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1 **The efficiency of DNA extraction kit and the efficiency of recovery techniques to release**  
2 **DNA using flow cytometry.**

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12 **Highlights**

- 13 • About 61% of DNA coming from 100 cells, keratinocytes, is recovered using the  
14 Lyse&Spin-QIAamp DNA Mini Kit.
- 15 • About 23% of DNA is recovered using the combination of the QIAshredder and  
16 QIAamp DNA Mini kit and the Microcon® 30 column.
- 17 • The extraction efficiencies of the Lyse&Spin-QIAamp DNA Mini Kit obtained by two  
18 different laboratories are similar.
- 19 • The FLOQSwab™ allows releasing about 97% of the cells attached to it.

20 **Novelty Statement**

21 This research was carried out in the context of evidence evaluation considering activity level  
22 propositions when the findings are a low level of DNA obtained from touched surfaces. In such  
23 cases, knowledge of the DNA extraction kit efficiency and the efficiency of instrument to  
24 release DNA is required to evaluate the significance of DNA quantity results.

25 Only a few studies dealt with these efficiencies. However, in these studies, the sole efficiency  
26 of the swab alone or the sole efficiency of the extraction kit alone usually remains unknown.  
27 This study aims at showing how the efficiency of DNA extraction kits and the yield of release  
28 of cells from swabs can be measured.

29 We also reports on the impact of the laboratory, since DNA extraction using Investigator®  
30 Lyse&Spin Basket-QIAamp DNA Mini kit from Qiagen were performed by two different  
31 persons, operating manually, from two different laboratories.

## 32 **Abstract**

33 This research was carried out in the context of evidence evaluation considering activity level  
34 propositions when the findings are a low level of DNA obtained from touched surfaces. In such  
35 cases, knowledge of the extraction efficiency of the kit used by the laboratory is required to  
36 evaluate the significance of DNA quantity results.

37 Flow cytometry has been used to investigate and measure DNA extraction efficiency. Flow  
38 cytometry allows the scientist to obtain a fixed number of cells, so that the initial quantity of  
39 DNA, before performing any extraction, is known. Small amounts of DNA compatible with the  
40 quantity of DNA left by a hand touch were obtained using a number of 100 cells.

41 We report on the extraction efficiency of two commercial DNA extraction kits (QIAshredder-  
42 QIAamp DNA Mini Kit using Microcon® 30 column, and Investigator® Lyse&Spin Basket-  
43 QIAamp DNA Mini Kit) used to extract and purify low quantities of DNA. The impact of the  
44 laboratory's performance on the extracted quantity has been assessed on the best performing  
45 kit (Investigator® Lyse&Spin Basket-QIAamp DNA Mini Kit). This research also provides  
46 data on the efficiency of a swab (FLOQSwab™ from COPAN) to release cells.

47 The results show that for the Investigator® Lyse&Spin Basket-QIAamp DNA Mini Kit, about  
48 61% of DNA coming from the 100 cells is recovered with no difference between the extracts  
49 obtained by two different laboratories. For the QIAshredder-QIAamp DNA Mini Kit, only  
50 about 23% of the initial quantity of DNA is recovered. We also show that the FLOQSwab™  
51 releases about 97% of the cells attached to it.

52 Flow cytometry proves to be a very efficient technique to obtain adequate estimates of DNA  
53 extraction efficiency.

54 **Keywords:** Extraction efficiency, Flow cytometry, DNA swabs, DNA evidence evaluation.

55

56

## 57 **Introduction**

58 In forensic investigations, low levels of DNA are often recovered from touched surfaces. As  
59 recommended by the ENFSI Guideline for Evaluative Reporting in Forensic Science [1], the  
60 evaluation of these DNA traces should be carried out using activity-level propositions which  
61 involves a relative assessment of the expected quantities of recovered DNA under the alleged  
62 activity depending on the propositions of interest. In order to do so, the quantity of the recovered  
63 DNA plays an important role and the efficiency of DNA extraction kit is one of the variables  
64 that should be considered [2]. Without knowledge of the extraction efficiency of the kit used  
65 by the laboratory, a meaningful evaluation of the findings would not be possible for DNA  
66 expertise or research. This study aims at showing how the efficiency of DNA extraction kits  
67 and the yield of release of cells from swabs can be measured.

68 Only a few studies dealt with the efficiency of extraction kits for traces of low levels of DNA  
69 [3, 4]. In Browlow *et al.* [3] the obtained measure of extraction efficiency jointly considered  
70 the type of surface and the efficiency of the swab used to collect and then release the cells and  
71 DNA; however, the sole efficiency of the extraction kit alone remains unknown because DNA  
72 traces were deposited on a surface. In Wood *et al.* [4], the efficiency of recovery techniques  
73 was evaluated from recovery up to the release of cells and DNA. While this considers the  
74 ability of the DNA swabs to release cells and DNA, which is a variable that affects the overall  
75 efficiency of the DNA extraction process, the efficiency of the extraction kit itself remains  
76 unknown since it combines the extraction efficiency of the kit and that of the release of cells  
77 and DNA. To measure its specific efficiency of extraction, one needs to know the initial  
78 quantity of DNA to be extracted. Flow cytometry is cited by Butts [5] as the most appropriate  
79 method to select a low number of cells to be used as the starting material for the measure of the  
80 extraction yield. In this research, we used flow cytometry to prepare constant number of cells  
81 that will be directly submitted to the extraction procedures or deposited on swabs.

82 Extraction kits are used by different persons from different laboratories, operating manually or  
83 using automated platforms, which influences the extraction efficiency. The impact of the  
84 laboratory is reported as well.

85 This study has three objectives. The first is to measure the extraction efficiency of two  
86 commercial DNA extraction kits (Investigator® Lyse&Spin Basket-QIAamp DNA Mini kit,  
87 and QIAshredder-QIAamp DNA Mini kit from Qiagen with Microcon® 30 spin column) used

88 to extract and purify low quantities of DNA based on initial quantities of DNA obtained using  
89 flow cytometry. The second is to study the impact of the laboratory on the yield offered by the  
90 best performing kit (Investigator® Lyse&Spin Basket-QIAamp DNA Mini kit from Qiagen).  
91 The last is to report on the efficiency of a swab (FLOQSwab™ from COPAN) to release cells  
92 and show how to obtain it by the combined usage of a swab and an extraction kit (QIAshredder-  
93 QIAamp DNA Mini kit from Qiagen with Microcon® 30 spin column).

## 94 **Methodology**

### 95 *Type and number of cells*

96 The method adopted here starts from a given and known number of cells obtained by cell  
97 cytometry. The cells were selected using the P658282Z3001 FACS Aria IIu cytometer with  
98 FACSDiva 8.0.1 version application.

99 The type of cells chosen for this study is adult keratinocytes, which are typical of skin cells.  
100 Epidermal keratinocytes cell culture (Human Epidermal Keratinocytes – Neonatal) from Lonza  
101 was performed according to manufacturer’s instructions. In order to avoid cell differentiation,  
102 cells were passed before they reached 80% of confluence and we minimized the doubling  
103 population. Cells were sorted after two population doublings. Propidium Iodide staining was  
104 used to sort the nucleated, living, cells.

105 To select the number of cells representing a quantity of DNA obtained when touching a surface,  
106 different numbers of cells were tested. First, four samples of 50, 100, 500 and 5000 cells were  
107 prepared respectively twice, then directly introduced into a microtube of 1.5mL containing  
108 180µL of a tissue lysis buffer (ATL buffer from Qiagen). Cell concentration was around  
109 1million/ml and generates a flow rate of 900 events/sec. Given this concentration, the “Single-  
110 cell” as the mode of precision used was chosen.

111 The extractions of these eight samples were performed using the combination of two kits:  
112 QIAshredder and QIAamp DNA Mini kit from Qiagen, concentrated to a final volume of 25µL  
113 with Microcon® 30 spin column. To simplify, these kits will be denoted as QIAshredder-  
114 QIAamp DNA Mini kit. The quantities of results obtained on the four numbers of cells are  
115 given in Table 1.

116

117 *Table 1: Table representing the average extracted quantity of respectively 50, 100, 500, 5000 cells*

Number of cells obtained by cell cytometry [cell]	50	100	500	5000
The average quantity of DNA obtained using the QIAshredder-QIAamp DNA Mini kit [pg]	125	250	1200	15000

118 One-hundred cells have been selected for the experiments as it led to an amount of around 125  
119 pg of DNA, which corresponds to the average amount of DNA obtained in a previous study  
120 focusing on DNA traces, obtained when touching a surface [6].

### 121 *Extraction efficiency of the kits*

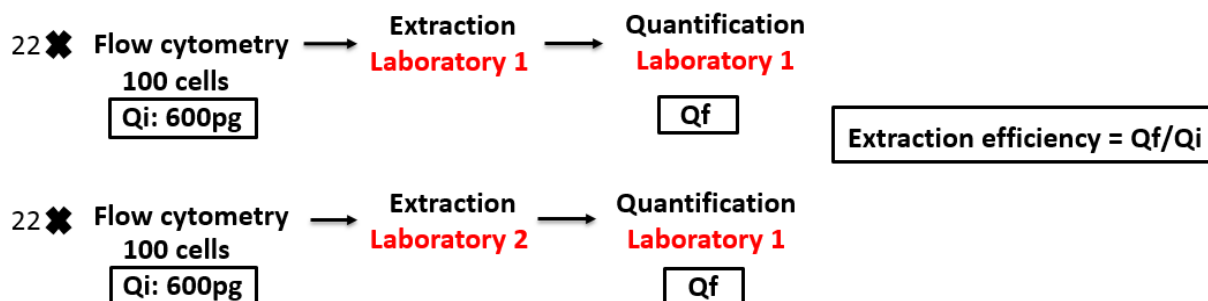
122 For each kit, extractions were made based on an initial preparation of 100 cells. Cell  
123 concentration was low, generating a flow rate of around 20-40 events/sec. The “Purity”  
124 precision mode was selected in order to increase the probability where a cell of interest could  
125 be sorted.

126 The cells were directly introduced into each of the baskets containing 60µl of Phosphate  
127 buffered saline (PBS) of pH 7.4, allowing the cells to be kept intact. The kits were used  
128 following manufacturer’s instructions. Quantifications were performed directly following the  
129 DNA extraction using the Investigator® Quantiplex kit from Qiagen on Rotor-Gene® Q  
130 according to the manufacturer’s protocols. 30 extractions were performed using the  
131 QIAshredder-QIAamp DNA Mini kit, following the body fluid protocol, concentrated to a final  
132 volume of 25µL with Microcon® 30 spin column, whereas 22 extractions were made with the  
133 Investigator® Lyse&Spin Basket-QIAamp DNA Mini kit from QIAGEN with a final volume  
134 of 60µL without using microcon® 30 spin column, due to laboratory constraints. The difference  
135 between the two kits is the use of Spin basket for the Investigator® Lyse&Spin Basket-QIAamp  
136 DNA Mini kit from QIAGEN instead of QIAshredder column and Microcon® 30 spin column.

### 137 *Effect of the laboratory*

138 The kit which was proven to be the best performing kit is the Investigator® Lyse&Spin Basket-  
139 QIAamp DNA Mini kit from Qiagen. To study the impact of the laboratory’s performance on  
140 the yield offered by this kit, the extractions were performed manually by two operators in two  
141 different laboratories (Figure 1). One-hundred cells were selected, using the “Purity” precision  
142 mode, then directly introduced into each of the 44 Lyse&Spin baskets containing 60µl of

143 Phosphate buffered saline (PBS) of pH 7.4, allowing the cells to be kept intact. Twenty-two  
144 extractions were made by each operator, with a final volume of 60µL. All the quantifications  
145 were performed together in the same run at the same time following the DNA extraction which  
146 was made two days after the flow cytometry.



147

148 *Figure 1: Illustration of the method used to study the impact of the laboratory on the yield offered by*  
149 *Investigator® Lyse&Spin basket-QIAamp DNA Mini kit. Qi is the initial quantity of DNA to be*  
150 *extracted, whereas Qf is the final extracted quantity of DNA.*

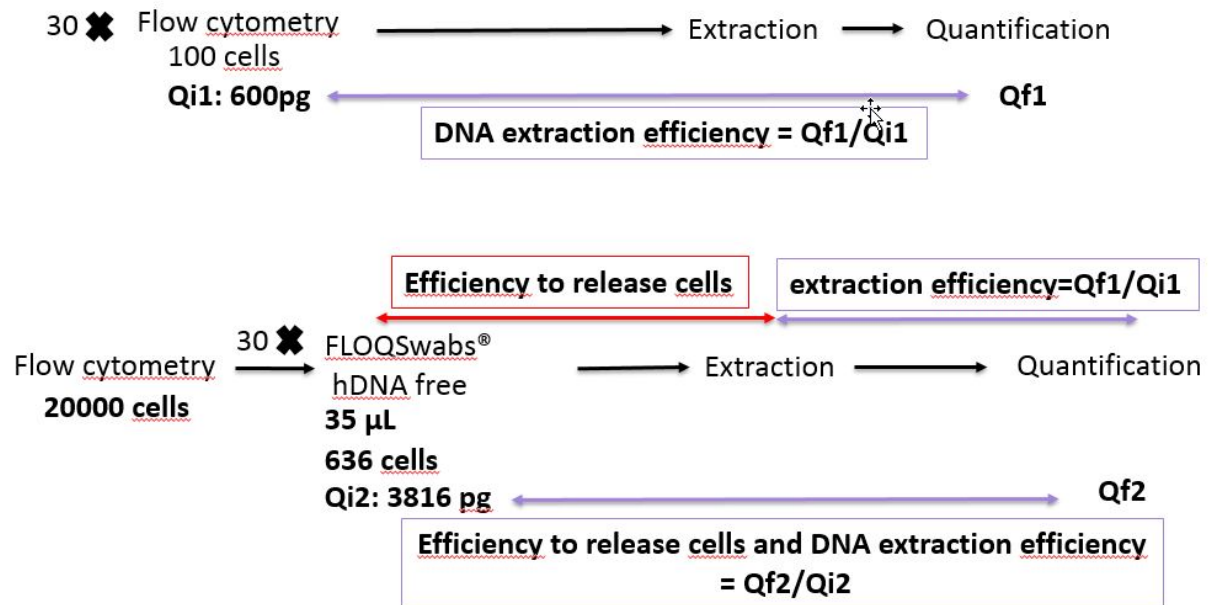
151 *Release of cells by the DNA swab*

152 Figure 2 describes the method used to study the efficiency of the FLOQSwab™ to release cells.  
153 The measure of the extraction efficiency for the QIAshredder-QIAamp DNA Mini kit has been  
154 already measured (see *Extraction efficiency of the kits*). In the following experiment, we will  
155 measure the joint yield (swab cells release and DNA extraction).

156 To measure it, 20000 cells were introduced into a microtube of 1,5mL containing 1.1 mL of  
157 PBS, to avoid the destruction of the plasma membranes. Because of the technical impossibility  
158 to directly deposit cells on the swab, the microtube was mixed by vortexing and 35µL (636  
159 cells) was pipetted on each 30 FLOQSwab™. To take into account the possible loss of cells  
160 being retained by the swab, the selected number of cells is higher than the number (100) used  
161 to study the extraction efficiency.

162 Swabs were dried during the afternoon before performing the DNA extraction using the  
163 QIAshredder-QIAamp DNA Mini kit. A concentrated final volume of 25µL was obtained at  
164 the end of the extractions using Microcon® 30 spin column. These 30 samples allowed for  
165 obtaining a joint measure of efficiency to release cells combined with the efficiency of the DNA  
166 extraction kit.

167



168

169 *Figure 2: Illustration of the method used to obtain the extraction efficiency of the kit and a joint*  
 170 *measure of efficiency to release cells combined with the efficiency of the DNA extraction kit (in*  
 171 *purple) in order to obtain the efficiency of the sampling device to release cells (in red).*

172 *Calculating efficiency*

173 The efficiency is measured by the ratio between the initial quantity of DNA (approximated in  
 174 pg) and the final quantity of DNA (measured in pg after quantification). The initial quantity of  
 175 DNA is related to the weight associated with 100 cells obtained by flow cytometry. There is an  
 176 average of 6pg per cell [7] based on the following formula:

177 Average DNA quantity per cell = Average number of base pair per cell  $\times 2 \times$  average molecular  
 178 weight of one base /  $N_A$

179 Hence: Average DNA quantity per cell =  $3 \times 10^9 \times 2 \times 660_{(g/mol)} / (6,022 \times 10^{23}_{(mol^{-1})})$

180 Using an average of 6pg of DNA per cell, the initial quantity of DNA was set to 600pg. The  
 181 final quantity of DNA is the product of the concentration obtained after quantification and the  
 182 volume left at the end of the extraction.

183 For the swab measure of release, the initial quantity of DNA is known: 636 cells were initially  
 184 deposited on the FLOQSwab™ from COPAN. The quantity of cells released by the swab  
 185 corresponds to the quantity of cells available for next extraction step (Figure 2). This quantity  
 186 is unknown, but will be measured indirectly after the measure of the extracted quantity of DNA  
 187 with the QIAshredder-QIAamp DNA Mini kit from Qiagen. The results obtained previously on



188 the extraction kit alone will be used to infer the swab cells release performance. This is  
 189 illustrated in Figure 3 below.

190 The choice of the Beta distributions is motivated by the nature of the measured variable (a  
 191 proportion). Beta distributions are ideally suited to model distributions between 0 and 1 (or 0%  
 192 to 100%).

193 Mean and standard deviation of the distribution of the DNA extraction efficiency of the kit itself  
 194 are known. Mean and standard deviation of the joint efficiency to release cells and extract DNA  
 195 are also known following the above measurements.



196  
 197 *Figure 3: Illustration of the extraction efficiency, of the efficiency to release cells and of the efficiency*  
 198 *to release cells then extract DNA, with the parameters associated with each distribution that is known*  
 199 *(in purple) or unknown (in red).*

200 By assuming that both extraction and release contribute jointly to the final product, it is easy to  
 201 find parameters c and d of the beta distribution representing the efficiency of the swab to release  
 202 cells. Dufresne [8] gives the equations of the moments for the product of two Beta distributions.  
 203 The parameters of a Beta distribution can be defined based on the mean and the variance of the  
 204 distribution [9]. Solving an equation with two unknowns, we obtain these parameters “c” and  
 205 “d” as follows:

206

207 
$$c = \frac{X^2 - XY}{XY + Y}$$

208

209 
$$d = \frac{X - Y}{XY + Y}$$

210 With:

211 
$$X = \frac{\text{mean3}/\text{mean1}}{1 - (\text{mean3}/\text{mean1})}$$

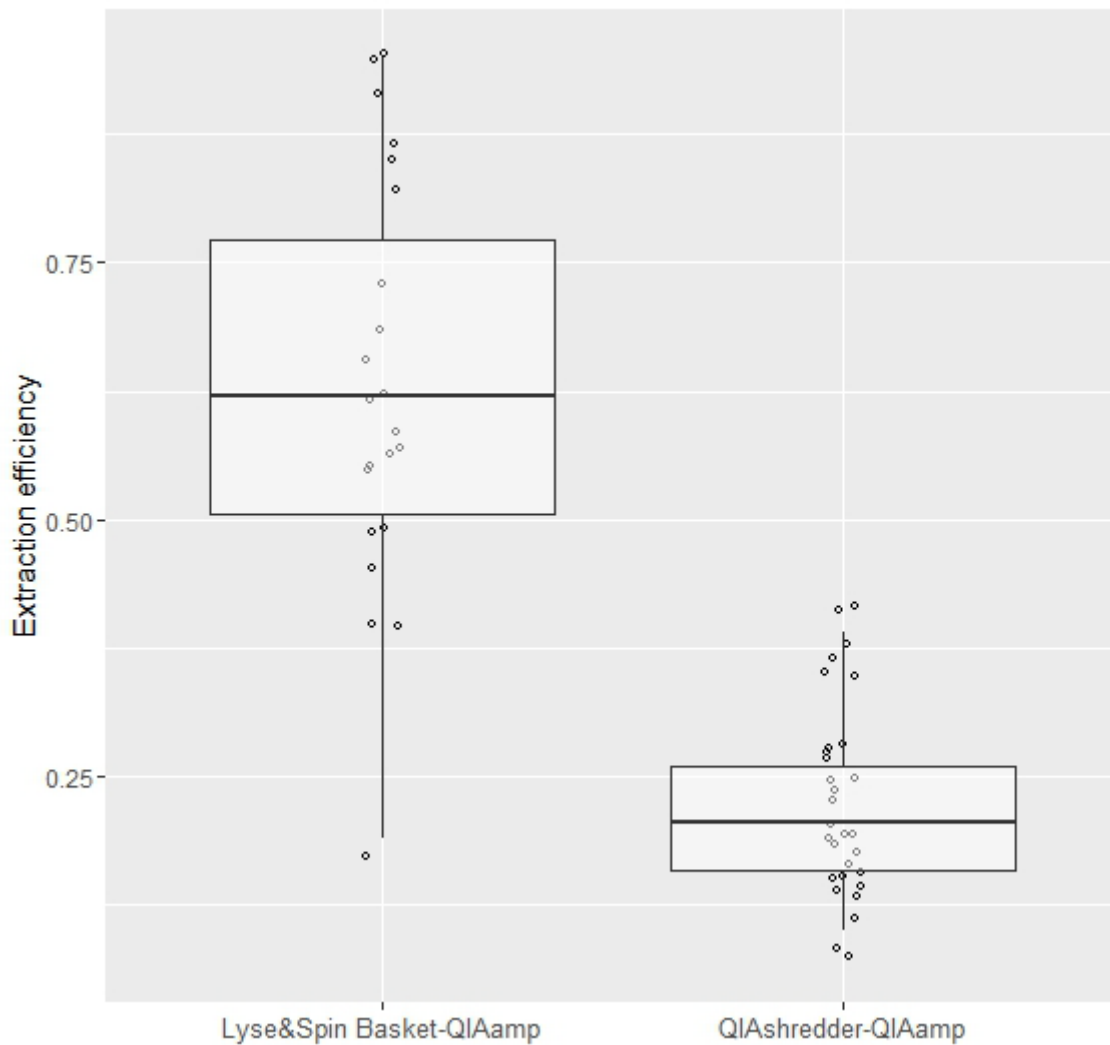
212 And

213 
$$Y = \frac{Sd3}{\text{mean3} * \text{mean1}} * \frac{(a + b + 1)}{a + 1}$$

214 **Results**

215 *Efficiency of the extraction kits*

216 Figure 4 presents the DNA extraction efficiency obtained on the 22 and 30, respectively,  
 217 samples following the extraction using each extraction kit:



218  
 219 *Figure 4: Extraction efficiency of the Investigator® Lyse&Spin Basket-QIAamp DNA Mini kit (Lyse*  
 220 *Lyse&Spin Basket-QIAamp) and QIAshredder-QIAamp DNA Mini kit (QIAshredder-QIAamp).*

221 An average of 63% and 23% of the DNA is recovered respectively with Investigator®  
 222 Lyse&Spin Basket-QIAamp DNA Mini kit and QIAshredder-QIAamp DNA Mini kit (Table  
 223 2). We can observe that the Investigator® Lyse&Spin Basket-QIAamp DNA Mini kit is more  
 224 efficient. Further, it shows the importance of considering the extraction kit used when assessing  
 225 a given amount of recovered DNA in an attempt to infer the initial quantity of DNA available.

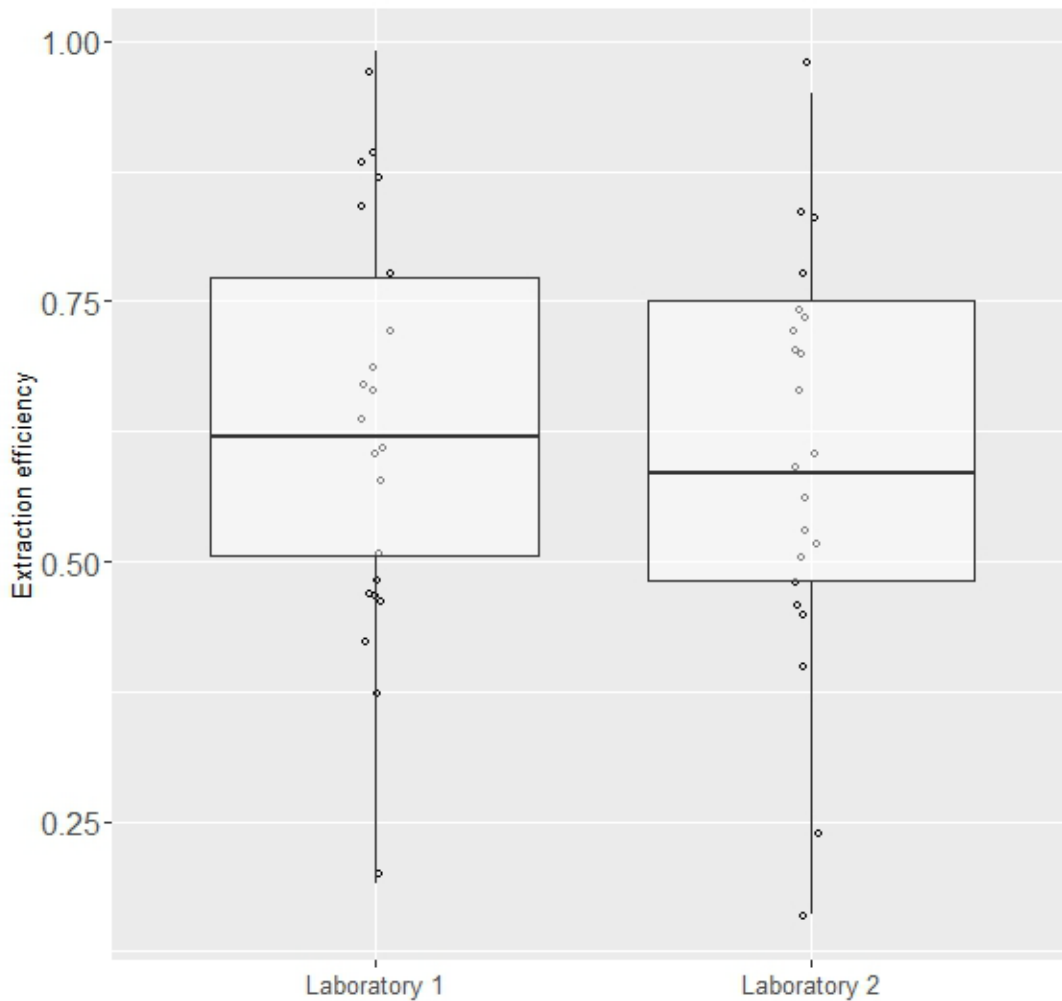
226 *Table 2: Summary statistics of the extraction efficiencies obtained using both kits following the*  
 227 *analysis of 30 samples respectively.*

Extraction kit	Min	0.05 percentile	Median	Mean	0.95 percentile	Max
Lyse&Spin Basket- QIAamp DNA Mini kit	0.19	0.41	0.62	0.63	0.92	0.99
QIAshredder-QIAamp DNA Mini kit	0.10	0.11	0.20	0.23	0.39	0.43

228 *Impact of the laboratory*

229 Figure 5 shows the DNA extraction efficiency of the 22 samples using Investigator®  
 230 Lyse&Spin Basket-QIAamp DNA Mini kit performed by each of the two laboratories.

231



232

233 *Figure 5: Extraction efficiency of the Investigator® Lyse&Spin Basket-QIAamp DNA Mini kit*  
 234 *performed by two laboratories.*

235 For the first laboratory, an average of 63% of the recovered DNA is observed. The efficiency  
 236 is an average of 59% for the second laboratory (Table 3). The difference between the two means  
 237 is not significant. The Bayes factor supports the hypothesis that there is no difference between  
 238 the two means [10].

239 *Table 3: Summary statistics of the extraction efficiencies obtained using the Investigator® Lyse&Spin*  
 240 *Basket-QIAamp DNA Mini kit performed by each of the two Laboratory. Laboratory 1 carried out the*  
 241 *analysis on 30 samples. Laboratory 2 worked on 22 samples.*

Laboratory	Min	0.05 percentile	Median	Mean	0.95 percentile	Max
Laboratory 1	0.19	0.41	0.62	0.63	0.92	0.99

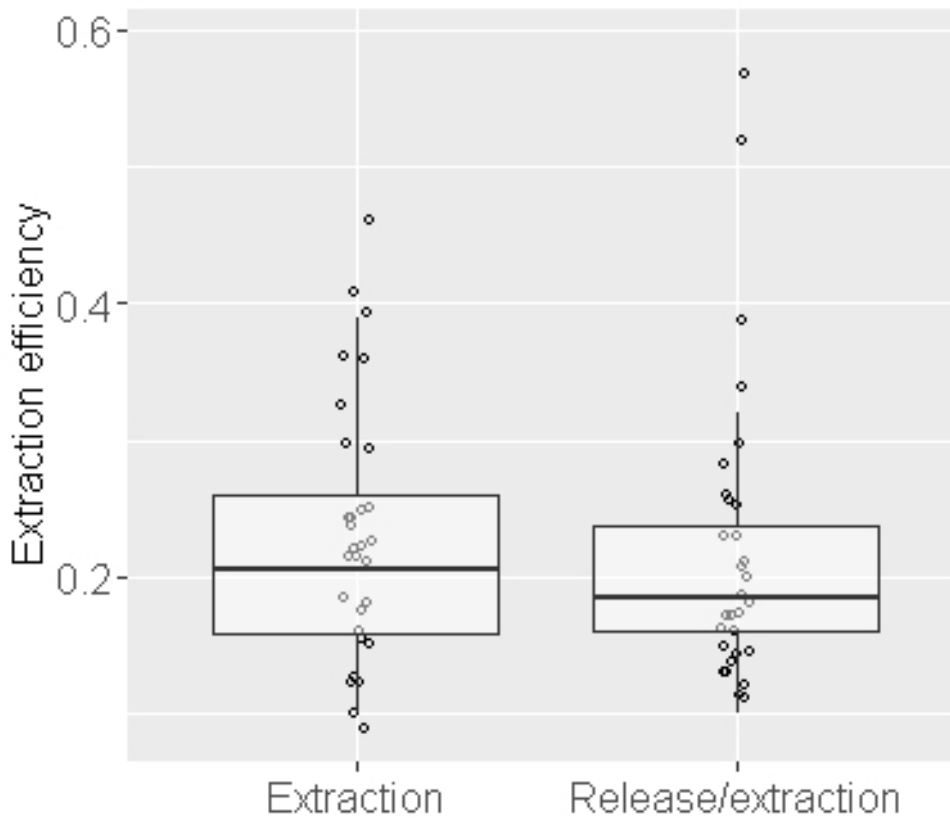
Laboratory 2	0.16	0.22	0.59	0.59	0.83	0.95
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242 Taken jointly, it means that, for the Lyse&Spin and QIAamp DNA mini Kit, about 61% of  
 243 DNA was recovered with no difference between the yields obtained by two different  
 244 laboratories.

245 *The efficiency of cells release from swabs*

246 The extraction kit used here is the QIAshredder-QIAamp DNA Mini kit for which the extraction  
 247 efficiency has been reported in the section *Efficiency of the extraction kits*. We recall that for  
 248 this kit, only about 23% of the initial quantity of DNA was recovered.

249 The efficiency results associated with the cell release and DNA extraction with the kit are shown  
 250 in Figure 6, jointly with the results on the DNA extraction kit only. It represents 30 samples  
 251 deposited on 30 FLOQSwab™ and subsequently extracted with the kit.



252

253 *Figure 6: Boxplot of the DNA extraction efficiency of QIAshredder-QIAamp DNA mini kit (left) with*  
 254 *the boxplot of the efficiency associated with the cell release by the FLOQSwab™ and DNA extraction*  
 255 *with the kit (right).*

256 About 22% of the initial quantity of DNA is recovered after the deposition on the FLOQSwab™  
 257 and the extraction using the QIAshredder-QIAamp DNA Mini kit. The detailed data summary  
 258 (Table 4) is below and compared the data obtained from the extraction kit alone.

259 *Table 4: Summary statistics of the extraction efficiency of the kit alone and of the efficiency associated*  
 260 *with the cell release by the FLOQSwab™ combined with the DNA extraction using the kit. In total 30*  
 261 *samples were analysed under both conditions.*

Efficiency	Min	0.05 percentile	Median	Mean	0.95 percentile	Max
Extraction kit alone	0.10	0.11	0.20	0.23	0.39	0.43
Release/Extraction	0.10	0.11	0.18	0.22	0.46	0.59

262 The average efficiency to extract DNA is close to the efficiency to release cells and to extract  
 263 DNA. It means that the cell release efficiency is close to 100%. How we estimate the cell release  
 264 efficiency is presented next.

265 Knowing the mean and the standard deviation of both distributions representing the DNA  
 266 extraction efficiency and the efficiency to release cells taking into account the DNA extraction  
 267 efficiency of QIAshredder-QIAamp DNA Mini kit, the parameter “c” and “d” of the beta  
 268 distribution  $Be(c, d)$  representing the efficiency of the swab release only can be calculated. A  
 269 Beta distribution  $Be(32.26, 0.98)$  was obtained.

270 To obtain simulated data for the efficiency of the swab to release cells, 1000 values were  
 271 randomly sampled from this Beta distribution  $Be(32.26, 0.98)$ . Each value is a theoretical result  
 272 of the efficiency – between 0 and 100% – to release cells by the swab.

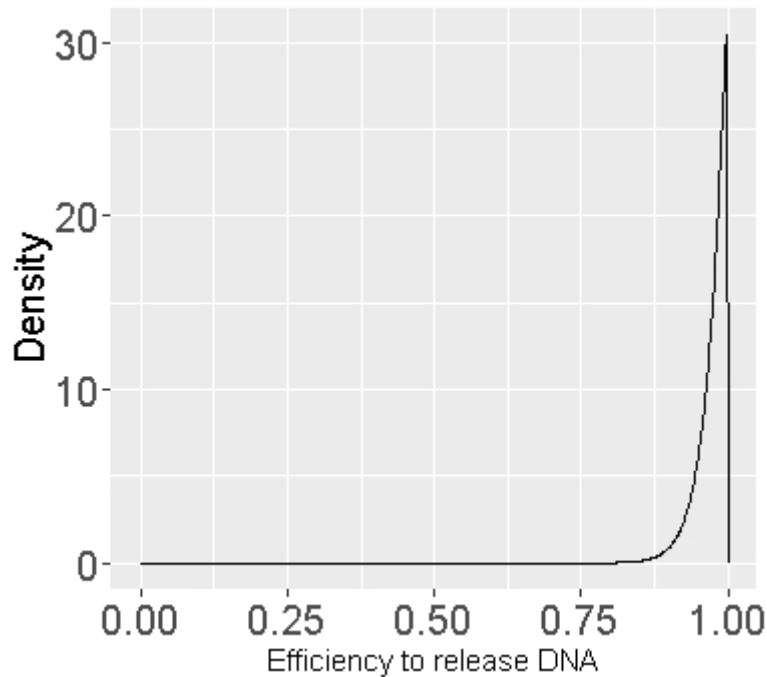
273 We can show that the FLOQSwab™ allows releasing about 97% of the cells on average.  
 274 Summary statistics of the simulations are given below (Table 5 & Figure 7).

275 *Table 5: Summary statistics of the efficiency of the FLOQSwab™ to release cells, based on 1000*  
 276 *simulated values taken from a  $Beta(32.26, 0.98)$ .*

Min	0.05 percentile	Median	Mean	0.95 percentile	Max
-----	-----------------	--------	------	-----------------	-----

0.82	0.92	0.98	0.97	1	1
------	------	------	------	---	---

277 The distribution representing these 1000 random samples is given in Figure 7.



278

279 *Figure 7: Beta probability distribution of 1000 simulated values taken from a Beta(32.26, 0.98)*  
 280 *representing the efficiency of the FLOQSwab™ to release cells.*

281 **Discussion**

282 This study had three objectives.

- 283 • To measure the extraction efficiency of two commercial DNA extraction kits  
 284 (Investigator® Lyse&Spin Basket-QIAamp DNA Mini kit, and QIAshredder-QIAamp  
 285 DNA Mini kit from Qiagen),
- 286 • To study the impact of the laboratory on the yield offered by the best performing kit  
 287 (Investigator® Lyse&Spin Basket-QIAamp DNA Mini kit),
- 288 • To report on the efficiency of a swab (FLOQSwab™ from COPAN) to release cells and  
 289 to show how to obtain it.

290 In the first part of the study, four DNA extractions were made using QIAshredder-QIAamp  
 291 DNA Mini kit showing an average efficiency of 41% (Table 1) against 23% (Table 2) with the  
 292 30 samples. Further, a large variation (Figure 4 & Table 2) from 10% to 43% in the efficiency

293 can be observed. These two observations show that a large number of experiments (greater than  
294 four) need to be done.

295 We report here a large difference of efficiency between both tested kits, despite the fact that the  
296 kits are quite similar regarding the laboratory protocols. The difference between the two kits is  
297 the use of Spin basket and no Microcon® 30 spin column for the Investigator® Lyse&Spin  
298 Basket-QIAamp DNA Mini kit from Qiagen instead of the use of QIAshredder and microcon®  
299 30 spin column for the QIAshredder-QIAamp DNA Mini kit. This observation can be a warning  
300 regarding the evaluation considering proposition at the activity level if specific data of the  
301 extraction kit should be used. In order to do this assumption, the impact of this different set of  
302 data on the result of evaluation should be studied. A lab can perform experiments on efficiencies  
303 with respect to its own method. If a lab is relying on data obtained using another kit, the impact  
304 on the result of the evaluation (on the likelihood ratio) of these other data, compared to the  
305 specific data of the laboratory, should be studied.

306 The large difference of efficiency between both tested kit could be explained by the different  
307 number of the DNA pipetting. QIAshredder-QIAamp DNA mini kit (QIAamp DNA Mini kit  
308 combined with QIAshredder and using the Microcon® 30 column) requires three DNA  
309 pipetting operations, including the pipetting into the microcon® 30 column, whereas the  
310 Investigator® Lyse&Spin Basket-QIAamp DNA Mini kit need only one. At each pipetting of  
311 the total volume, a loss of DNA could occur with DNA being retained on the wall of the  
312 microtube or of the tips both made of polypropylene. Indeed, Gaillard [11] shows that  
313 adsorption of DNA to polypropylene tubes can occur. The large difference of efficiency  
314 between both tested kits could also be explained by the different number of spins used to retain  
315 DNA. Indeed, some DNA fragment could pass through the spin [12] instead of being retained.  
316 QIAshredder-QIAamp DNA Mini kit has more spins and microcon® 30 column than the other  
317 kit.

318 We have observed no significant difference between the DNA extraction efficiencies with the  
319 same kit used by two laboratories. This observation suggests that the effect of the laboratory is  
320 small compared to the variation due by the kit itself. However, given the limited number of  
321 laboratories involved (2), we ought to take this conclusion with the necessary caution.

322 We have also noticed that the maximum of the efficiency to release cells and to extract DNA is  
323 greater than the maximum of DNA extraction efficiency only. If the ratio of these two maximum



324 values were done, an efficiency of swab to release cells greater than 1 would be obtained.  
325 However, this observation is possible, knowing that experiments are independent and knowing  
326 the large variation between efficiencies. Therefore, taking the ratio of the two efficiencies  
327 values seems not ideal. All data allowing determining both extraction efficiency and efficiency  
328 to release cell and extract DNA should be used to estimate the efficiency of swab to release  
329 cells, as shown in Part 2 (Methodology- *Calculating efficiency*).

330 We have shown a large variation in efficiencies for a same kit in the same operator. This could  
331 be explained by the kit itself, but also by the flow cytometry. We suggest that the error  
332 introduced by flow cytometry is negligible. The calibration and quality controls performed on  
333 the instrument have shown that a variation on the cell number between 5 and 10% can occur,  
334 depending of the cell type and the cell concentration. It means that with a target number cells  
335 of 100, 90 to 110 cells will be selected. Therefore, the initial quantity of DNA may be slightly  
336 estimated. This effect is considered negligible compared to the ratio between initial quantity of  
337 DNA and final quantity of DNA. Because of this large variation, a distribution of efficiency  
338 values (and not a single point estimate such as the mean) should be taken into account when  
339 evaluating cases considering propositions at the activity level.

340 This study shows how flow cytometry can be a very effective tool to conduct DNA extraction  
341 and cell release efficiency research.

342 In Wood *et al.* [4], an extraction efficiency around 81% was reported, using QIAamp® DNA  
343 Investigator Kit (QIAGEN). This is higher than those reported in this paper: 23% and 63%,  
344 using respectively, QIAshredder-QIAamp DNA Mini kit and Investigator® Lyse&Spin Basket-  
345 QIAamp DNA Mini kit. However, when using QIAamp® DNA Investigator Kit (QIAGEN),  
346 EtOH is added in the first step of extraction protocol. This step may increase the recovery of  
347 DNA. Besides, the direct comparison between them has its limits. Indeed in *Wood et al.* [4],  
348 acellular DNA was used whereas keratinocyte cells were used in this study. DNA traces,  
349 obtained when touching a surface may be the results of a mix between acellular DNA, and cells  
350 [13]. Therefore, the extraction efficiency obtained in *Wood et al.* [4] or in this study may  
351 underestimate the extraction efficiency for DNA traces, obtained when touching a surface.  
352 Indeed, Propidium Iodide staining was used to sort the nucleated, living, keratinocytes cells. In  
353 that case, only porous cells are selected.

354 *Wood et al.* [4] obtained a lower efficiency of DNA release for nylon-flocked swabs (COPAN's  
355 FLOQSwabs™) that could also be due to the use of acellular DNA instead of cells. Free DNA  
356 and cell membranes could interact differently with the microfibers of the swab.

357 Regarding the ability of the swab to release cells, unfortunately, a fixed number of cells cannot  
358 be directly deposited on the swab. A volume of the cell suspension containing a known  
359 concentration of cells is pipetted onto the swab. A loss of cells and DNA could occur via the  
360 pipetting, but the adsorption of cells and DNA to polypropylene tubes is limited by taking a  
361 partial volume of 35 µL of a total volume mixed by vortexing. The efficiency of the swab to  
362 release cells could be underestimated. In addition, the chosen initial number of cells allowed  
363 obtaining quantity of DNA larger than the one obtained for touch DNA traces. In that case, the  
364 efficiency to release cell could be overestimated.

365 The nylon-flocked swabs (COPAN's FLOQSwabs™) have a higher efficiency to release cells  
366 than the two cotton swabs, Dryswab™ and Applimed SA [14]. However, samples of diluted  
367 blood were used in Rocque et al. [14] instead of a fixed number of keratinocytes.

368 To obtain the final quantity of DNA, a quantification needs to be performed. To perform this  
369 quantification, a loss of DNA could occur. However, the loss due to the use of a different  
370 quantification kit is supposed to be negligible (limited number of pipetting). Regarding the  
371 quantification, the quantity of DNA depends on the kit of quantification and the instrument of  
372 quantification. For consistency in this study, a single operator performed the quantification  
373 using the same kit and the same instrument in order to focus only on the impact of the laboratory  
374 on the extraction efficiency.

## 375 **Conclusion**

376 Knowledge of the extraction efficiency of the kit used by the laboratory has a bearing on the  
377 assessment of the expected quantities of DNA that could be the result of different types of  
378 activities. It will impact the evaluation of the DNA results considering propositions at the  
379 activity level, especially when the case involves a low level of DNA. We developed a method  
380 to measure the efficiency of DNA extractions kits and the release efficiency of DNA swabs can  
381 be measured using flow cytometry. Flow cytometry allows obtaining a fixed number of cells.  
382 Therefore, the initial quantity of DNA, before performing an extraction, is known and  
383 controlled. It proves to be a very efficient technique to obtain adequate estimates of DNA  
384 extraction kit efficiency.

385 We measured the extraction efficiency of two commercial DNA extraction kits, Investigator®  
386 Lyse&Spin Basket-QIAamp DNA Mini Kit, and QIAshredder-QIAamp DNA Mini Kit used to  
387 extract and purify low quantities of DNA.

388 Results have shown that for the Lyse&Spin and QIAshredder-QIAamp DNA Mini Kit, about  
389 61% of DNA is recovered with no difference between the extracts obtained by two different  
390 laboratories. For the QIAshredder-QIAamp DNA Mini Kit, only about 23% of the initial  
391 quantity of DNA is recovered.

392 Furthermore, we measured the efficiency of a swab, the FLOQSwab™ from COPAN, to release  
393 cells and have shown that the FLOQSwab™ releases about 97% of the cells.

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