Estimating the quantity of transferred DNA in primary and secondary transfers

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Highlights

- A single label to describe a donor's ability to leave DNA should not be used.
- DNA shedding ability should be considered as a *distribution* of a quantity of DNA.
- The transfer proportion depends on the donor and on the type of the transfer.
- Deconvolution of the DNA profiles is required, depending on the type of transfer.

Novelty Statement

- We have three objectives: to characterize the distribution of the quantity of DNA observed on the hands and directly or secondarily transferred on surfaces; to assess if deconvolution of the DNA profiles is required to estimate the quantity of DNA of the POI; to test if the transfer proportion is similar across individuals and can be used to predict the quantity of transferred DNA.

- We propose, when assessing the probability of observing a given quantity of DNA, for a given donor, that whole distibution should be accounted for.

- We show that the total quantity of DNA can be used to study primary transfer without resorting to a mixture deconvolution process. However, the deconvolution is required when considering secondary transfers.

- Finally, we show that the transfer proportion may vary between participants and will depend on the type of the transfer (primary versus secondary).

Introduction

According to the ENFSI guideline on evaluative reports [1], the evaluation of biological stains, especially traces with a low quantity of DNA, should be carried out considering activity-level propositions. It involves a relative assessment of the expected quantities of recovered DNA under the alleged activities put forward by the parties. In order to do so, the respective shedder status (or shedding ability) of the person of interest and of the alternative offender should be investigated.

Previous studies have dealt with the shedder status of donors [2, 3, 4 and 5]. They all reported large variations between individuals in the amount of contact DNA that each donor may leave on a receiving surface; some individuals transfer more DNA than others. In addition, Pesaresi *et al*. [6], van Oorschot *et al*. [7] and Bright and Petricevic [8] show that variations can be observed in the amount of DNA a given individual may deposit. These studies show that variation within an individual should be taken into account to assess the probability of observing a given quantity of contact DNA.

In the present study, we will show that the DNA shedding ability of an individual should be characterized as a *distribution* of the quantity of DNA present on hands or transferred on surfaces. Individuals do not have fixed shedder status (such as “good” or “bad”) regardless of the circumstances. Indeed, a given individual may deposit a mean quantity of DNA, but due to the inherent within-source variability, may also, at times, deposit, much more or less than this quantity. So, the probability of observing a given quantity of DNA should account for this distribution. We will inform this distribution by a measure of its mean and spread. In addition, the amount of DNA available to be shed from a hand to a surface depend on the conditions of the hands at the time of transfer (e.g. sweaty or dry). Lacerenza *et al*. [9] indicated that life habits have no impact on the recovered DNA quantity on hands except for the habit of touching the hairy surfaces. Touching his/her hairs increases the quantity of DNA recovered on hands. Our experimental design will consider a range of quantities of DNA on hands.

The above literature on the shedder status is mostly concerned with primary transfer and not with secondary or subsequent transfers. In this study, we will deal with two situations involving a knife handle; the first is a primary transfer from a hand to a knife handle and the

second is a secondary transfer from a Person of Interest (POI) to the hand of an intermediate person who then took the knife handle. This is not the first time that transfer on surfaces is studied [3, 10, 11, 12, 13, 14], but these studies have some limitations. All researchers studied the probabilities of primary or secondary transfer of DNA but without considering the inherent variability due to the donor.

After the touch a surface by a POI, it is frequent to observe in addition to his/her DNA contribution, the DNA contribution of additional individuals [9, 14]. Modern probabilistic genotyping systems (such as STRmix, https://www.strmix.com/) allows to deconvolute these mixtures and, from the estimated mixing proportion, derive the effective quantity corresponding to the POI. That approach was already adopted by [11, 12]. In this study we will explore if such deconvolution is required to assess the quantity of DNA left by the POI or if the total quantity of DNA is sufficiently informative.

We will also investigate if the quantity of transferred DNA on an object can be predicted from the measure of the DNA quantity available on the hand and the application of a transfer proportion (TP) that will be fixed for each individual. Quantifying the amount of DNA on the hands has been made by McColl et al. [15] but only looking at the variability between donors and not reporting on the variability within donors.

To sum up, this study has three objectives: (1) to characterize the distribution of the quantity of DNA observed on the hands of individuals and transferred on surfaces either through primary or secondary transfer; (2) to assess if deconvolution of the DNA profiles is required to estimate the quantity of DNA of the POI; and finally (3) to test if the transfer proportion (quantity transferred on the surface over the initial quantity on the hand) is similar across individuals and can be used to predict the quantity of transferred DNA.

Methodology

Transfer Experiments

Six consenting participants, three men and three women, were randomly selected to deposit contact DNA following activities of primary or secondary transfer.

For primary transfer, each participant was asked to rub their hands during around five seconds [13] with a view to redistribute surface DNA evenly on both of them [16], then took a knife (Stainless Steel, X50 Cr Mo V15) handle with their usual hand and, immediately after, stab

three times a ballistic soap (from Mettler SA). 30 stabbing experiments were performed for each participant, leading in total to 180 experiments. Before each experiment, the knife was thoroughly cleaned, using bleach and ethanol.

The duration of the contact, the type of contact and the force of the stabbing were not specified in order to simulated conditions as closed as possible than casework. The ballistic soap allowed mimicking the physical properties of a human body. This direct transfer on the knife handle is what will be considered as primary transfer. The entire surface of the knife handle and the inside part of the other hand, meaning the palm and the fingers inside the hand, not used for the activity, were swabbed just after the stabbing to collect DNA using the FLOQSwab™ from COPAN. One FLOQSwab™ was used per sample, following the procedure of the laboratory. The knife handle being a smooth surface, the FLOQSwabs™ were moist.

The stabbing conditions used for the experiments were adapted in order to increase or decrease the quantity of DNA initially on the surface of the hand and subsequently transferred. These variations aimed at reflecting an extreme range of life conditions for a given individual. For a first set of ten experiments out of thirty, each participant was asked to wash their hands just before performing the stabbing. For the next set of ten experiments, they were asked to wear gloves for 30 minutes to increase sweating. For the last set of experiments, no specific indication was given to the participants. Each set were performed on different days. However, within each set, some experiments were conducted on the same day. For the washing and glove wearing conditions, it has no bearing. For the last condition (no specific indication), a sufficient time between experiments (about an hour) was allowed. The above-described experimental design, as performed by the six participants, is illustrated in Figure 1.

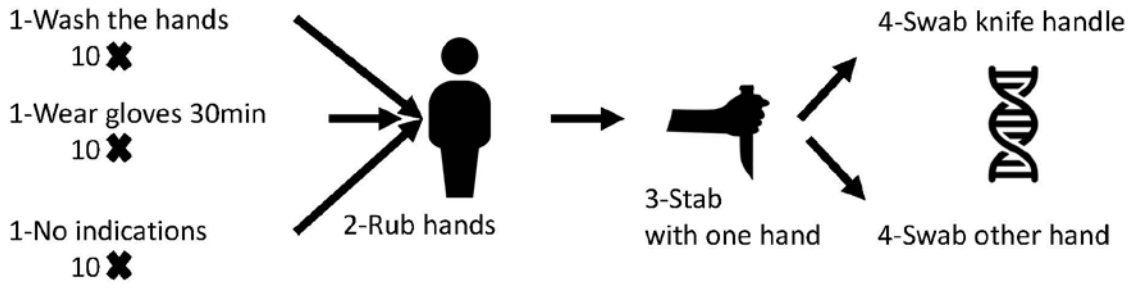

Figure 1: Illustration of the experimental design to study the quantity of DNA on hands and the quantity of DNA transferred during the primary transfer.

To study secondary transfer, only two participants were chosen in the light of the first set of experiments. Based on their mean quantities of transferred DNA, participant 2 and participant 6 have shown to be the "best" and the "worst" DNA donor respectively (See Figure 3, Figure 4, Figure 7, Table 1 and Table 2). Two identical knives, one for each participant, were used for all their experiments. Before each experiment however, the knife was thoroughly cleaned, using bleach and ethanol. Both participants were first asked to shake hands and then to stab the ballistic soap with the knife. No indication on the duration of the handshake was given to the participants in order to mimic real life conditions as closely as possible, the contact though didn't exceed by few seconds. The entire surface of the two knife handles were then swabbed for DNA just after the stabbing using one moist COPAN's FLOQSwab™, following our laboratory procedure. Thirty experiments were performed for each of the two participants (leading to 60 experiments in total). Experiments were subsequently performed with a minimum delay of five minutes between them. This experimental design is illustrated in Figure 2.

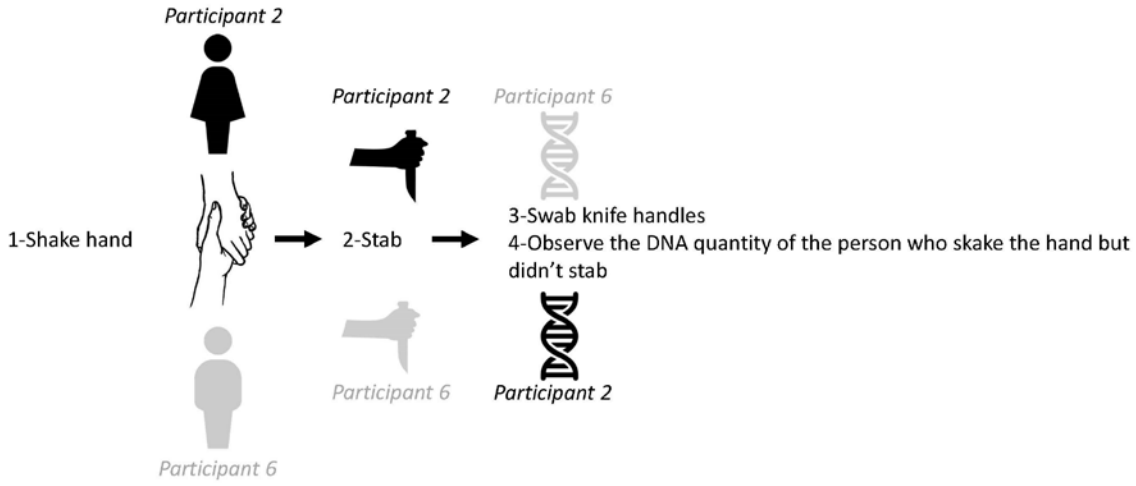

Figure 2: Illustration of the experimental design to study the secondary transfer of the first participant's DNA (Participant 2, in black) and the second participant's DNA (Participant 6, in gray), respectively.

Quantification of DNA

DNA was extracted from the swabs using a combination of two kits: QIAshredder and QIAamp DNA mini kit from Qiagen, concentrated to a final volume of 25µL with microcon® 30 spin column. Quantifications were performed directly following the DNA extraction using the Investigator® Quantiplex kit from Qiagen on Rotor-Gene® Q. DNA was then amplified at 30 cycles using 10 µL of DNA extract per sample and the NGM SElect (Applied Biosystem™-Thermofisher) kit with a PCR system 9700 (Applied Biosystem™), analyzed on a 3500 Series Genetic Analyzers (Applied Biosystem™-Thermofisher Scientific) coupled with GeneMapper1IDX Software (Applied Biosystem™-Thermofisher Scientific). The kits were used as per manufacturer's instructions.

DNA quantification allows to obtain information about the total quantity of DNA recovered from the knife handle. That quantity may result from a mixture of DNA of the POI and of other contributors. To estimate the proportion of DNA corresponding to the POI, STRmix™ v2.5.11 software is used to assess the mixing ratio from each donor in the mixture. The number of contributors entered in the software for each case is based on the number of the peaks detected at each locus, peak height balance information and how the experiments were designed (i.e., we expected one, two or three person's DNA).

Deriving the parameters of the transfer proportion

For primary transfers, the parameters of the distribution for the log10 of the transfer proportion (log10(TP)) for each individual is obtained by combining the results of the initial quantity of DNA on hands (Qi) and the results of the quantity of DNA observed on the knife handle (Qf), under the assumption that both Qi and Qf are Normally distributed [17]. When transformed in log10, the parameters of the distribution for log10(TP) are obtained as follows [18]:

$$\text{mean}\left(\log10(\text{TP})\right) = \text{mean}(\log10(\text{Qf}) - \text{mean}(\log10(\text{Qi})$$

and

$$\text{SD}\left(\log10(\text{TP})\right) = \text{SD}\left(\log10(\text{Qf})\right) + \text{SD}\left(\log10(\text{Qi})\right) - 2 * \sqrt{\text{SD}(\log10(\text{Qf})) * \text{SD}(\log10(\text{Qi}))}.$$

The same transfer proportion parameters can be computed for the secondary transfers taking the quantity of DNA matching the POI left on the knife handle following secondary transfer as Qf.

Results

Quantity of DNA present on the hand and following primary transfer

The initial quantity of DNA on hands and the quantity of DNA directly transferred on the knife handle are shown in Figure 3 and Figure 4, distinguishing the total quantity and the quantity corresponding to the POI (adjusted using the mixing proportions estimated using STRmix™).

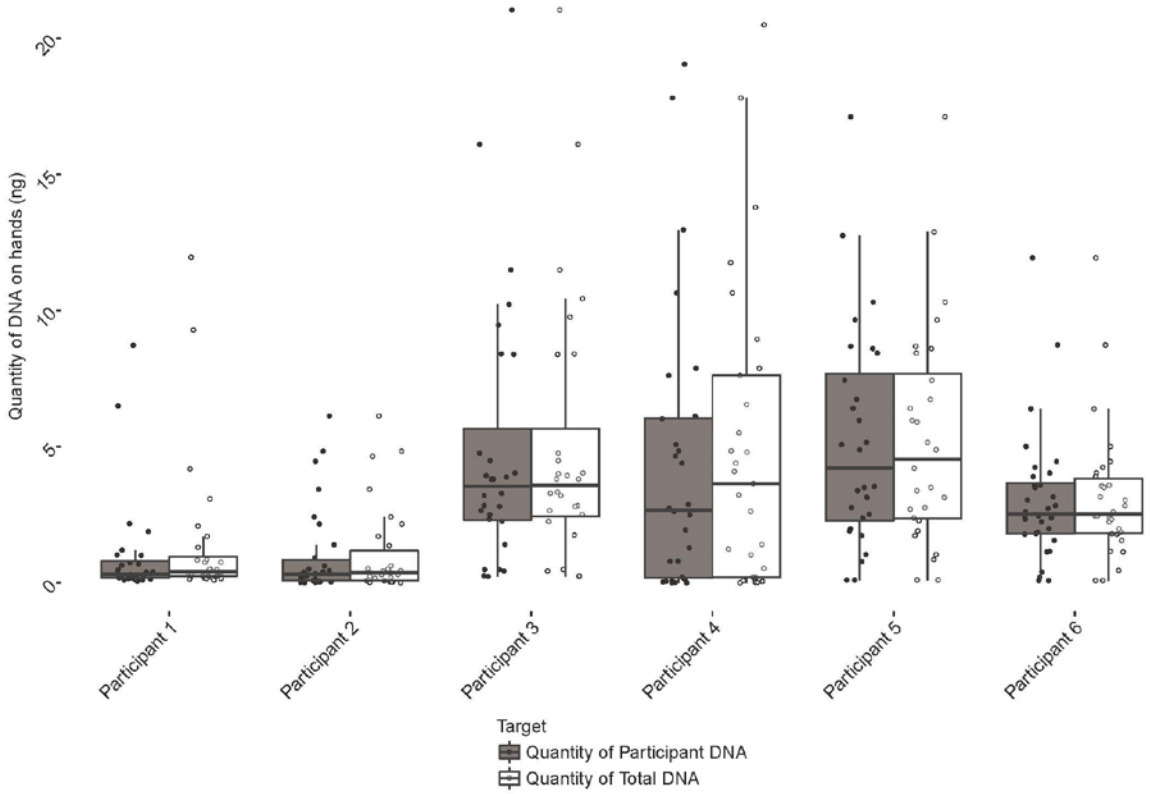

Figure 3: Boxplots of the total quantity of DNA (and the quantity corresponding to each participant) recovered from the hand. Each dot corresponds to the corresponding quantity obtained after each experiment.

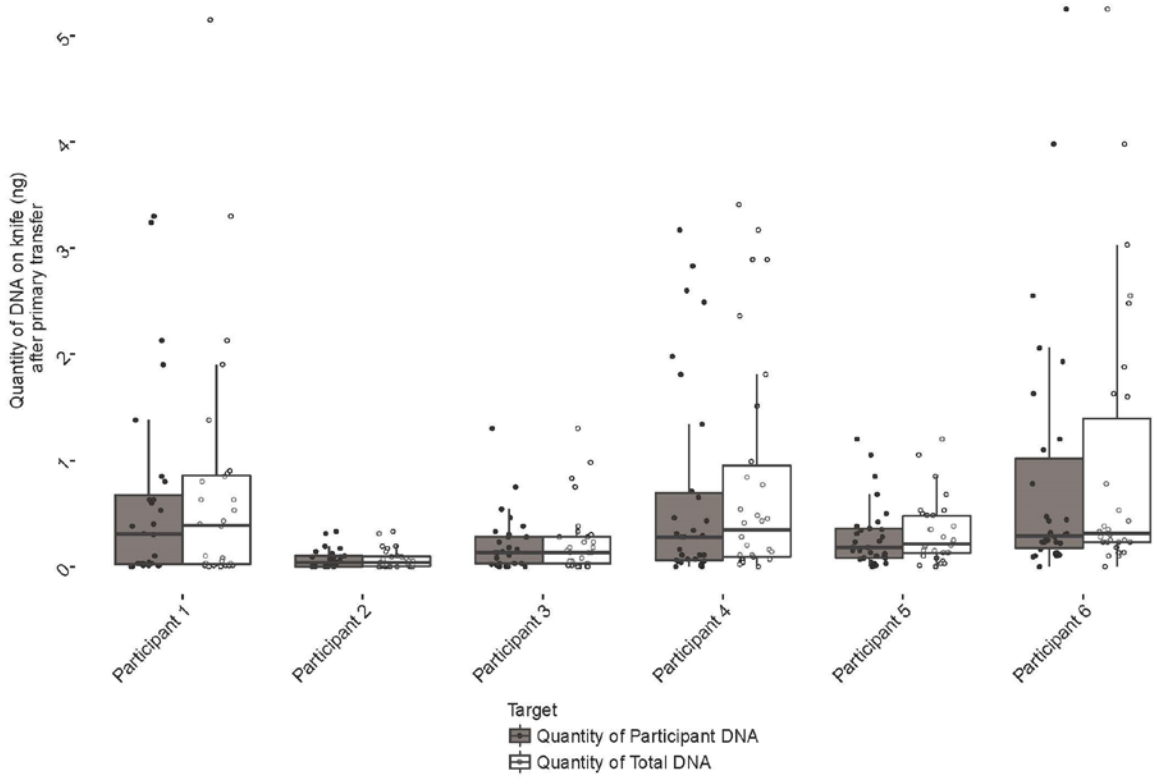

Figure 4: Boxplots of the total quantity of DNA (and the quantity corresponding to each participant) recovered from the knife handle. Each dot corresponds to the corresponding quantity obtained after each experiment.

A large variation of the quantity of DNA collected on participants' hand (Table 1) and the quantity recovered from the knife handle after a direct transfer (Table 2) is observed between participants. Indeed, the mean value of total DNA range from 1 ng to 5 ng. A large variation for each participant is also observed as can be seen from the ranges (max-min) of DNA quantities. For participant 1 for example, between 0 and more that 5ng of DNA can be recovered after directly handling the knife handle depending on the experiment (and between 0 and more than 11ng directly from his hand).

Table 1: Summary statistics of the quantities of total DNA and of the participant's DNA (obtained following mixture deconvolution) recovered on his hand.

The quantity of DNA on the other hand (ng)		Min	0.05 percentile	Median	Mean	SD	0.95 percentile	Max
Participant 1	Total DNA	0.10	0.11	0.42	1.47	2.78	7.50	11.95
	Participant 1 DNA	0.06	0.10	0.31	1.02	1.96	4.98	8.72
Participant 2	Total DNA	0.00	0.02	0.38	1.06	1.63	4.76	6.12
	Participant 2 DNA	0.00	0.01	0.31	1.02	1.62	4.66	6.12
Participant 3	Total DNA	0.23	0.31	3.57	5.03	4.94	14.48	21.03
	Participant 3 DNA	0.23	0.31	3.54	4.96	4.95	14.48	21.03
Participant 4	Total DNA	0.00	0.01	3.64	4.94	5.54	16.19	20.48
	Participant 4 DNA	0.00	0.00	2.68	4.39	5.23	16.01	19.04
Participant 5	Total DNA	0.10	0.36	4.54	5.29	4.04	11.97	17.10
	Participant 5 DNA	0.10	0.34	4.20	5.21	4.06	11.89	17.10
Participant 6	Total DNA	0.08	0.25	2.53	3.15	2.45	7.67	11.93
	Participant 6 DNA	0.08	0.22	2.53	3.15	2.43	7.67	11.93

Table 2: Summary statistics of the quantities of the total DNA and of participant's DNA (obtained following mixture deconvolution) recovered on the knife handle after the participant directly stabbed a ballistic soap with the knife (primary transfer).

The quantity of DNA recovered on the knife handle after direct transfer (ng)		Min	0.05 percentile	Median	Mean	SD	0.95 percentile	Max
Participant 1	Total DNA	0.00	0.0035	0.39	0.74	1.17	2.89	5.15
	Participant 1 DNA	0.00	0.00	0.31	0.63	0.93	2.82	3.30
Participant 2	Total DNA	0.00	0.00	0.04	0.08	0.09	0.27	0.33
	Participant 2 DNA	0.00	0.00	0.04	0.07	0.09	0.29	0.33
Participant 3	Total DNA	0.00	0.00	0.13	0.24	0.32	0.92	1.30
	Participant 3 DNA	0.00	0.00	0.13	0.21	0.28	0.67	1.30
Participant 4	Total DNA	0.00	0.03	0.35	0.82	1.07	3.04	3.41
	Participant 4 DNA	0.00	0.00	0.28	0.70	0.97	2.73	3.17
Participant 5	Total DNA	0.00	0.02	0.22	0.32	0.30	0.96	1.20
	Participant 5 DNA	0.00	0.02	0.18	0.29	0.30	0.96	1.20
Participant 6	Total DNA	0.00	0.10	0.32	0.95	1.30	3.55	5.25
	Participant 6 DNA	0.00	0.10	0.29	0.84	1.23	3.34	5.25

Quantity of DNA following secondary transfer

The quantities of DNA obtained following the secondary transfer experiments are given in Figure 5 and Table 3. For participant 2 for example, about 0.2 ng of total DNA can be recovered on the knife handle after a secondary transfer with 0.03 ng of DNA corresponding to the participant's 2 DNA profile. POI. Whereas, for participant 6, 0.1 ng of total DNA can be recovered on the knife handle after a secondary transfer with only 0.003 ng of DNA corresponding to his DNA profile. A marked difference is observed for the two participants between the total quantity of DNA and the quantity of DNA corresponding to the POI.

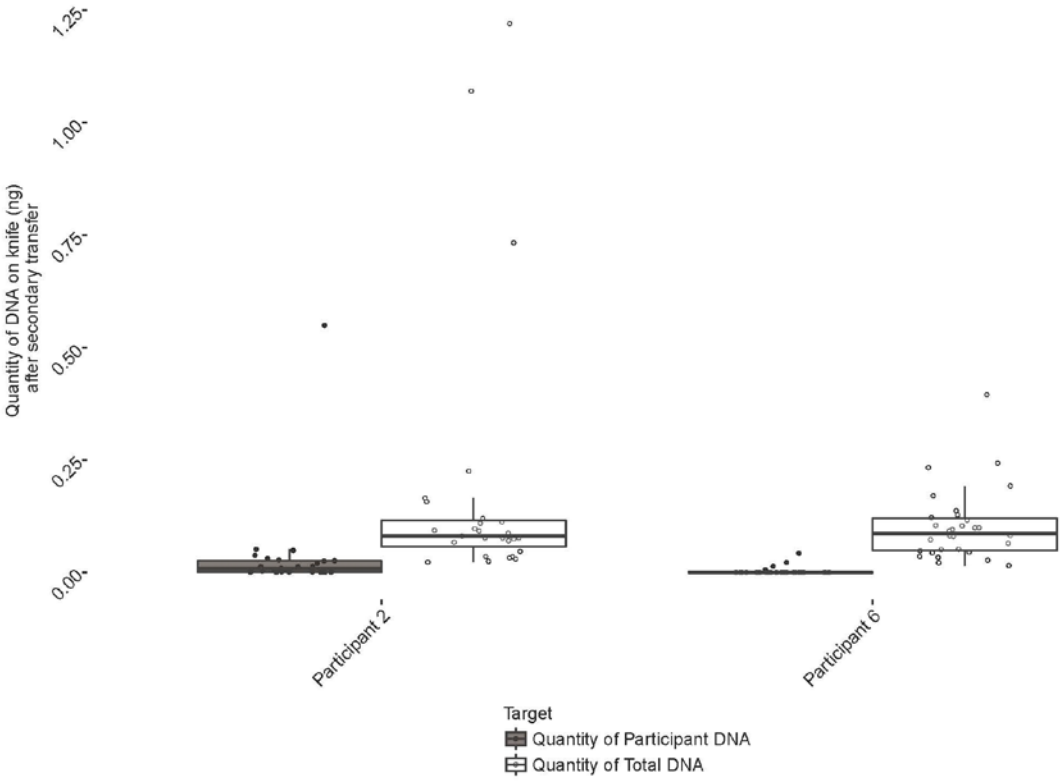

Figure 5: Boxplots of the DNA quantities for participant 2 and 6 obtained indirectly on the knife handle following secondary transfer. Each dot corresponds to the corresponding quantity obtained after each experiment

Table 3: Summary statistics of the quantities of the total of DNA and participant's DNA recovered on the knife handle after this participant shook hands with another participant who stabbed a ballistic soap with the knife. In this situation, Participant 2 shook hands with participant 6 then Participant 6 stabbed the ballistic soap and vice versa.

The quantity of DNA recovered on the knife handle after secondary transfer (ng)		Min	0.05 percentile	Median	Mean	SD	0.95 percentile	Max
Participant 2	Total DNA	0.02	0.03	0.08	0.19	0.31	0.97	1.22
	Participant 2 DNA	0.00	0.00	0.01	0.04	0.11	0.05	0.55
Participant 6	Total DNA	0.02	0.02	0.09	0.10	0.08	0.24	0.40
	Participant 6 DNA	0.00	0.00	0.00	0.00	0.01	0.02	0.04

POI's DNA: comparing hands, primary, and secondary transferred quantities

If we focus our attention on the quantity of DNA corresponding to the POI for the three cases studied (hand, primary transfer and secondary transfer), we recorded large variations of that quantity within participant and between participants. Figure 6 and Table 4 bring together these data (already shown in part before).

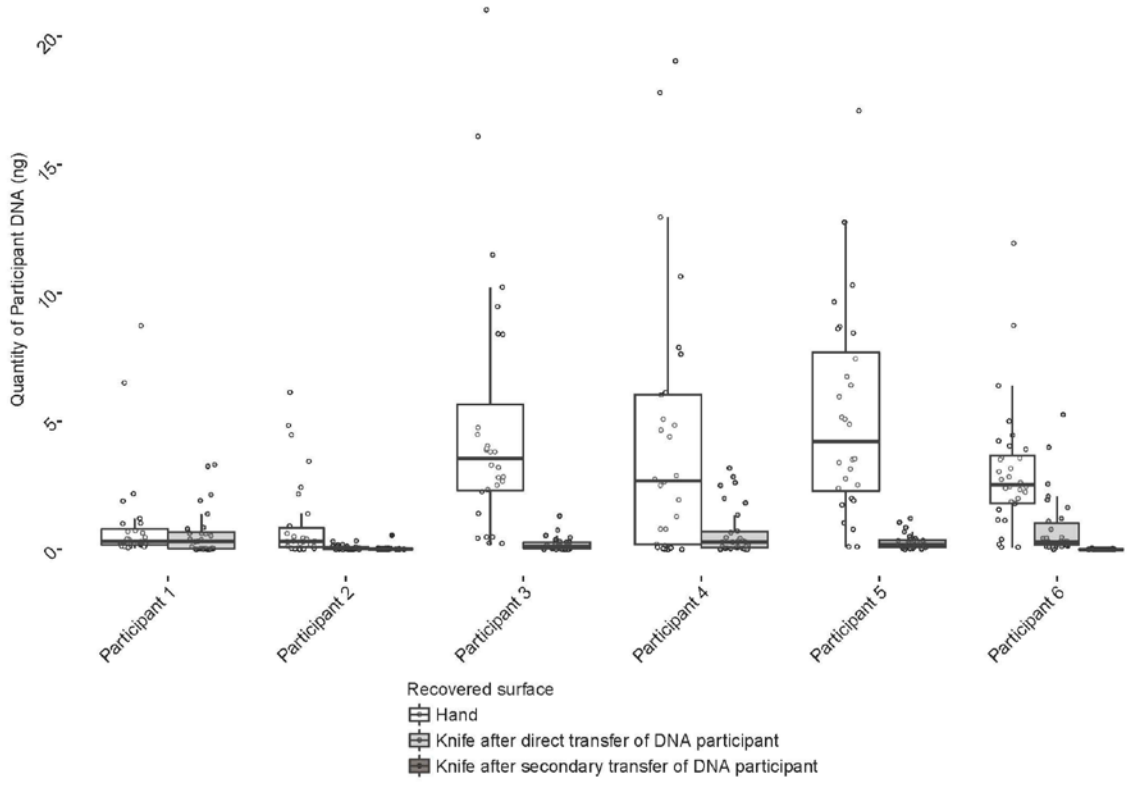

Figure 6: Boxplots of the DNA quantities for each participant recovered on the participant's hand, on the knife handle after primary transfer and secondary transfer (only for participants 2 and 6). Each dot corresponds to the corresponding quantity obtained after each experiment

Table 4: Summary statistics of the quantities of participants' DNA on participant's hands, participants' DNA on the knife handle after direct transfer and participants' DNA on the knife handle after secondary transfer respectively for each participant.

POI's quantity of DNA (ng)		Min	0.05 percentile	Median	Mean	SD	0.95 percentile	Max
Participant 1	On hands	0.06	0.10	0.31	1.02	1.96	4.98	8.72
	After direct transfer	0.00	0.00	0.31	0.63	0.93	2.82	3.30
Participant 2	On hands	0.00	0.02	0.38	1.06	1.62	4.74	6.12
	After direct transfer	0.00	0.00	0.04	0.07	0.09	0.29	0.33
	After secondary transfer	0.00	0.00	0.01	0.04	0.11	0.05	0.55
Participant 3	On hands	0.23	0.31	3.54	4.96	4.95	14.48	21.03
	After direct transfer	0.00	0.00	0.13	0.21	0.28	0.67	1.30
Participant 4	On hands	0.00	0.00	2.68	4.39	5.23	16.01	19.04
	After direct transfer	0.00	0.00	0.28	0.70	0.97	2.73	3.17
Participant 5	On hands	0.10	0.34	4.21	5.21	4.06	11.89	17.10
	After direct transfer	0.00	0.02	0.18	0.29	0.30	0.96	1.20
Participant 6	On hands	0.08	0.22	2.53	3.15	2.42	7.67	11.93
	After direct transfer	0.00	0.10	0.29	0.84	1.23	3.34	5.25
	After secondary transfer	0.00	0.00	0.00	0.00	0.01	0.02	0.04

The standard deviation (SD) observed on the quantity of DNA generally reduces for each donor when we move from hand, to primary transfer and subsequently to secondary transfer.

We have observed no obvious relationship between the quantity of POI's DNA recovered on the hand and the quantity of transferred DNA. For example, participant 3 has, in general, a large quantity of DNA on his hand compared to the other participants. However, this donor transferred a very small quantity of DNA on the knife handle through primary transfer. On the contrary, small quantities of DNA are recovered from the hand of the participant 1, compared to other participants, but he transferred a large part of that DNA on the handle. Hence, for primary transfer, there is no fixed transfer proportion (TP) for all participants as shown in Table 5. Participant 1 and participant 6 proportionally left more of their DNA than the other

participants. They both gave an average of 20% on the knife handle, whereas, participant 3 for example transferred an average of 7% only.

Primary TP	Mean	SD
Participant 1	0.20	0.25
Participant 2	0.13	0.19
Participant 3	0.07	0.14
Participant 4	0.14	0.23
Participant 5	0.11	0.19
Participant 6	0.20	0.25

Table 5: Means and standard deviations computed for the primary transfer proportion for each participant.

Figure 7 illustrates these differences in TPs between participants. Each boxplot represents 1000 data points that have been randomly selected from a Beta distribution with parameters set from the mean and the standard deviation specified in Table 5.

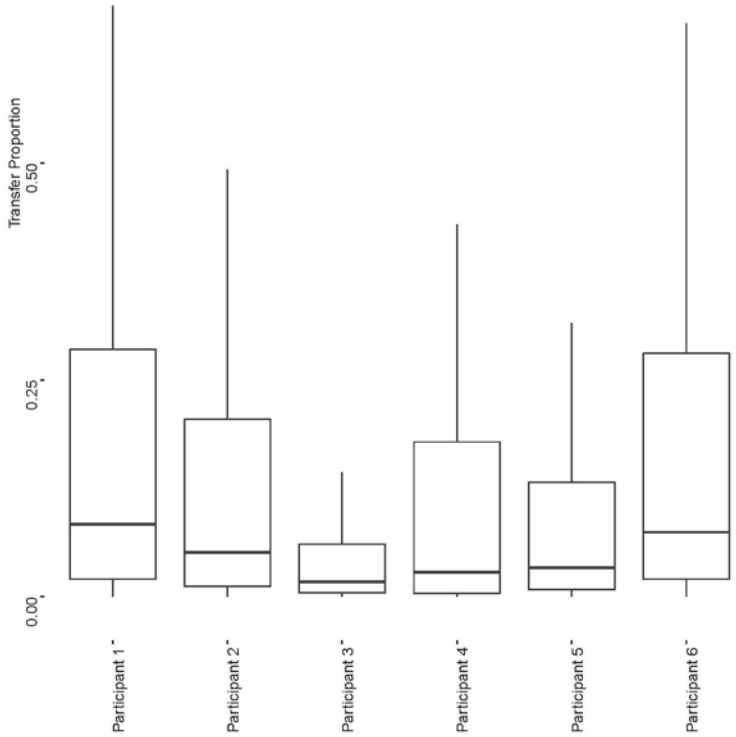

Figure 7: Boxplots of 1000 direct transfer proportions of DNA simulated from each participant corresponding distribution.

The proportions of transfer can also be computed for secondary transfers (against the quantity on the hand) as shown in Table 6. The mean secondary transferred TP for participant 2 has an average of 1% whereas it is 3% for participant 6.

Secondary TP	Mean	SD
Participant 2	0.01	0.03
Participant 6	0.03	0.11

Table 6: Means and standard deviations computed for the secondary transfer proportion for the two participants

When these proportions are compared to the primary TP, they differ largely for the two participants. As before, these differences are illustrated graphically by re-sampling from the respective distributions (Figure 8).

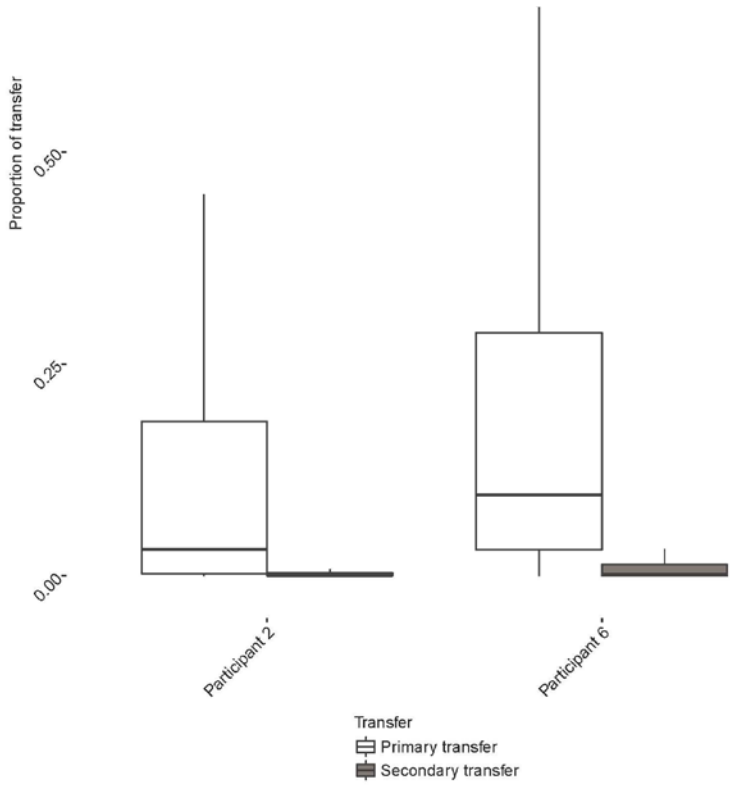

Figure 8: Boxplots of the direct transfer proportions and secondary transfer proportions for each participant (1000 data points randomly generated from the corresponding distributions).

Discussion and conclusion

Comparison of the results with other studies.

On the hands of the participants to this study, we observed a total quantity of DNA between 0 and 21 ng, made in majority of the donor's DNA with, on average, less than 8% of non-self DNA. That percentage of non-self-DNA can vary substantially between donors. Take

participant 1, for example, the total quantity of DNA obtained from his hand is contributed by only 70% of his own DNA. These quantities can be comparable to those obtained by Szkuta *et al.* [14]. They observed between 0.1 and 85.5 ng of DNA on 70 hands.

McColl *et al.* [15] observed higher quantities between 0 and 585ng. However, they studied a larger number of hands (120 hands), and that could explain the difference. However, the percentage of non-self DNA recovered on hands is similar to the percentage observed in the present study (an average of 8.5% of non self DNA and maximum less than 30%).

Following the primary transfers of DNA on the knife handles, we observed a total quantity of DNA ranging from 0 to 5ng. We observed (Table 2) that on average less than 8% of the total quantity originates from a different contributor than the donor. There are variations between donors with regards to the non-self DNA present on their hands and transferred on the handle. For example, 40% of the total quantity of DNA on the handles used by participant 1 comes from someone else. These results are in line with those obtained by Goray *et al*. [5], Samie *et al.* [10] and Szkuta *et al*. [14]. They reported recovered quantities of DNA between around 0 and 5ng [5], 0 and 5 ng [10] and 0 and 7ng [14].

We note however that other researchers have reported higher quantities of DNA transferred on knife handles, namely:

- Meakin *et al.* [11]: They reported between 3 and 10 ng of total DNA recovered on the knife handles with less than 3% of non-self DNA for 3 donors and 25% with one donor.
- Butcher *et al.* [12]: They reported between 1 and 10 ng of total DNA recovered on the knife handles with less than 16% of non-self DNA.

In our opinion, the differences observed may be due to the fact that in our experiments (as in others [5, 10, 14]), the surfaces were cleaned before each experiment, whereas in [11, 12] the handles were swabbed after the knife being used regularly for some time. In these conditions we could expect an accumulation of DNA, hence a higher yield.

Novelty of the results.

We set out three objectives to this study that we recall here:

(1) to characterize the distribution of the quantity of DNA observed on the hands of individuals and transferred on surfaces either through primary or secondary transfer;

(2) to assess if deconvolution of the DNA profiles is required to estimate the quantity of DNA of the POI and;

(3) to test if the transfer proportion (quantity transferred on the surface over the initial quantity on the hand) is similar across individuals and can be used to predict the quantity of transferred DNA.

We were able to characterise for 6 individuals the distributions of the quantity of DNA observed on their hand and subsequently transferred on a knife handles either through primary contact or by a secondary mechanism. As already mentioned we have recorded very different quantities of DNA recovered on hands and on the knife handles after direct transfer for each participant and between participants. One person could then be judged as “good shedder” overall, but when considering a single experiment, that same person could be a very “poor shedder”. The shedder status, or for a better word the “shedding ability”, is better described by a distribution than by a single mean quantity. Our observations question the use, for a given individual, of a fixed label such as “good shedder” or “bad shedder”, irrespectively of time and circumstances. We propose alternatively to characterise a donor’s shedding ability by the parameters (mean and standard deviation) of the distribution of his/her quantities of DNA. Hence, when assessing the probability of observing a given quantity of DNA, for a given donor, that whole distibution should be accounted for and not only its mean (or a single shedder status label associated to it).

Regarding the second objective and the need to apply a deconvolution technique to mixed DNA profiles, we noted that, for each participant, both quantities (total DNA and POI’s DNA only) do not differ very much for primary transfers. It means that the total quantity of DNA can be used to study primary transfer without resorting to a mixture deconvolution process. However, in the experiments involving secondary transfers, we observed a marked difference between the total quantity of DNA and the quantity of DNA corresponding to the POI. It shows, as expected, that the total quantity of DNA left on the surface is dominated by the DNA coming from the handler. The POI’s DNA, who, in the secondary transfer scenario, did not touch the object but only the hand of the handler, is a minor contributor to the recovered mixed DNA profiles. Hence, the deconvolution is required when considering secondary transfers.

Regarding the third hypothesis postulating constant transfer proportions (TP) between donors, we have shown that TP may vary between participants and will depend on the type of the transfer (primary versus secondary). It means that we cannot simply resort to a quantification of DNA on one hand to infer the shedder status and assess what will be transferred on a surface. Ideally, the measure of the distribution of the quantity of DNA should be carried out for a given person depositing on a given target surface following the alleged transfer mechanism.

We conclude in saying that in order to properly evaluate a given quantity of DNA considering different activities, the whole variation of DNA quantity should be accounted for. This can be done by using or measuring empirically the appropriate underpinning distribution that will be dependant on the donor, the substrate and the transfer mechanism.

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