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# Speed of Accumulation of DNA in a Fingermark

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## Abstract

Variation has been reported in the amount of DNA accumulating on the skin of individuals. A shedder status is the propensity of a person to transfer DNA to a substrate by touch. In previous tests of shedders, individuals washed their hands and after 15 minutes made contact with substrates at time points up to 180 minutes after handwashing. No examination has looked at the accumulation of cellular material between time zero and this 15 minute time point. Here we present the results of an examination of cellular material within thumbprints at time points 0, 2, 5, 15 and 60 minutes after hand washing using donors who are representative of heavy, intermediate and light shedders. The rate of accumulation of cellular material and the total amount detected in thumbprints showed a difference between these donors, but for all donors the initial rate of accumulation is surprisingly fast. Informative STR profiles can be generated only 2 minutes after handwashing from 100%, 33% and none of the heavy, intermediate and light shedders, respectively. These results confirmed that there was a correlation between the cellular material present on the thumbprint and the percentage success of an STR profile for each individual and time point.

**Keywords:** Diamond™ Nucleic Acid Dye; DNA binding dye; direct PCR; STR; shedder

## 1. Introduction

Whenever a person makes direct contact with an object, they have the potential to deposit their DNA. This type of 'touch DNA' for many years[1] has been reported as a source for generating DNA Short Tandem Repeat (STR) profiles. While touch DNA can be highly informative, the amount of DNA transferred by touch to a substrate has been shown to be highly variable between individuals. This led to the designation of a shedder status with a person either being a shedder and non-shedder[2], or good shedder and bad shedder[3]. A good shedder is someone who has a higher propensity to transfer their DNA on to an object compared to a bad shedder. There are many factors reported to affect shedder status such as gender, hand-dominance and time after hand-washing before touching[3-5]. The standard shedder test required volunteers to wash their hands, thus removing free DNA and skin cells loosely adhering to the dermis, then hold an object such as a plastic tube for 15 seconds after defined time periods post-handwashing; these time periods were 15 minutes, 30 minutes, 60 minutes and up to 180 minutes[2]. The amount of DNA transferred was estimated after taking a swab from the plastic tube, performing a DNA extraction and quantifying the amount of DNA collected. Recently, a novel means to determine the shedder status of an individual has been reported that uses a fluorescent nucleic acid dye which binds to the external minor grooves of the DNA molecule[6]. The amount of DNA present on a surface can therefore be determined directly (i.e. without resorting to the use of real-time PCR or genetic profiling) *in situ* and permits an accurate assessment of the amount of DNA transferred to an object and hence the shedder status of the individual[6]. In the initial report, cellular material within a fingermark was scored at time intervals post-handwashing of 0, 15, 60 and 180 minutes.

The detection of DNA within cellular material transferred by touch has been transformed by using fluorescent nucleic acid dyes that are normally used for detection of nucleic acid in agarose gel electrophoresis. Examples include SYBR® Green I (SG),

EvaGreen™, RedSafe™, GelGreen™ and Diamond™ Dye (DD) to target latent DNA within fingermark[7], hair, skin, saliva[8] and touched DNA on IED components[9]. Diamond™ Dye has many advantages: 1) there is little or no background fluorescence when no nucleic acid is present, 2) the dye does not inhibit PCR during direct PCR amplification[6,10], 3) the dye does not interfere in analysis by capillary electrophoresis (CE)[6,10], 4) there is good stability of the fluorescent signal after staining ( > 90 days)[11], 5) DD costs approximately five-fold less than SG, 6) DD is an external binder and consequently less mutagenicity than the intercalating dyes and 7) DD does not bind effectively with micro-organism DNA[6,12,13].

In the initial report on shedders using DD to examine the transfer of cellular material, it was noted that sufficient cellular material was transferred by both heavy and intermediate shedders after only 15 minutes post-handwashing to generate informative DNA profiles[6]. The question arises therefore as to why this happens so quickly and what might be the source of the DNA (e.g. exuded by pores such as sweat glands or part of the flaking dermis sloughed off in only 15 minutes). Here, we report on the accumulation of cellular material from both heavy, intermediate and light shedders starting at time 0 and then over a narrow time window (2, 5, 15, and 60 minutes) post-handwashing.

## 2. Materials and methods

### *2.1 Substrate, equipment and working area preparation*

All equipment and consumables were cleaned with 3% bleach, followed by wiping with absolute ethanol and irradiation with UV light by placing into a Spectrolinker XL-1000 UV Crosslinker (Stratagene, NSW, AUS) for 15 minutes to ensure no DNA was present on surface. The laboratory bench was cleaned with bleach (3%) prior to use.

### *2.2 Thumbprint deposition*

Donors were selected based on their designated shedder group as determined by Kanokwongnuwut *et al.* (2018). These were a heavy (male), an intermediate (male) and a light (female) shedder. The donors were asked to wash their hands thoroughly under running water, with no soap used, and dry with a paper towel. They then deposited a thumbprint on to a clean glass slide at time intervals of 0, 2, 5, 15, and 60 minutes post-handwashing. Contact with the slide was with medium pressure for 15 seconds. All time points were performed in triplicate.

### *2.3 Thumbprint detection*

Diamond™ nucleic acid dye (DD) (Promega, Madison, WI, USA) was prepared to a 20x solution by diluting a 10,000x DD stock (ratio 1: 500) with 75% ethanol. The 20x DD solution (5 µL) was pipetted onto a thumbprint deposited on glass slide. The stained thumbprint were visualised and recorded using a Dino-Lite fluorescent digital microscope (AnMo Electronics Corporation, New Taipei City, TWN) under blue light (480 nm) on a black background. The position of cellular material was recorded using the software DinoXcope 2 for Mac OS. The microscope was calibrated under 220x magnification with a standard provided by the company. The measurement was performed on stained cellular material within thumbprints (n = 30) under 220x magnification. The stained cellular material was scored by counting within a 1 $mm^2$ frame under 220x magnification; all recording was performed in triplicate.

To compare cell-free DNA detection, DNA, RNA and heated RNA (40ºC for 10 min) 10 ng/µL (Qubit® dsDNA and RNA HS standard, Life technologies) were stained with 20x DD 1 µL on clean glass slides and visualised under the microscope at 50x magnification in ambient light; all testing was performed in triplicate.

### *2.4 Thumbprint DNA collection*

Clean Ultrafine swabs (City Dental, SA, AUS) were moistened with 2 µL of 0.1% Triton-X (Sigma, VIC, AUS). A thumbprint stained with DD was swabbed during visualisation under the fluorescent microscope to ensure the entire stained cellular material within a thumbprint was collected.

### *2.5 Reference DNA*

A sterile cotton swab (Livingstone International Pty, NSW, AUS) was rubbed inside the inner cheek of three donors. The swabs were extracted by QIAamp® DNA Mini kit (QIAGEN, VIC, AUS) following DNA purification from buccal swabs (spin protocol). Extracted DNA was quantified by Qubit® 2.0 Fluorometer (ThermoFisher Scientific, VIC, AUS) and diluted to 1 ng/µL with nuclease-free water for STR profiling as reference.

### *2.6 Direct PCR amplification*

The Ultrafine swab head was cut off using clean scissors and placed directly into a 0.2 mL thin-walled PCR tube (BioRad, NSW, AUS). Direct PCR was performed using either the AmpFlSTR® NGM SElect kit (Thermo-Fisher Scientific) for samples of time interval 0, 2, 5, 15 minutes or the AmpFlSTR® Identifler Plus™ kit (Thermo Fisher Scientific) for 60 minutes after hands were washed. The amplification was performed in a total volume of 25 µL composed of Master Mix 10 µL, Primer Set 5 µL and Low TE Buffer 10 µL, and amplified for 29 cycles following the validated procedure. All reactions were amplified using a ProFlex thermal-cycler (Thermo Fisher Scientific).

### *2.7 Capillary electrophoresis and data analysis*

PCR product (2 µL) was added to 9.5 µL Hi-Di formamide and 0.5 µL 500 LIZ™ Size standard (Thermo Fisher Scientific) and separated on a 3500 Genetic Analyser (Thermo Fisher Scientific). Samples were injected and separated using ThermoFisher's validated recommended method and no increase in PCR cycle was used. Data were analysed by using GeneMapper ID-X (version 1.4) (Thermo Fisher Scientific), and a peak detection threshold of 50 relative fluorescence unit (RFU) was used for homozygotes and 150 RFU used to designate alleles from a heterozygote. The percentage of successful STR profiles (%) was calculated from the number of designated alleles that matched the reference DNA profile of the donor. An informative STR profile, being one that can be uploaded to the Australian National Criminal Investigation DNA databases (NCIDD), was defined as a STR profile containing at least 12 alleles (plus amelogenin).

## 3. Results and discussion

### *3.1 Stained cellular material within thumbprint detection*

Within a thumbprint collected 2 minutes after handwashing, stained material appears under the blue light of the microscope as bright green spots (Figure 1). The stained material showed as an irregular round and flat shape with average size 33.44 µM (n = 30), with a minimum size of 27.14 and a maximum of size 43.49 µM, suggesting that it is cellular. This suggestion is supported by a recent study[14] that reported the nanoscale characterization of two cells type (non-keratinized buccal cells and the keratinized epidermal cells) where palm cells were approximately 40 µM by 2 µM in size. Our previous study described stained cellular material of similar shape and size[6].

Within the thumbprint on the glass slide, ridges and sweat pores can be observed; this is under white light and before staining with DD (Figure 1, A). After staining with DD, the

fluorescent material is more abundant where the ridges were present, although the ridge detail can no longer be observed (Figure 1, B). Furthermore, Figure 1B shows that the fluorescent material is more often found associated with, or close to, the sweat pores on the ridge rather than elsewhere.

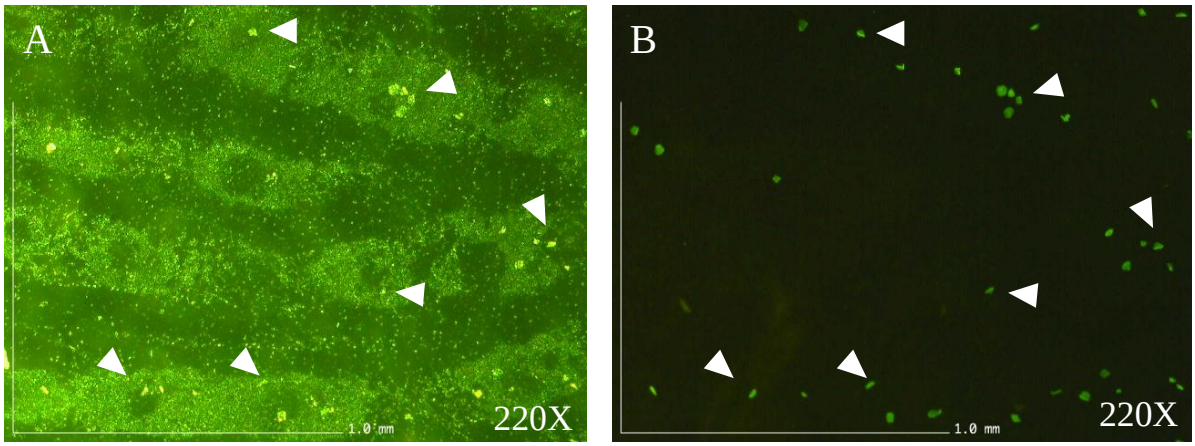


**Figure 1** photomicrograph illustrating the stained cellular material located near the sweat pores on finger ridges compared by visualisation under white light prior to staining (A) and blue light after staining (B).

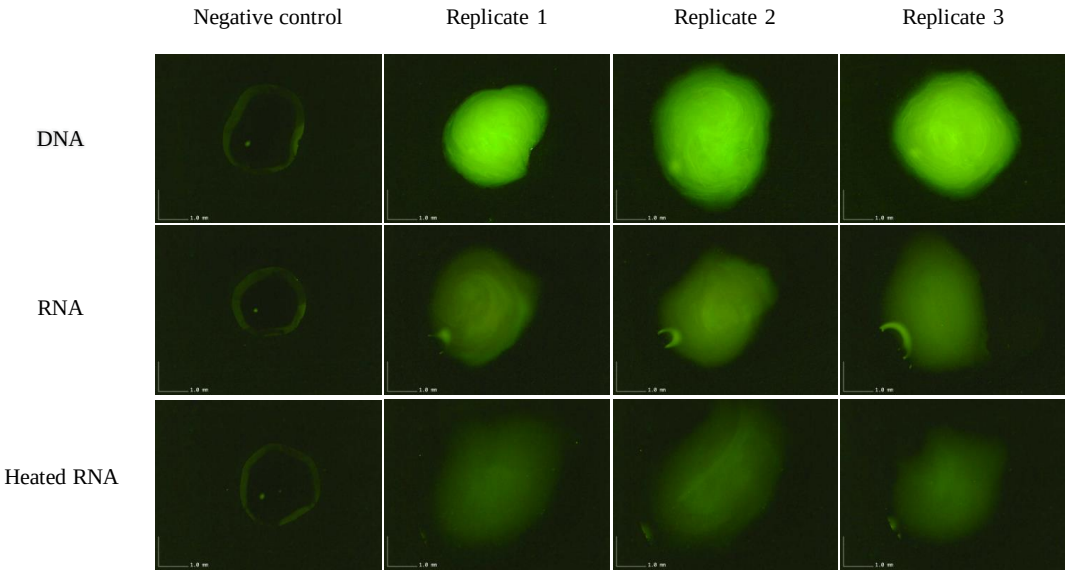


**Figure S1** illustrating the DD staining within 10 ng DNA, RNA and heated RNA (40°C for 10 minutes) in three replicates. The fluorescent signal was visualised under the fluorescent microscope at 50x magnification in ambient light.

Confirmation that the visualised material is from double-stranded DNA and not RNA was performed by comparing 10 ng of both DNA and RNA stained with DD (Figure S1). The result shows far greater intensity of fluorescence when double-stranded DNA is stained compared to single-stranded RNA. In theory DD cannot bind effectively to RNA and this is demonstrated as faint fluorescence in both unheated and heated RNA after DD staining. Single-stranded RNA, such as mRNA, showed the lowest intensity of fluorescence. While cell-free DNA may be present in very low amounts on the thumbprint and therefore may a component of the total fluorescence, staining of single molecule of free-DNA is unlikely to be detected by using only 220x magnification at the relatively low resolving power of the microscope.

### *3.2 Scoring stained cellular material within thumbprints*

The average amount of cellular material present was scored based on three frames of 1 $mm^2$ (as seen in Figure 2) for each of the five time intervals (0, 2, 5, 15, and 60 minutes after handwashing). This was performed in triplicate using three donors (to create a total of 135 frames). The results displayed a distinct difference in the amount of cellular material and the speed of accumulation of the cellular material for the three representatives of shedder status (Figure 2). The average speed of accumulation on the thumbprint of the three donors is displayed clearly in Figure 3A and Table 1.

The accumulation of cellular material was most rapid for donor 1, who was designated as a heavy shedder, and this individual also exhibited the highest amount of cellular material (e.g. at 5 minute time point donor 1 has an average of 24.78 cells/$mm^2$ as compared with intermediate donor having 15.22 cells/$mm^2$). At time point 0 where a thumbprint is made directly after handwashing, there were on average 2 cells per 1 $mm^2$ but after 2 minutes this had increased to 20 stained cells. There then was a doubling in cellular accumulation to about 40 cells per 1 $mm^2$ after 60 minutes (Figure 2, B1 and E1).

Donor 2, an intermediate shedder, showed a steady increase in the accumulation at all five time intervals from approximately 7 to 11, 15, 18, and 28 cells per 1 $mm^2$. A stained thumbprint from donor 2 under 220x magnification is shown in Figure 2, 2A - 2E and Figure 3A.

Donor 3, who was a light shedder, provided the lowest number and slowest speed of accumulation of cellular material. Few cells per square millimetre were found at time point 0 and at 2 minutes after handwashing and at time points 5, 15 and 60 there was still a low amount of cellular material of about 10, 12 and 16 cells in 1 $mm^2$, respective, as seen in Figure 2, 3A - 3E and Figure 3A.

***3.3 STR profile from a thumbprint of three shedder groups***

The percentage of STR alleles amplified correlated with the appearance and scoring of the stained cellular material (Figure 3, B). For donor 1, an average of 82% of alleles were generated after only 2 minutes post handwashing with the STR data ranging approximately from 70% of alleles amplified to a full DNA profile. A full STR profile was generated from a stained thumbprint of donor 1 where approximately 29 cells per 1 $mm^2$ were scored. A full STR profile was also generated from donor 2, but was from a time point post handwashing later than donor 1, at 15 minutes. Here the amount of scored cellular material was approximately 25 cells per 1 $mm^2$. The success of STR DNA amplification was lower for donor 3, a poor shedder. After 5, 15, and 60 minutes, the percentage of STR alleles amplified to a detectable level increased from about 21% to 35% to 40% at a maximum. The data for all STR typing from the 5 time points for the 3 donors can be seen in Table 1.

The correlation between the scored stained cellular material and STR results observed is illustrated in Figure 4. Here the $R^2$ was 0.863 showing a close correlation and supports the data shown in Figure 3, A. This graph shows that if the score for the amount of stained cellular

material within a thumbprint is 25 or equal within 1 $mm^2$ then a full STR profile has the potential to be generated.

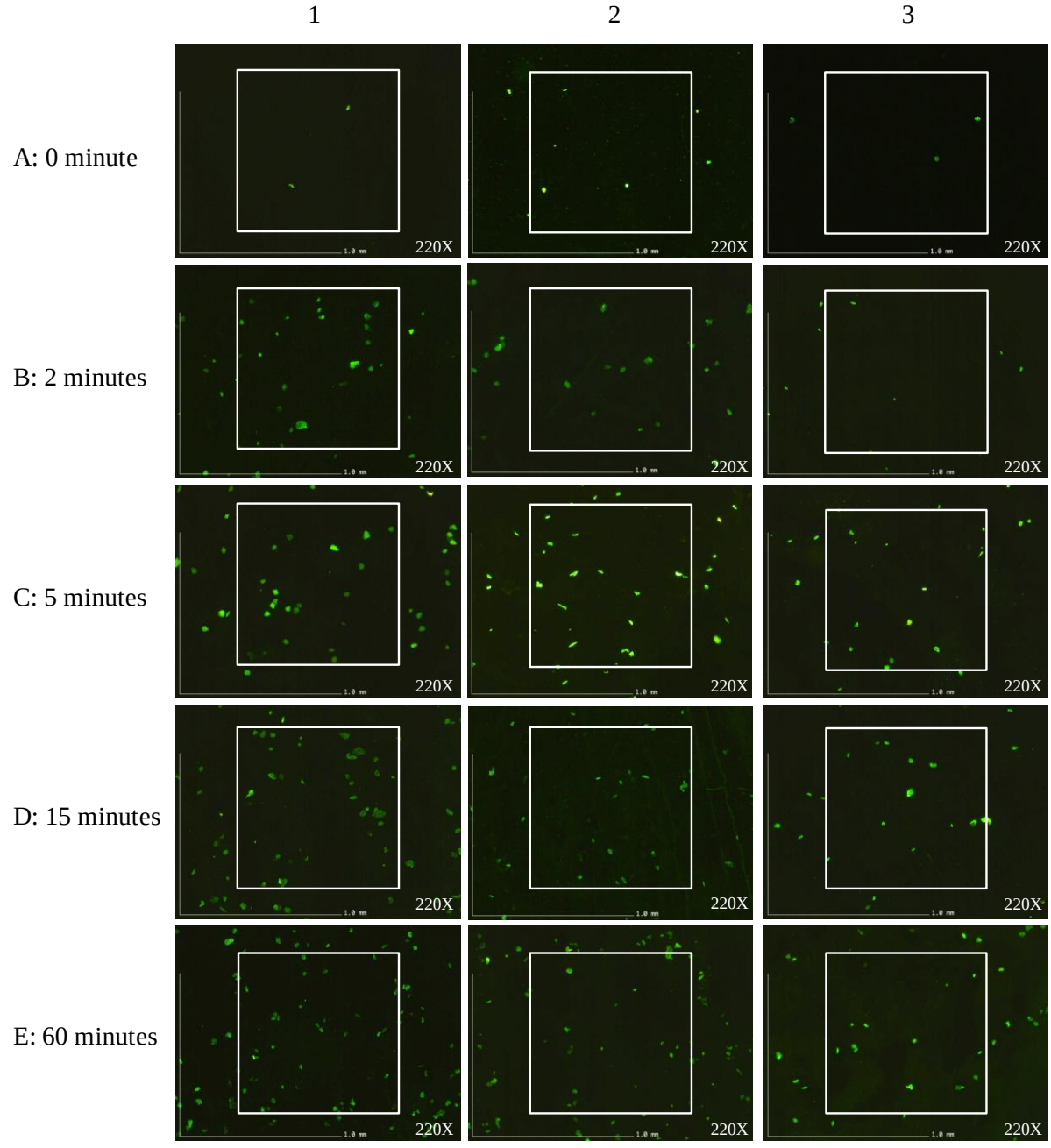


**Figure 2** illustrating the DD stained cellular material on a glass slide visualised by a digital fluorescent microscope under 220x magnification. The fingermarks were collected after hand-washing at time intervals; 0, 2, 5, 15, 60 minutes from heavy (1), intermediate (2) and light (3) shedders. The amount of cellular material was scored in 1 $mm^2$ area (□).

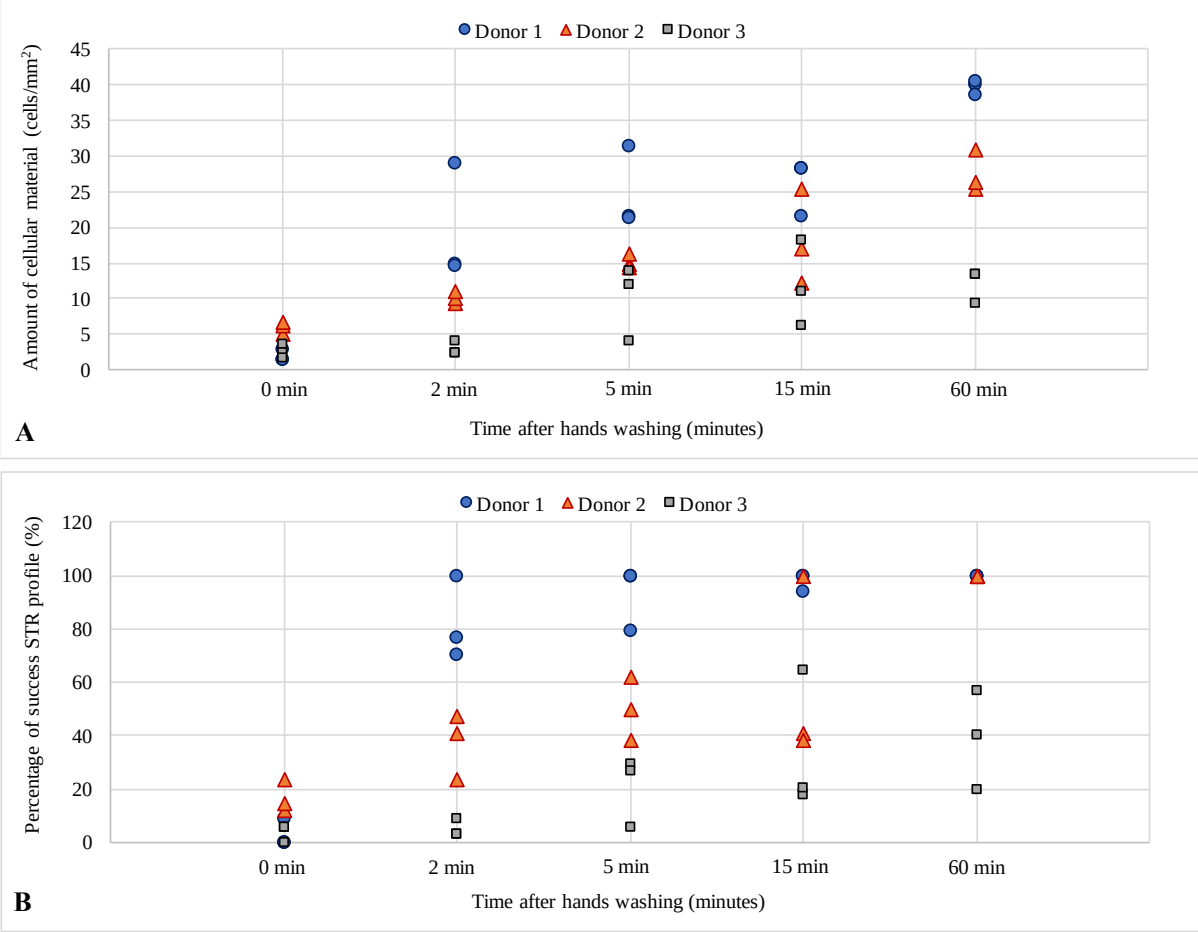


**Figure 3** illustrating the amount of cellular material (A) and percent of successful STR DNA profiling (B) on the y-axis at each of the five time intervals (0, 2, 5, 15 and 60 minutes after hand-washing). The experiment was performed in triplicate and average values are shown. Data points were plotted using 'scatter" options using Excel.

**Table 1** listing of the data present in Figure 3A and 3B in average of triplicate.

| **Time after hand washing (minute)** | **Donor 1** (Heavy shedder) | | | **Donor 2** (Intermediate shedder) | | | **Donor 3** (Light shedder) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Amount of cellular material (cell/mm²) | % success of STR | % informative STR | Amount of cellular material (cell/mm²) | % success of STR | % informative STR | Amount of cellular material (cell/mm²) | % success of STR | % informative STR |
| 0 | 1.89 | 2.94 % | 0 | 6.00 | 16.67 % | 0 | 2.56 | 3.92 % | 0 |
| 2 | 19.56 | 82.35 % | 100.00 % | 10.11 | 37.25 % | 33.33 % | 2.89 | 4.90 % | 0 |
| 5 | 24.78 | 93.14 % | 100.00 % | 15.22 | 50.00 % | 66. 67 % | 10.00 | 20.59 % | 0 |
| 15 | 26.11 | 98.04 % | 100.00 % | 18.22 | 59.80 % | 66. 67 % | 11.89 | 34.31 % | 33.33 % |
| 60 | 38.67 | 100.00 % | 100.00 % | 27.56 | 100.00 % | 100.00 % | 12.00 | 40.00 % | 33.33 % |

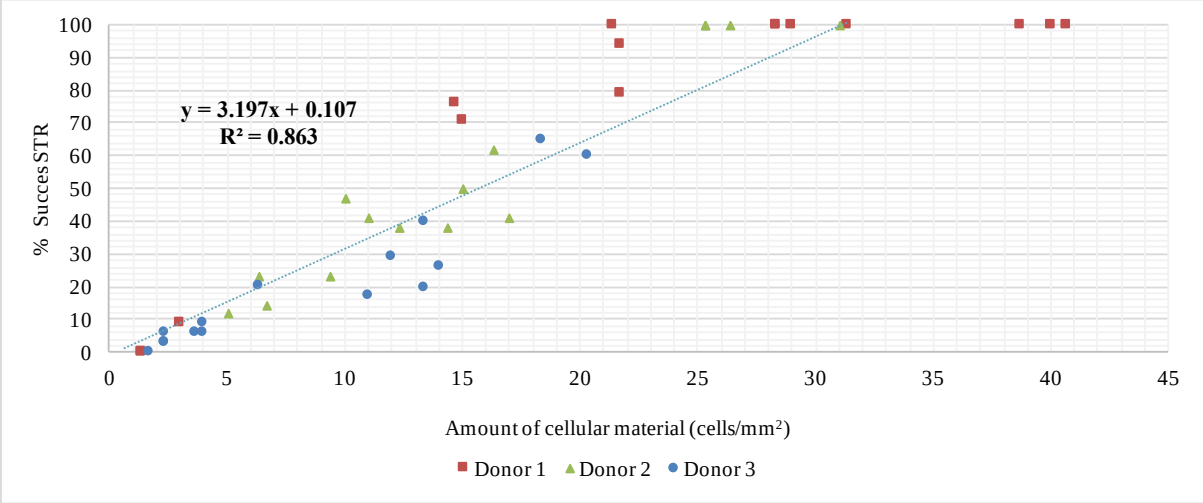


**Figure 4** illustrating a correlation of the amount of cellular material and % success STR. The number of cellular material in 1 $mm^2$ is shown on the x-axis. Data points were plotted using 'X Y scatter' options using Excel.

## 4. Conclusion

The capability to stain and visualise cellular material transferred by touch is a new process[6]. DD has been used extensively in the last few years to detect DNA and more recently the assumed presence of DNA *in situ*[7,8,13]. The more recent paper on shedder status correlated the amount of stained cellular material using DD with the relative number of STR alleles generated from a fingermark[6]. However, until now the question remained as to how quickly DNA appears on the finger and can be transferred to a fingermark.

While it is still not possible to definitively state that the stained material in fingermarks is cellular, the data in this report shows that what appears to be cellular material is transferred from skin very soon after handwashing. Previously published studies examined the presence of DNA at time point 0 and after 15 minutes post-washing[2,6] but this study looks at intervals closer to time point zero and it was found that some persons have on their thumb sufficient DNA to generate a full DNA profile after only 2 minutes. Further, we can show that it is possible to predict if there is sufficient stained material on a substrate such that a full or highly informative STR profile can be determined.

The volunteers were chosen as they represented the two extremes, a heavy and poor shedder, along with someone who was in the middle of those we have tested previously to be intermediate shedders[6]. Adding further volunteers within the range of shedder status may add further clarity to the speed at which cellular material accumulates on the skin. The three volunteers chosen do however show a trend and differentiates heavy from poor shedders effectively.

Being able to indicate the presence of cellular material on items has the real potential to allow targeted sample collection. Further, the approach described here is a simple targeting method on swabs to show that DNA has been collected successfully[15], and is thus a real opportunity to save wastage arising from processing crime-scene swabs devoid of DNA. Our current study highlights the potential usage of a non-toxic, simple to apply stain that rapidly determines if a source of DNA is, or is not, present. It should be noted that this technique has limitations in making any decision on the approximate quantity of cellular material on a swab and further any triage currently requires much manual processing. In future a computer software program might be generated that would help to reduce human resources and processing time. The approach described here also offers the possibility to demonstrate an individual's activity in regards to a crime. For example, if cellular material can be seen within the ridges of a fingermark on an object but not beyond the fingermark, and if that cellular material yields a DNA profile (but the region surrounding the fingermark does not) there is strong support for the hypothesis that the object had been touched. The genetic profile may be sufficient to identify the individual who touched the object even if the fingermark is devoid of an informative ridge pattern.

The source of the stained cellular material is unclear. While the staining was predominantly close to what appears to be pores of the ridges of the thumbprint, without greater magnification it is not possible to provide any further detail. The work described here builds

on our understanding of touch DNA and the implications for DNA typing from items of forensic interest.

**Disclosure statement**

There are no conflicts of interest.

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