Development of RNA profiling tools and the implementation in forensic casework

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Chapter 4

Prevalence of human cell material on public objects

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Abstract

With the availability of DNA and RNA typing techniques, forensic researches are able to obtain information on both donors and cell types present in biological traces found at a crime scene. Nowadays, forensic analyses have extended to minute amounts of biological material such as present in touch samples. Especially with this type of samples, it is conceivable that redundant cellular material not relevant to the case is present, next to crime-related biological material. Transfer, persistence and recovery of touch DNA have been studied but prevalence of specific cell types (e.g. blood, saliva, semen, skin, vaginal mucosa or menstrual secretion) has not yet been addressed systematically. We examined ten different public objects (six specimens each), which are frequently handled by a variety of persons using DNA and RNA analyses. Our data indicate that from many public objects analysable amounts of DNA and RNA can be retrieved. Some objects tend to carry more cellular material such as for instance (paper) money. The objects often contain contributions from several people who appear to donate predominantly skin cells. Occasionally other cell types are found and this coincides partly with relatively high DNA yields. Although variation between samples in this preliminary study is seen, some information regarding general trends for “background” levels DNA and RNA such as contributor numbers and observed cell types is obtained.
Introduction

Technological advances in the field of forensic genetics have been aimed at, amongst others, increasing the chance of obtaining useful DNA profiles from minute amounts of cellular material residing in for instance touch specimens [1-4]. The application of highly sensitive methods to minute samples has the consequence that (low level) cell deposits unrelated to the crime may become detected as well. We refer to these depositions as background cell material. Background material may complicate forensic genetic analyses as non-relevant signals may mask (in strength) or outnumber the crime-related signals, and discriminating both types of signals can be troublesome. Handled or touched objects are most likely to contain background cell material, which can be introduced through direct or secondary means of transfer [2,4]. Research regarding DNA transfer has shown that various factors influence the amount of cellular material deposited such as substrate type [5,6], contact intensity and frequency, time of contact [2], shedder status (which shows variation not only between persons but also for individuals when tested on distinct days) [4,6,7]. Irrespective whether cell material was deposited directly by an individual or through a second person, persistence levels may vary [4,8,9] which can affect both the amount (yield) and the number (exemplified by donor number) of cell depositions residing in the background. The third factor (beside deposition and persistence) determining background levels is the efficiency with which cell material is recovered from a substrate as illustrated in a study on skin sampling in case of mimicked strangulation by de Bruin et al. [10]; here tape lifts recover relatively more of the ‘perpetrator’ DNA than the double swab technique. Although various studies investigating touch DNA have shown that measurable quantities of DNA and full DNA profiles can be obtained [2,4,11-13], the amounts of cell material are generally limited [6]. Information regarding the type of cells that tend to be present in background material could assist interpreting forensic evidence, not so much by providing clear-cut answers on the course of events but rather by informing on the probability of the observations for different hypotheses. Direct contact with objects is likely to cause deposition of epithelial cells [14], but it is perceivable that also other cell types (blood, saliva, semen, vaginal mucosa, menstrual secretion) are left and since these body fluids generally have high DNA levels (semen of sterile men excluded) they may contribute relatively much to a DNA profile. Saliva may be the body fluid most prone to be present in touch specimens as we regularly touch our face, cough, sneeze and speak. On the other hand, other cell types (like keratinized epidermal cells or spermatozoa) may have a structure that protects them better against degradation by external factors. Also the density of (nucleated) cells
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may be higher for other body fluids than for saliva due to which smaller amounts of these fluids can be detected in genetic analyses. To date, no information on the occurrence of cell types in background material is available. Techniques to study this include presumptive testing and the recently developed RNA profiling methods of which the RNA analyses have several advantages as these address a wider range of cell types, are human-specific and for some body fluids more sensitive [15-17].

Besides the afore mentioned importance for inferring the probability of specific forensic hypotheses, knowledge on the prevalence of cell types in touch specimens could be used to optimise sampling strategies on future crime scenes. This could be by, for instance, limiting the sample area or applying a more superficial sampling methodology to minimise the collection of background material. In this study we investigated ten different public objects, assumed to be frequently touched by multiple individuals, with the aim to make an inventory regarding cell types and donors present. The ten public objects had a different surface structure and were each sampled in six-fold. Each of these 60 specimens was analysed for total of human DNA yield and subjected to STR genotyping and RNA profiling. From the DNA profiles the type of profile was monitored and the minimum number of contributors was estimated. From the RNA profiles the number and identity of observed cell types was assessed using the six different cell types accommodated in the multiplex [17].

Materials and methods

Samples and collection

Ten different objects were selected that are presumed to be regularly touched by multiple persons and which display a variety of different surface properties. For all object types, samplings were collected from six distinct objects using DNA-free 12 mm² adhesive tape-lifts. Procedures for preparation of the tape-lifts were followed as described previously [18]. The target area was sampled until the adhesive strength of the tape-lift was saturated. Samples were stored at room temperature until further processing. Objects and sample characteristics are shown in Table 1.

DNA and RNA profiling

For DNA/RNA co-isolations the protocol previously described by Lindenbergh et al. [17] was used. During isolations, the tape-lifts were used in its entirety. The elution volumes were halved as nucleic acid yields were expected to be low; DNA
was eluted in 50 µL 25 % AE buffer (QIAGEN, Venlo, The Netherlands) and RNA in 30 µL of nuclease free water (Ambion®, Austin TX, USA). DNA was quantified on an ABI 7900HT real-time PCR system (Applied Biosystems™ (AB) by Life Technologies, Nieuwerkerk a/d IJssel, The Netherlands) using Alu repeat amplicons as previously described by Nicklas and Buel [19] (with minor modifications to the protocol as we used 200 nM Alu forward and DYZ5 reverse primer).

DNA extracts determined to have a total DNA yield below 1 ng were concentrated to a final volume of 10 µL by ethanol precipitation [17]. For DNA profiling, the AmpFL®STR® NGM™ PCR Amplification Kit (AB) was used. Concentrated DNA extracts were fully used for DNA profiling. For the remaining extracts, input was 500 pg, when available in a maximum volume of 10 µL. Amplified DNA fragments were separated and detected on a 3130x/ ABI Prism® Genetic Analyzer™ (AB) using performance optimized polymer 4 (POP-4) (AB) as separation matrix. NGM PCR products were injected at 3 kV for 15 seconds. A detection threshold of 50 relative fluorescence units (rfus) was used.

RNA extracts were subjected to DNase treatment as described previously [17]. RNA extracts from samples for which the DNA extract was concentrated were also subjected to ethanol precipitation using the same protocol. Full RNA concentrates (10 µL) were used for cDNA synthesis. For the remaining samples a fixed volume of 10 µL RNA extract was used. For RNA analysis, a 20-plex was applied which identified blood, saliva, semen, menstrual secretion, vaginal mucosa and skin. The multiplex consisted of all 19 markers from our previously published multiplex [17], supplemented with a primer set for skin marker LCE1C [20] (Forward (5’ to 3’): TGTGACCCCGCTCCTGAATCCG, Reverse (5’ to 3’): CTTGGGAGGGCACTTGGGGGTG, both at 0.02 µM). Each investigated sample was amplified in 5-fold: first exploratory inputs of 5 µL and 2 µL were used which were complemented with three additional amplifications that used 2 µL of cDNA. The four RNA profiles based on 2 µL input were used for the interpretation of RNA profiling results. Amplified DNA fragments were separated and detected on a 3130x/ ABI Prism® Genetic Analyzer™ (AB) using POP-7 (AB) as separation matrix. RT-PCR samples were injected at 3 kV for 10 s, and a detection threshold of 150 rfus was used. For each sample, tissues were scored according the procedure described by Lindenbergh et al. [21].
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Table 1. Selected objects and sampling information.

<table>
<thead>
<tr>
<th>Object</th>
<th>Sampling surface</th>
<th>Sampling location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Handle of a shopping cart (indoor storage)</td>
<td>Plastic</td>
<td>Top side of the handle</td>
</tr>
<tr>
<td>Handle of a shopping basket</td>
<td>Plastic</td>
<td>Lower side of the handle</td>
</tr>
<tr>
<td>Coin money (random value)</td>
<td>Metal</td>
<td>Both sides and whole surface</td>
</tr>
<tr>
<td>Banknotes (random value)</td>
<td>Paper</td>
<td>Both sides and whole surface</td>
</tr>
<tr>
<td>Handrail of stairs at a train station</td>
<td>Painted metal</td>
<td>All round the handrail</td>
</tr>
<tr>
<td>Handrail of escalators at a train station</td>
<td>Rubber</td>
<td>Top side and side edges of</td>
</tr>
<tr>
<td></td>
<td></td>
<td>the handrail</td>
</tr>
<tr>
<td>Door handle toilet</td>
<td>Smooth metal</td>
<td>Top and lower side</td>
</tr>
<tr>
<td>Flush button toilet</td>
<td>Plastic</td>
<td>Top side</td>
</tr>
<tr>
<td>Handle bars indoors</td>
<td>Smooth metal</td>
<td>All round the handrail</td>
</tr>
<tr>
<td>Library book</td>
<td>Laminated carton</td>
<td>Back, front and aft side</td>
</tr>
</tbody>
</table>

Results

DNA profiling results

To investigate the prevalence of human cell material on public objects, ten items, assumed to be frequently handled by multiple individuals, were sampled. These included shopping cart handles, shopping basket handles, coins, banknotes, handrails of stairs at a train station, handrails of escalators at a train station, toilet door handles, toilet flush buttons, handle bars located indoors and library books. The objects have different surfaces (Table 1), and six independent samplings were collected for each object type. Thereby, a total of 60 samples was obtained.

A sensitive human-specific DNA quantification system was employed to determine the DNA yield for each sampling. This Alu system gives reliable concentration measurements down to 0.5 pg/µL *i.e.* accurate yield determinations down to 0.025 ng in this study. For 54 of the 60 samplings reliable DNA yields were obtained that ranged from 0.027 to 41.103 ng. The six samples presenting a yield below reliable measurement were derived from one shopping cart handle, three handrails (one of stairs and two of escalators), one toilet door handle and one toilet flush button. In Figure 1, the DNA yield of each sample is shown. Most samples (47 of 60) had a yield below 1 ng (of which 42 samples had a yield even below 0.5 ng), and only three samples had a yield above 5 ng. When the DNA yields were plotted for the samples grouped per object type (Fig. 1A), it became apparent
that a large variation both within and between the various object types occurs and that higher yields occur relatively often for coins and banknotes (Fig. 1). From all 60 DNA extracts one DNA profile was generated using the sensitive NGM system [1]. When samples had a DNA yield below 1 ng, the full extract was concentrated and completely used (resulting in DNA inputs up to 0.92 ng). With yields above 1 ng (concentrations above 0.02 ng/µL), a maximum input volume of 10 µL was taken which resulted in inputs ranging from 0.20 to 0.54 ng. The DNA profiles of these public objects were analysed for general profile type and estimated minimum number of contributors. No comparative DNA studies were performed. The profiles were typed as: (i) profiles with no or only few (less than eight) allele calls (11 samples), (ii) incomplete DNA profiles with allele calls under the stochastic threshold (determined to be 400 rfus [1]; 37 samples) or (iii) DNA profiles presenting a clear single major contributor (possibly with additional allele calls below stochastic threshold from extra contributors; 12 samples).

To examine the relation between DNA yield and profile type, the plots depicted in Figure 1A and 1C were generated; the 12 samples presenting a distinguishable major contributor represent the majority (nine) but not all (13) of the samples giving higher yields (above 1 ng), and occur for six of the ten object types. The 11 samples giving few allele calls all had very low DNA yields.

To estimate the minimum number of individuals who donated cellular material in the samplings, the Maximum Allele Count (MAC) method was used [22]. In Figure 1C, samples are grouped by the estimated minimum number of contributors and DNA yield and profile type are indicated per sample. Most frequently, the samples showed an estimated minimum number of contributors of two or three, although up to six contributors were found. When comparing the minimum estimated number of contributors to the total DNA yield, the samples estimated to have more contributors tended to have higher DNA yields: for one estimated contributor 91% of the samples (10/11 samples) had a yield below 1 ng while for estimates of two, three and four contributors this percentage reduced to 78%, 72% and 70% respectively (14/18, 13/18 and 7/10 samples). When the estimated minimum number of contributors is regarded per object type (Fig. 1A), the handles of shopping baskets appear to have relatively many contributors (three to six) even though the yield of five of the six samples is below 1 ng. Lowest numbers of contributors were found for handrails of escalators on a train station (Fig. 1A/C) that in addition displayed low yields. The surface (rubber-like material) may have a role in the recovery of cell material by tape lifts. Profiles with a distinguishable major were observed with estimates of one, two, three and four contributors (but for estimates of five or six the data set comprised only a single profile). We infer that a clear major may occur with any number of contributors.
Fig. 1. All panels (ABCD) show the DNA yield for each sampling.
Fig. 1 (continued). All panels (ABCD) show the DNA yield for each sampling.
Fig. 1. All panels (ABCD) show the DNA yield for each sampling. Yields below 0.025 ng are too low to be determined accurately. In panels A and B, samplings are grouped by each of the ten object types; in panels C and D, samplings are grouped by the estimated minimum number of contributors (zero to six). For some samples the DNA yield exceeds the Y-axis scale (set to 7 ng for panels A and B and to 2 ng for panels C and D). In these cases the yield is indicated at the top of the bar. Below each bar or groups of bars the estimated minimum number of contributors and object type are indicated. The bars in panels A and C have three colours which depict type of DNA profile: light yellow bars reflect profiles with no or only few allele calls; green bars indicate incomplete profiles (alleles occurring below stochastic threshold level); dark green bars mark profiles that have a distinguishable single major contributor. If more than one contributor occurs for the samplings marked in dark green, the genotyping of the other component(s) appears incomplete (peaks below stochastic threshold). The bars in panels B and D have four colours which indicate the number of observed cell types (besides housekeeping markers): white bars indicate no observed cell types; blue bars indicate one observed cell type (in all cases skin); dark blue bars indicate skin and saliva, dark purple bars indicate skin and vaginal mucosa.

RNA profiling results

Next to DNA profiling, samples were subjected to RNA profiling to infer the biological origin of the cellular material present. First, 2 and 5 µL were amplified and analysed using the cell typing 20-plex. From the results (similar number of marker peaks, higher r fus and sometimes overloaded signals for the 5 µL inputs) it was decided to base RNA profile interpretation [21] on four replicates having 2 µL cDNA input. Thus, three more of these amplifications were generated. For this study, we focused our interest on cell types categorised as “observed”. Thus, the categories “sporadically observed” and “(sporadically) observed and fits with” [21] were regarded as “not observed”. For the identification of vaginal mucosa, only signals for MUC4 were considered and HBD1 was excluded since this marker performs suboptimal in multiplex [17]. Housekeeping marker signals were scored “observed” in 55 of the 60 samples; skin was regarded “observed” in 56 samples (with two samples overlapping i.e. neither skin nor housekeeping observed). For the samples having skin scored as “not observed”, no other cell types were categorised as “observed”. Actually, only eight samples showed an additional cell type beside skin in the “observed” category: four contained saliva (two handles of a shopping basket, one banknote and one library book) and four had vaginal mucosa (two banknotes, one toilet door handle and one toilet flush button). Samples with more than two cell types were not observed. When
the relation between observed cell type and DNA yield was plotted (either grouped per object type -Fig. 1B- or by estimated minimum number of contributors -Fig. 1D-), some of the samples having high DNA yields had saliva or vaginal mucosa observed. The sample with the highest DNA yield (41 ng), however, was categorised as “observed” for skin only. The four samples for which no cell types were observed had low but not the lowest DNA yields. Rather, a relation to object type may exist as two of the six library book samples fall into this category (which may mean that RNA is less preserved on this object type). For all eight samples for which a second cell type is observed, the estimated minimum number of contributors is two or more. Since for mixed samples it is inadvisable to associate donor and cell type (Chapter 3), a suggestion like ‘skin and saliva may be donated by distinct donors’ cannot be made as a donor could have left multiple cell types and multiple donors could have given the same cell type.

The specifics of the DNA and RNA profiling results, such as profile type and observed cell types, are indicated in distinct panels of Figure 1. Since panels A and B (and C and D) use the exact same order of the samples, the relation between profile type and observed cell types can be assessed. For half of the samples containing a second cell type (either saliva or vaginal mucosa), profiles show a distinguishable major component (which was for the two samples with vaginal mucosa observed once a female and once a male component). In these four cases DNA yields were above 1 ng. The other samples, for which a second cell type is observed, showed incomplete profiles of at least two contributors and have DNA yields below 1 ng. When examining the four samples for which no cell types were observed, only one sample resided among the ten samples showing few allele calls in the DNA profile (the other three samples had incomplete profiles).

Discussion

In this study, we performed DNA and RNA analysis on 60 public objects to get an improved understanding of the occurrence of cellular material on such items. From each object, we assessed the DNA quantity, estimated minimum number of contributors and number and identity of observed cell types and evaluated trends between these aspects.

For collection of traces from touched objects, tape-lifts were used. Tape-lifts are mostly used for the collection of biological material from clothing, as they allow one-sided sampling for the specific recovery of the cell material on the external or wearer side [2]. In recent years, forensic researchers have found additional applications
and substrates for tape-lifts [18,23], but the general sampling methodology involves swabs. For touched objects, the double swab technique has been recommended by several studies [24-26], but we chose to use tape-lifts in this study as they can be applied swiftly in a public environment and are straightforwardly stored after sampling. For most samples (90 %), yields were sufficient to obtain reliable DNA quantifications although most yields (78 %) were lower than 1 ng. Lowest yields were collected from the rubber handrails from escalators on train stations. This may have several causes: the object type could be less touched, cell material may be removed due to the rolling escalator system, deposition of cellular material may be less efficient as these handrails are touched rather lightly or the rubber surface may saturate the glue layer of the tape-lift earlier limiting the collection of cell material (rubber-like fragments were noticed on the tape-lift after sampling). For 78 % of the samples, ethanol-precipitation was used to concentrate the DNA and corresponding RNA extracts. For these samples, all genetic material needed to be used to generate DNA profiles or synthesise cDNA which is an uncommon strategy with casework as (in the Netherlands) a portion of DNA extract needs to remain for contra expertise. For some samplings, full DNA profiles were obtained which is in concordance which literature that reported, among others, full genetic profiles of samples from briefcases, pens, telephones, mugs and glasses with total DNA yields ranging between 2 – 150 ng [4]. Because of the low genetic content, the majority of the DNA profiles showed signals below stochastic threshold [1] and drop-out of markers. When such incomplete DNA profiles are obtained in casework it is advised to perform replicate analysis in order to obtain a consensus profile to confirm observed alleles as these may represent drop-in alleles [27]. In this study, only one profile was used for analysis as for the majority of samples no extract remained to perform replicate analysis. We believe this strategy suffices for the exploratory purposes of this study that include the analysis of profile type and estimation of minimum number of contributors and not profile comparison to reference samples. Each sample was investigated by RNA analysis as well using an updated version of our previously described multiplex [17] to which skin marker LCE1C was added [20]. LCE1C appeared more sensitive than the other two skin markers (LOR and CDSN), and the primer concentrations for LCE1C were 30-fold lower than LOR and CDSN to achieve a balanced RNA profile (Bhoelai, van den Berge and Sijen, unpublished results). With that, LCE1C is a useful supplement to the multiplex for identifying skin.

When examining the DNA and RNA profiling results, the first aspect that stood out was the high success rate: for 90 % of the specimens a DNA yield could be reliably measured (i.e. yield was above 0.025 ng) and for 93 % RNA cell typing results were obtained. This suggests that the applied methodology (which included concentration of nucleic acids prior to analysis) is sensitive.
However, for all assessed aspects (yield of human DNA, estimated minimum number of contributors, type of DNA profile, number and identity of observed cell types) large variation was observed between the individual samplings and for the various object types. Due to this large variation, strict relations were not obvious, but general trends were observed as specified in the results section.

A striking observation was that skin was far-out the most prominent cell type observed in 93 % of the samplings, while an additional cell type was only observed in 13 % of the samples. Thus, skin appears the most prominent source of biological material on touched public objects. Presence of skin is best explained by direct deposition as DNA depositions for touch samples are generally low [28], while secondary/tertiary transfer rates are only a fraction of the deposited cell material [29,30]. Thus, the finding that 80 % of the specimens appear to have two or more contributors, implies that multiple individuals touched the analysed public objects. Saliva and vaginal mucosa were seen as a second cell type in four samplings each. Saliva may have been deposited directly by sneezing, speaking or coughing, but secondary transfer cannot be excluded; for instance the occurrence of saliva on a library book may be due to ‘finger-wetting’ to assist page turning. Alternatively, tertiary transfer could be involved in saliva deposition, for instance when person A coughs in his hand, then shakes hands with person B who next touches one of the public objects. For vaginal mucosa, deposition through secondary/tertiary transfer seems most likely.

This study has shown that most public objects contain variable, but measurable, amounts of cell material. This finding may complicate analysis of such objects and complex the interpretation of casework results, especially when the crime-related traces represent low-level depositions. High template depositions are expected to reduce the background signals to noise level in both the DNA and RNA profiles as such samples require much lower inputs in the DNA and RNA analyses. Information on the time since deposition could be of assistance in assessing the relevance of cell material in relation to the crime. Currently, the status is that much more research on this subject is needed to allow reliable estimations [31]. Also, the analysis of mixed traces containing cells deposited at various moments in time will be a true challenge (and suffer problems analogous to those described in Chapter 3 on the association of donors and cell types in samples comprising two cell types). Visualisation of human cell material would be very useful [32,33], especially when combined with precise sampling methods (e.g. mini-tip swabs, mini tape-lifts), in order to reduce the relative amounts of background material collected.

Information on the prevalence of human cell material on public objects can assist the evaluation of hypotheses as performed by reporting officers during casework. Clearly, more extensive studies are opportune and such studies could be extended
to private objects like clothing or household items (e.g. door handles, light switches in private places). Beside cell material from the wearer, clothing can carry biological material from other persons for instance when clothing is touched in relation to a crime (e.g. violent or sexual assault cases, which can be a firm type of contact). Cell material may also be deposited by house mates (e.g. from holding or touching each other or from handling the cloths as laundry) or from the environment (e.g. sitting in a train). Thus, it is of interest to study items from various types of households (1, 2 and 3 or more person households). An additional subject for further research is the assessment of cell types in designed secondary/tertiary transfer experiments. This subject has not been investigated previously.

Concluding remarks

The objective of this study was to gather knowledge on the occurrence of six different cell types on public objects through combined DNA and RNA profiling. From these objects, mostly low DNA yields (below 1 ng) were obtained and the majority of the subsequent DNA profiles showed alleles with signals under the stochastic threshold. Generally, two or more contributors were involved. Skin cells were found to be most prevalent, occurring in almost every investigated sample. Next to skin, saliva and vaginal mucosa could be identified but only in a small percentage of the samples. Blood, menstrual secretion and semen were not observed. These experiments have shown that for touched objects, both DNA and RNA profiles should be interpreted in context of sample contamination. Especially when skin cells are observed there is a possibility that these are not crime-related. Further research concerning DNA and RNA typing of trace evidence is necessary to improve the accuracy of proposed hypotheses and assessment of case scenarios.

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References

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