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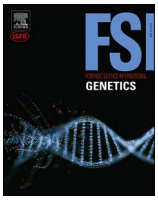

An evaluation of the RapidHIT™ ID system for hair roots stained with Diamond™ Nucleic Acid Dye

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ABSTRACT

The RapidHIT™ ID (RHID) system was evaluated for its suitability in processing a single hair root to obtain informative DNA profiles. Hair samples were assessed for nuclear DNA prior to DNA analysis using Diamond™ Nucleic Acid Dye (DD) and real-time Extended Depth of Field (EDF) imaging to visualise and count nuclei if present. Hairs were viewed under an Optico N300F LED Fluorescent Microscope and imaged using a MIchrome 5 Pro camera. Hair roots were processed through both the ACE GlobalFiler™ Express sample cartridge and the RapidINTEL™ sample cartridge. A total of 44 hairs including shed hairs (9) and plucked hairs (35) from 8 donors were evaluated in this study. The processing of hairs using the RHID system required the modification of a standard swab that allowed for hairs to be easily collected and placed into the cartridge but also allowed for the re-collection of hair roots post RHID analysis (for potential standard DNA workflow). 90% of plucked hairs with a high nuclei count (>100) resulted in a high partial or full DNA profile, with the remaining 10% resulting in a low partial profile. 44% of shed hairs resulted in a low partial profile, with the remaining hairs resulting in a null profile. This study demonstrated that the RHID system could successfully obtain a DNA profile from a single hair root with nuclei present post-DD staining. According to these results, it is suggested that when dealing with hairs containing fewer than 50 nuclei, using the RapidINTEL™ cartridge can enhance allele recovery.

1. Introduction

The standard forensic DNA analysis workflow consists of multiple steps to generate a DNA profile, these processes must be performed by experienced and trained scientists and require the use of specialised kits and instruments in a controlled laboratory environment [1,2]. In some cases, this can be time consuming and labour-intensive [1]. Recent advancements in DNA analysis have led to rapid DNA systems which can reduce turn-around times by performing all aspects of DNA analysis with minimal human intervention [3,4]. This technology also enables the processing of samples in the field, which can be advantageous for cases necessitating prompt results, like disaster victim identification. Human remains are prone to faster degradation, and reducing the processing time for samples would be particularly valuable in such scenarios [1,5,6].

The RapidHIT™ ID system (RHID) (Thermo Fisher Scientific, VIC, AUS) is a single instrument that performs DNA analysis in approximately 90 min and provides an STR profile using the GlobalFiler Express Kit (GFE) and GeneMarker HID software [1,7,8]. It requires minimal human intervention and uses the 'sample in – profile out' approach [1,3,4]. The system uses two cartridges that incorporate the GFE kit for amplification: ACE GFE cartridges for higher template samples and RapidINTEL™ cartridges for lower template samples. Initially, rapid instruments were developed to process reference DNA material, which is currently applied in police booking stations [7,8]. Forensic laboratories have also investigated the use of these instruments to process DNA samples from mass disasters and crime scenes [7]. Depending on the urgency of the case and samples, studies have demonstrated that the RHID system enables rapid DNA typing of forensic samples. These can be obtained in as little as 90 min in contrast to standard DNA analysis workflow, which can provide results in one day [2]. Other studies have evaluated the RHID system for 'one-cheek-touch' swab type samples, which yielded full DNA profiles [8]. Through a series of validation studies using reference buccal swabs, the RHID system produced reliable results with swabs that were analysed more than once and were free of sample-to-sample contamination [9]. Comparable results for blood and saliva samples were found using

Abbreviations: DD, Diamond™ Nucleic Acid dye; GFE, GlobalFiler Express; STR, Short Tandem Repeats; nDNA, Nuclear DNA; RHID, RapidHIT ID; RFU, Relative fluorescent unit.

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the RHID system versus the ANDE™ 6 C Rapid DNA Analysis™ System. The sensitivity limits of the instruments were also suggested to be around 5–10 ng of DNA (0.5 μL of blood) [10,11]. Rapid DNA has also been evaluated for crime scene blood and saliva samples; however, sensitivity was reported as one of the disadvantages of this technology [12]. To the best of the authors' knowledge, there have been no studies conducted employing rapid DNA systems on minimal DNA samples, specifically those containing less than 5 ng of DNA, such as hair samples.

Hair is one of the most encountered types of evidence at crime scenes, as humans shed between 150–200 hairs per day [13,14]. Hair samples encountered at crime scenes may vary, potentially including both plucked and shed hairs. Plucked hair requires forced removal from the body tissue (i.e., scalp) and can contain high amounts of DNA nuclei, often resulting in full DNA profiles [14]. Shed hairs are more commonly found at crime scenes; however, most shed hairs lack DNA nuclei, which is not ideal for DNA analysis [15]. Shed hairs with follicular tags or cellular material are more likely to produce profiling results [16].

Forensic laboratories receive many hair samples with no possible indication of the amount of DNA present [17]. This will not become evident until later stages of the DNA analysis process, which can waste time and resources [17]. Determining the suitability of the hair prior to DNA analysis is an important step in the examination of hairs. Using fluorescent or histological stains are techniques commonly used to assist in this process [16,21–25].

Fluorescent dyes that bind to nucleic acids, including SYBR Green, DAPI, and Diamond™ Nucleic Acid Dye, have undergone evaluation in various studies as a screening method for visualising DNA material on a range of forensic samples. These samples include fingermarks, hairs, dead skin, blood, semen, saliva, tape lifts, swabs, and improvised explosive devices [17–20]. Fluorescent dyes work by binding to DNA, which allows for the visualisation of DNA nuclei or cellular material when examined under a fluorescent microscope [21]. This can help in identifying the presence of DNA in forensic samples prior to commencing DNA analysis, as it indicates the ability to obtain a DNA profile based on the number of nuclei present or through the observation of fluorescence [17,18].

Hematoxylin and Eosin (H&E) is a standard histological staining technique for hairs that does not require the use of a fluorescent microscope [22,23]. However, this technique has disadvantages, such as multiple washing steps, which could potentially remove DNA and is also time intensive [24]. Additionally, H&E staining on hair samples has resulted in issues in hair visualisation as it was difficult to distinguish between the roots and shafts [24]. DAPI, a minor groove binding dye, has also been assessed for its suitability in identifying the presence of DNA nuclei in hairs [16,22,24,25]. Similar to H&E, the staining technique for DAPI is time consuming as it involves multiple steps and long incubation times [16,22,25].

Diamond™ Nucleic Acid Dye (DD) is a fluorescent dye that has been evaluated in multiple studies for its ability to visualise DNA [17–19,21]. The visualisation of DNA when stained with DD is immediate, which makes it a quick and easy technique in comparison to other dyes or histological stains. The use of DD as a screening technique has been assessed on items such as metal, glass, cartridge cases and mobile phones [19]. In previous studies, DNA nuclei were visualised in plucked hair samples after staining with DD, which indicated that a sufficient amount of DNA was present and therefore allowed for subsequent DNA analysis and the generation of full DNA profiles [18,21]. This also indicates that DD appeared to have minimal effects on downstream DNA analysis [18].

Hair samples that exhibit a substantial number of nuclei (>100) generally yield quantification values below the previously reported rapid DNA sensitivity threshold of approximately 5–10 ng [10,11,20]. This study will determine the suitability of the RHID system for these lower-level DNA samples, post DD staining. Being able to assess the presence of DNA in hairs using DD while obtaining rapid DNA profiles could potentially cut down on turn-around times and provide the ability to examine samples from high profile cases quicker.

2. Materials and methods

2.1. Sample collection

Reference buccal swabs were collected from 8 donors (4 male, 4 female) on sterile rayon swabs (COPAN, 155 C, Interpath, VIC, AUS). A total of 130 plucked and shed hairs were self-collected from the donor's scalp, with the quantity of hairs obtained varying, typically ranging from 5 to 10 hairs for each type. Each donor was provided with a new hair-brush and was asked to brush their hair several times and collect the shed hairs using sterile tweezers into a paper-boat (a folded A4 piece of paper) and then sealed in a snap seal plastic bag. For the collection of plucked hairs, donors were provided with sterile tweezers and were instructed to use them to pluck approximately 5 hairs. The plucked hairs were then collected into a paper-boat and sealed in a snap seal plastic bag.

This study was approved by Western Sydney University Human Research Ethics Committee (H15115).

2.2. Fluorescent staining and imaging of hair samples

130 hairs collected from donors were stained using a 20x solution of DD (Promega, Madison, WI, USA) in 75% Ethanol. Hairs were placed onto a clean microscope slide, and an adhesive flag was used to keep the hair in place on the slide. 1 μL of 20x DD solution was applied to the root end of the hair sample and visualised under an N300F LED Fluorescent Microscope (Optico, Microscopes Australia) with blue excitation (465–475 nm) and imaged using a MIchrome 5 Pro camera (Optico) using the live extended depth of field (EDF) function. The number of nuclei, if present, were estimated manually and sorted into either < 50, 50–100 and > 100 nuclei (see supplementary data Fig. S1).

2.3. Swab modification for RHID processing

To allow for ease of sampling of hairs using the RHID cartridges, a swab was modified, so that it was fit for purpose (see Fig. 1). This involved the removal of the rayon swab head from the plastic shaft (COPAN, 155 C) and placing a portion of double-sided tape (Scotch® Removable Poster Tape 1.9 cm x 3.8 m) ~2 mm × 19 mm at the end of the plastic shaft. The swab was then placed back into the swab casing

Fig. 1. Modified swab for hair samples using a rayon swab and ~2 mm × 19 mm of double-sided tape and the RHID ACE GFE cartridge with a hair sample inserted.

and UV treated for 60 min prior to use. The backing of the double-sided tape was removed using sterile tweezers prior to hair sampling. A swab negative control was processed to determine whether the modified swabs were free from contamination. Please note that RapidHIT™ ID ACE GlobalFiler™ Express cartridges (Thermo Fisher Scientific) will henceforth be referred to as RHID ACE GFE cartridges, and the RapidINTEL™ Sample Cartridge (Thermo Fisher Scientific) will be referred to as INTEL.

2.4. Sample preparation for RHID processing

Approximately one third of the reference buccal swab was excised and placed into the RHID ACE GFE cartridges for processing, along with the shaft of a sterile swab to keep the sample in place.

Hairs longer than 20 mm were cut for sampling, with approximately 10–20 mm of the root end excised. The adhesive section of tape on the swab shaft was then affixed to the root of the hair sample, ensuring the root was positioned at the end of the swab shaft.

Due to the limited number of RHID cartridges available (37 ACE GFE and 15 INTEL), sample selection had to be conducted to determine which hairs were to be processed. The number of hairs selected for RHID processing from each donor varied depending on the amount of DNA present, however, at least one plucked, and one shed hair were chosen from each donor. Due to the limited number of INTEL cartridges available, these were used specifically for shed hairs (with visible cellular material if possible) and hairs with low-level visible nuclei.

2.5. Data analysis

Automatic data analysis was performed with RapidLINK™ (v1.3.3) and GeneMarker™ HID software (Soft Genetics). The software flagged alleles that did not meet specified thresholds, marking them in yellow or red. The analytical threshold was set at 50 RFU for both cartridges, unless otherwise specified for specific loci. Additionally, a stochastic threshold of 1600 RFU for INTEL and 100 RFU for ACE GFE was employed, except in cases where different thresholds were specified for certain loci, as outlined in the User bulletin [26]. DNA profiles were defined as one of four categories based on profile percentage; null, 0%; low partial, 1–49%; high partial, 50–99% or full, 100%, these boundaries were based off of previous literature [5]. The profile percentage is determined by the count of observed alleles relative to the reference profile, considering homozygotes as two alleles.

3. Results

Shed and plucked hairs (130 in total) from eight donors were stained with DD and assessed for the presence of nuclei and cellular material. Out of this sample pool of 130 hairs, 44 hairs (35 plucked and 9 shed) were analysed using the RHID system as they presented the highest level of nuclei and/or cellular material when compared to other hairs. The quantity of hairs chosen from each donor was contingent on the DNA content, but, as a minimum, one plucked hair and one shed hair were selected from each donor. Considering the limited availability of INTEL RHID cartridges in this study, these cartridges were predominantly employed for processing at least one shed hair from each donor. The remainder of the available INTEL cartridges were used to process an additional six hairs with varying nuclei counts. The total number of alleles and calculated profile percentages of each processed hair sample are presented in Table 1 below.

The results, specifically regarding shed hairs and plucked hairs with nuclei counts above 100 and below 50, are depicted in Fig. 2. Shed hairs resulted in the most null profiles, with only four shed hairs exhibiting some allelic peaks. The highest profile percentage achieved from a shed hair (with a present follicular tag) was only 14% (refer to Table 1). Please see the supplementary data for hair images and the RHID profile (Fig. S3). Nevertheless, it was noted that the existence of a follicular tag

Table 1
Results of hair samples stained with DD (20x) and analysed with the RHID system. Shed hairs and a few selected plucked hairs with varying nuclei counts were processed through the RHID INTEL sample cartridge, all other samples were analysed with the RHID ACE GFE sample cartridge.

Donor	Hair Type (sample #)	Cartridge type	Number of visible nuclei	Numbers of alleles	Profile %
A	Plucked (1)	ACE GFE	> 100	44/44 (Female)	100
	Plucked (2)	INTEL	< 50	37/44 (Female)	84
	Plucked (3)	ACE GFE	> 100	33/44 (Female)	75
	Plucked (4)	INTEL	< 50	44/44 (Female)	100
	Plucked (5)	ACE GFE	> 100	44/44 (Female)	100
	Plucked (6)	INTEL	< 50	39/44 (Female)	89
	Plucked (7)	ACE GFE	> 100	26/44 (Female)	59
	Shed (8)	INTEL	FT	6/44 (Female)	14
B	Plucked (9)	ACE GFE	> 100	44/44 (Female)	100
	Plucked (10)	ACE GFE	> 100	43/44 (Female)	98
	Plucked (11)	ACE GFE	> 100	43/44 (Female)	98
	Plucked (12)	ACE GFE	> 50	37/44 (Female)	84
	Plucked (13)	ACE GFE	< 50	0/44 (Female)	0
	Plucked (14)	ACE GFE	< 50	0/44 (Female)	0
	Plucked (15)	ACE GFE	> 50	32/44 (Female)	73
	Shed (16)	INTEL	FT	0/44 (Female)	0
C	Plucked (17)	ACE GFE	> 100	41/46 (Male)	89
	Plucked (18)	ACE GFE	> 100	30/46 (Male)	65
	Plucked (19)	ACE GFE	> 100	46/46 (Male)	100
	Plucked (20)	ACE GFE	> 100	8/46 (Male)	17
	shed (21)	INTEL	CM	0/46 (Male)	0
	Shed (22)	INTEL	FT	0/46 (Male)	0
D	Plucked (23)	ACE GFE	> 100	37/46 (Male)	80
	Plucked (24)	INTEL	< 50	33/46 (Male)	72
	Plucked (25)	ACE GFE	< 50	0/46 (Male)	0
	Shed (26)	INTEL	N/A	0/46 (Male)	0
E	Plucked (27)	ACE GFE	< 50	42/46 (Male)	91
	Plucked (28)	ACE GFE	> 100	12/46 (Male)	26
	Plucked (29)	ACE GFE	> 100	46/46 (Male)	100
	Shed (30)	INTEL	FT	3/46 (Male)	7
F	Plucked (31)	ACE GFE	< 50	26/44 (Female)	59
	Plucked (32)	ACE GFE	< 50	9/44 (Female)	20
	Plucked (33)	ACE GFE	< 50	0/44 (Female)	0
	Shed (34)	INTEL	FT	0/44 (Female)	0

(continued on next page)

Table 1 (*continued*)

Donor	Hair Type (sample #)	Cartridge type	Number of visible nuclei	Numbers of alleles	Profile %
G	Plucked (35)	ACE GFE	> 100	46/46 (Male)	100
	Plucked (36)	ACE GFE	< 50	45/46 (Male)	98
	Plucked (37)	INTEL	< 50	46/46 (Male)	100
	Plucked (38)	ACE GFE	> 100	45/46 (Male)	98
	Shed (39)	INTEL	FT	5/46 (Male)	11
H	Plucked (40)	INTEL	> 100	44/44 (Female)	100
	Plucked (41)	ACE GFE	> 100	34/44 (Female)	77
	Plucked (42)	ACE GFE	> 100	40/44 (Female)	91
	Plucked (43)	ACE GFE	> 100	44/44 (Female)	100
	Shed (44)	INTEL	FT	4/44 (Female)	9

FT = Follicular tag, CM = Cellular material, N/A = no cellular material or follicular tag present

or cellular material does not inherently guarantee DNA profiling results, as four out of the five shed hairs with these characteristics produced null profiles. As shown in Fig. 2, profiles were successfully obtained from plucked hairs with nuclei counts above 100, as the majority (17 out of 19) resulted in a high partial or full DNA profile. No instances of null profiles were identified, with only two samples resulting in a low partial profile. The ACE GFE cartridge was less effective in obtaining DNA profiling results for hairs with a nuclei count of 50 or below, as four hairs yielded null profiles (Fig. 2). Conversely, the INTEL cartridge demonstrated greater success in obtaining DNA profiling results, yielding two full profiles from hairs (<50 nuclei), while the remaining three produced high partial profiles.

Fig. 3. shows an electropherogram produced from a plucked hair stained with DD and processed through the ACE GFE cartridge. The nuclei stained can be easily seen in the close-up fluorescent images of the hair (Fig. 3 A & B). The DD staining and visualisation process is rapid, with the entire procedure, including image capture, taking only a few minutes to complete. The number of nuclei present (Fig. 3) vastly exceeds the count of 100 nuclei; however, due to time constraints, the counting was limited to 100 to streamline the process.

Artefacts were visible in the electropherograms including the supplied negative control cartridge (Fig. 4a) and the modified swab negative control (Fig. 4b). Three non-specific peaks were produced consistently in the blue dye channel only (~80 bp, 100 bp and 155 bp) and the peak heights varied across the different sample types. No other peaks were observed in the modified swab negative control, indicating the modified swab for hair collection had no detectable signs of contamination. These non-specific peaks were below the analytical threshold (50 RFU) in the plucked hair sample (Fig. 4d).

4. Discussion

Shed and plucked hairs (44 in total) were analysed using the RHID system post DD staining. Hair roots were assessed for the presence of DNA and/or cellular material. Hair samples were chosen based on the cellular material present, either the number of nuclei for plucked hairs or the presence of a follicular tag for shed hairs. The cartridges designed for the RHID workflow allow for the recollection of the original sample (i.e., swab), which allows for subsequent analysis of the sample through standard DNA workflow, as evidenced in previous research [8,28]. With this consideration in mind, a modification was made to a standard swab to facilitate the sampling of hairs. Given the static properties of hairs, collecting, and placing into the RHID cartridge can be challenging. As explained in Section 2.4 and depicted in Fig. 1, a section of adhesive tape was affixed to the swab shaft that facilitates the collection of hair, which can then be conveniently transferred into the RHID cartridge. The plastic shaft of the swab ensures that the hair, adhering to the adhesive tape, remains situated at the bottom of the chamber throughout the extraction process. After the extraction process is finished, sterile tweezers can be used to remove the plastic swab shaft, allowing the hair to be recollected into a 1.5 mL tube for further analysis. The purpose behind this re-collection process was to facilitate the subsequent processing of the hair through standard DNA analysis. Subsequent analysis is achievable because the extraction procedure within the RHID system does not break

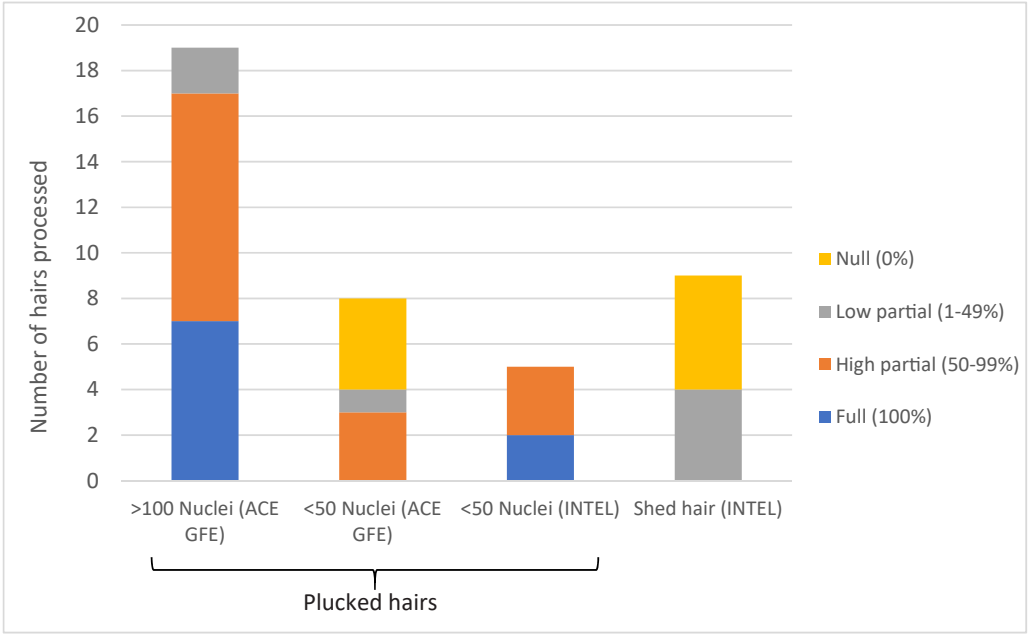

Fig. 2. Summary of the DNA profiling results obtained from hairs processed through the RHID system using both ACE GFE and INTEL cartridges, post DD staining. The displayed results from plucked hair samples only includes hairs classified with more than 100 nuclei and less than 50 nuclei.

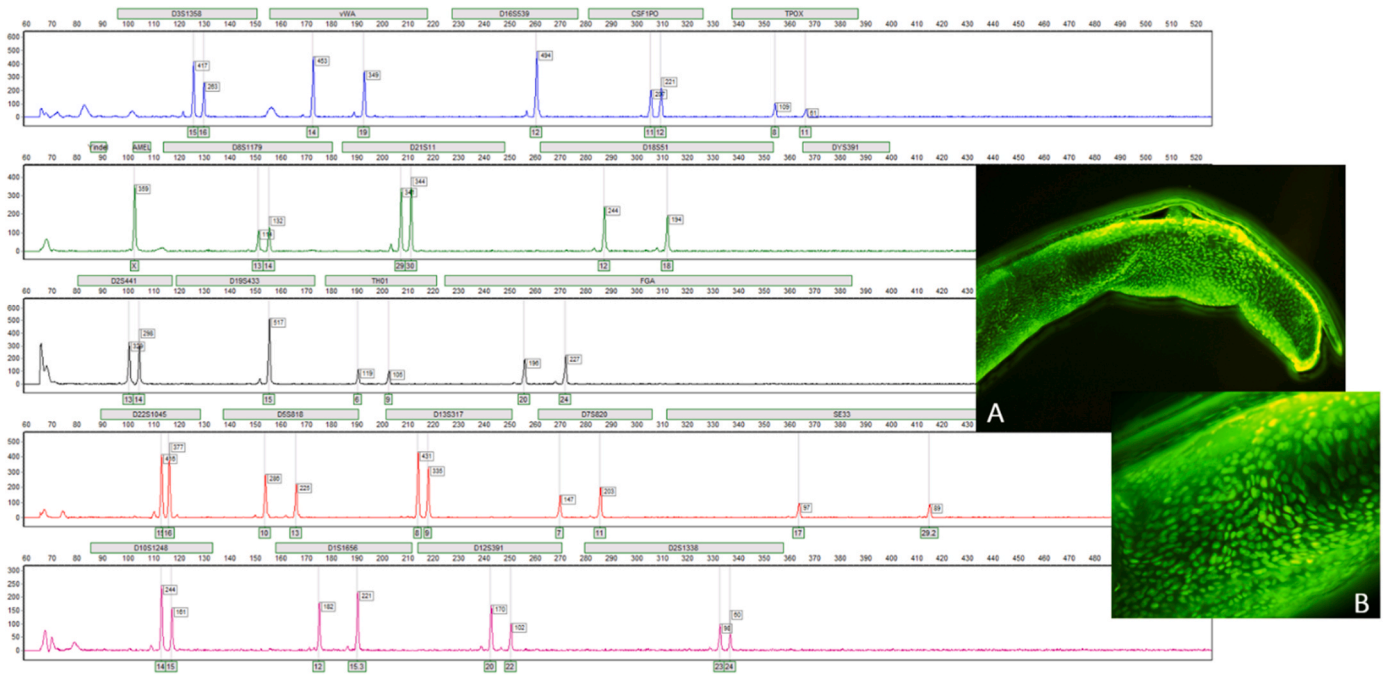

Fig. 3. Electropherogram of a plucked hair (#1) treated with 20x DD stain and processed using the RHID ACE GFE cartridge, images captured via the Optico N300F LED Fluorescent Microscope and MIchrome 5 Pro camera employing the EDF function. Image (A) showing 100x magnification, while Image (B) displays 400x magnification, showcasing the visible nuclei after DD staining.

down the hair matrix, likely because it involves a relatively crude extraction process.

Since INTEL cartridges are specifically engineered to improve sensitivity compared to ACE GFE cartridges [26], this research aimed to evaluate the potential for obtaining DNA profiling results from low-level sample types, specifically, shed hairs. Nine shed hairs were processed through the INTEL cartridges, with at least one shed hair per donor being processed. Due to the low allele coverage obtained, no further shed hairs were tested, the remaining six were used to process low nuclei plucked hairs. All other plucked hairs (29 out of 35) were processed through the RHID system using ACE GFE cartridges.

Table 1 demonstrates the number of alleles and profile percentage of each sample processed. Most of the hairs produced DNA profiles that were consistent with the amount of DNA nuclei/cellular material observed, with some exceptions, as two plucked hairs resulted in no profile. The two hair samples, originating from the same donor, were relatively small, measuring less than 5 mm in length, which could potentially have led to incomplete immersion of the hair in the reagents during the RHID extraction process. See supplementary data (Fig. S4) for an example of one of the hair roots with stained and visible nuclei that resulted in a null profile. In addition to these two samples, another two plucked hairs, from different donors also resulted in a null profile. These four plucked hairs were designated with a nuclei count below 50, and this factor could have played an additional role in influencing the null result. A possible reason, in addition to hair length and nuclei count, could be that the plastic shaft of the swab was trimmed too short, creating excessive space between the end of the plastic shaft and the upper surface of the cartridge lid. For optimal results, it is advisable to trim the plastic shaft to around 80 mm, as doing so helps prevent potential complications in the extraction process resulting from cutting the plastic shaft too short. Moreover, the adhesive tape on the plastic shaft might adhere to the inner surface of the swab chamber, potentially leading to complications during the extraction process. However, further investigation would be required to confirm these two explanations for the observed results.

In addition to the factors listed above, the RHID process only utilizes a crude extraction technique in comparison to standard DNA analysis workflow. The sample within the swab chamber is incubated at 75 °C in a buffer solution with volumes that vary depending on the cartridge used [8,26]. Therefore, the hair matrix remains intact during this process, contrary to the usual breakdown that occurs during DNA extraction from hair. Generally, reagents like dithiothreitol (DTT) and proteinase K are commonly utilized in the digestion process to facilitate the breakdown of the hair matrix [16,21,23]. Therefore, the inefficiencies observed in utilizing the RHID system for certain hairs, leading to null profiles, may stem from the absence of hair matrix breakdown including that of the nuclei visible through DD staining. It's important to highlight that obtaining complete DNA profiles from plucked hairs after direct PCR post-DD staining, excluding an extraction step, is also feasible [21].

Consistently, in the blue dye channel, three non-specific peaks were generated, with sizes around 80 bp, 100 bp, and 155 bp, and the peak heights exhibited variation among different sample types (Fig. 4). In the RapidHIT® ID User guide, it states that an 80 bp dye artifact peak has been observed previously at D10S1248 [29]. However, in this study the 80 bp peak was observed outside the boundary of the D3S1358 locus. Previously, Salceda, S. et al., identified peaks in the blue channel around ~80 bp and ~95 bp, which were attributed to primer flare peaks, as well as a dye blob around ~130 bp [8]. In the plucked hair sample (Fig. 4d), the heights of these non-specific peaks dropped below the analytical threshold (50 RFU). This suggests that these peaks are likely primer flare peaks, in line with earlier published findings [8], due to the higher presence of DNA in the sample.

Fig. 3 shows profiling results of a plucked hair that was processed through the RHID system with images of the hair post DD staining. A full DNA profile was obtained from the plucked hair sample, which had visible nuclei (>100 nuclei count). Based on the fluorescent images, it is evident that this hair sample contained a substantial number of nuclei. Assuming there is 6 pg of DNA per cell [27], obtaining a 1 ng input would require over 167 cells, which this image clearly exceeds (Fig. 3A). Refer to the supplementary data for an additional example illustrating a hair with a high nuclei count (Fig. S2). The precise count of nuclei in the images was not performed beyond 100 due to time constraints. Future research could explore the utilization of cell counting software to enhance the counting process; however, this aspect was not undertaken

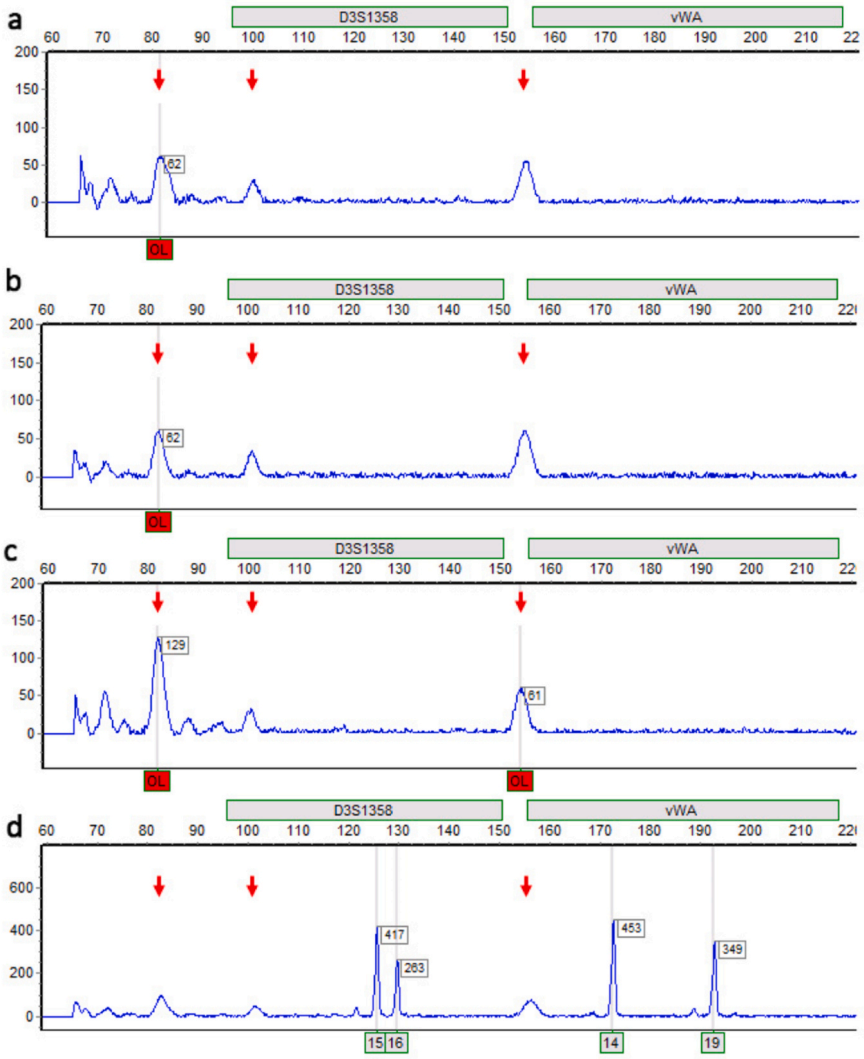

Fig. 4. Artefacts/non-specific peaks (red arrows) were observed with samples analysed using the RHID ACE GFE and INTEL cartridges in the blue dye channel, (a) RHID ACE GFE negative control cartridge, (b) swab negative control (ACE GFE), (c) example shed hair (INTEL) and (d) example plucked hair (ACE GFE). The vertical axis is intensity in RFU, and the horizontal axis is fragment length from 60 bp to 220 bp.

within this study.

Using the RHID system, plucked hairs with a high nuclei count (>100) that were processed with the ACE GFE cartridge, showed that approximately 37% (7 out of 19) of these hairs produced full profiles. Moreover, about 59% (10 out of 17) generated high partial profiles, and the remaining hairs (2 out of 19) resulted in low partial profiles, as illustrated in Fig. 2. Two plucked hairs had a nuclei count of 50–100 which both resulted in high partial profiles. These findings indicate that the ACE GFE cartridge is effective for processing hair samples that display a high nuclei count exceeding 100. This aligns with previous research, which indicated that 55% of hairs containing over 100 nuclei produced complete profiles. However, these results were from hairs that were directly amplified using a different STR amplification kit (AmpF®STR NGM™ kit) compared to the RHID chemistry employed in this study [21].

For hairs with a lower nuclei count (<50 nuclei), it was demonstrated that the INTEL cartridge was more suitable for these hair samples, as all five hairs produced either a full or high partial profile. In contrast, four hairs in the same nuclei range processed with the ACE GFE cartridge resulted in null profiles, with no full profiles obtained. This is to be expected due to the improved sensitivity of the INTEL cartridge, with an increase in amplification cycle number (from 28 to 32 PCR cycles), and a decrease in lysis buffer volume (from 500 μL to 300 μL) [26]. These results also align with a previous study, which noted that hairs with fewer than 100 nuclei rarely yielded an allele recovery rate exceeding 20% [24], however, recovery of alleles within this study was higher. Out of the nine shed hairs processed, four of the samples gave a low partial profile, and the remainder of the samples gave a null result (Fig. 2). These findings suggest that presently, the RHID system is not suitable for processing shed hairs. These findings from shed hairs without nuclei correspond to earlier research that demonstrated when hairs lacking nuclei were directly amplified, 72% (23 out of 32 hairs) led to null profiles [21].

Variation was observed in the results obtained for plucked hairs (>100 nuclei), where some donors predominately provided full or high partial profiles compared to other donors where hairs only gave low partial profiles. This may be sample specific, or donor variation may have contributed to the variability in the obtained results. Analysing the recollected hairs from the RHID cartridge through standard DNA analysis would help determine if the observed study findings stem from donor variation or potential sample issues during the RHID process. However, this was beyond the scope of this study; nevertheless, it stands as an important area for future research.

Out of the 35 plucked hairs that were analysed using the RHID

system, 80% of samples, regardless of nuclei count and type of cartridge used, resulted in high partial or full DNA profiles. This demonstrates the usability of the system to process hair roots for identification purposes. This study has also highlighted the significance of staining hairs to visualize the presence of DNA before analysis, aiding in determining the most appropriate cartridge for use.

5. Conclusions

A modified swab was effectively designed to enable the collection of hair samples, making their convenient placement into the RHID sample cartridge possible, while also facilitating sample retrieval for potential subsequent standard DNA workflow if required. Consequently, in cases where there is an urgent need for the immediate processing of hair samples (with suitable nuclei counts), it is possible to utilize the RHID system and, if necessary, employ standard DNA processing procedures at a later date. Reanalysis of the hair samples using the conventional DNA analysis workflow was not undertaken in this study; however, it represents an essential focus for future research.

The RHID system showed the ability to produce full DNA profiles from plucked hairs that have been stained with DD to determine nuclei count. From the 35 plucked hair samples analysed using both cartridge types, 80% (28 out of 35) yielded a high partial or full DNA profile, 9% (3 out of 35) yielded a low partial profile and 11% (4 out of 35) yielded a null DNA profile. This study builds upon previously published research on the type of forensic samples that have been deemed suitable for rapid DNA processing [2–6,8–12]. This research also demonstrates that forensic samples that have been stained with DD, are able to produce profiling results using the RHID system.

Based on these findings, it is advisable that when dealing with hair samples containing fewer than 50 nuclei, the INTEL cartridge is used to enhance the likelihood of achieving DNA profiling results. If a substantial number of visible nuclei is present, then processing through the ACE GFE cartridge can be considered. The findings in this study also suggest that, currently, the RHID system is not suitable for processing shed hairs. Refer to the supplementary data (Fig. S5) for a diagram illustrating the proposed process for handling hair samples in urgent scenarios.

CRediT authorship contribution statement

Tabarek Aljumaili: Conceptualization, methodology & investigation of study. Data visualization, writing – original draft and writing – review & editing. Alicia M. Haines: Conceptualization of study, methodology & investigation of study. Data visualization, writing – original draft and writing – review & editing, Supervision, and Funding acquisition. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fsigen.2023.103003.

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