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A preliminary investigation of transfer of condom lubricants in the vaginal matrix

Nikola Saric, Loïc Fabien, Julia Fischer, Anaïs Hermelin, Geneviève Massonnet, Céline Burnier*

Ecole des Sciences Criminelles, University of Lausanne, Switzerland

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ABSTRACT

Condom evidence has become in the past years a very relevant item of evidence in sexual assault or rape cases, being an objective help in the reconstruction of the activity. Traces recovered from a vaginal swab might help to identify whether a condom or other lubricants were used, and thereby possibly confirming or infirming allegations of the parties. However, the interpretation of condom traces can be challenging and requires a detailed understanding of various factors like condom lubricant chemical composition and occurrence on the market, transfer and persistence parameters and background. Herein, we aimed at improving our understanding of factors affecting the transfer variability of condom residues recovered from vaginal matrix.

This work employed Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS) to provide new data for a characterization of condom lubricants and their traces after a transfer in a vaginal matrix has occurred. Condom traces were recovered from volunteers and the traces characteristics were investigated and analyzed. The effects of donor (condom) and receiver (vaginal matrix) were firstly evaluated, as they are known factors, and these data that could be obtained in real caseworks. Using principal component analysis (PCA), this study highlighted that the effect of the donor was more important than the receiver effect. Vaginal matrix residues were not detected in transferred extracts. The discrimination pattern amongst the donor was found to be indistinguishable from the one obtained on reference material using ATR-FTIR (Attenuated Total Reflectance).

1. Introduction

Condom traces, more specifically silicone residues coming from condom lubricants, have recently been more investigated for forensic purposes. The issue of condom traces in forensic science has been illustrated since the 1980s, especially in cases of sexual assaults on women [1–3]. According to the information obtained during an investigation, it may become necessary to determine whether a trace of a condom is present, in order to confirm or deny the allegations of the parties: it is not the occurrence of a sexual act but the way it happened that is questioned [4]. The condom trace is also used as associative evidence to establish the *corpus delicti* and verify penetration [2,5,6].

Multiple researches have focused on different, but complementary, approaches to be able to exploit the condom evidence. Most studies focused on the composition of the condoms and the discrimination of samples present on various markets [4,7–12,12,13]. More rarely, chemical markers in the vaginal matrix were investigated to identify which compounds were the most easily detected and what could be found in traces [14]. Another important aspect seems to have been neglected by researchers: the question of interpretative parameters. This approach generally takes into account parameters such as occurrence, background, transfer and persistence parameters. Despite the recurrence of such questions, there's is no existing model to assist the forensic scientist to understand what happens during the transfer as well as after a sexual intercourse, and thus to allow adequate interpretation of the evidence. This is likely due to barriers related to this type of study. Indeed, the variations observed in a profile are influenced by various factors which are difficult or even impossible to control [15,16], and whose effects are for the most part unknown (e.g. the effects of the contact itself (duration, intensity.), or the influence of the vaginal matrix) [15]. Added to this are ethical considerations, which make these studies long and tedious but demonstrate the need to increase research on the subject.

As described by Burnier and Massonnet [15], several factors are likely to influence the trace and its recovery by influencing the transfer and

* Correspondence to: Ecole des Sciences Criminelles, Quartier UNIL-Sorge, Bâtiment Batochime, CH-1015 Lausanne, Switzerland.
E-mail address: celine.burnier@unil.ch (C. Burnier).

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persistence of the evidence. Both the initial composition and recovered composition of the evidence are significantly affected by different influence factors, classified in five groups: the donor, the receiver, the contact, the elapsed time between intercourse and sample and the activity of the receiver between the elapsed time [15].

Donor characteristics can easily be assessed using market studies. In a recently published ATR-FTIR market study [17], silicone lubricants were found to be the more common lubricant on condoms, whereas most of the other types of lubricants were containing water- based compounds. The classification model built in this study, as in many other publications, was based on pure lubricants, and the classification of real traces has so far not been investigated, to see whether they classify in the same way than pure lubricant samples do. Indeed, chemical profiles coming from traces are likely to be affected by the vaginal matrix, and in a forensic context, it is important to understand how the target compounds react when in contact with the matrix, and the effects of the donors (condoms) and receivers (human vaginal matrix). Receiver characteristics and contact have yet never been reported, although these are more than likely to affect the evidence as illustrated in multiple caseworks [5,18].

As such, this study aims to provide data to investigate the transfer of silicone lubricants in the vaginal matrix using DRIFTS [14] as a tool to detect condom residues. This study sought to contribute to an empirical evidence base to establish the nature of the transfer of condom lubricants and develop a scientific basis to develop further interpretation model when considering the detection of condom evidence.

2. Material and methods

2.1. Chemicals

PDMS 200 cSt was purchased from Sigma Aldrich (USA) and was diluted in hexane (analytical grade; Sigma Aldrich (USA)) at concentrations of 0.1, 1.0, 2.0 and 3.0 mg/mL. KBr used for DRIFTS analysis was purchased from Acros Organics. Human sample collection was led by self-sampling procedure, using COPAN 150C cotton swabs (Copan Inc., USA). All the sample preparation and spiking were realized using a 5 µl syringe eVol XR ® from SGE Analytical Science (Australia).

2.2. Samples

To investigate the donor (i.e. condom) effect, 2 volunteers had sexual intercourse using 11 different condoms, listed in Table 1. Volunteers self-sampled 3 blank swabs prior to intercourse, and 3 samples right after intercourse. To avoid any cross-contamination, the volunteers were asked to wait one week between each protected intercourse. Each sample was analyzed 3 times, resulting in 9 replicates for a same donor. As each volunteer provides 3 swabs and each swab is analyzed in triplicates, a total of 180 analyses (blank included) were obtained for volunteer 1, whereas 90 were obtained for volunteer 2. This leads to a total of 270 analyses run for the aim of this paper.

To investigate the receiver (i.e., volunteer) and therefore matrix effect, the donor was fixed as the Ceylor Blue condom (D1-latex silicone lubricated condom). In the eventuality the volunteer asked for a latex free condom, the selected condom was Manix Skyn (D9). These choices were made as they are common condoms found on the market, and they are not statistically distinguishable based on their chemical composition. 9 volunteers were asked to have sexual intercourse using the provided condom, and to self-sample using the cotton swabs right after the intercourse. Volunteers R2, R3 and R8 asked for a latex-free condom (D9-Manix Skyn), and all the other volunteers used the latex silicone lubricated condom (D1-Ceylor Blue). Here again, blanks were collected prior to intercourse to ensure there are no silicones in the vaginal matrix, and therefore that the traces detected come from the given intercourse and not from previous use.

Sample collection was led in accordance with the Swiss Federal Act on Research involving Human Beings (Ordinance on Human Research with the Exception of Clinical Trials) and approved by Swissethics (2018-00690). Informed consent was obtained from all donors, and collected data were entirely anonymized.

2.3. Extraction procedure and sample preparation

Sample extraction procedure was taken from Refs. [3,5,10], with cotton swabs separated from the wooden stick and put in a glass vial. 1 mL of hexane was added prior to vortexing for 1 min and sonicating for 15 min. Extracts were analyzed in triplicate.

KBr pellets were prepared by filling sample cups with finely manually ground KBr powder. Cups were then dried for 15 min at 100 °C. 10 µl of extract solution were spiked on the pellets, and one pellet was spiked with hexane only. Three cups were left unspiked for blank analysis. Samples were then put back in the oven for 15 min at 100 °C to ensure solvent evaporation. Samples were left to cool down in a Pyrex® dish, filled silica beads to absorb exceeding humidity.

2.4. Data acquisition

Data were acquired using a Digilab FT3000 FTIR spectrometer equipped with Spectra-Tech 0030-05 Collector II Diffuse Reflectance Accessory. Agilent's Resolution Pro v.4 software was used for data collection. Spectra were collected over the 4000–400 cm^{-1} resolution, using 64 co-added scans and a 4 cm^{-1} resolution. KBr background was collected every 3 analysis and solvent blanks were run to account for interferences.

2.5. Data analysis

Qualitative analysis and visualization of the spectra was led on ThermoFisher Omnic32™ software (v. 8.2.0.387). The Unscrambler®

Table 1
List of the donors used for the donor effect investigation and their features, with the number attributed and which receiver used which samples. Composition is known based on observations from Ref. [17].

No	Donor	Type	Lubricant	Other components	Receiver 1 (R1)	Receiver 2 (R2)
D1	Ceylor Blue	latex	silicone	no	X	X
D2	Ceylor Gold	latex	Water based + silicone	Glycerin, PEG, nonoxynol-9	X	X
D3	Ceylor Ultrathin	non latex	silicone	no	X	X
D4	Ceylor Green	latex	none	no	X	
D5	Manix Contact	latex	silicone	no	X	X
D6	Durex Natural	latex	silicone	no	X	
D7	Durex Gefühlsecht	latex	silicone	no	X	
D8	Manix Orgazmax Plus	latex	silicone	Propylene Glycol	X	
D9	Manix Skyn	non latex	silicone	no	X	
D10	Prix Garantie	latex	silicone	no	X	
D11	Manix Fraise	latex	silicone	no		X

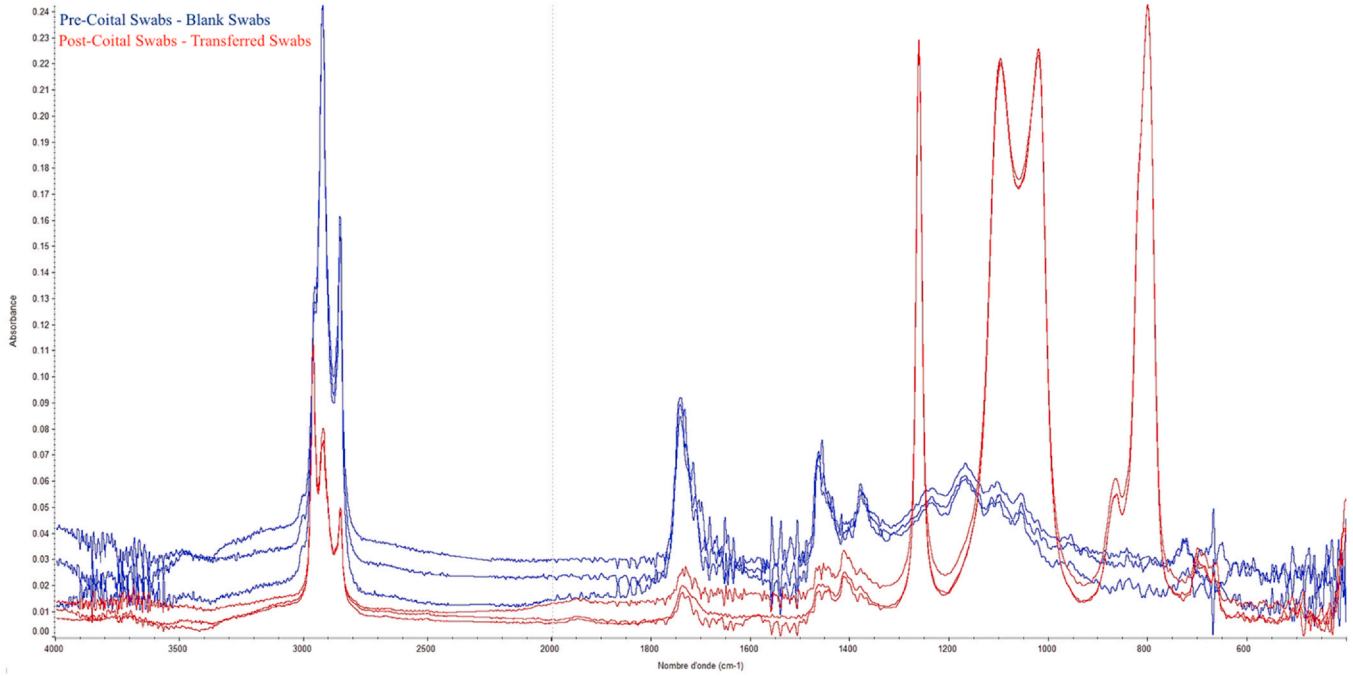

Fig. 1. Illustration of the vaginal secretions (blank swabs – Blue spectra) and an overlay of the transferred spectra (PDMS containing – Red spectra). There is no interference noted between the blanks and the transferred residues. Blanks do not present any signs of a presence of PDMS.

X 10.1. software from Camo Software AS (Oslo, Norway) was used to perform all the automated data preprocessing and chemometric analysis. Spectra were truncated to omit the 2340–1880 cm^{-1} region and range normalized before processing principal component analysis (PCA).

PCA was run using non-linear iterative partial least square (NIPALS) algorithm, with a total of 1000 iterations. This allows to visually explore the structure of the data and evaluate the variation within the latest. Loadings, known as linear combination coefficients, are used to help understanding which variables affect the most the sample separation. Sample replicates presenting a lower within-sample variability (intra) than between-sample variability (inter) should be clustered together and separated from other sample replicates on the first principal components. PCA was run using the Unscrambler® X 10.1.

3. Results and discussion

3.1. Preliminary considerations

As illustrated in Ref. [15], transfer is affected by three main categories of factors, the donor, the receiver and the contact, which lead to an initial post-coïtal transferred composition of the trace. The donor is here assessed as the condom, and its characteristics can easily be known based on the different published market studies. Similarly, the receiver characteristics, in this study the vaginal matrix, can be investigated, although the cyclic evolution of the menstrual cycle [19–22], specific medication, the use of toiletries or genetics are likely to influence the composition. Contact parameters includes the pressure and the duration of the contact between the donor and the receiver, and more broadly various parameters linked to the sexual intercourse itself [15]. If both donor and receiver parameters can be monitored within such a study, the contact parameter cannot be monitored without causing ethical issues. Therefore, the contact parameter will not be discussed further in this study.

All the volunteers were asked to collect 3 blank swabs prior to sexual intercourse. All the blanks were run before the rest of the samples to ensure that there were no silicone residues within the blanks. This allows to ensure that the detected compounds come from the questioned sexual intercourse and not from previous use of silicone containing products. All the blanks were found to be blank for all the volunteers. Fig. 1 illustrates the results of the blank swabs and swabs with silicone collected from one volunteer.

These observations confirm the observations of [14] who indicated that silicone bands were well resolved from the vaginal secretions and were indicative of the presence of PDMS in the vaginal matrix when some is present.

Prior to investigating the donor and receiver parameters, it is necessary to evaluate the vaginal matrix effect on the collected PDMS traces, the influence of different concentrations of pure lubricant as well as the influence of diluted lubricant extracts from the cotton swab. Three different types of samples were used: a standard reference PDMS diluted in hexane at a concentration of 1 mg/mL, with no substrate, cotton swabs spiked with various concentrations of PDMS between 1.0 and 3.0 mg/mL, introducing the cotton support, and cotton swabs with residues from self-sampled volunteers, thus including the vaginal matrix.

This allows evaluation of whether there are any specific patterns linked to dilution, to cotton swabs and/or to the human matrix. No visual differences were observed as illustrated in Fig. 2.

Principal component analysis was performed to see if the samples were statistically distinguishable. Results are presented on Fig. 3.

Three clusters can be observed from Fig. 3:

- Cluster A contains 3 replicates from the PDMS traces in the vaginal matrix separated from the rest of the dataset
- Cluster C contains 7 replicates from the PDMS standards
- Cluster B contains the rest of the samples analyzed.

These data highlight the significant variability that can be encountered in casework. Indeed, samples from Cluster A were found to originate from a single volunteer, whereas all the rest of the receivers were found to be clustered together (Cluster B). Therefore, the separation of Cluster A from the rest of these replicates might be

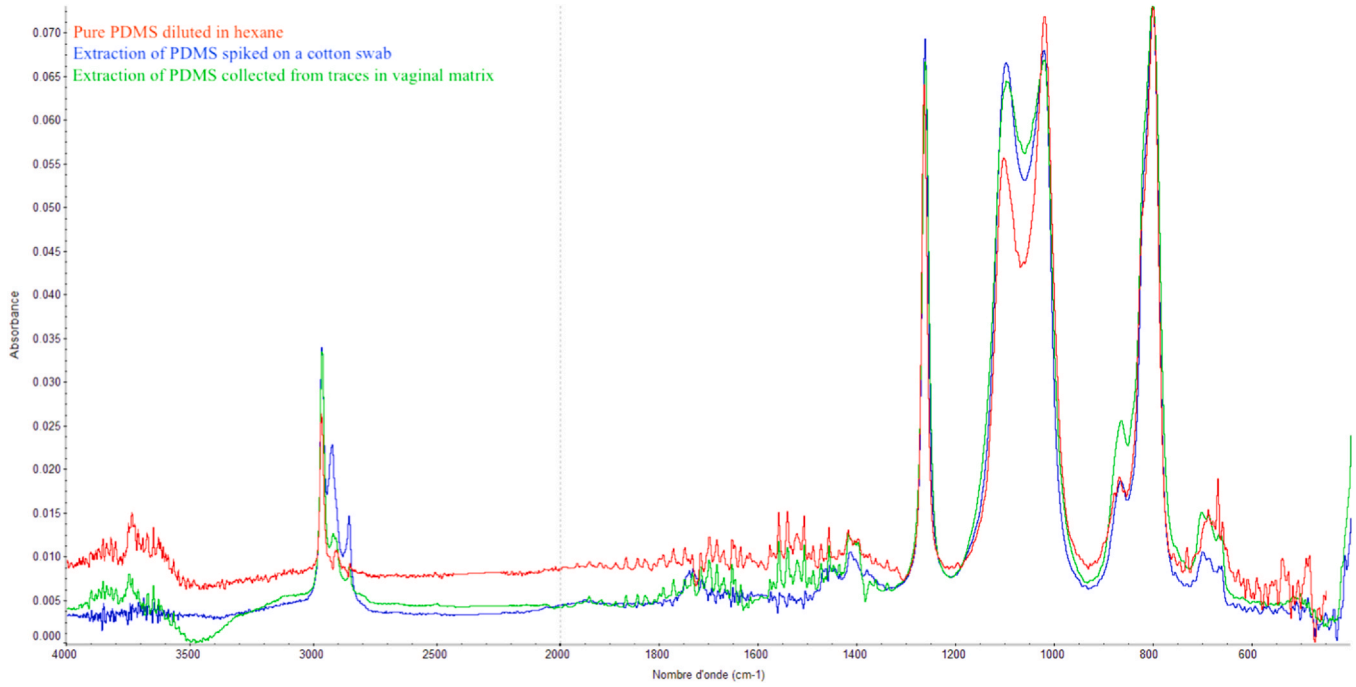

Fig. 2. Illustration of the chemical profile obtained from pure PDMS diluted in hexane at a concentration 1.0 mg/mL (red spectrum), extract of PDMS spiked on a cotton swab at concentration 3.0 mg/mL (blue spectrum) and extract of PDMS collected as trace evidence in a vaginal matrix after intercourse (green spectrum).

due to the receiver rather than to the matrix effect. Investigation of the loading's plots indicated that the separation of this cluster from the rest of the data was due to the CH_2/CH_3 content, which was previously assigned to cell membrane components and sex hormones. Variation in the concentrations of these compounds in this sample type may contribute to this clustering. These variations could originate from a specific contraceptive use as well as from the hormonal cycle variation. No information on the type of contraceptive used by the volunteers was asked, therefore this hypothesis cannot be confirmed. Regarding the hormonal cycle variation, there is, to the authors' knowledge, no publication monitoring these variations of the vaginal matrix over multiple cycle period, but such studies would definitely help assisting further interpretation of transfer and persistence results of other type of evidence in the forensic area.

Samples constituting Cluster C were found to all come from the reference PDMS samples. Loadings plots highlighted that this cluster was separated from the rest of the samples due to its silicone content. Variations in concentration would explain such clustering. However, as previously outlined in regarding DRIFTS analysis, significant variability can be observed for this type of analysis due to the sample preparation [23,24]. The particle size of the ground KBr used for the analysis might generated variable adsorption of the silicone molecules and variable coating of the KBr while depositing the extract on the cell, as previously described by Ref. [14]. If grain size varies, the distribution of PDMS around KBr grains will differ and so will the resultant coating. The contact area is maximized when the KBr particles are small, which increases the infrared signal. Moreover, it is impossible to control how the particles percolate through the KBr, and whether they remain on the surface or not. If the KBr particles are too small, adsorption may not occur, whereas if they are too large, the results will not be repeatable. These phenomena were reported to significantly affect the variability and thus are more likely to be the reason of the separate cluster.

Multiple concerns can be raised regarding the variability implied by the sample preparation and the implication on the overall feasibility of the results when applied to casework samples, where concentrations would vary. DRIFTS-FTIR was reported as a valuable screening method in several papers [10,25], as FTIR is available in most forensic laboratories

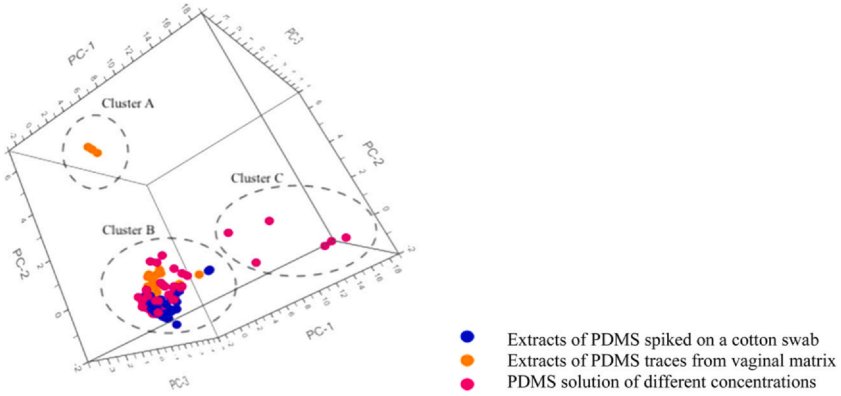

Fig. 3. 3D-Scores plot obtained from the PCA on the data used to investigate matrix effect. Several clusters are outlined, but more importantly is outlined the variability of the samples. PC 1–3 are presented. Separation is made according to the type of sample: extracts of PDMS on a cotton swab (blue), PDMS traces from vaginal matrix (orange) and PDMS solution (pink).

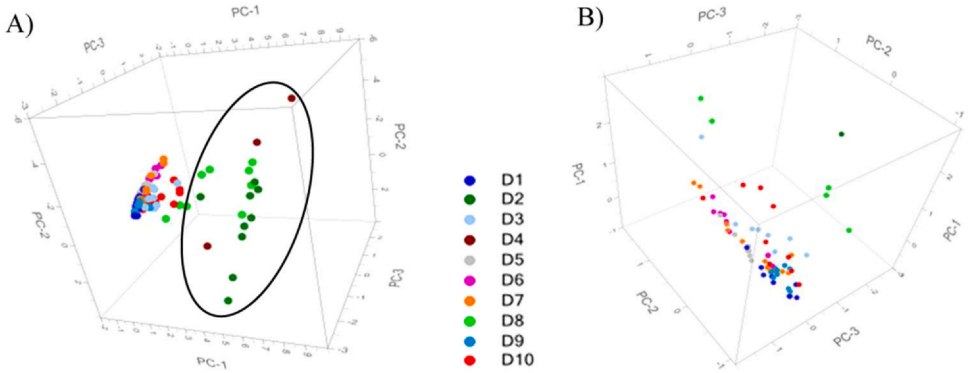

Fig. 4. 3D-Scores plot obtained from the PCA on the data used to investigate donor effect with **receiver 1**. PC 1–3 are presented. Samples are colorized according to donor (i.e. condom). For each donor, replicates are n = 9, except for D4 which has n = 3 replicates due to complications in sampling. A) considering the whole dataset B) after removing samples circled on Fig. 4A.

and offers the possibility of a rapid detection. When used in casework, a confirmation method such as for example py-GC/MS [16,26] or mass spectrometric techniques [4,5] should be used to infirm or confirm the results. FTIR is also very valuable in order to know which technique to use for further investigations. For example, the use of py-GC/MS is only relevant on silicone-containing samples, whereas GC/MS is more relevant on water-based samples [27]. However, more studies on the application to casework are needed for most of the confirmation techniques.

Regarding Cluster B, it was not found to be possible to differentiate the samples prepared from different substrates. These observations show that hexane extraction might not be affected to the same extent as methanol extraction in terms of matrix residues, as most of the matrix is expected to be aqueous/hydrophilic, and thus not soluble in hexane [15,21,22,25]. This might also reinforce that the chemical model presented in Ref. [17], could be directly applied to trace evidence without any major concerns. The preprocessing methods previously used to discriminate samples [17] were found to be unable to account for variability due to sampling types, as samples were not projected according to their sampling procedure in PCA scores plots. Investigation of further principal components (up to 7) did not enable further separation of the samples.

3.2. *Influence factor I: donor*

To investigate the effect of the donor, 11 donors from the market dataset published in Ref. [17] were used (Table 1). Two volunteers (receivers) had protected intercourse, the volunteer 1 doing the experiments with 10 out of 11 condoms while volunteer 2 only used 5 out of the 11 different condoms (donors). The volunteers self-sampled immediately after transfer (T = T0).

Given that the receiver might be affecting the results, PCA was performed separately on the data acquired for receiver 1 and receiver 2. Results are presented in Fig. 4 for Receiver 1, and in Fig. 5 for Receiver 2. As this section aims to observe the difference between the donors, and its consistency, data acquired for both volunteers will not be computed together.

Fig. 4A shows the PCA plots obtained when discriminating donors according to their chemical profiles. Donors D2, D4 and D8 were found to be statistically differentiated from other donors. Although reported as non-lubricated, D4 was found to present a very light silicone composition, which suggest that manufacturers need to put silicone to avoid the latex to stick to itself, no matter the type of condom [15,17]. Visually, sample D2 and D8 were found to present a different chemical profile than the other PDMS lubricated samples

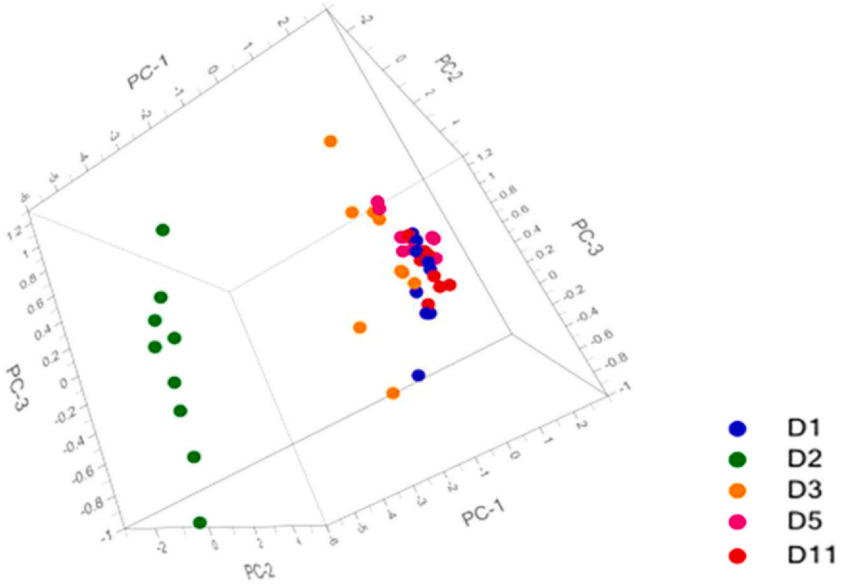

Fig. 5. 3D-Scores plot obtained from the Principal component analysis on the data used to investigate donor effect on **receiver 2**. PC 1–3 are presented. Separation is made according to the donor. For each donor, replicates are n = 9.

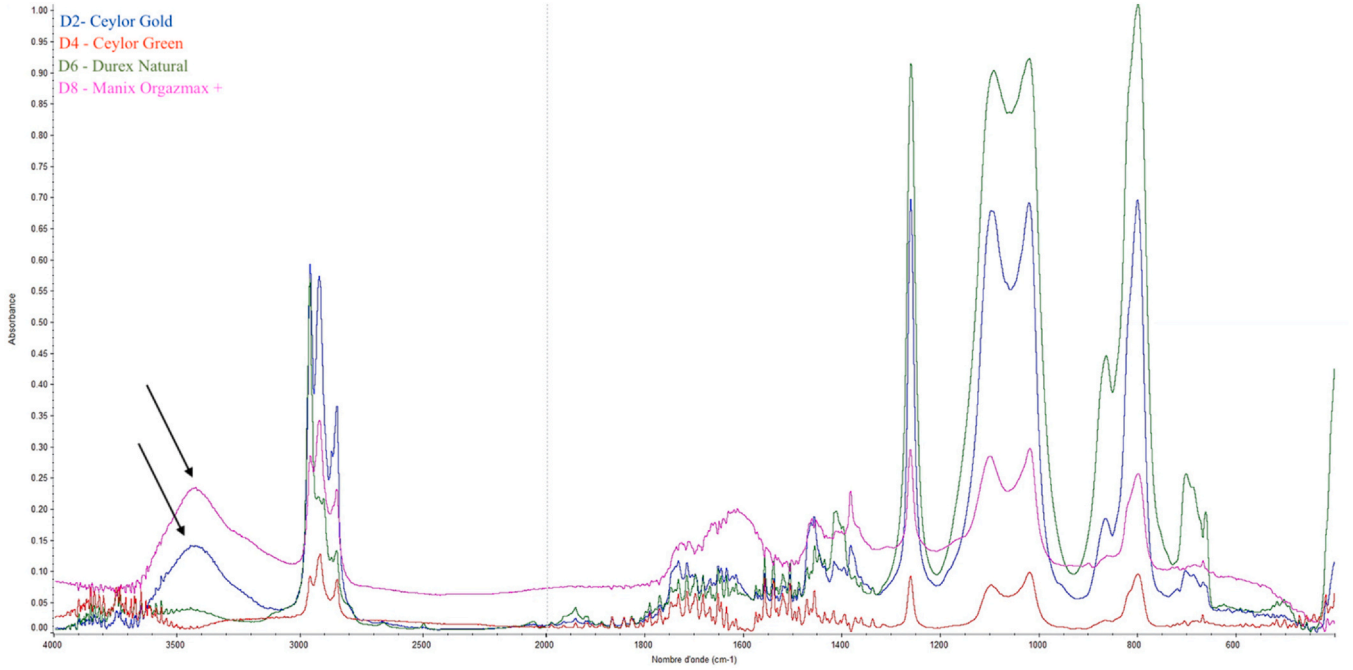

Fig. 6. Illustration of the different DRIFTS-FTIR chemical profile obtained for Durex Natural condom (D6 in green), Ceylor Gold condom (D2 in dark blue), Ceylor Green condom (D4 in red) and Manix Orgazmax Plus condom (D8 in pink). The black arrows indicate the OH bond, sign of the presence of water-based components. These chemical profiles perfectly match the ones previously observed when leading the market study using ATR-FTIR.

like D6, as illustrated on Fig. 6, due to the presence of OH bonds. This is due, for D2- Ceylor Gold sample to the presence of OH-siliconated lubricants and presence of PEG and nonoxynol-9 in the composition of the sample and for D8-Manix Orgazmax + to the presence of propylene glycol [17].

These observations are consistent with the market survey [17] as donors D2 and D8 were already found cluster separately from the rest of the condom samples. Regarding donor D4, such a classification is not surprising as it is a dry condom type, which means it is not lubricated. It looks like D4 is clustered with samples D2 and D8 and such an observation was previously observed with FairSquared Sensitive Dry sample, which was clustered with non-silicone-based samples [17].

Further observations on Fig. 4A and 5 also indicate that the composition of the body of the condom does not affect the chemical profile of the trace. This observation was already noted by Ref. [17] in their paper but is now also confirmed to be the case for trace evidence.

As donors D2, D8 and D4 significantly impacted discrimination, these samples were removed from the dataset and PCA was rerun on the remaining samples. Fig. 4B illustrates the scores plot generated from the reduced dataset. Samples from donors D10 and D3 were found to present significant variability. However, this variability can be considered normal and more likely to be linked to the analytical instrumentation or the self-sampling procedure than to other factors. The variability observed in the data is in accordance with the variability observed in the ATR dataset from the market study.

In both cases, further principal components up to PC7 were used to project the data, but none of them enabled improved separation of the samples in the dataset.

The same procedure was applied to samples collected from receiver 2. As illustrated on Fig. 5, donor D2 was again found to be clearly separated from the rest of the dataset, which confirms previous observations in the market study and receiver 1. The pattern observed on Fig. 4 strongly resembles the pattern observed in Ref. [17] which illustrated the various types of chemical profiles observed within the condom population. According to Ref. [17], Donor D2 is known to belong to another category than the rest of the samples. Therefore, the clustering of donor D2 in Fig. 4 and 5 is due to the variability within the condom samples.

Discrimination patterns are in agreement with previous observations on the discrimination of condom profiles conducted with another instrument (i.e. ATR) and applied on raw samples (i.e. non extracted condom rubbed on ATR crystal) [17].

Observations of donor variation were found not to be distinguishable from those made in the market study presented in Ref. [17], although two different instruments were used. This suggests that the model created on the condom dataset in presented in Ref. [17], can be considered as relevant and representative as the trace samples presented in this study exhibit exactly the same discrimination patterns.

3.3. *Influence factor II: receiver*

To investigate the effect of the receiver, the donor was fixed (here, D1-Ceylor Blue or D9-Manix Skyn, both silicone-lubricated condoms), and 9 volunteers (here after receiver) were asked to self-sample post-coïtal residues.

Typical spectra obtained from real samples were already presented in Ref. [14]: no interaction with the matrix was noted, and spectra presented clear and well-defined Si-O-Si and Si-C stretching bands, thus confirming the efficacy of DRIFTS analysis for case work. PDMS was detected in all the swabs that were collected right after coitus. From a qualitative point of view, abundances were found to vary between different volunteers but also within the same volunteer (Fig. 7). Such variation is likely to happen due to self-sampling or parameters linked to the contact itself and cannot be reasonably monitored.

The PCA scores plot shown in Fig. 8 contains two main clusters, one consisting of replicates from receiver R7, the second containing the rest of the samples. Investigation of PCs 4–7 did not produce any further enhancement of sample clustering.

Such a strong separation of the samples can originate from variation in self-sampling procedure or from the analytical instrumentation. Samples coming from receivers R7, R8 and R9 were run on the same

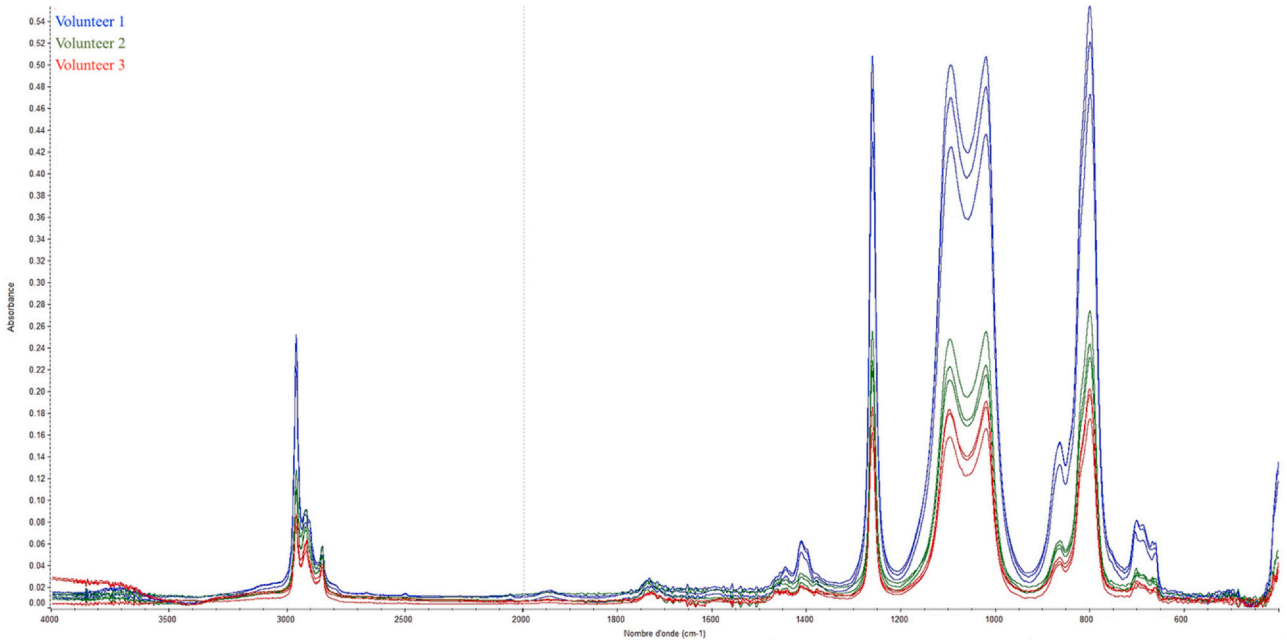

Fig. 7. Infrared spectra of post-coïtal transferred residues from 3 different volunteers. For each volunteer, the 3 replicates acquired are presented.

analysis day, which means it is unlikely that the variability of the sample preparation would be the main reason for R7 to be separated from the rest of the samples. Observation of the spectra obtained from R7 compared to the rest of the dataset coupled to the analysis of the loading plots obtained from the PCA computation allowed to note that the difference of abundance between the two bands coming from the Si-O doublet (Fig. 9) was the source of the variation.

Although Fig. 8 shows no significant differences between the volunteers when it comes to the relative abundance of the Si-O doublet band, similar difference of abundance was observed on Fig. 2, with pure PDMS samples analyzed after dilution in hexane. The first hypothesis considered for such difference was a variation of concentration. However, as illustrated on Fig. 9, not all the peaks are affected, only the silicone doublet, which does not make this hypothesis valid. The second hypothesis is that the variability induced in the DRIFTS results is partly due to the sample geometry: KBr particle size, influence of a manual KBr grinding or influence of a manual spiking on the pellet. A possible contribution from specular component, which differs depending on the KBr surface, can not be neglected. All these can induce a relative intensity changes.

Receiver R7 was clustered significantly separately from the rest of the samples. The corresponding samples were removed from the dataset and PCA was rerun on the remaining samples in order to investigate the variability between the other receivers. Fig. 10 illustrates the results of the new PCA. Receiver 3 was found to be clustered separately from the rest of the samples. Other samples were found to cluster together. Spectra were found to be visually similar, with no distinguishable features that could be used for sample separation according to the receiver. As the algorithm is able to detect a significative difference between spectra coming from Receiver 3

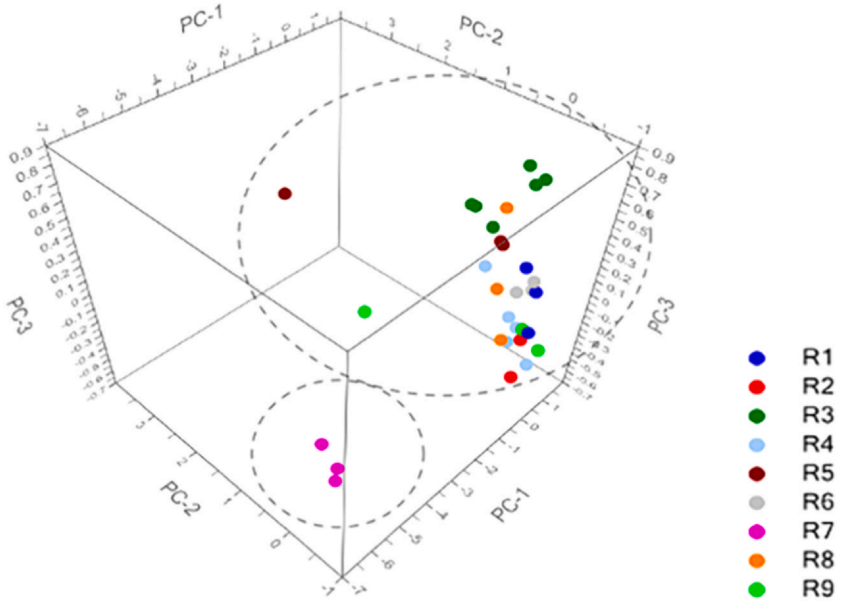

Fig. 8. 3D-Scores plot obtained from the Principal component analysis on the data used to investigate receiver effect. PC 1–3 are presented. Separation is made as a function of the receiver. Transfer was generated with silicone-lubricated condom D9-Manix Skyn for R2, R3 and R8, and with D1-Ceylor Blue condom for the other receivers.

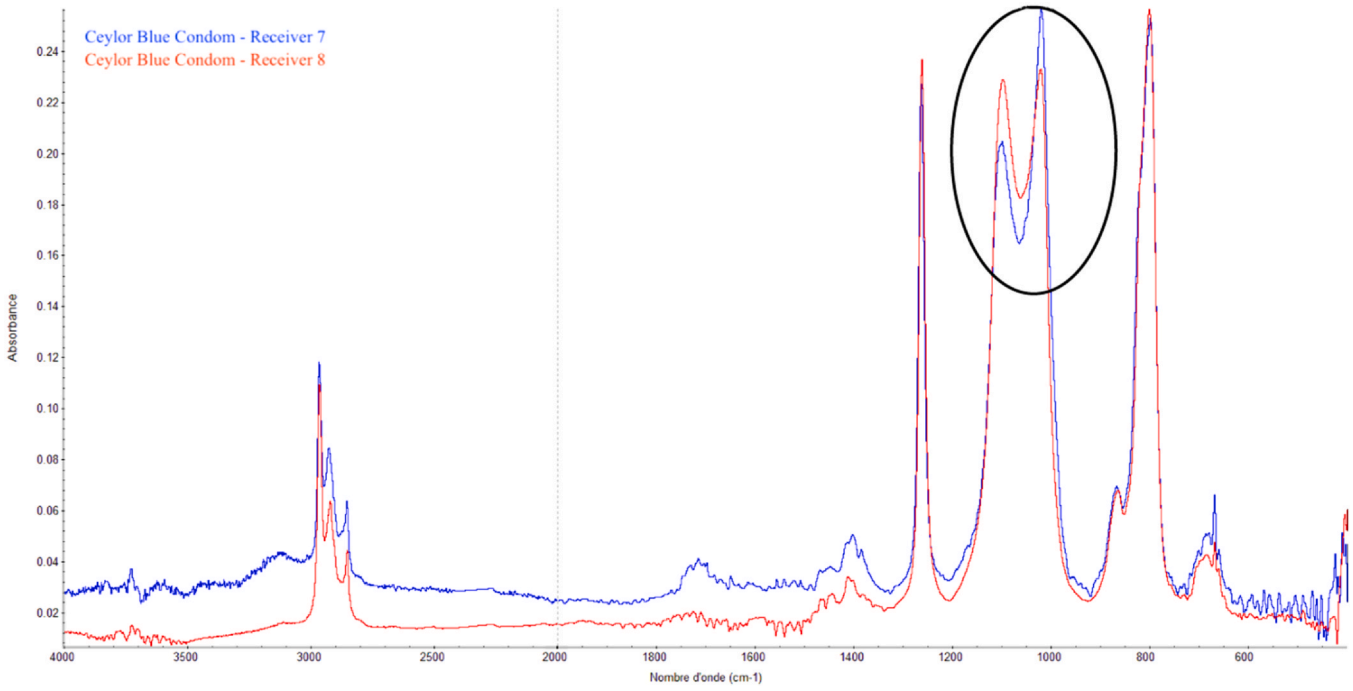

Fig. 9. Illustration of the difference of the abundance of the Si-O doublet peaks, highlighted in the black circle, between Receiver 7 and 8, samples collected at T = T0.

and the rest of the receivers, multiple hypothesis can be drawn, the main ones being:

1. A different initial concentration. This can result from variations during contact or during self-sampling.
2. Interactions between the matrix and the lubricants have generated chemical changes. Although these interactions have not really been reported and investigated and knowing that PDMS is highly nonpolar and not supposed to be reactive to most of the microbiota, this hypothesis is not very likely.

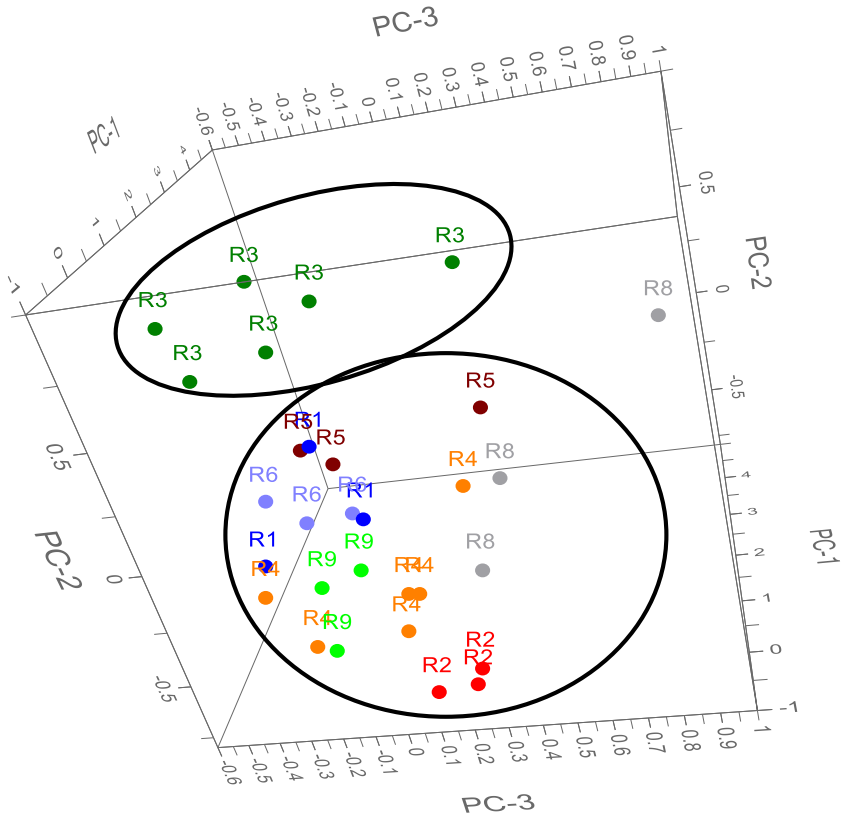

Fig. 10. 3D-Scores plot obtained from the Principal component analysis on the data used to investigate receiver effect. Receiver 7 removed from dataset. PC 1–3 are presented. Transfer was generated with silicone-lubricated condom (D1 -Ceylor Blue and D9- Manix Skyn).

3. The matrix has overcome some important changes at the time of the experiments, which explains the separation. Given the information obtained from the volunteer, there is no way to confirm that statement.

The clustering of the data is consistent with the results obtained in the background publication of Hermelin et al. [28] who showed that all the donors were not presenting different chemical profile nor were statistically differentiated. The present data illustrate a similar dispersion than the one observed in Ref. [28] and most of the data were found to cluster together independently of the receiver. Based on the indication discussed in Burnier and Massonnet [15], this strongly illustrate that the discrimination of the transferred residues does not seem to be less affected by the receiver than by the contact. The impact of the contact itself cannot be investigated and will remain an "uncontrolled" factor, as it implies to get information on the length of the penis, the intensity of the contact, or the duration of the sexual intercourse. Self-sampling also adds some variability, but such variations will be observed even though sampling is realized by a health care professional /SANE nurse, regardless of standardized training. Finally, some variations within the sample preparation, especially due to the manual preparation of the samples, which adds undesired additional variation to the data.

The great variability observed amongst the DRIFTS-FTIR results does not make it the gold method for an application in casework. Indeed, reproductibility is one the important parameters to control when considering a validation procedure in the eventuality of an accreditation for use in court. However, DRIFTS is a very interesting and successful screening method, fast and easily available. However as described in Refs. [10,25], FTIR is a screening method that should be coupled to an adequate confirmation method, such as GC/MS or py-GC/MS for example.

4. Conclusion

This study investigated the classification of condom traces after their transfer in a vaginal matrix as well as factors impacting transfer, such as the donor and the receiver, using human collected samples.

Visually, none of the condom extracts containing silicones were found to be significantly distinguishable, whereas non lubricated or water-based containing samples were found to be distinguishable. Clustering patterns observed using unsupervised statistical analysis were found to be similar to the ones obtained using ATR-FTIR in previous studies [17]. Traces were found to be not visually distinguishable from the reference material they originated from. Statistically, the algorithm created 4 clusters, which were found to be consistent with groupings created in previous publications. The results indicate that reference condom material and transferred traces do not present distinct chemical profiles. The experiments described in this study illustrate the potential to distinguish condoms in a similar way to what was observed during the construction of the infrared profile database.

Transfer was found to be affected mainly by the donor (i.e. the condom) rather than by the receiver (i.e. the vaginal matrix), which is not surprising when considering an apolar extraction of the cotton swabs. The contribution of the support after extraction of the traces from the cotton support proved to be practically nonexistent: the diluted silicone standards and spiked cotton having statistically indistinguishable profiles from the trace samples from a living vaginal matrix.

The experiments also highlighted the current difficulties in reducing the variability of the parameters due to different volunteers. If the procedure were to be limited to the use of known factors, the use of comparison material from the identified victim would be required, but this practice would be ethically questionable. These ethical questions were already highlighted in fingermark ageing works, where authors showed out that a model would need to be built using the suspect (if you can find them) and replicating the exact environment of the evidence [29–32].

The results obtained are no less interesting, but it is clear that additional studies must be carried out in order to assess the impact of the various influence factors and whether it is possible to construct a more relevant statistical model. Investigation of persistence of the traces as well as the ability to visually and statistically distinguish background from transferred and persisted evidence are still needed so as to allow a proper interpretation of the evidence in a Bayesian framework.

CRediT authorship contribution statement

Nikola Saric: Writing - original draft, Methodology, Validation, Data curation, Visualization. **Loïc Fabien:** Writing - original draft, Methodology, Validation, Data curation, Visualization. **Julia Fischer:** Writing - original draft, Methodology, Validation, Data curation, Visualization. **Anaïs Hermelin:** Writing - original draft, Methodology, Validation, Data curation, Visualization. **Geneviève Massonnet:** Writing - review & editing, Resources. **Céline Burnier:** Writing - review & editing, Conceptualization, Methodology, Investigation, Resources, Data curation, Visualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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