Science & Justice 59 (2019) 405–410

Contents lists available at ScienceDirect

Science & Justice

journal homepage: www.elsevier.com/locate/scijus

The efficiency of DNA extraction kit and the efficiency of recovery techniques to release DNA using flow cytometry

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1. Introduction

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

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

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

This study has three objectives. The first is to measure the extraction efficiency of two commercial DNA extraction kits (Investigator® Lyse& Spin Basket-QIAamp DNA Mini kit, and QIAshredder-QIAamp DNA Mini kit from Qiagen with Microcon® 30 spin column) used to extract and purify low quantities of DNA based on initial quantities of DNA obtained using flow cytometry. The second is to study the impact of the laboratory on the yield offered by the best performing kit (Investigator® Lyse&Spin Basket-QIAamp DNA Mini kit from Qiagen). The last is to report on the efficiency of a swab (FLOQSwab™ from COPAN) to release cells and show how to obtain it by the combined usage of a swab and an extraction kit (QIAshredder-QIAamp DNA Mini kit from Qiagen with Microcon® 30 spin column).

2. Methodology

2.1. Type and number of cells

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

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

To select the number of cells representing a quantity of DNA obtained when touching a surface, different numbers of cells were tested.

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https://doi.org/10.1016/j.scijus.2019.02.003
Received 25 June 2018; Received in revised form 22 September 2018; Accepted 17 February 2019

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	15,000

First, four samples of 50, 100, 500 and 5000 cells were prepared respectively twice, then directly introduced into a microtube of 1.5 mL containing 180 µL of a tissue lysis buffer (ATL buffer from Qiagen). Cell concentration was around 1million/ml and generates a flow rate of 900 events/s. Given this concentration, the "Single-cell" as the mode of precision used was chosen.

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

One-hundred cells have been selected for the experiments as it led to an amount of around 250 pg of DNA, which corresponds to the average amount of DNA obtained in a previous study focusing on DNA traces, obtained when touching a surface [6].

2.2. Extraction efficiency of the kits

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

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

2.3. Effect of the laboratory

The kit which was proven to be the best performing kit is the Investigator® Lyse&Spin Basket-QIAamp DNA Mini kit from Qiagen. To study the impact of the laboratory's performance on the yield offered by this kit, the extractions were performed manually by two operators in two different laboratories (Fig. 1). One-hundred cells were selected, using the "Purity" precision mode, then directly introduced into each of the 44 Lyse&Spin baskets containing 60 µl of Phosphate buffered saline (PBS) of pH 7.4, allowing the cells to be kept intact. Twenty-two extractions were made by each operator, with a final volume of 60 µL. All the quantifications were performed together in the same run at the same time following the DNA extraction which was made two days after the flow cytometry.

2.4. Release of cells by the DNA swab

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

To measure it, 20,000 cells were introduced into a microtube of 1,5 mL containing 1.1 mL of PBS, to avoid the destruction of the plasma membranes. Because of the technical impossibility to directly deposit cells on the swab, the microtube was mixed by vortexing and 35 µL (636 cells) was pipetted on each 30 FLOQSwab™. To take into account the possible loss of cells being retained by the swab, the selected number of cells is higher than the number (100) used to study the extraction efficiency.

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

2.5. Calculating efficiency

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

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

Hence: Average DNA quantity per cell = $3 \times 10\hat{}9 \times 2 \times 660_{(g/mol)}/(6022 \times 10^{23}_{(mol)^{-1}})$

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

For the swab measure of release, the initial quantity of DNA is known: 636 cells were initially deposited on the FLOQSwab™ from COPAN. The quantity of cells released by the swab corresponds to the quantity of cells available for next extraction step (Fig. 2). This quantity is unknown, but will be measured indirectly after the measure of the extracted quantity of DNA with the QIAshredder-QIAamp DNA Mini kit from Qiagen. The results obtained previously on the extraction kit alone will be used to infer the swab cells release performance. This is illustrated in Fig. 3 below.

The choice of the Beta distributions is motivated by the nature of the

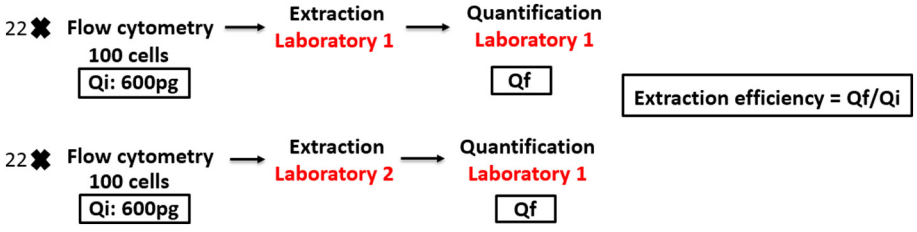

Fig. 1. Illustration of the method used to study the impact of the laboratory on the yield offered by Investigator® Lyse&Spin basket-QIAamp DNA Mini kit. Qi is the initial quantity of DNA to be extracted, whereas Qf is the final extracted quantity of DNA.

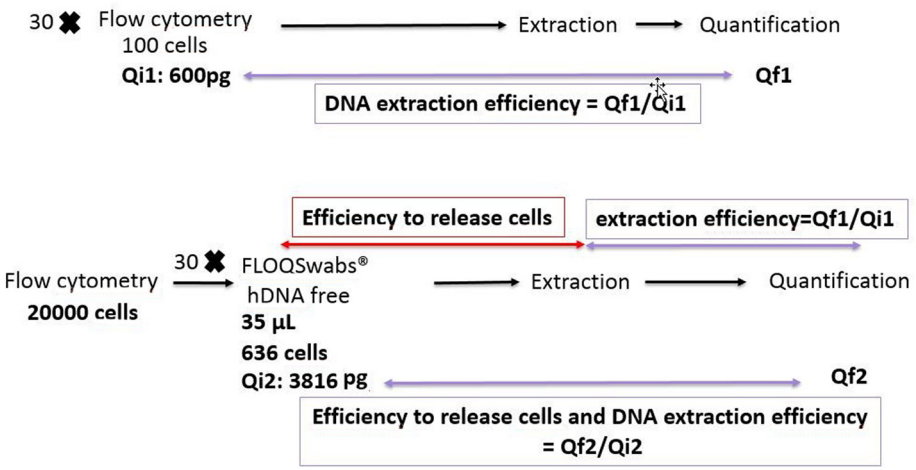

Fig. 2. Illustration of the method used to obtain the extraction efficiency of the kit and a joint measure of efficiency to release cells combined with the efficiency of the DNA extraction kit (in purple) in order to obtain the efficiency of the sampling device to release cells (in red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 3. Illustration of the extraction efficiency, of the efficiency to release cells and of the efficiency to release cells then extract DNA, with the parameters associated with each distribution that is known (in purple) or unknown (in red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

measured variable (a proportion). Beta distributions are ideally suited to model distributions between 0 and 1 (or 0% to 100%).

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

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

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

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

with:

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

And

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

3. Results

3.1. Efficiency of the extraction kits

Fig. 4 presents the DNA extraction efficiency obtained on the 22 and 30, respectively, samples following the extraction using each extraction kit:

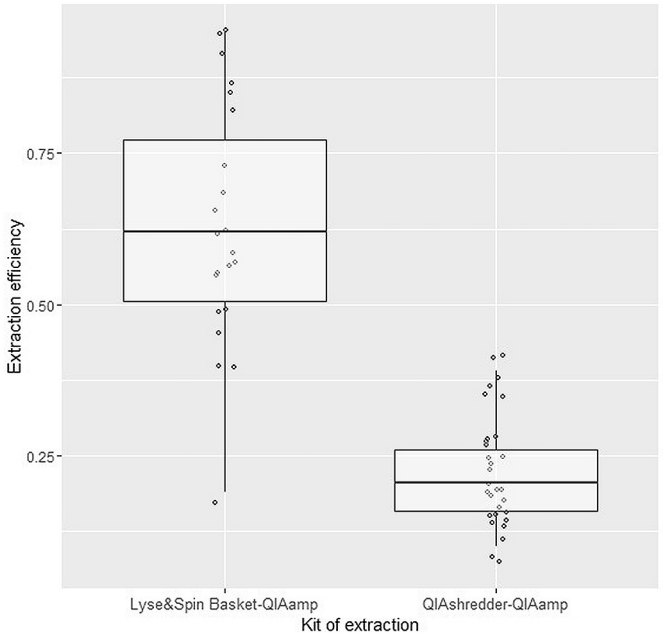

Fig. 4. Extraction efficiency of the Investigator® Lyse&Spin Basket-QIAamp DNA Mini kit (Lyse Lyse&Spin Basket-QIAamp) and QIAshredder-QIAamp DNA Mini kit (QIAshredder-QIAamp).

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

3.2. Impact of the laboratory

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

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

Taken jointly, it means that, for the Lyse&Spin and QIAamp DNA mini Kit, about 61% of DNA was recovered with no difference between

Table 2
Summary statistics of the extraction efficiencies obtained using both kits following the 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

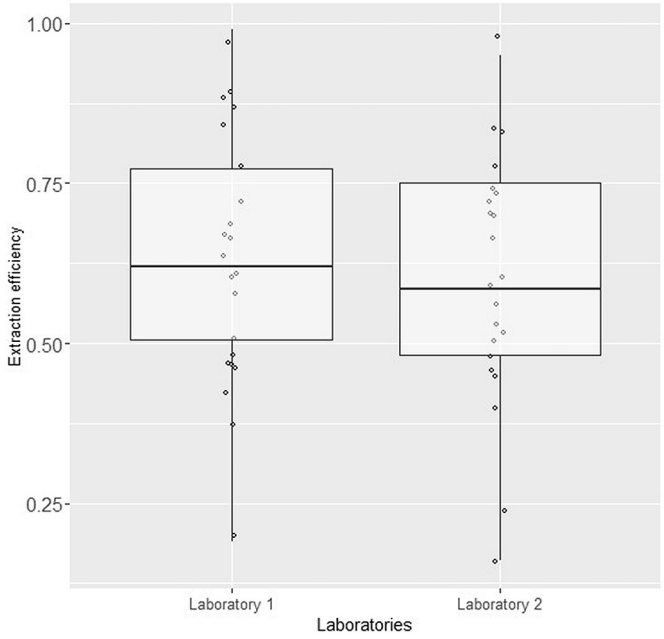

Fig. 5. Extraction efficiency of the Investigator® Lyse&Spin Basket-QIAamp DNA Mini kit performed by two laboratories.

Table 3
Summary statistics of the extraction efficiencies obtained using the Investigator® Lyse&Spin Basket-QIAamp DNA Mini kit performed by each of the two Laboratory. Laboratory 1 carried out the 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

the yields obtained by two different laboratories.

3.3. The efficiency of cells release from swabs

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

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

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

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

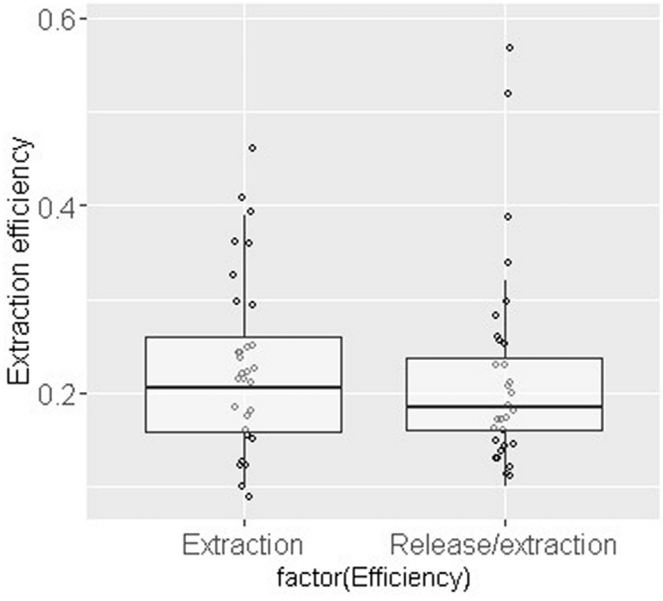

Fig. 6. Boxplot of the DNA extraction efficiency of QIAshredder-QIAamp DNA mini kit (left) with the boxplot of the efficiency associated with the cell release by the FLOQSwab™ and DNA extraction with the kit (right).

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

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

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

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

4. Discussion

This study had three objectives.

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

In the first part of the study, four DNA extractions were made using QIAshredder-QIAamp DNA Mini kit showing an average efficiency of 41% (Table 1) against 23% (Table 2) with the 30 samples. Further, a

Table 4
Summary statistics of the extraction efficiency of the kit alone and of the efficiency associated with the cell release by the FLOQSwab™ combined with the DNA extraction using the kit. In total 30 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

Table 5
Summary statistics of the efficiency of the FLOQSwab™ to release cells, based on 1000 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

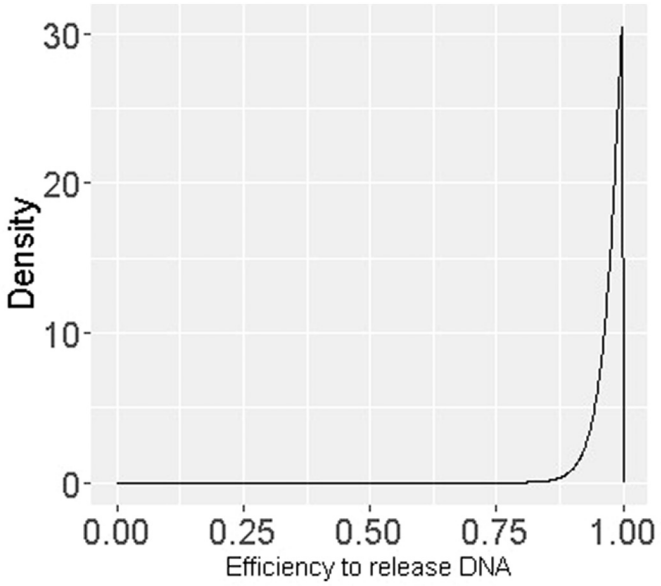

Fig. 7. Beta probability distribution of 1000 simulated values taken from a Beta (32.26, 0.98) representing the efficiency of the FLOQSwab™ to release cells.

large variation (Fig. 4 & Table 2) from 10% to 43% in the efficiency can be observed. These two observations show that a large number of experiments (greater than four) need to be done.

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

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

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

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

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

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

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

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

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

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

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

5. Conclusion

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

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

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

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

Acknowledgements

We wish to thank Patrick Basset for his help and the two persons for their participation to the laboratory part of this study, Magali Stoudmann and Delphine Nivard. We would like to express our gratitude to Henry Swofford for the insightful comments.

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