100 °C for 8 min. Finally, samples were centrifuged for 3 min at 14,000 × *g* [14]. Samples were amplified using 30 μl DNA-template and fragments were separated and analyzed as described earlier [14,15]. Each STR profile of an analyzed hair root was compared to the STR profile of the donor of the hair. Profiles were subdivided into full (all loci gave interpretable results), partial (result for one or more loci did not meet the minimum thresholds) or no profile.

2.7. Statistical analysis

Level of significance was calculated by SPSS (IBM, New York, US) using the McNemar test. A p-value <0.05 was regarded as significant.

3. Results and discussion

3.1. 1 h incubation in DAPI (part I)

58 hair roots incubated in DAPI for 1 h, were subdivided into 4 groups depending on the number of visible nuclei (Table 1). An example of a hair root without visible nuclei is shown in Fig. 1A while an example of a hair root with more than 50 nuclei is shown in Fig. 1B. If 20 or more nuclei were observed, at least partial profiles could be obtained. STR profiling of hair roots containing more than 50 nuclei resulted in full STR profiles. All 38 hair roots without any visible nuclei resulted in no STR profile (Table 1).

3.2. Direct staining of hair roots with DAPI on microscope slides (part II)

To reduce the incubation time in DAPI even further, 23 hair roots were stained directly on microscope slides and images were acquired immediately afterwards. An example of a hair root without visible nuclei after direct DAPI-staining on microscope slides is shown in Fig. 1C; Fig. 1D shows a hair root with more than 50 nuclei. Results of this fast staining method were comparable with those described above. Even more, in all cases where nuclei were observed, full STR profiles could be obtained. All hair roots without visible nuclei resulted in no STR profile (data not shown).

To be sure whether the immediate staining on the microscope slides would lead to the detection of the same number of nuclei compared to the staining with 1 h incubation, the two staining methods were performed on the same hair roots and compared. Focus has been put on naturally shed hairs, mimicking forensic situations. There were no significant differences between the two staining methods (McNemar test, $p = 1.00$), except for one hair in which direct staining of the hair root on a microscope slide resulted in detection of less nuclei compared to the longer incubation method. Counting less than 20 nuclei, all hair roots but one resulted in full STR profiles. From the 49 hair roots without any visible nuclei, 3 resulted in a partial STR profile and 1 even in a full STR profile (Table 2). One of the hair roots which resulted in a partial profile, showed presence of adhering material, presumably dandruff. Adhering material can contain DNA and could therefore result in a STR profile.

In an optimal situation, hair roots without visible nuclei could be discarded. In 96% (94/98) of all cases where no nuclei were observed, no STR profile was obtained. However, in 4% of these

Table 1
STR profiling of 58 hair roots stained with DAPI for 1 h.

Number of visible nuclei	Total root *N*	*N* roots with STR profile		
		Full profile	Partial profile	No
0	38	0	0	38
<20	4	2	0	2
$20 < n < 50$	4	3	1	0
>50	12	12	0	0

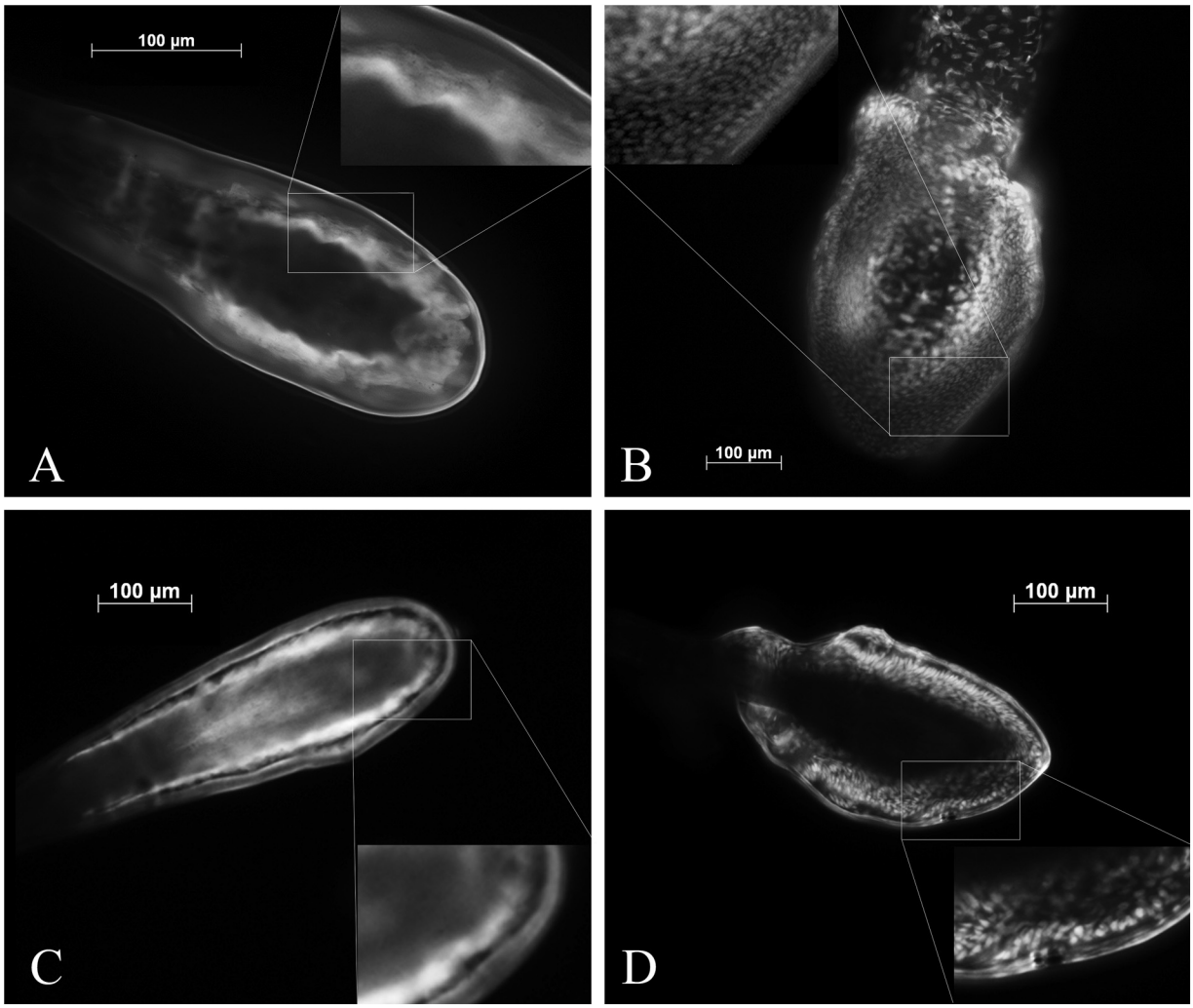

Fig. 1. Hair root without visible nuclei (A) and with more than 50 nuclei (B) stained with DAPI for 1 h (part I). Hair root without visible nuclei (C) and with more than 50 nuclei (D) stained directly on microscope slides (part II).

cases, a full or partial STR profile could be obtained. Therefore, results of DAPI-staining should always be considered in function of the importance of the evidential value of the found hair. If the hair is the only biological evidence in the forensic case, one might consider to submit the hair to STR analysis anyway, even if the staining is considered to be negative. If necessary, multiple hair roots showing the same characteristics can be pooled for STR analysis. In case the hair root did not yield a STR profile, the remainder of the hair can still be submitted to mitochondrial DNA analysis [16,17]. However, as STR analysis has a higher discriminative power compared to mitochondrial DNA analysis, the former is preferred.

Table 2
Evaluation of the staining and STR analysis of 54 hair roots.

Number of visible nuclei	Total root *N* after direct staining	Total root *N* after staining with 1 h incubation	*N* roots with STR profile		
			Full profile	Partial profile	No
0	49	49	1	3	45
<20	5	4	4 (3[a])	0	1
>50	0	1	0 (1[a])	0	0

[a] Number of roots with STR profile after staining with 1 h incubation.

3.3. Hairs on adhesive tapes

Ten hairs plucked from 1 donor were collected using the tape lifting kit, subsequently removed from the adhesive tape and directly stained on microscope slides. In 8 of 10 cases, 21–50 nuclei were counted while in the remaining 2 cases, more than 50 nuclei were observed. In all cases, full STR profiles were obtained (data not shown). However, loss of nuclei after removing the hair root from the adhesive tape could be observed as the adhesive tape was re-examined under the fluorescence microscope and nuclei were found on the tape. Therefore, if adhesive tapes are used for collecting hairs from a crime scene, it can be interesting for STR analysis to include that part of the tape where the hair root was located.

3.4. Forensic cases

The presented fast screening method was applied in 36 forensic cases in which 279 hair roots were stained with DAPI directly on microscope slides (part II). 263 hair roots were quoted as negative. Although hair roots without visible nuclei were not selected for STR analysis, 8 of these hair roots were submitted to STR analysis because adherent material was present around the hair root. However, no STR profile could be obtained on these hair roots. All hair roots containing any nuclei ($n = 16$), were submitted to STR

Table 3
STR profiling of hair roots stained with DAPI directly on microscope slides in forensic cases.

Number of visible nuclei	Total root *N*	*N* roots with STR profile		
		Full profile	Partial profile	No
<20	6	1	2	3
20 < *n* < 50	4	2	2	0
>50	6	6	0	0

analysis. Full STR profiles could be obtained on the 6 hair roots with more than 50 visible nuclei. Two hair roots containing 20–50 nuclei, (one of them collected from an adhesive tape), resulted in a full STR profile, while the other 2 resulted in a partial STR profile. From the 6 hair roots with less than 20 visible nuclei, 1 resulted in a full STR profile, 2 in a partial STR profile and the other 3 in no profile (Table 3). For PCR however, only 30 μl of the 200 μl DNA extract is used, which could provide an explanation for this observation.

Using the proposed fast screening method, all hair roots containing any nuclei should be submitted to STR analysis. However, one needs to keep in mind that the success rate of STR analysis of hair roots collected from a crime scene could be lower than the observed experimental success rate as adverse environmental condition prior to collection could influence the results.

4. Conclusion

In conclusion, a fast screening method using DAPI to stain nuclear DNA in hair roots collected at a crime scene can be used to predict STR analysis success. This non-destructive, quick and inexpensive screening method which does not require an incubation time, allows the forensic DNA laboratory to analyze only the most promising hair roots, containing any nuclei. Therefore, judiciary costs can be reduced.

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