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# Reprocessing of hair roots using conventional forensic DNA analysis following RapidHIT® ID processing

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ABSTRACT

Hair traces are frequently recovered at crime scenes and represent a valuable source of forensic DNA, although the cellular content of hair roots varies substantially depending on growth stage, with shed hairs typically containing limited DNA. Rapid DNA platforms such as the RapidHIT® ID (RHID) system enable expedited STR profiling; however, the impact of RHID processing on downstream laboratory-based analyses remains an important consideration. In this study, the ability to reprocess hair roots following RHID analysis was evaluated, and Diamond™ Nucleic Acid Dye (DD) was employed to assess cellular retention and degradation associated with the RHID workflow. Hair roots representing a range of nuclei counts were initially assessed using DD staining and fluorescence microscopy, processed using the RHID system, and subsequently recovered from the cartridge for conventional forensic DNA analysis. Post-RHID assessment using DD enabled visualisation of remaining cellular material and provided an indication of substrate integrity following rapid DNA processing. Recovered hair roots were subjected to standard lysis, extraction, and STR amplification protocols to determine the feasibility of downstream profiling. Results demonstrated that hair roots, particularly those with higher initial nuclei counts, could be successfully reprocessed following RHID analysis, yielding DNA profiles suitable for comparison and further forensic applications. DD staining indicated that cellular material was retained post-RHID, supporting the suitability of recovered hair roots for additional testing. In contrast, shed hairs were less likely to yield usable profiles after reprocessing, reflecting their inherently limited cellular content rather than RHID-induced degradation. These findings demonstrate that RHID processing does not preclude subsequent laboratory analysis of hair roots and highlight the value of hair root visualisation prior to processing, as demonstrated here using Diamond™ Dye (DD). Collectively, this approach supports the use of rapid DNA workflows while preserving evidentiary material for confirmatory and extended forensic analyses.

## 1. Introduction

Conventional DNA workflow of forensically relevant samples generally can be time consuming and laborious due to the many steps involved [1,2]. The RapidHIT™ ID system (RHID) (Thermo Fisher Scientific, VIC, AUS) requires minimal human intervention and can generate a DNA profile within approximately 90 min, potentially improving turnaround times compared to conventional workflows [3,4]. Studies have demonstrated the value of the technology across a range of scenarios, including the rapid processing of samples in mass disaster contexts, where degradation is common and timely analysis is critical [1, 5–7]. RHID instruments can be placed in controlled laboratories and non-controlled laboratory environments which further highlights the versatility of using such instruments [5]. Furthermore, the addition of a Rapid DNA instrument to a laboratory allows for priority samples to be analysed quickly and efficiently without disturbing regular laboratory procedures.

Originally developed for the analysis of reference buccal swabs, Rapid DNA instruments have since been evaluated for use across a broader range of forensic sample types, including blood and saliva [8]. While effective for higher-template samples, several studies have shown that Rapid DNA analysis performs less reliably than conventional

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

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laboratory workflows when applied to low-template material such as trace or touch DNA [5,9], a finding that is consistent across evaluations of Rapid DNA suitability for touch DNA analysis [10].

Hair samples represent a common forensic exhibit recovered from crime scenes and may provide probative information regarding the presence of individuals through DNA profiling [11,12]. Hair is also frequently used for identification purposes in missing persons investigations and disaster victim identification (DVI), particularly as an ante mortem reference sample [13]. However, the nuclear DNA yield from hair roots varies considerably depending on the hair growth stage, with shed hairs typically containing limited cellular material [14]. As a result, pre-assessment techniques are often employed to determine whether a hair root is suitable for nuclear DNA analysis or should instead be preserved for mitochondrial DNA testing [15].

Previous research has demonstrated that staining hair roots, assessed through light or fluorescence microscopy, can provide an indication of cellular content and suitability for nuclear DNA analysis [16–24]. Diamond™ Nucleic Acid Dye (DD) is a DNA-binding dye widely recognised for effective hair visualisation [21–24], its primary role in this workflow is to support sample selection rather than downstream interpretation. Building on this approach, a recent study demonstrated that hair roots stained with DD to visualise morphology and cellular content could be successfully processed on the RHID platform using a modified standard swab [24]. The modified swab consists of a conventional swab with the head removed and adhesive tape applied to the shaft to facilitate hair collection and introduction into the cartridge. This study looked at both the RapidHIT™ ID ACE GlobalFiler™ Express cartridges (Thermo Fisher Scientific), and the RapidINTEL™ Sample Cartridges (Thermo Fisher Scientific) [24]. Both cartridge types were available to the research team; however, due to differences in cartridge availability, hairs assessed as having higher nuclear content were preferentially processed using ACE cartridges, whereas those with lower cellular content, including shed hairs, were processed using RapidINTEL™ cartridges. The RapidINTEL™ cartridge has since been replaced with the RapidINTEL™ Plus cartridge with improved sensitivity and peak height balance [25].

The RHID system enables recovery of biological samples from the cartridge following processing, allowing reanalysis using conventional laboratory workflows. This capability has previously been demonstrated for buccal swabs, with full DNA profiles successfully generated from samples that had already undergone RHID analysis [26]. Such recoverability is particularly important in forensic casework, where confirmatory testing, optimisation, or court-admissible analysis may be required following the generation of rapid investigative intelligence.

When applied to hair samples, this feature is especially valuable. Following RHID processing, the hair root remains intact within the cartridge, allowing the same sample to be retrieved and reprocessed using standard extraction and amplification protocols [24]. Subsequent analysis may generate additional genetic information to complement the initial Rapid DNA profile, support database submission, or enable specialised testing such as familial searching or Y-STR profiling. Preserving the hair root also mitigates the risk of irreversible sample consumption, which is a critical consideration given the limited and variable cellular content typically associated with hair roots.

The ability to recover and reanalyse DNA samples following RHID testing is therefore of particular significance for low-template or limited forensic samples. Sample recovery supports downstream re-extraction, quantification, optimisation, and profile regeneration, thereby preserving evidentiary value and enabling verification of initial results. This capability is especially important in jurisdictions where RHID analysis is currently restricted to intelligence purposes and Rapid DNA profiles are not admissible in court, ensuring that samples used to generate investigative leads remain available for subsequent confirmatory and court-admissible testing.

In this study, the feasibility of reprocessing hair roots previously stained with DD and analysed using the RHID system was evaluated. The impact of RHID processing on the integrity of the hair sample was also assessed by examining potential cellular material loss. Hair samples once retrieved from the cartridge were re-stained with DD and examined using fluorescence microscopy to assess changes in morphology and cellular content following RHID processing. The hair samples were then subjected to a conventional DNA extraction and amplification workflow. Collectively, these findings provide practical guidance for best-practice workflows aimed at maximising DNA recovery and preserving evidentiary value from hair samples that have previously undergone Rapid DNA analysis.

## 2. Materials and method

### 2.1. Sample collection

Sterile rayon swabs (COPAN, 155 C, Interpath, VIC, AUS) were employed to collect reference buccal swabs from eight donors (four males, four females). Scalp hair samples, previously collected, Diamond™ Dye (DD)-stained, and processed through RHID as described by Aljumaili, T., & Haines, A. M. [24], were subsequently retrieved from the RHID cartridges. Recovered hairs were fluorescently stained (Section 2.2 below), and placed into a DNA LoBind®, 1.5 mL tube (Eppendorf, Macquarie Park, NSW), then stored at −20 °C until DNA extraction.

From the original sample pool of 130 hairs across eight donors, a subset of 44 hairs (35 plucked, 9 shed) was selected for RHID processing [24]. As sample selection in the previous study was based on visible nuclei count, the number of hairs processed per donor varied. All shed hairs (n = 9) were processed using the RapidINTEL™ cartridge. The majority of plucked hairs (n = 29) were processed using the ACE GlobalFiler™ Express cartridge, while the remaining plucked hair samples with lower nuclei counts (n = 6) were processed using RapidINTEL™. A detailed breakdown of sample type, cartridge type, and nuclei count for each donor is provided in the Supplementary Material (Table S1).

The collection and analysis of samples used in this study were approved by the Western Sydney University Human Research Ethics Committee (H15115).

### 2.2. Fluorescent staining and image capture

Hair samples were removed from the adhesive tape attached to a sterile plastic swab shaft, initially used to facilitate the hair sample processing in the RHID cartridge. Utilizing sterile tweezers, the hair was carefully lifted and positioned onto a clean microscope glass slide. To secure the hair in place, an adhesive flag was employed. Out of the 44 hair samples processed through RHID system, only 43 were able to be recovered from the adhesive tape (Donor B #10 not recovered). Subsequently, a 20x DD solution (1 in 500 dilution) in 75% ethanol was applied to the root end of the hair sample. The visualisation was conducted under an N300F LED Fluorescent Microscope (Optico, Microscopes Australia) with blue excitation (465–475 nm), and images were captured using a MIchrome 5 Pro camera (Optico) with the live extended depth of field (EDF) function.

Based on the availability of donor shed hair samples (from the original pool of 130 hairs), five hairs from three donors (C, E, and F) were selected, stained as described above, and processed exclusively using conventional DNA analysis. These samples were specifically chosen to represent comparable cellular content to those previously analysed using RHID. This approach enabled evaluation of DNA quality obtained through direct conventional processing, compared with samples subjected to reprocessing following RHID analysis.

### 2.3. DNA sample processing and analysis

Reference buccal swabs corresponding to the donors [24], previously

processed using RHID with the ACE cartridge, were stored at −20 °C until DNA extraction. These samples were subsequently analysed using conventional DNA analysis to confirm donor allele designations and to facilitate further testing across different STR kit chemistries, such as VeriFiler™ Plus. The swabs were extracted using the PrepFiler™ Extraction Kit (Thermo Fisher Scientific, VIC, AUS) following the manufacturer's protocol.

Hair samples were extracted using the PrepFiler™ BTA Extraction Kit (Thermo Fisher Scientific) following the manufacturer's protocol, incorporating an elevated Proteinase K volume (14 μL) and an increased 1 M Dithiothreitol (DTT) volume, of 14 μL. Hairs were incubated for up to two hours or until hair roots were visibly dissolved, with a final elution volume of 50 μL.

Extracts were quantified by the NSW Forensic and Analytical Science Service (FASS) using Quantifiler® Trio (Thermo Fisher Scientific) with a half reaction volume (as per internal validation) following the manufacturer's protocol and performed on the QuantStudio™ instrument (Thermo Fisher Scientific).

DNA profiling was performed using GlobalFiler™ (GF) Amplification PCR Kit (Thermo Fisher Scientific), to provide concordant loci for comparison to the RHID generated profiles. Amplification was performed using a GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems, Thermo Fisher Scientific) for 29 cycles and a total input of 1 ng if available. Half reaction volume was performed to conserve reagents and has been previously documented in the literature [27,28]. A Master Mix volume of 3.75 μL with 1.25 μL of the Primer Set was used per sample. For extracts with quantification values lower than 1 ng/μL, a maximum sample volume of 7.5 μL was used with a final reaction volume of 12.5 μL. For DNA extracts with higher than 1 ng/μL concentration, a dilution was performed to obtain an optimal input of 1 ng total DNA, using low TE buffer (Thermo Fisher Scientific).

Hairs that did not result in a full profile using conventional DNA analysis with GF (13 out of 42 hair samples) were amplified with VeriFiler™ Plus (Applied Biosystems, Thermo Fisher Scientific), following manufacturer's protocols. VeriFiler™ Plus (VF+) was chosen due to the amplification kit being specifically designed for challenging and inhibited samples and may increase the profiling results previously obtained with GF.

Amplified products were analysed using a SeqStudio™ Genetic Analyser (Thermo Fisher Scientific) following manufacturer's protocol with an injection voltage of 1.2 kV and an injection time of 10 s. A 9.5 μL aliquot of Hi-Di™ Formamide (Applied Biosystems™, Thermo Fisher

Scientific) and 0.5 μL of GeneScan™ 600 LIZ™ dye Size Standard (Applied Biosystems™, Thermo Fisher Scientific) was added to each sample.

### 2.4. Data analysis

RHID-generated DNA profiles were analysed in accordance with the approach described by Aljumaili, T., & Haines, A. M. [24]. Extracted profiles were analysed using GeneMapper™ ID-X Version 1.7 Software (Thermo Fisher Scientific), with an analytical threshold of 50 RFU and a homozygous threshold of 500 RFU (in-house laboratory threshold). Data visualisation and analysis was performed using R (version 4.5.1) and RStudio (version 2025.05.1, "Mariposa Orchid"). The Shapiro-Wilk test was conducted to assess the normality of the data, followed by the Kruskal-Wallis test (if non-parametric) for comparison and Wilcoxon signed-rank test for comparing between paired groups (RHID versus conventional extraction).

Sample DNA profiles were directly compared against the donor profiles to obtain a profile percentage and ensure that contamination was not present. DNA profiles were categorized into one of four groups based on the profile percentage: null (0%), low partial (1–49%), high partial (50–99%), or full (100%). These boundaries were established according to previous literature [5,24]. The profile percentage is determined by counting the observed alleles in relation to the reference profile, with homozygotes considered as two alleles.

## 3. Results and discussion

The RHID platform uses self-contained cartridges that incorporate all required consumables and employ a basic extraction method that only partially disrupts the sample matrix. This design enables residual material to be recovered and processed through a conventional DNA workflow, preserving DNA-containing material within hair roots for subsequent analyses. Of the 44 hairs previously examined [24], 42 were successfully processed using a conventional workflow and matched the donor profile. One sample could not be recovered from the RHID cartridge, and one produced a donor-inconsistent profile, resulting in 42 usable hair samples. Careful handling during the transfer step is essential to minimize the risk of misplacement, sample loss and prevent contamination. Although this study utilised earlier generation cartridges, this does not diminish the importance of establishing whether hair roots can be successfully reprocessed through conventional forensic DNA workflows following RHID analysis.

### 3.1. Visualisation of re-stained hair samples

Macroscopic examination indicated that the hair matrix remained largely intact following RHID processing, in contrast to conventional DNA analysis where enzymatic digestion results in substantial matrix disruption. Preservation of the hair matrix enables recovery of the hair root for subsequent conventional DNA lysis and extraction. Hair roots collected from the RHID cartridge using a modified swab were removed from the adhesive and re-stained with Diamond™ Dye to assess any degradation to the hair matrix attributable to RHID processing and to determine whether cellular material remained available for downstream DNA extraction.

The hair samples (42/44) that were re-stained and imaged exhibited a reduction in the visibility of cell nuclei for the plucked hair samples and a decrease in DNA-containing material for the shed hair samples. Fig. 1 illustrates an example of a plucked hair both before and after RHID processing and a shed hair before and after RHID. The follicular tag present on the shed hair prior to RHID processing appears to have been degraded during the RHID extraction process and was not observed post-RHID. The use of Diamond™ dye in this process provides insights into the extent of sample degradation.

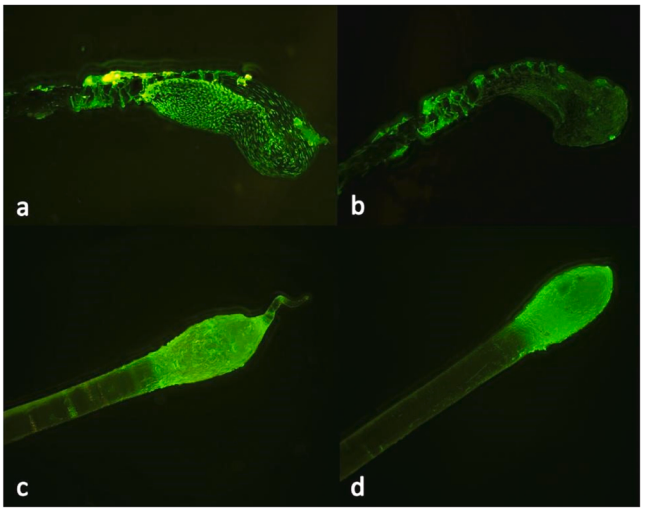



**Fig. 1.** Hair root images were captured using the Optico N300F LED Fluorescent Microscope and MIchrome 5 Pro camera, utilizing the EDF function at 100x magnification, after DD staining. Image (a) displays a plucked hair (donor A #5) before RHID processing, (b) after RHID processing, (c) shows a shed hair (donor C #22) before RHID processing, and (d) after RHID processing.

In four plucked hairs, the root was dislodged from the hair shaft and was not present following RHID processing (see Supplementary Figures S1 and S2). Three of these hairs originated from Donor B (hairs #9, #11, and #14), and one from Donor C (hair #19). It is unclear whether this reflects increased hair fragility in these donors or whether root loss occurred during removal from the adhesive substrate, although no visible root material was observed on the adhesive.

Only three of the 34 plucked hairs exhibited clearly visible cell nuclei following RHID processing; however, these samples still showed evidence of degradation, including reduced cellular material and diminished fluorescence intensity (Fig. 2).

Table 1 summarises the appearance of shed hairs from each donor before and after RHID processing. Although the crude extraction method employed within the RHID cartridge did not result in complete breakdown of the hair matrix, observable degradation of the hair root was evident. In particular, shed hairs that exhibited pronounced follicular tags prior to RHID processing showed markedly reduced follicular material post-processing, most notably among samples from Donors B, F, and G.

#### 3.1.1. *Morphological changes and DD staining*

Re-staining of hair roots following RHID processing demonstrated that the crude extraction conditions impacted the integrity of the root matrix, while the hair shaft remained largely preserved. Diamond™ Dye enabled detailed visualisation of structural features within the root, including matrix disruption and the presence or absence of cell nuclei. Consistent with previous studies using DD [20,24], hair samples with higher nuclei counts were associated with improved DNA recovery potential. Together, these observations support the use of DD as a practical tool for visualising post-processing degradation and for informing triage decisions by identifying hair samples with sufficient residual cellular material for downstream DNA profiling.

### 3.2. *DNA Allele recovery: RHID vs. Conventional extraction*

All DNA profiles recovered from hair samples were single source, and all but one were consistent with the corresponding donor reference profiles. No mixed DNA profiles were observed, indicating no evidence of contamination during processing. One sample from Donor A (plucked 2) was excluded from further analysis after conventional profiling produced a profile inconsistent with the indicated donor and instead matched a different donor. Although the precise cause of this discrepancy is unknown, the most likely explanation is sample misplacement following RHID processing. This observation underscores the importance of stringent handling and tracking procedures when processing hair samples following RHID workflow.

DNA profiling outcomes were optimised when hair samples were subjected to conventional DNA extraction following RHID processing. In this study, profiling success was evaluated based on allele recovery (i.e., proportion of expected alleles detected) as a measure of overall profile completeness. Assessment of data distribution using the Shapiro–Wilk test indicated that profile recovery values were not normally distributed ($p = 2.9 \times 10^{-5}$); therefore, results are reported as medians and analysed using nonparametric tests.

When processed using RHID alone, samples yielded a median allele recovery of 74%, with substantial variability observed across individual samples. Following conventional DNA extraction post-RHID, median allele recovery increased to 100%, indicating enhanced profiling success. Recovery rates following RHID ranged from 20% to 81% across donors, while conventional extraction yielded recovery rates between 55% and 98%. These comparisons reflect differences in overall profiling outcomes between processing workflows rather than extraction efficiency in isolation.

Statistical comparisons were restricted to evaluating differences between RHID and conventional processes applied to the same samples (paired workflow comparison) and were not intended to assess the influence of hair type or nuclei count. A Kruskal–Wallis test showed no statistically significant differences in recovery rates between donors for either processing method ($p > 0.05$). In contrast, a paired Wilcoxon signed-rank test demonstrated a statistically significant improvement in allele recovery following conventional extraction compared with RHID processing alone ($p = 0.00026$). This improvement indicates that downstream reprocessing can enhance the completeness of DNA profiles obtained from the same sample. It should be noted that the interpretation thresholds used to analyse sample profiles for RHID and conventional DNA analysis differed between approaches, which may have contributed to the observed differences in recovery outcomes; however, the analytical threshold was consistent for both methods at 50 RFU.

To further characterise DNA profiling performance, allele recovery outcomes were grouped into four categories: null (0%), low (1–49%), partial (50–99%), and full (100%). The distribution of samples across these categories differed markedly between RHID and conventional extraction methods (Fig. 3). Conventional extraction yielded full DNA profiles in 29 samples, compared with 10 samples processed using RHID (7 with ACE cartridges and 3 with RapidINTEL™ cartridges), indicating greater overall reliability. This difference is consistent with the more extensive breakdown of the hair matrix achieved during conventional extraction relative to the comparatively limited extraction process

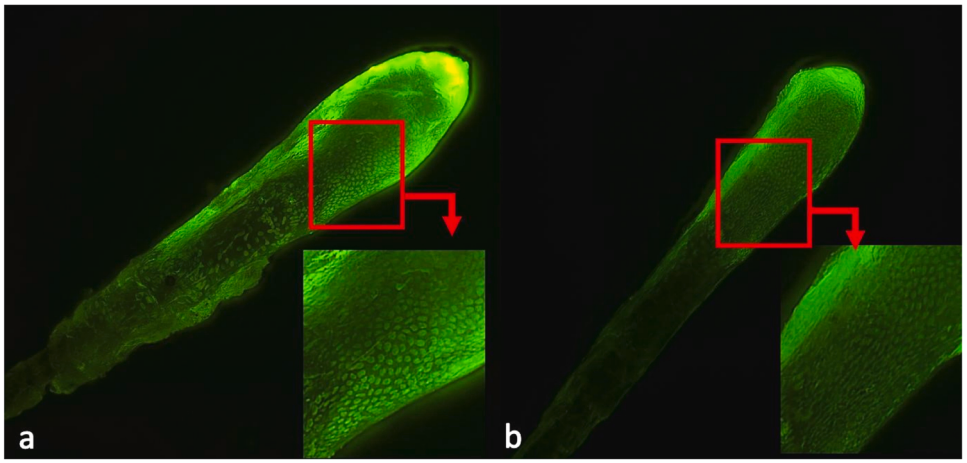
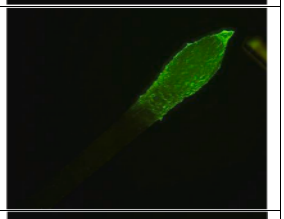
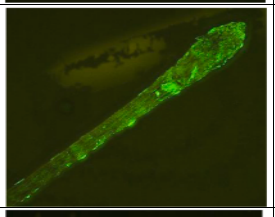
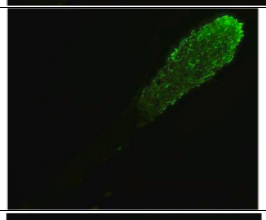
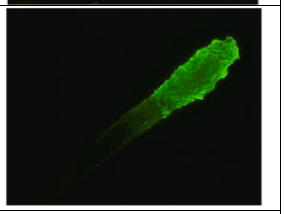
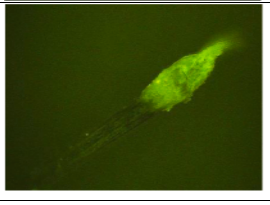
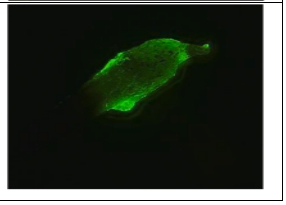



**Fig. 2.** Hair root images were captured using the Optico N300F LED Fluorescent Microscope and MIchrome 5 Pro camera, utilizing the EDF function at 100x magnification, after DD staining. Image (a) displays a plucked hair (donor A #3) before RHID processing, (b) after RHID processing. Insert images illustrate the cellular material present in the hair root before and after RHID processing.

**Table 1**
Shed hairs from each donor were observed both before and after RHID processing. Images were captured using the Optico N300F LED Fluorescent Microscope and MIchrome 5 Pro camera, employing the EDF function at 100x magnification, after DD staining.

| Donor | Pre-RHID | Post-RHID |
|---|---|---|
| A |  |  |
| B |  |  |
| C |  |  |
| D |  |  |
| E |  |  |
| F |  |  |
| G |  |  |
| H |  |  |

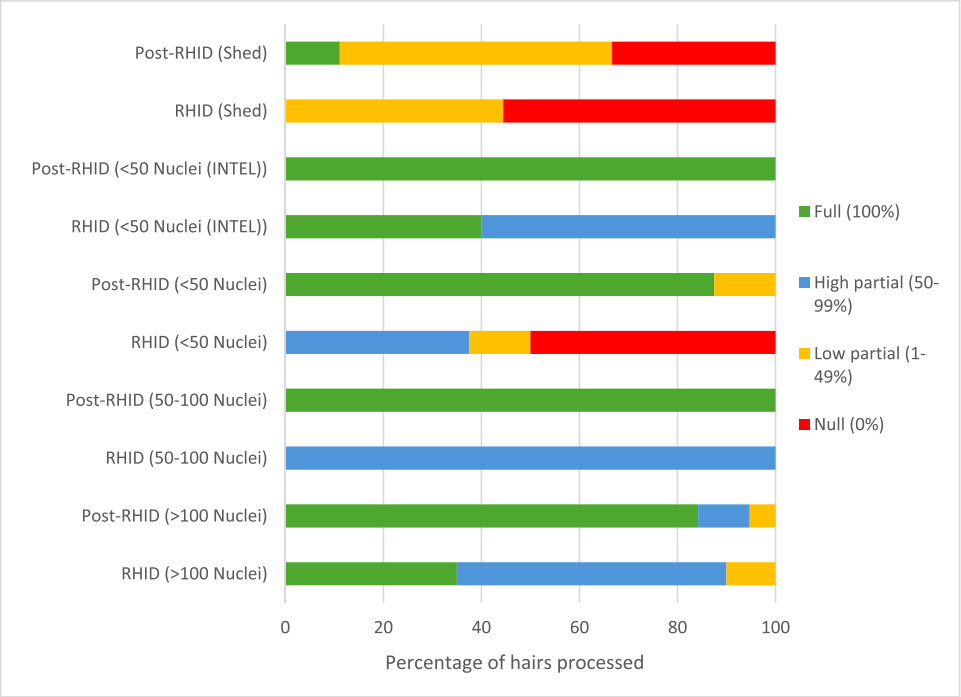



**Fig. 3.** Distribution of DNA profiling outcomes following RHID and post-RHID processing across hair samples stratified by nuclei count and cartridge type. Outcomes are shown as percentages of hairs yielding full (100%), high partial (50–99%), low partial (1–49%), or null (0%) STR profiles. Panels represent nuclei count categories (>100, 50–100, <50, <50 INTEL, and shed hairs), with direct comparison between RHID and post-RHID processing within each category.

employed by RHID.

Partial profiles were more frequently observed following RHID processing, with 16 samples falling within this category, compared with only three samples following conventional extraction, suggesting that RHID more often yields intermediate profiling outcomes. Both methods produced seven samples with low allele recovery, indicating that certain samples may be inherently limited in DNA yield irrespective of extraction approach. Overall, conventional extraction produced a bimodal distribution of outcomes, with samples tending toward either full recovery or failure, whereas RHID profiling exhibited a more graded distribution across recovery categories.

A Bland–Altman analysis was used to assess agreement between the two workflows at the individual sample level (Supplementary Figure S3). The results showed a consistent trend towards lower recovery following RHID, with wide limits of agreement (−91.5% to 53.1%), indicating that the difference in profile recovery varies substantially between samples. This demonstrates that while conventional reprocessing generally improves profile completeness, the extent of improvement is sample-dependent, and the two methods are not interchangeable.

### 3.3. *Influence of nuclei count on profiling success*

Grouping of hair samples by nuclei category was previously examined using Diamond™ Dye microscopy [20,24]. In the present study, nuclei counts were not directly assessed; therefore, analyses were limited to comparing DNA profiling outcomes following RHID processing and subsequent conventional DNA extraction.

Within these previously defined cellular content categories, conventional DNA extraction consistently produced higher allele recovery than RHID processing alone. Although samples with greater apparent cellular content tended to yield good profiling outcomes using both methods, RHID performance declined in samples classified as having low or trace cellular material. This contrasts with conventional extraction, which maintained relatively high recovery across categories.

While nuclei count was not directly assessed in this study, these findings are consistent with earlier work demonstrating an association between cellular content and DNA profiling success in hair samples [20]. The reduced performance of RHID in low cellularity samples may reflect the non-destructive nature of the RHID extraction process, whereby limited cellular material is not fully released, in contrast to the more comprehensive matrix disruption achieved during conventional extraction.

### 3.4. *Comparative Allele recovery from shed hairs*

Five shed hairs with comparable levels of cellular material were analysed to compare allele recovery following conventional DNA extraction, RHID processing, and post-RHID extraction. Allele recovery was consistently higher when shed hairs were processed using conventional DNA extraction alone. As shown in Fig. 4, all shed hairs subjected solely to conventional extraction achieved allele recovery of at least 10%, whereas the majority of profiles generated through RHID or post-RHID yielded recovery rates below 10%.

These results demonstrate that shed hairs, even when follicular tags or cellular material are present, are most effectively processed using conventional DNA analysis rather than RHID. While these findings are based on the ACE and RapidINTEL™ cartridges used in this study, improvements in newer RHID RapidINTEL™ Plus cartridge designs may influence the suitability of RHID processing for shed hairs and warrant further evaluation. Supplementary images and allele recovery data for all shed hairs are provided in Table S2 and Figure S6.

### 3.5. *Allele recovery using VeriFiler™ Plus*

Hair samples, including both shed and plucked hairs, that yielded partial or null STR profiles were selected for further analysis using the VeriFiler™ Plus (VF+) amplification kit to assess whether increased amplification sensitivity could improve allele recovery. VF + was selected based on manufacturer-reported performance improvements for low-template, degraded, or inhibited DNA. As most incomplete profiles were obtained from shed hairs, which are inherently challenging

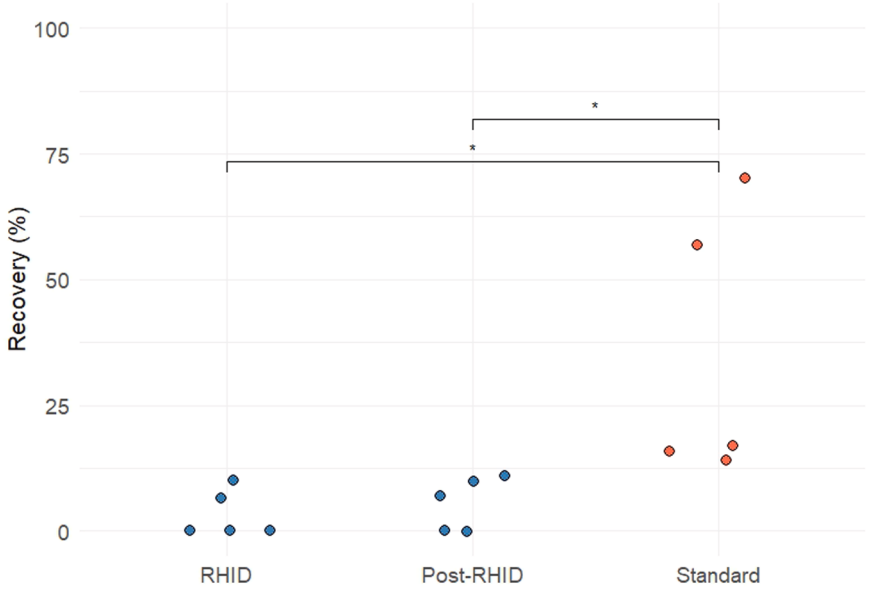



**Fig. 4.** Allele Recovery (%) across the DNA extraction protocols (RHID, Post-RHID (standard extraction), and Standard extraction only. RHID and post-RHID values were derived from the same shed hair samples, where the shed hair after RHID processing was put through conventional DNA analysis. Significant differences (*) were observed between the Standard method and both RHID and post-RHID samples (Wilcoxon rank-sum test, Bonferroni-adjusted $p < 0.05$).

due to limited DNA content, VF + was evaluated to determine whether additional alleles could be recovered.

Among the thirteen samples analysed, no statistically significant differences in profile recovery were observed between RHID profiling, GlobalFiler™ (GF) amplification following extraction, and VF + amplification (Fig. 5).

Importantly, the ability to recover samples from the RHID cartridge and reprocess them using a conventional DNA workflow represents a practical advantage, enabling the recovery of additional alleles beyond those obtained through RHID profiling alone. This finding supports the use of RHID as a rapid screening approach while preserving material for subsequent laboratory-based DNA analysis where required.

### 3.6. Forensic applications and limitations

The RHID platform offers clear operational advantages in time critical forensic contexts by enabling rapid profiling of samples to generate investigative intelligence at crime scenes and mass disaster locations. Crucially, the ability to recover samples from the RHID cartridge preserves evidentiary material for subsequent conventional laboratory analysis. In disaster victim identification (DVI) scenarios, hairs can be readily collected from deceased individuals, rapidly profiled using RHID to support preliminary identification, and then reprocessed using standard forensic workflows to obtain more complete profiles or undertake specialised analyses such as Y-STR profiling. This capacity to combine rapid intelligence led screening with downstream confirmatory testing enhances both efficiency and evidentiary robustness in operational forensic workflows. Further research is required to determine whether

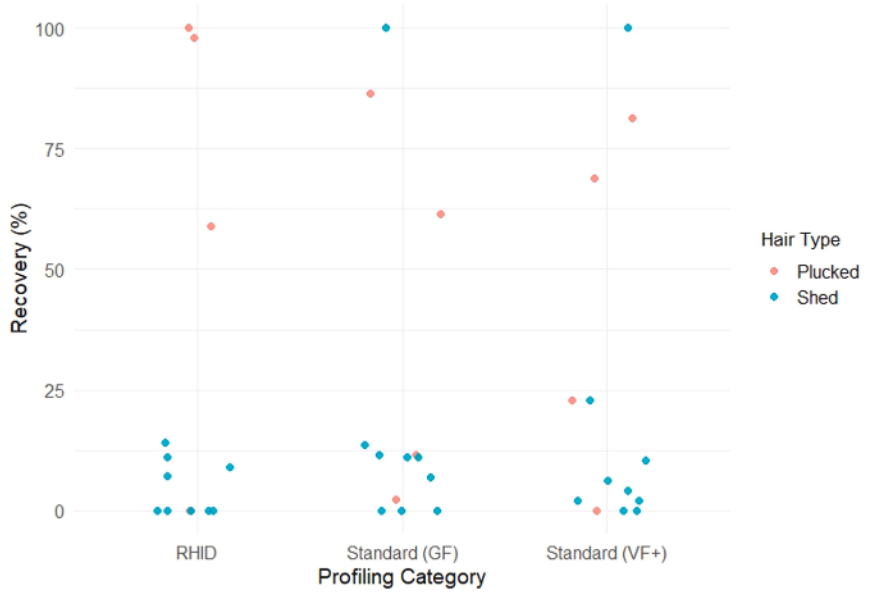



**Fig. 5.** Comparison of Profile Recovery (%) across RHID, Standard (GF), and Standard (VF+) amplification kits by hair type. Only shed hairs and plucked hairs that resulted in less than 100% recovery through Standard (GF) were analysed through Standard (VF+), n = 13.

RHID-processed hair roots are suitable for mitochondrial DNA analysis [29,30], which demands stringent contamination control [31].

While RHID performs well for high yield samples such as plucked hairs, telogen (shed) hairs remain inherently challenging due to limited and often fragmented nuclear DNA content, making them less suitable for routine STR profiling [32]. In some cases, however, shed hairs may retain residual cellular material or follicular tags capable of supporting nuclear DNA analysis. In this study, Diamond™ Dye staining was used to visualise hair root morphology and cellular integrity both before and after RHID processing, enabling assessment of the degree of degradation associated with the RHID extraction. Observations indicated that the basic extraction process employed by RHID removed a proportion of the already limited cellular material present in telogen roots, thereby reducing the likelihood of successful downstream conventional DNA profiling. The relatively small number of shed hairs analysed represents a limitation of the current study, and evaluation using a larger sample set may further refine these findings.

DD staining provided a practical means of assessing residual cellular material and the impact of RHID processing on hair root integrity, allowing informed consideration of appropriate downstream analytical pathways for shed hairs. Where DD staining indicated the presence of cellular material, conservation of telogen hairs for complete lysis and extraction, direct PCR workflows [20], or mitochondrial DNA analysis [29,30,33] may be more appropriate than RHID processing. Future studies should also examine the performance of the RapidINTEL™ Plus Sample Cartridge, as the earlier generation cartridges used here are no longer manufactured, to determine whether modifications to cartridge design result in improved retention and recovery of DNA from low yield samples. Overall, these findings highlight that while RHID offers clear advantages for rapid profiling of high DNA samples, conventional laboratory workflows remain essential for maximising DNA recovery from low-level DNA samples.

## 4. Conclusion

This study demonstrates that hair samples recollected after RHID processing can still yield recoverable DNA, with extraction methods generally improving recovery outcomes. The improved performance is attributed to the basic extraction approach used by the RHID system, which does not fully disrupt the hair matrix. Of the 44 previously tested samples, 42 were successfully recovered, processed through conventional DNA analysis and matched the donor profile. The ability to obtain a DNA profile through either RHID or conventional processing was strongly influenced by the nuclear content of the hair, with greater nuclei counts correlating with better DNA allele recovery. Reference buccal swabs processed through RHID exhibited variability in downstream extraction and amplification results, particularly when amplified using VeriFiler™ Plus. Given the limited number of swabs analysed and the absence of systematic investigation into contributing factors, no definitive cause for this variability can be established. Accordingly, for reference buccal swabs, conventional DNA profiling using the GlobalFiler™ kit remains the more reliable approach within this study.

## CRediT authorship contribution statement

**David Bruce:** Writing – review & editing, Writing – original draft, Supervision, Resources. **Tabarek Aljumaili:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Conceptualization. **Alicia Mary Haines:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

## 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.2026.103572.

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