



Research paper

Detection of latent DNA

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ARTICLE INFO

Keywords:

Diamond dye
Fluorescent microscopy
Forensic science
Latent DNA
STR profiling

ABSTRACT

Touch DNA is one of the most common sample types submitted for DNA profiling. There is currently no process to visualise the presence of such DNA deposited when a person makes direct contact with items of forensic relevance.

This report demonstrates the effective use of Diamond Dye to bind to DNA and allow visualisation of deposited cellular material using a mini-fluorescence microscope. Volunteers made contact with a range of items typical of those submitted as part of a forensic investigation. Contact was for less than 5 s and occurred either 15 min after hands were washed to remove any traces of DNA, and therefore under controlled conditions, or at an undefined time post handwashing to mimic real-world scenarios. Diamond Dye bound to cellular material on all the items used and in all cases it was clear where the volunteers had made this brief contact. It was also clear where no contact had been made. DNA profiling was performed on a sub-set of samples to confirm that the cellular material viewed under the microscope was human in origin and deposited by the person contacting the item; this was the result obtained in every sample tested.

Diamond dye is relatively inexpensive, simple to apply, binds to the DNA in 3 s or less, has no mutagenic effects at the concentrations used, does not affect subsequent DNA profiling, and does not bind effectively to bacterial DNA. In combination with a mini-fluorescence microscope, this proof-of-concept study shows that otherwise invisible DNA deposited by touch can be visualised. The position and amount of cellular material deposited during even brief contact can be recorded allowing targeted sampling in any further DNA typing of forensically-significant items.

1. Introduction

When people touch surfaces at crime scenes or items of forensic significance, they can potentially deposit their DNA. Subsequent analysis of this ‘touch DNA’ can be highly informative in further investigations [1–4]. DNA deposited by touch is not visible and therefore detecting where it is on items is not possible currently. The net result is that collecting DNA from such substrates is not targeted but based on testing the most likely areas of contact. Typically, swabs are used to collect this latent DNA by moistening the swab material (cotton, foam or nylon) and rubbing the substrate where DNA might be present. This blind sampling, based on best assumptions, may lead to samples collected at scene that contain no DNA, simply because there had been no recent contact at the position sampled. The presence, or lack of, DNA on the swab will not be known until the swab head is subjected to a DNA extraction process and DNA quantification. These are costly and time consuming processes.

Diamond™ Nucleic Acid Dye (DD) binds to the backbone of the DNA molecule as it is an external groove-binding molecule [5]. This dye has

been used to detect the presence of cellular material associated with hairs [6,7] and shown to have little inhibitory effects on further DNA profiling [8]. Due to the mode in which the dye binds to DNA, it will not bind effectively to supercoiled bacterial DNA. This is highly advantageous as the signal arising from human DNA deposited by touch will not be swamped by background signal arising from bacterial DNA. Previous studies required the use of a conventional fluorescence microscope and were not appropriate to use outside a specialist laboratory [6,8,9]. The Dino-Lite fluorescence digital microscope is highly portable and examination of DD can be performed in ambient light, making it highly applicable to mobile use and examination outside of the laboratory. A recent study has used this same combination to assess the shedder status of individuals [10]. In this shedder assessment study volunteers touched glass slides at defined time points after handwashing and DD was applied such that for the first time cellular material deposited by touch could be observed and scored [10].

We report here on the detection of latent DNA deposited by touch on a wide range of substrates using DD in combination with a Dino-Lite fluorescence digital microscope. The items chosen are relevant to

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Received 8 March 2018; Received in revised form 13 July 2018; Accepted 7 August 2018

Available online 08 August 2018

1872-4973/ © 2018 Published by Elsevier B.V.

forensic investigations and deposition of cellular material was at both defined period after handwashing or at non-defined time periods to mimic real-world scenarios. A means by which DNA, such as that deposited by touch, turns from latent to visible can transform the process of sample collection, guide the analyst to collect at areas where DNA is present and save the wastage of processing samples where no DNA is present.

2. Materials and methods

Approval from the Social and Behavioural Research Committee (reference 7569) was obtained prior to initiating this project.

2.1. Deposition of DNA

A range of substrates were used comprising: glass slides, credit cards, mobile phones, SIM cards, zip-lock bags, nickel cartridge cases and aluminium cartridge cases. These items were cleaned with 3% bleach, followed by wiping with absolute ethanol, and were then irradiated with ultraviolet (UV) light for 15 min before use to ensure no DNA was present. Total removal of any DNA was confirmed by staining with DD followed by fluorescence microscopy (described in Section 2.2).

A total of five volunteers comprising 4 males (designated M1–M4) and 1 female (designated F1) were used in this study. Contact was made on a glass slide by placing a finger on the glass for 5 s with medium pressure. Contact was made on the home button of the mobile phone and on the centre front for 5 s. The credit card, SIM cards and cartridge cases were held in the hand for 5 s. The zip-lock bag was opened and then sealed mimicking the action of placing an item inside. For all sample types, contact was made either 15 min post handwashing as used in other reports [10–13] or at unknown times after handwashing to simulate real-world scenarios. Both the 15 min time intervals and items touched at unknown time intervals were performed in duplicate by each of the 5 volunteers. This was for each of the 7 items giving a total, including the items collected at any time, of 140 touched items. One complete duplicate set of items (14) from one volunteer was retained at room temperature for 7 days before examining. All the other items were examined within 24 h of deposition of cellular material.

2.2. Staining of substrate with Diamond dye

The substrates were stained with 20-fold dilutions of the stock (10,000 x) solution of DD (Promega, Madison, WI, USA). Dilutions were made in 75% ethanol (v/v). An aliquot (5 µL) of the dye dilution was pipetted onto each substrate and left to incubate for 2–3 s either where contact was made or at areas of non-contact to act as a negative control. A Dino-Lite fluorescence digital microscope (AnMo Electronics Corporation, New Taipei City, TWN) equipped with an emission filter of 510 nm and a blue LED excitation light source (480 nm) was used to visualise the presence of cellular material. Black background was used for colourless surfaces such as glass slides and plastic bags. Scoring of cellular material was performed by counting the number of stained cells in a frame (each 1 mm²) under the microscope at 220× magnification.

2.3. Staining of fingerprint on glass slide with haematoxylin and eosin

Haematoxylin was added (5 µL) onto the glass slides at the same position of a DD stained fingerprint. The stain was left for 5 min before washing gently with sterile H₂O. Eosin (5 µL) was added and left for 3 min before washing gently with sterile H₂O. The slide was then air-dried.

2.4. Bacteria staining

Single colonies of *E. coli* (DH5a) were picked and dissolved in

200 µL of sterilized water. From this solution, 5 µL was pipetted onto a glass slide, stained with 5 µL of 20 x DD, covered with a cover slip, and visualised under the microscope. Epithelial cells from a buccal swab were mixed with the *E. coli* dissolved solution in ratio of 1:1 (v/v), stained with 5 µL of 20 x DD and visualised under the microscope.

2.5. Collection of DNA from touched items

A micro-applicator (ultra-fine) swab (City Dental, SA, AUS) was used to collect material from the touched items. Touched items from two volunteers (M1 and M3) were tested. The volunteers touched a glass slide after waiting 15 min after handwashing. At an undefined time interval post handwashing, volunteers touched a glass slide, plastic bag and nickel cartridge. This was performed in duplicate giving a total, including clean items as a negative control, of 22 samples. The swabs were moistened with 2 µL of 0.1% Triton-X (Sigma, VIC, AUS) solution and applied to the stained entire fingerprint on each slide.

2.6. Direct amplification of DNA

Direct PCR was performed using the AmpFISTR® NGM Select™ kit (Thermo Fisher Scientific, VIC, AUS) by removing the swab head directly into a 0.2 mL thin-walled PCR tube. Amplification was performed in 25 µL following the manufacturer's validated protocol of 29 cycles. PCR product (2 µL) was added to 9.5 µL Hi-Di formamide and 0.5 µL 500 LIZ™ (Thermo Fisher Scientific) and separated on a 3500 Genetic Analyser (Thermo Fisher Scientific). Data were analysed using GeneMapper ID-X (version 1.4).

2.7. DNA profiling of volunteers

DNA extracts were obtained from the 5 volunteers using the QIAamp® DNA Mini kit (QIAGEN, VIC, AUS) following the 'buccal swab' protocol. Extracted DNA was quantified by Qubit® 2.0 Fluorometer (ThermoFisher Scientific). Approximately 500 pg of DNA from each of the 5 volunteers was used and STR amplification performed as described in 2.6.

3. Results and discussion

Cellular material was observed for all 7 items (glass slides, credit cards, mobile phones, SIM cards, zip-lock bags, nickel cartridge cases and aluminium cartridge cases) handled by all 5 volunteers. Fig. 1A–G shows examples of the presence of cellular material on these items. Fig. S1 shows these samples before any contact was made to illustrate that the cellular material was deposited at the time of touching each of these items. Table 1a shows the number of detected cells in 1 mm² of each item after the volunteers touched the items 15 min after handwashing. Table 1b shows data from the same volunteers but after no defined time period after handwashing (both under 220× magnification).

The ability to detect where a person has touched an item has clear benefits. Windows, and bottles and glasses made of glass, are items that are encountered in a forensic investigation and glass was also chosen to follow on from previous studies [14,15]. The fingerprints on the glass slide contained cellular material at the areas where contact was made. No such stained material was noted on the same surfaces where no contact was made (Figs. 1A and S1).

The credit card was predominantly plastic in composition with a metallic chip. The images (Fig. 1B and C) show the cellular material on the chip and a part of the plastic area respectively and the number of cells was recorded. The embossed numbers on the credit card collected more cellular material than on the smoother plastic surface (Fig. S2). The back side of the credit card also was tested and showed the presence of cellular material on the security hologram (Fig. S3). The credit cards used in this study had different coloured covering (black, white, pink, red, yellow, blue, and silver grey). The stained cellular material

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