

1 **Estimating the quantity of transferred DNA in primary and secondary transfers**

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9 **Declarations of interest: none**

10 **Highlights**

11

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

16 **Novelty Statement**

- 17 • We have three objectives: to characterize the distribution of the quantity of DNA
18 observed on the hands and directly or secondarily transferred on surfaces; to assess if
19 deconvolution of the DNA profiles is required to estimate the quantity of DNA of the
20 POI; to test if the transfer proportion is similar across individuals and can be used to
21 predict the quantity of transferred DNA.
- 22 • We propose, when assessing the probability of observing a given quantity of DNA, for
23 a given donor, that whole distribution should be accounted for.
- 24 • We show that the total quantity of DNA can be used to study primary transfer without
25 resorting to a mixture deconvolution process. However, the deconvolution is required
26 when considering secondary transfers.

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

29 **Introduction**

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

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

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

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

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

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

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

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

81 **Methodology**

82 *Transfer Experiments*

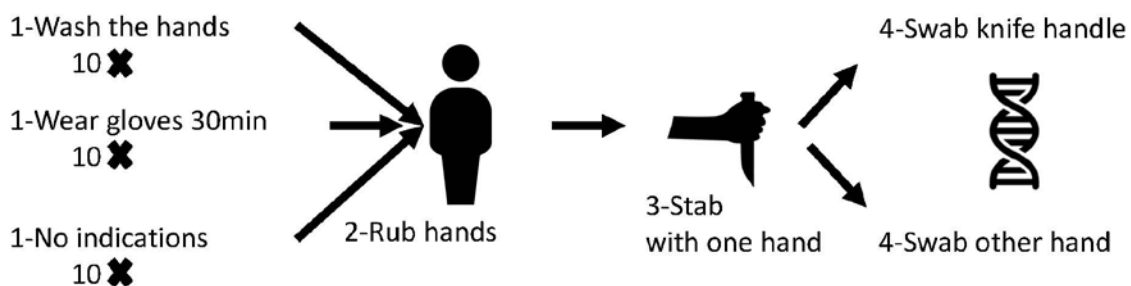
83 Six consenting participants, three men and three women, were randomly selected to deposit
84 contact DNA following activities of primary or secondary transfer.

85 For primary transfer, each participant was asked to rub their hands during around five seconds
86 [13] with a view to redistribute surface DNA evenly on both of them [16], then took a knife
87 (Stainless Steel, X50 Cr Mo V15) handle with their usual hand and, immediately after, stab

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

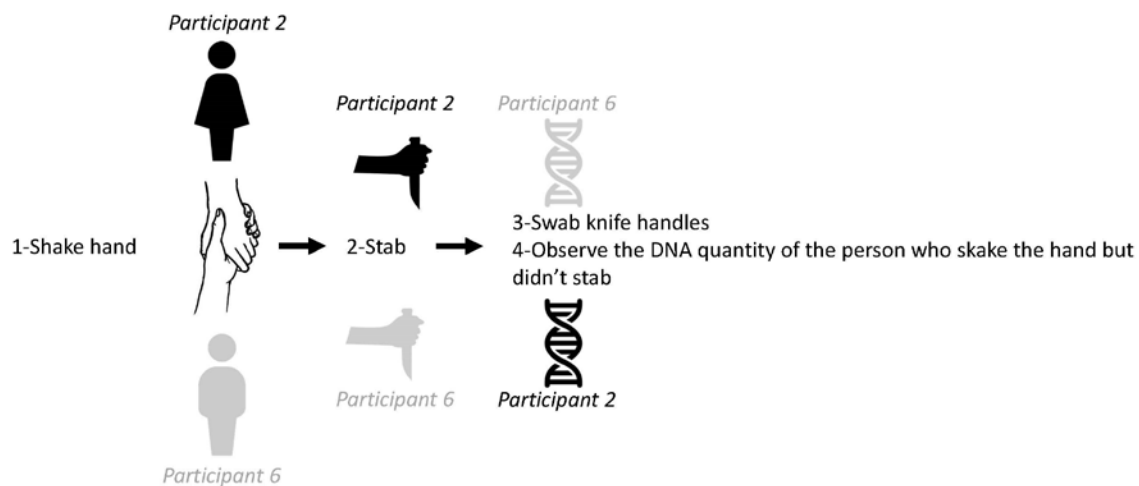
91 The duration of the contact, the type of contact and the force of the stabbing were not
92 specified in order to simulated conditions as closed as possible than casework. The ballistic
93 soap allowed mimicking the physical properties of a human body. This direct transfer on the
94 knife handle is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

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



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

115 To study secondary transfer, only two participants were chosen in the light of the first set of
116 experiments. Based on their mean quantities of transferred DNA, participant 2 and participant
117 6 have shown to be the “best” and the “worst” DNA donor respectively (See Figure 3, Figure
118 4, Figure 7, Table 1 and Table 2). Two identical knives, one for each participant, were used
119 for all their experiments. Before each experiment however, the knife was thoroughly cleaned,
120 using bleach and ethanol. Both participants were first asked to shake hands and then to stab
121 the ballistic soap with the knife. No indication on the duration of the handshake was given to
122 the participants in order to mimic real life conditions as closely as possible, the contact though
123 didn’t exceed by few seconds. The entire surface of the two knife handles were then swabbed
124 for DNA just after the stabbing using one moist COPAN’s FLOQSwab™, following our
125 laboratory procedure. Thirty experiments were performed for each of the two participants
126 (leading to 60 experiments in total). Experiments were subsequently performed with a
127 minimum delay of five minutes between them. This experimental design is illustrated in
128 Figure 2.



129

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

133

134

135 *Quantification of DNA*

136 DNA was extracted from the swabs using a combination of two kits: QIAshredder and
137 QIAamp DNA mini kit from Qiagen, concentrated to a final volume of 25µL with microcon®
138 30 spin column. Quantifications were performed directly following the DNA extraction using
139 the Investigator® Quantiplex kit from Qiagen on Rotor-Gene® Q. DNA was then amplified at
140 30 cycles using 10 µL of DNA extract per sample and the NGM SElect (Applied
141 Biosystem™-Thermofisher) kit with a PCR system 9700 (Applied Biosystem™), analyzed on
142 a 3500 Series Genetic Analyzers (Applied Biosystem™-Thermofisher Scientific) coupled
143 with GeneMapper1IDX Software (Applied Biosystem™-Thermofisher Scientific). The kits
144 were used as per manufacturer's instructions.

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

152 *Deriving the parameters of the transfer proportion*

153 For primary transfers, the parameters of the distribution for the log10 of the transfer
154 proportion (log10(TP)) for each individual is obtained by combining the results of the initial
155 quantity of DNA on hands (Qi) and the results of the quantity of DNA observed on the knife
156 handle (Qf), under the assumption that both Qi and Qf are Normally distributed [17]. When
157 transformed in log10, the parameters of the distribution for log10(TP) are obtained as follows
158 [18]:

159

160
$$\text{mean}(\log_{10}(\text{TP})) = \text{mean}(\log_{10}(\text{Qf})) - \text{mean}(\log_{10}(\text{Qi}))$$

161 and

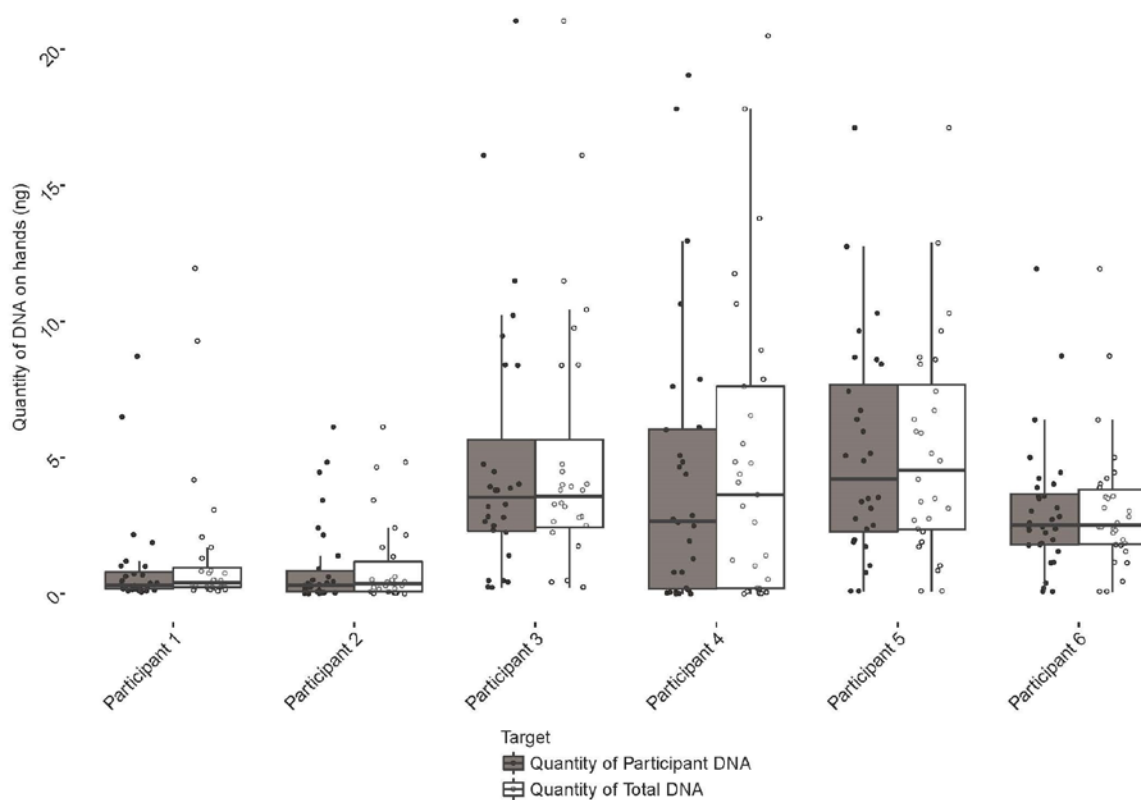
162
$$\text{SD}(\log_{10}(\text{TP})) = \text{SD}(\log_{10}(\text{Qf})) + \text{SD}(\log_{10}(\text{Qi})) - 2 * \sqrt{\text{SD}(\log_{10}(\text{Qf})) * \text{SD}(\log_{10}(\text{Qi}))}$$

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

166 Results

167 *Quantity of DNA present on the hand and following primary transfer*

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



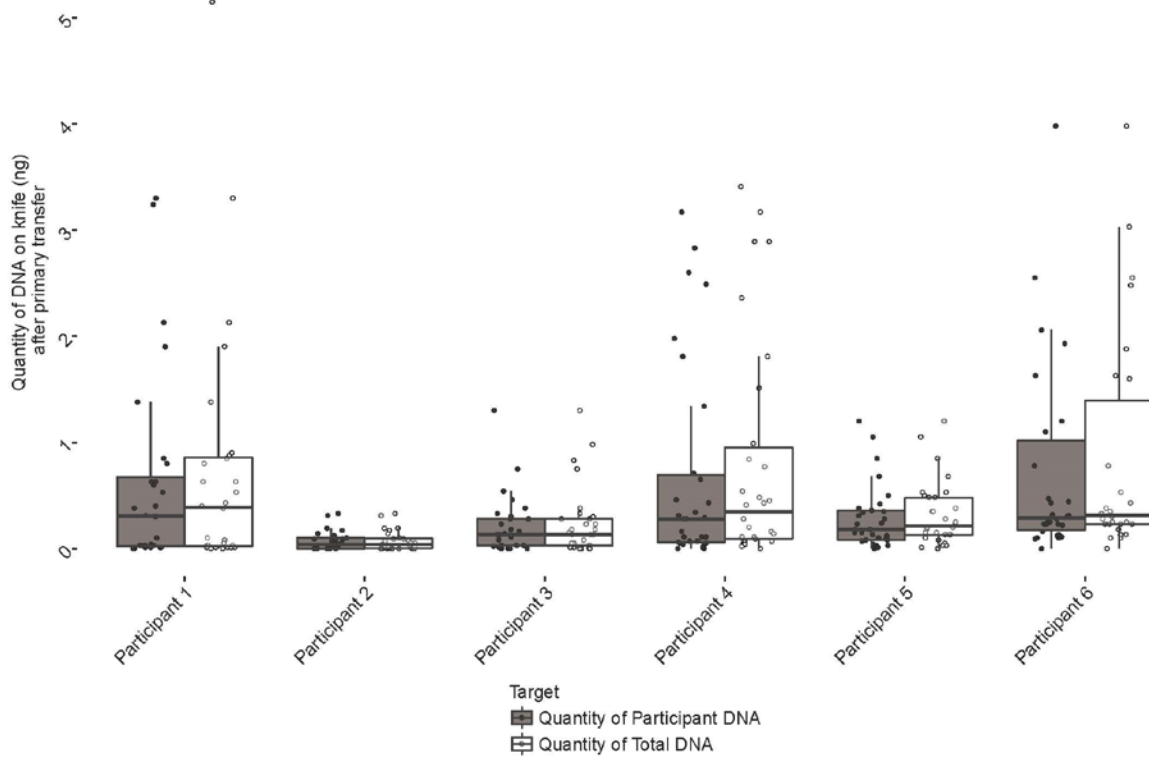
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173 *Figure 3: Boxplots of the total quantity of DNA (and the quantity corresponding to each participant)*
174 *recovered from the hand. Each dot corresponds to the corresponding quantity obtained after each*
175 *experiment.*

176

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179

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

183 A large variation of the quantity of DNA collected on participants' hand (Table 1) and the
 184 quantity recovered from the knife handle after a direct transfer (Table 2) is observed between
 185 participants. Indeed, the mean value of total DNA range from 1 ng to 5 ng. A large variation
 186 for each participant is also observed as can be seen from the ranges (max-min) of DNA
 187 quantities. For participant 1 for example, between 0 and more that 5ng of DNA can be
 188 recovered after directly handling the knife handle depending on the experiment (and between
 189 0 and more than 11ng directly from his hand).

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195 *Table 1: Summary statistics of the quantities of total DNA and of the participant's DNA (obtained*
 196 *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

197

198 *Table 2: Summary statistics of the quantities of the total DNA and of participant's DNA (obtained*
 199 *following mixture deconvolution) recovered on the knife handle after the participant directly stabbed a*
 200 *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

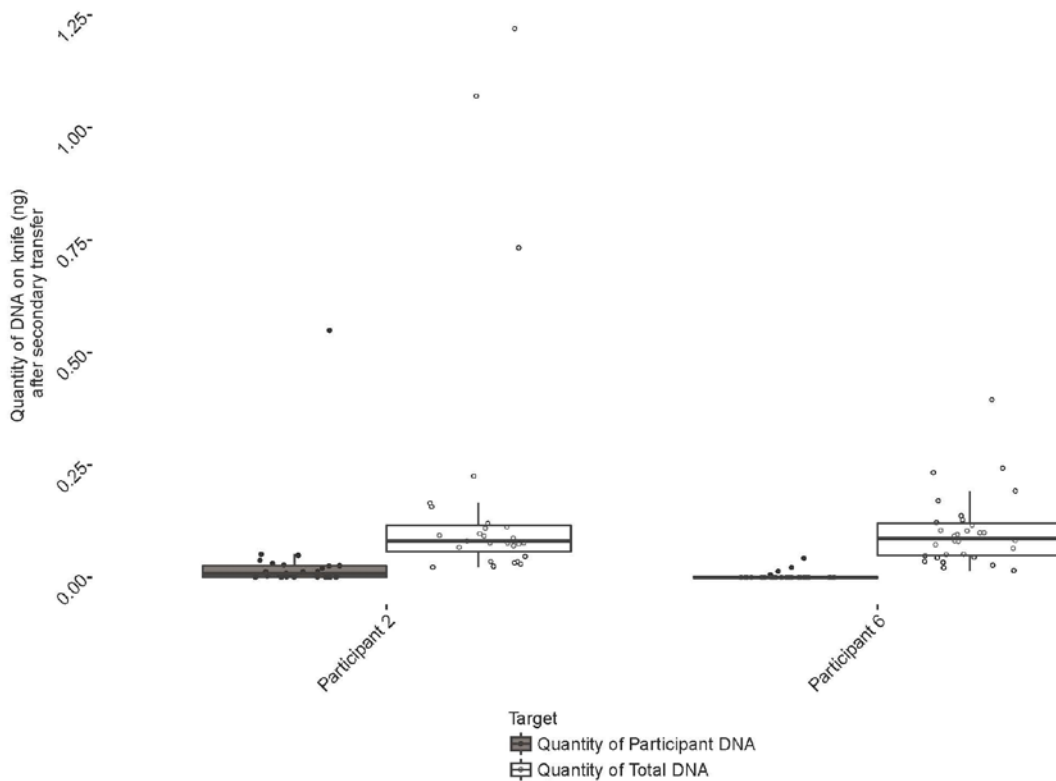
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202

203 *Quantity of DNA following secondary transfer*

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

211



212

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

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

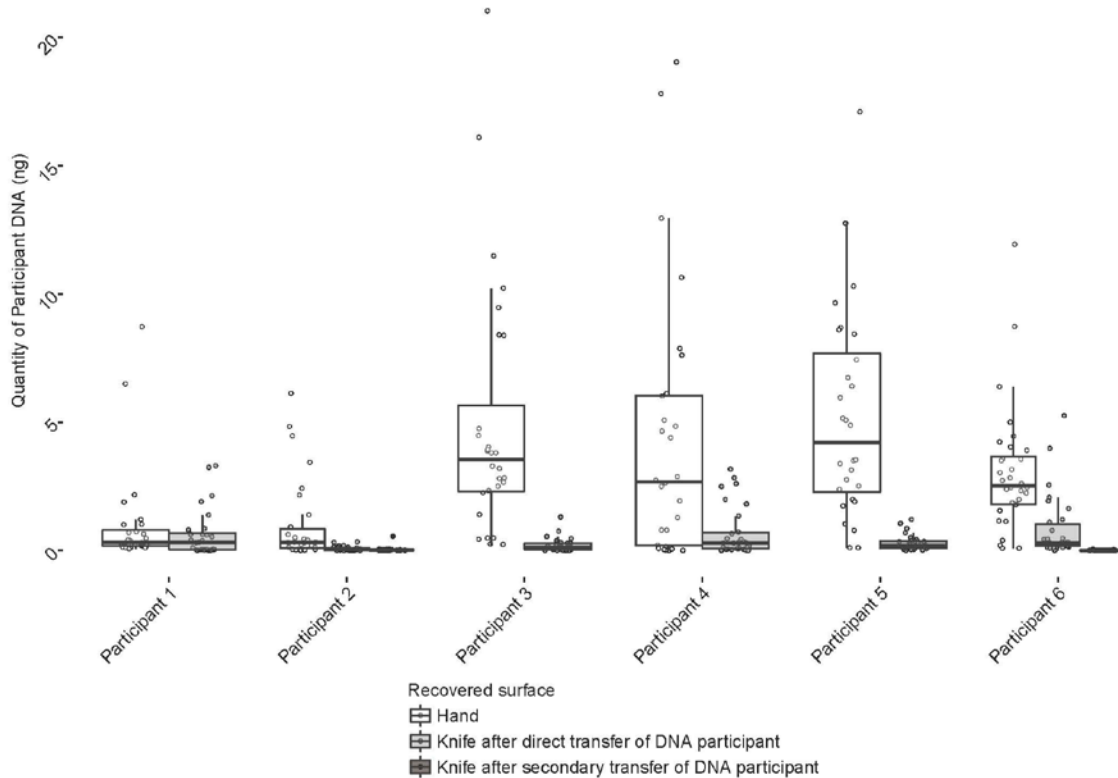
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The quantity of DNA recovered on the knife handle after secondary transfer (ng)		Min	0.05 percentile	Median	Mean	SD	0.95 percentile	Max
Participant 2	Total DNA	0.02	0.03	0.08	0.19	0.31	0.97	1.22
	Participant 2 DNA	0.00	0.00	0.01	0.04	0.11	0.05	0.55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

221

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

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



227

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

231 *Table 4: Summary statistics of the quantities of participants' DNA on participant's hands,*
 232 *participants' DNA on the knife handle after direct transfer and participants' DNA on the knife handle*
 233 *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

234

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

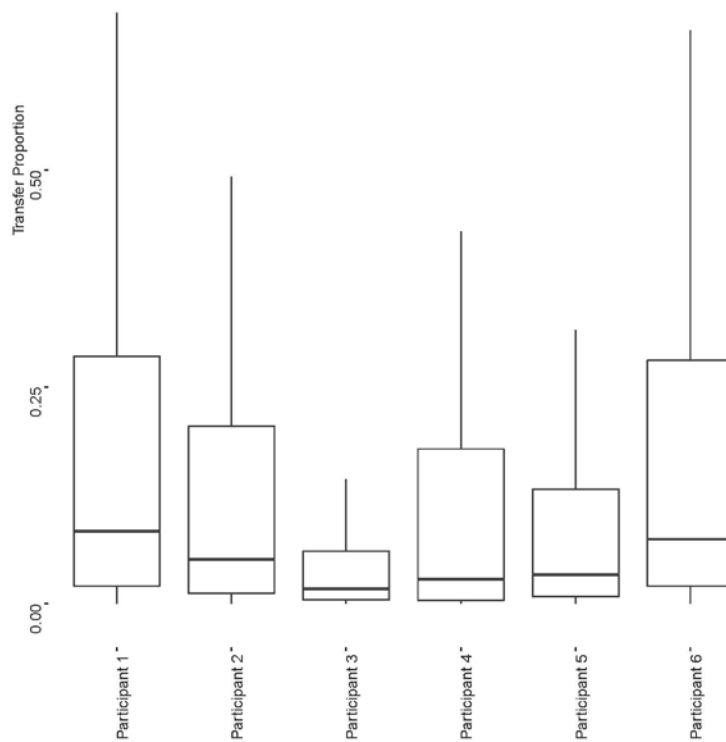
237 We have observed no obvious relationship between the quantity of POI's DNA recovered on
 238 the hand and the quantity of transferred DNA. For example, participant 3 has, in general, a
 239 large quantity of DNA on his hand compared to the other participants. However, this donor
 240 transferred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

245 participants. They both gave an average of 20% on the knife handle, whereas, participant 3 for
 246 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

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

249 Figure 7 illustrates these differences in TPs between participants. Each boxplot represents
 250 1000 data points that have been randomly selected from a Beta distribution with parameters
 251 set from the mean and the standard deviation specified in Table 5.



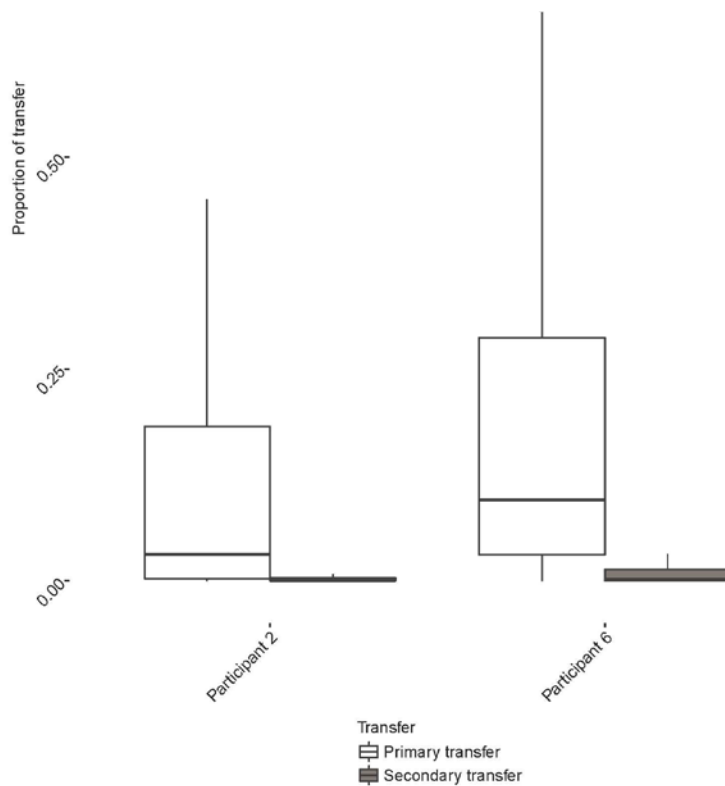
252
 253 *Figure 7: Boxplots of 1000 direct transfer proportions of DNA simulated from each participant*
 254 *corresponding distribution.*

255 The proportions of transfer can also be computed for secondary transfers (against the quantity
 256 on the hand) as shown in Table 6. The mean secondary transferred TP for participant 2 has an
 257 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

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

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



264

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

267 **Discussion and conclusion**

268 *Comparison of the results with other studies.*

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

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

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

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

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

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

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

298 *Novelty of the results.*

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

- 300 (1) to characterize the distribution of the quantity of DNA observed on the hands of
301 individuals and transferred on surfaces either through primary or secondary transfer;
- 302 (2) to assess if deconvolution of the DNA profiles is required to estimate the quantity of
303 DNA of the POI and;
- 304 (3) to test if the transfer proportion (quantity transferred on the surface over the initial
305 quantity on the hand) is similar across individuals and can be used to predict the
306 quantity of transferred DNA.

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

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

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

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

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