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LESSONS FROM A STUDY OF DNA CONTAMINATIONS FROM POLICE 1 SERVICES AND FORENSIC LABORATORIES IN SWITZERLAND 2 3 Patrick BASSET a* and Vincent CASTELLA a# 4 5 ^a Unité de Génétique Forensique, Centre Universitaire Romand de Médecine Légale, Centre 6 7 Hospitalier Universitaire Vaudois et Université de Lausanne, Ch. de la Vulliette 4, 1000 8 Lausanne, Switzerland 9 10 * Corresponding author, email address: Patrick.Basset@chuv.ch 11

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1 ABSTRACT

2

In Switzerland, the DNA profiles of police officers collecting crime scene traces as well as forensic genetic laboratories employees are stored in the staff index of the national DNA database to detect potential contaminations. Our study aimed at making a national inventory of contaminations to better understand their origin and to make recommendations in order to decrease their occurrence. For this purpose, a retrospective questionnaire was sent to both police services and forensic genetic laboratories for each case where there was a contamination.

Between 2011 and 2015, a total of 709 contaminations were detected. This represents a mean 10 11 of 11.5 (9.6 – 13.4) contaminations per year per 1'000 profiles sent to the Swiss DNA database. 12 Feedbacks were obtained from the police, the laboratory or both for 552/709 (78%) of the contaminations. Approximately 86% of these contaminations originated from police officers 13 whereas only 11% were from genetic laboratories employees and 3% were associated to other 14 15 sources (e.g. positive controls, stain-stain contaminations). Interestingly, a direct contact 16 between the stain and the contaminant person occurred in only 51% of the laboratory contaminations whereas this number increased to 91% for police collaborators. The high level of 17 indirect DNA transfer in laboratories might be explained by the presence of "DNA reservoirs" 18 19 suggesting that cleaning procedures should be improved. At the police level, most contaminations originated from the person who collected the trace and likely occurred directly at 20 the crime scene. Improving sampling practices could be beneficial to reduce these 21 22 contaminations.

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24 KEYWORDS: Forensic DNA analysis; DNA contamination; transfer; recommendations.

1 INTRODUCTION

2 With the current sensitivity of profiling STR kits, it is more common to detect minute amount of 3 contaminating DNA left by persons collecting or analyzing crime scene traces [1-3]. These 4 contaminations represent one of the most frequent source of error in forensic genetics and may have serious consequences on the result of an analysis [1, 4]. First, the contaminant profile 5 6 might mask the DNA profile of a crime stain and prevent a relevant profile to be sent to the national database. Second, if unidentified, a contaminant profile might create erroneous 7 8 investigative leads as illustrated in the classical example of the "Heilbronn phantom" [5]. This increases the risk of wrongfully discarding correct investigative leads and might have costly 9 10 consequences (e.g. increase resources needed to process comparisons, delay the process of 11 other cases) [4]. Third, contaminations may also create mixed DNA profiles and may therefore 12 decrease the evidential value of a match with the DNA profile of a person [1]. Finally, if contaminations are not detected early enough, they may generate a lot of public or justice 13 14 attention and may damage the reputation of forensic actors (i.e. police services or genetic laboratories) [1]. For these different reasons it is necessary to take all possible actions to keep 15 16 the risk of contaminations as low as possible.

17 Contamination may occur through different modes (e.g. through direct or indirect transfer, as a 18 result of ineffective cleaning procedures or as a result of contaminated material used to collect traces) and at any stage of the analysis of a DNA sample (i.e. from the collection at the crime 19 20 scene to the analysis in the laboratory) [4]. Therefore, it is important to increase our understanding of the factors involved in contaminations. Although several recent studies tried to 21 22 list and evaluate the occurrence of contaminations (e.g. [1, 2, 4, 6]) or focus on specific modes of contaminations (e.g. [7-11]), few studies tried to address contaminations from both the police 23 and laboratory perspectives. Thus, several general important questions about contaminations 24 25 are still open. These include knowing (i) the nature of the contaminated stains, (ii) the relative

1 frequency of direct or indirect contaminations, (iii) the consequences of contaminations on the 2 exploitation of a stain, (iv) where and when do contaminations occur the most likely, and (v) 3 whether there are differences between laboratory or police contaminations. Answering such 4 questions might help improve procedures, design good forensic practices to prevent DNA 5 contaminations both at crime scene and in the laboratories and provide better education to the 6 persons involved in the collection and the analysis of DNA stains.

7 Switzerland is a country with approximately 8 million inhabitants. It is divided into 27 police 8 services and has 7 accredited forensic DNA laboratories independent of the police. Although 9 some variability exists among services and laboratories regarding how crime scene samples are to be processed, stains or crime scene items are mostly collected directly at the crime scene by 10 11 police collaborators. In other cases, these items are sent to police or forensic DNA laboratories for stain collections by scientific collaborators. The stains are then analyzed in the ISO 17025 12 accredited laboratories using various STR kits and protocols. According to the Swiss law [12], at 13 least two PCR amplifications are necessary to validate a DNA profile. Based on the concordance 14 15 between replicates, the result at a locus can be validated. The profiles are generally 16 characterized into several categories: (i) no DNA profile, (ii) profile not interpretable, (iii) profile appearing as single source, (iv) mixed DNA profile appearing as 2 person mixture, (v) mixed 17 DNA profile with a major component of one or two contributors and a minor component not 18 19 interpretable or available for local comparisons, (vi) mixed DNA profile of more than two contributors available for local comparisons. Both the profiles of one contributor (single or major 20 contributor) and the mixtures of two contributors can be sent to the Swiss national DNA 21 22 database if at least six, respectively eight, loci have been validated. In contrast, mixtures of more 23 than two contributors and minor components of mixtures cannot be sent to the database. The Swiss database has been initiated in 2000 and is based on the CODIS software. At the end of 24 the study period (2015), approximately 62'000 stain profiles and 175'000 person profiles were in 25 the database. Since 2012, the database accepts profiles of new generation kits such as for 26

example NGM SElect or PowerPlex ESI 17. At the end of 2015, the DNA profiles of 2018 police 1 2 collaborators collecting crime scene stains, 429 forensic genetic laboratories collaborators, as 3 well as 10 profiles of other types (such as positive controls) were stored in the staff index of the 4 national DNA database. However, no nationwide legislation requires Swiss crime scene officers and laboratory employees to submit their DNA profile to the staff index. Therefore, each police 5 6 service and laboratory have their own regulations on whose DNA profile must be included or not in the staff index. Each new profile transferred to the national DNA database is not only 7 8 compared to the person and stain indexes, but also to the staff index to detect potential 9 contaminations. Once a potential contamination has been detected and validated, the laboratory and the head of the police department that handled the stain are informed so that appropriate 10 11 measures can be taken. However, each entity generally addresses their contaminations 12 independently.

In such a context, we decided to conduct a large DNA contamination study in Switzerland. In this regard, a retrospective questionnaire was sent to both police departments and forensic genetic laboratories for each contamination detected between 2011 and 2015. Our aims were to (i) make a national inventory of DNA contaminations, (ii) increase our understanding of their origins and of the mechanisms involved in these contaminations and (iii) identify potential measures to minimize their occurrences. In addition, this study aimed at increasing communications about forensic errors such as contaminations as recently recommended by [1].

20

21 MATERIAL AND METHODS

After a local pilot study done in our laboratory, a questionnaire was designed to address questions relative to DNA contaminations. This survey was designed to study three main topics: (i) the nature of the contaminated stain, (ii) what group of person acted as a contaminant, and (iii) the mechanisms likely involved in the contamination process. Representative questions as

well as the type of expected answers can be found in Table 1. In addition, a full version of the 1 2 questionnaire in table format can be found in the supplementary Table 1. This questionnaire was 3 built in three parts. The first part contained basic information about the contaminated stain such 4 as the category of the contaminant profile (police collaborator, laboratory employee, other) as well as the identification number (PCN) of each contaminated stain. After this first part, the 5 6 person who answered the questionnaire was directed either to a questionnaire addressed to the 7 police department which handled the contaminated stain or to the forensic DNA laboratory which 8 analyzed the stain depending on their affiliation. The identification number of the stain was used 9 to link the police and laboratory parts of the questionnaire. As our aim was not to compare the different laboratories or police services in terms of contamination numbers, the data were 10 11 globally and anonymously analyzed.

12 To make this questionnaire as user friendly as possible, an online version was created (available 13 upon request). The access link (in French or German according to the main language of the 14 service) was sent in spring 2016 to the corresponding police services and to the DNA laboratories which agreed to take part in the study. Each participant was asked to fill the 15 16 questionnaire for each potential contamination detected by the staff index database between 17 2011 and 2015. In addition, to investigate contaminations which had not been identified by the 18 database, each participant was also asked to give information on contaminations which had 19 been detected locally during the same period.

Answers were automatically stored in an Excel file which allowed further analyses of the data. For some data (e.g. nature of the contaminated stain, DNA concentration recovered on contaminated stain, characterization of contaminated profiles), the raw answers were directly used to estimate the characteristics of each parameter, whereas for other information (e.g. where and when the contamination likely occurred, factors potentially involved in the contaminations) answers to several questions were combined. Finally, the police services and

DNA laboratories had the possibility not to answer if they had insufficient information. For this
reason we decided to take into account only the cases for which an answer was obtained.
Therefore, the number of data available varies depending on the different questions addressed
in this study.

5

6 **RESULTS**

7 Data collected and frequency of contaminations. Five out of the 7 DNA laboratories and 23 8 out of the 27 police services of Switzerland took part in the study. The lack of participation of some laboratories and services was mostly justified by time constraints to answer the 9 questionnaire. For 3 of these police services, no contaminations were detected on the 356 10 profiles submitted to the database during that period (2011 - 2015). Feedbacks were received 11 12 from either the police service only (N = 6), the DNA laboratory only (N = 159) or both laboratory 13 and police services (N = 387). Out of the 709 contaminations detected by the national database between 2011 and 2015, information was received for 552 (78%) cases (Table 2A). This 14 15 represented a mean of 11.5 (minimum 9.6 – maximum 13.4 across years) contaminations per 16 year per 1'000 profiles sent to the Swiss DNA database (Table 2B). Most contaminations were associated to police staffs (86%; N= 476), whereas 11% (N = 62) were associated to laboratory 17 employees and 3% (N = 14) were associated to other type of profiles (Table 2A) such as positive 18 19 controls used during analyses (N = 5). Unknown profiles likely to be present on the swabs as background were also detected (N = 7; e.g. phantoms) as well as stain-stain contaminations (N 20 = 2). In general, contaminations appeared as sporadic, independent, events as contamination by 21 a single person of multiple stains belonging to the same case occurred in only 4% of the cases 22 (N = 22).23

Detection of the contamination. The majority of contaminations (N = 444; 90%) analyzed in 1 2 this study have been detected through the use of the staff index of our national database. This 3 staff index identified 417 (95%) out of the 438 contaminations by police collaborators and 27 4 (51%) out of the 53 contaminations by laboratory employees. Other contaminations were detected after a control requested by the police (e.g. following unexpected match between DNA 5 profiles) (5% of the contaminations involving police collaborators (N = 21) and 2% laboratory 6 employees (N = 1)) or through controls with local databases (47% of the contamination involving 7 8 laboratory employees (N = 25)).

9 Characteristics of contaminated stains. Contaminated stains were described either as "trace" 10 DNA (i.e. touch DNA) in 96% (N = 334) of the cases or as blood in 2% (N = 6), saliva in 2% (N = 6) or semen in less than 1% (N = 1) of the cases. No clear difference could be noticed between 11 12 the stains described as trace DNA and the other categories (i.e. blood, saliva or semen). When 13 quantified, the DNA concentration recovered from these contaminated stains ranged from undetectable level to 2.1 ng/ul, averaging at 0.14 ng/ul with a median concentration of 0.02 14 ng/ul. The number of validated loci for contaminated stains analyzed with "older" generation STR 15 16 systems (e.g. SGM Plus or SEFiler with a maximum number of respectively 10 or 11 loci) varied 17 between 5 and 11 with an average of 9 loci. The number of loci validated for stains analyzed with current STR systems (e.g. NGM Select or ESI with a maximum of 16 loci) varied between 3 18 and 16 with an average of 13 loci (Figure 1). 19

Characterization of contaminated profiles. Contaminated profiles corresponded to profiles appearing as single source in 145 cases (27%), mixture profiles with a major component sent to the database and a minor component not interpretable in 271 cases (50%) or a minor component available for local comparisons in 26 cases (5%), mixture of two contributors in 87 cases (16%), profiles only available for local comparisons in 7 cases (1%) and complex mixtures of more than two contributors in 3 cases (1%) (Figure 2A). **Consequences of contaminations**. No profile, other than the contaminant, could be identified in 420 (78%) of the stains (Figure 2B). The contaminant could be filtered out of 48 mixtures profiles (9%) which were subsequently submitted to the database, and it was part of a two contributors mixtures submitted to the database in 37 cases (7%). For 34 of the contaminated profiles (6%), the profile could not be sent to the database and was only available for local comparisons.

Once the contamination was detected, a new sampling was performed and analyzed in 18
cases. In 12 out of these 18 cases (66%), the new analysis resulted in a profile different from the
contaminant profile.

Direct versus indirect contaminations. Overall contaminations for which there is a clear direct explanation (i.e. direct contact with an item and/or activities such as speaking, sneezing or coughing near that item) represented 88% (N = 385) of the cases. Indirect contaminations (i.e. including one or more intermediary vector between the contaminant and the item) represented only 12% (N = 55) of the cases. Indirect contaminations were observed in only 7% (N = 28) of the contaminations involving police collaborators and 46% (N = 27) of those involving laboratory employees.

17 Hypotheses on where and when contaminations occurred For contaminations involving police collaborators, 72% (N = 227) likely occurred on the crime scene, 24% (N = 75) in the 18 19 police's examination room or 4% (N = 14) during transport or storage of the item (Table 3). For 20 these cases, the contaminant person was involved in the collection of the stain in 79% of the 21 cases (N = 176 for the crime scene contaminations and N = 73 for contaminations in the 22 examination room). The contaminant persons were also involved in either the collection (N = 27; 9%) or transport of the item (N = 3; <1%), present on the crime scene (N = 23; 7%), manipulated 23 the object during the storage (N = 9; 3%), photographed the items (N = 1 on crime scene and N 24

1 = 2 in the examination room of the police; <1%) or were the main users of the desks on which 2 the items were stored (N = 2; <1%).

In cases of contaminations by laboratory employees, 2% (N = 1) of the contaminations likely occurred during the collection of the stain, 81% (N = 37) during the extraction process or 17% (N = 8) during the amplification as the contaminant person was generally in charge of these steps in cases of direct contaminations.

Other types of analyses (e.g. search for fibers, fingermarks) had been performed on
contaminated stains in 15% (N = 60) of the cases but it is generally not clear if the contamination
occurred during these analyses.

10 **Examples of explanations given about contaminations.** To increase our understanding of the 11 different factors involved in these contaminations, the possible explanations reported for some 12 contaminations were grouped into 8 categories (Table 4). Although this list should be taken as qualitative indications (some explanations are easier to identify than others) and other categories 13 14 are also possible, we sorted these explanations according to their reported relative frequencies : (i) direct contact with an item or with the surface to swab, (ii) potential contaminations of gloves 15 16 in their package, (iii) potential contaminations of gloves before the collection or handling of a 17 stain, (iv) accidently spitting or sneezing over an item or a stain, (v) multiple handling of an 18 object to perform other analyses (e.g. fiber search, fingermarks, photographs) or because of the difficulty of opening packages, (vi) contamination of the swabbing kits after multiple use, (vii) 19 20 transport or storage of items in open bags and (viii) presence of potential DNA reservoirs in laboratories. Interestingly most of these explanations referred to both police and laboratory 21 22 contaminations (e.g. contaminations of gloves through contact with swab boxes, multiple handling to perform several activities, DNA reservoirs). 23

24

1 DISCUSSION

Using a retrospective questionnaire sent to both police forensic services and forensic genetic 2 3 laboratories in Switzerland, we collected information about 552 contaminations detected between 2011 and 2015. This number represents 78% of the 709 contaminations recorded by 4 the Swiss DNA database during the same time period and suggests that our data represent a 5 6 good picture of the contaminations occurring in Switzerland. The remaining contamination events for which no data was received may be explained by the fact that several laboratories 7 8 and police services did not take part in this survey. To our knowledge this represents the largest study of DNA contaminations published so far [1, 2, 4]. Nevertheless, the number of 9 10 contaminations detected in our study most likely represents an underestimation of the real 11 number of contamination in Switzerland for several reasons. First, not all the persons in contact 12 with crime scene stains have their DNA profile in the staff index database. The frequency of the 13 representation of crime scene workers in the staff index database strongly varies among police 14 services and among the categories of crime scene workers. For example, all forensic police services have introduced some collaborators in the staff index but five of these services have 15 16 introduced only a part of their collaborators. In addition, most police officers that are not part of 17 forensic services do not have their profile in the staff index. Therefore, this frequency can only 18 roughly be estimated to be around 60-80% in Switzerland suggesting that at least about 20-40% of the contaminations have not been detected in our study because of the lack of profiles in the 19 20 national database. The benefit of having a comprehensive elimination database is illustrated by the high proportion (> 90%) of contaminations that have been detected within the national 21 22 database. Increasing the number of relevant profiles in the staff index database is therefore of primary importance to detect more contaminations as early as possible as already highlighted [2, 23 4]. The frequency of contamination detected by the national staff index database was lower for 24 contaminations by laboratory employees than by police collaborators (51% vs. 95%). An 25

explanation is that laboratories can use local elimination database to search for possible staff 1 2 contaminations before the profiles are transmitted to the national database. It is interesting to 3 note that most laboratory contaminations detected by local databases would have also been 4 detected by the national database as all laboratory employees have also their profile in the national staff index database. It should nevertheless be recommended to send every 5 contaminated profiles to the national database to allow a better global picture of contaminations 6 7 as well as for transparency reasons [1]. Second, the number of contaminations reported in this 8 study is most likely underestimated because most mixtures of more than two contributors, as 9 well as minor profile only available for local comparisons, are not sent to the database and only compared if there is a good reason to do so. This is illustrated by the low proportion of profiles 10 11 which do not meet the required criteria to be submitted to the database (e.g. complex profiles) 12 identified as contaminated in this study (~ 2% of all the contaminated profiles) although this type of profiles represents for example about 10% of the DNA profiles obtained in our laboratory. 13 Such is also the case for profiles that were not exploitable which are absent from our data but 14 15 which could also be explained by the addition of contaminant alleles. Finally, contaminations 16 involving other types of profiles (e.g. positive controls, phantoms, stain-stain contaminations) represent only 3% of the contaminations reported in this study. This probably also reflects an 17 underestimation and may be explained by the difficulty to detect these contaminations as such, 18 19 as they are not easily detected by database comparisons. Additionally, stain-stain 20 contaminations might be difficult to differentiate from situations where a single person is involved in multiple cases. The lack of data about stain-stain contamination is alarming as this type of 21 22 contamination can result in misleading evidences (in contrast to police or laboratory employees 23 contaminations) and can therefore result in the most serious type of errors (e.g. false associations) and miscarriage of justice as illustrated by the Jama or Scott cases [13, 14]. 24 Therefore, it is recommended to always keep in mind the "contamination hypothesis" in cases of 25 26 unexpected results and when evaluating DNA evidence in general.

During the study period, the frequency of contaminated profiles was stable and represented 1 2 approximately 1% of the ~ 48'000 profiles submitted to the Swiss database by the participating 3 services. This is a non negligible proportion and it highlights the importance of improving 4 procedures to minimize contaminations (see below). Although the comparison among the few studies which addressed the frequency of contaminations is difficult (e.g. the definition of the 5 6 contaminations is not always the same), the frequency of 1% reported here is similar to those reported in recent years studies in Norway and Austria [2, 6] but approximately one order of 7 8 magnitude larger than the frequency reported in a study in Québec (31 cases among ~ 31'000 9 profiles $\approx 0.1\%$) [4]. This difference may find an explanation in the higher number of profiles of police collaborators and laboratory employees present in the staff index within the national 10 11 database in Switzerland (2457) compared to the Québec study (327). It is important to specify 12 that the rates reported in our study are irrelevant in the context of a particular case [1]. In such situations, only case-specific contamination probabilities are relevant and these probabilities 13 need to be determined for each individual case. 14

Characteristics of contaminated stains. Most contaminated stains (> 96%) were trace stains 15 (i.e. touch DNA) and the concentration of DNA recovered on these stains was often very low (the 16 concentration recovered on half of the contaminated stains was below 20pg/µl). Although the 17 generally low concentration, the quality of contaminated profiles was relatively good as the 18 19 number of validated loci (i.e. reproducible) was often close to the maximum expected number 20 (Fig. 1). This is in favor of the hypothesis that the increased sensitivity of new STR profiling systems increases the probability to detect very small quantities of contaminants [2, 3]. 21 22 However, we cannot really evaluate the influence of the sensitivity of new generation kits since most of the contaminated stains were analyzed with new generation kits, as the criteria for the 23 24 Swiss database changed in 2012 and the new kits were introduced within the different 25 laboratories between 2011 and 2013.

Consequence of the contamination on the exploitation of the stain. The contaminant profile 1 2 represented the only exploitable profile (as single or as major contributors) in more than 75% of 3 the cases (Fig. 2A). Two contrasting hypotheses may explain that. First, the quantity of the 4 contaminant DNA might be too large compared to the DNA already present on the stain. In particular when the contaminant DNA represents more than about 10 times the minor DNA [15, 5 16], the contaminant profile can mask other relevant profiles. In such situations, the concerned 6 7 stains might can be ruined by the contamination. Second, the quality and quantity of the DNA 8 already present on the stain before the contamination was too low and/or not good enough to 9 give an interpretable profile. In such situations, even without the contamination, the concerned stains would not have given an interpretable profile and the contamination would thus have no 10 11 real impact on the exploitation of the stain. It is difficult to distinguish between the two 12 hypotheses for cases with high DNA concentrations. However, the generally low DNA concentrations measured for contaminated profiles suggest that the second hypothesis could be 13 favored in most cases and therefore the contamination did not really affect the informativeness 14 15 of these stains.

16 For about 15% of the cases, a DNA profile different from the contaminant profile could be sent to the database, either as a mixture of two contributors including the contaminant profile or as a 17 single profile after filtering out the contaminant profile (Fig. 1B). In these cases, the 18 19 contamination may have complicated the exploitation of the stain (e.g. probabilistic interpretation of a DNA mixture profile instead of a single-source profile) but it did not really prevent a search 20 in the database as it is possible to search DNA mixtures assigned as being from two contributors 21 22 in the Swiss national database. Contaminations probably prevented profiles from being sent to 23 the database in less than 6% of the cases. Thus, although contaminations might have dramatic consequences on individual cases, in most situations, it apparently did not fully compromise the 24 exploitation of the contaminated stain. 25

Contamination can however have other important consequences such as creating erroneous investigative leads and increasing the risk of wrongfully discarding appropriate investigative leads [4], decrease the evidential value of a trace [1] and/or damage the reputation of forensic actors [1]. For example, during the study period, the Swiss staff index database detected about 100 contaminations per year. This represents ~100 cases for which erroneous investigative leads were quickly avoided.

Finally, collecting a new sample when this is feasible and adequate might reduce the impact of
contamination. In 12 out of the 18 cases (66%) of our study for which a new sample had been
collected, profiles different from the contaminant profiles were produced. This highlights the
added value of such a strategy.

Direct vs. indirect contaminations. Contaminations might be explained by a direct contact with 11 12 an item and/or some behaviors such as speaking, sneezing or coughing near that item [17]. 13 Personal communications of several police officers indicated that crime scene investigators do 14 not always wear gloves and face masks when collecting and/or processing crime scene samples (Table 4). Some contaminations were also explained by gloves contaminations (in their package 15 16 or before the collection of the stain) (Table 4). Protective gloves used during crime scene 17 investigation have already been shown to transfer DNA efficiently highlighting the need to 18 frequently change gloves [8, 18]. This further supports the observation that protective clothing alone is not sufficient and can give a false sense of security [18, 19]. 19

Such direct contaminations are generally easily understandable and can globally be prevented by strict applications of appropriate DNA collection and/or good laboratory practices. In contrast, in some situations the contaminant matches with a person who was not in proximity with the contaminated item. Thus, the contamination can only be explained by one or more transfers involving unknown vectors. These indirect contaminations are much more difficult to understand and prevent. As expected, most of the contaminations analyzed in our study (88%) enter in the

direct category. However, the number of cases for which there is no clear explanations on how 1 2 the contaminant DNA was found in the DNA sample (i.e. indirect contamination) is significant 3 and represented 12% of all cases. Interestingly, the frequency of those indirect contaminations 4 increases to 46% for contaminations by laboratory employees. This value is consistent with recent studies which reported likely indirect contaminations in approximately 35% of the cases 5 [2, 20]. Although each individual case is difficult to explain, the high level of indirect DNA transfer 6 7 in laboratories might be explained by the occurrence of "DNA reservoirs" such as surfaces, tools and equipment that are regularly used by one or several persons in the closed laboratory 8 9 environment [20]. To prevent these contaminations, it is necessary to improve cleaning procedures as well as clear physical separations between living environment (e.g. offices) and 10 11 laboratories facilities [21, 22]. In such a context, it is also recommended that laboratories 12 implement environmental DNA monitoring programs for the detection of DNA reservoirs [3, 9, 21]. 13

14

15 Who was the source of the contamination? Contaminations by police collaborators were 16 detected 8 times more often than contaminations by laboratory employees (86% vs. 11%). This difference might be explained by several factors. First, the police are generally in charge of 17 collecting the stains (roughly more than 95% of the stains are collected by police collaborators). 18 19 This collection might require multiple handlings and a close proximity with the sampled item which increases the contamination risk. Second, the collection of the stain is generally done on 20 crime scene in generally more than 60% of the time (this number can even be larger than 90% 21 depending on the police services) which is a complex environment compared to the laboratory. 22 23 For example, many persons might be present on the crime scene before or during the collection of the stains, which increases the risk that one or more of these persons deposit their DNA. In 24 addition, weather, light or stress conditions as well as disturbance by other persons on crime 25 26 scene (e.g. victims, paramedics) might further increase these risks compared to the laboratory.

The difficulty of the crime scene environment is illustrated in our study by the fact that 72% of the 1 2 police contaminations likely occurred on the crime scene. Finally, good laboratory practices are 3 fundamental parts of the education of laboratory technicians whereas police staffs are maybe 4 less aware of important practices to minimize contamination risks (e.g. changing gloves and wearing face masks). In 79% of the police contamination cases, the contaminant person was 5 involved in the collection of the stain (either on crime scene or in the examination room) 6 highlighting the importance of careful and efficient collection practices. Training to increase 7 8 knowledge in biological transfer mechanisms of all the persons involved in the collection or 9 examination of crime scene samples is essential [2]. The persons involved in the transport and/or the storage of an item should also be involved in these trainings since they are 10 11 associated to almost 20% of the contaminations (Table 3 and 4). This highlights the need to 12 establish good practice procedures also for such activities (e.g. transport and storage of evidence in adapted closed package, dedicated storage rooms with restricted access, 13 minimization of unnecessary handling and transport). Multiple handlings of exhibits looking for 14 15 evidence other than DNA (e.g. fingermarks, fibers, pictures) could also explain other 16 contaminations. For example, in 15% of the contaminations within our study, other examinations had also been performed. In such a context, sampling DNA evidence before other examinations 17 seems essential and it might be useful to split specific activities (pictures, collection of stains) 18 19 among different persons to prevent a single person from touching simultaneously an object 20 (such as a camera) and the crime scene item.

21

Conclusions and recommendations. DNA contaminations have always been part of the forensic genetic domain and should be prevented as much as possible. The high sensitivity of current STR profiling systems makes amplification of trace amount of contaminating DNA easier and therefore increases the protection required when handling crime scene items. However, because contaminations can have serious consequences on individual cases, it is essential to

inform each person potentially in contact with crime scene items about these risks. Our study 1 2 improves the knowledge about DNA contamination and we hope it will contribute to an open and 3 blame-free research culture in forensic science that promotes criminal justice and public trust [1]. 4 These contaminations can occur at each step along the chain of analysis; from the collection on crime scene or in the examination room, to the transport or the storage, up to the DNA analysis 5 6 in the forensic genetic laboratory. However, our analysis highlights that some of the steps, such as the collection of the DNA on crime scene, require special attention. Although it is impossible 7 to fully eliminate contaminations, the different issues reported in this study point to the 8 9 importance of training as well as improving the compliance with practical recommendations proposed to reduce the risk of contaminations [2, 3, 21]. In such as context and following our 10 11 results we can make the following recommendations:

- The DNA profile of all the persons potentially in contact with DNA samples, and the items
 they are collected from, should be introduced in a centralized elimination database to
 detect contamination as much as and as early as possible.
- Each detected contamination (even locally) should be sent to the national database thus
 allowing a better global picture as well as for transparency reasons.
- The knowledge in biological transfer mechanisms, while handling crime scene samples,
 should be increased through training courses and/or continuous education of all involved
 persons, with special attention to those involved in stains collection.

4. Appropriate protective clothing (such as uncontaminated gloves and face masks) should
 be worn systematically while collecting or handling evidences. Gloves have to be
 changed frequently, ideally before the collection of each new item of evidence and every
 time an object has been touched

- 5. Whenever possible, DNA evidence should be collected before other examinations. If
 that's not the case, other examinations should follow procedures compatible with DNA
 contamination prevention.
- 6. Best practice procedures to handle, transport and store DNA evidence should be
 established. Transport and storage of DNA evidence should be done in appropriate
 conditions within dedicated clean and protected spaces.
- 7 7. DNA reservoirs should be reduced as much as possible through efficient cleaning
 procedures as well as clear physical separations between living environment,
 laboratories or storage facilities; in this context, specific activities (e.g. pictures, collection
 of stains) should be split among different persons to interrupt the chain of contamination.
- 11 Environmental DNA monitoring programs might also be useful.
- Finally, each entity (service, laboratory, surveillance authorities) should develop its own
 guidelines or adapt existing ones [23-26] in order to minimize contaminations.
- 14

1 CONFLICT OF INTEREST

- 2 None
- 3

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1 FIGURE LEGENDS

- 2 Figure 1. Number of validated loci per contaminated profiles analyzed with older generation
- 3 systems (e.g. SGM, SEFiler; maximum 10-11 loci) and current generation systems (e.g. NGM
- 4 Select, ESI; maximum 16 loci).
- 5 Figure 2. Characterization of contaminated profiles before (A) and after (B) the detection of the
- 6 contamination.

- **Table 1.** Representative questions and expected answers of the questionnaire sent to (a) the
- 2 police services and (b) to the forensic genetic laboratories (a full version of the questionnaire is
- 3 also available in supplementary Table 1).

4 (a) Police services

Questions	Expected answers
Date of the collection of the contaminated stain?	Date
Nature of the contaminated stain?	Trace DNA, blood, saliva, semen, unknown
Do you have an explanation about this	Yes, no
contamination? If yes, which one?	Text answer
Has the contaminant person been in direct	Yes, no, unknown
contact with the stain? If yes, where did the contact take place?	e.g. during collection of the stain, during handling of the box, during labeling, during storage, unknown
Where does the contaminant person work?	Only in the laboratory, only on crime scenes, in the laboratory and on crime scenes, other
Has another stain been collected on the same	Yes, no
item? If yes, did that allow to get another DNA profile different than the contaminant profile?	Yes, no
General remarks?	Text answer

(b) Forensic genetic laboratories

Questions	Expected answers		
How was the contamination detected?	National database (CODIS), local staff database, other		
Quantification value [ng/ul]?	Concentration value		
Kit used?	e.g. NGM select, ESI, Global filer, SGM		

	Plus, SEfiler, minifiler, ESX
Characterization of the contaminated profile?	profile appearing as single source; mixture profile, major for CODIS, minor not interpretable; mixture profile, major for CODIS, minor for local comparison; mixture profile of 2 contributors; reduced profile; profile kept for local comparison; other
After the detection of the contamination, how was it possible to use the profile?	Profile other than the contaminant profile sent to CODIS as a mixture; profile other than the contaminant profile sent to CODIS as a reduced profile; profile kept for local comparison; no other profile than the contaminant profile; other
At what step do you think the contamination occured?	Storage; reception/control/registration; collection of the stain; extraction; quantification; amplification; During the handling of the stain by the police; unknown; other
In cases of contamination by a laboratory employee, has the person worked with the sample?	Yes, no, unknown
If yes, at which step?	Storage; reception/control/registration; collection of the stain; extraction; quatification; amplification; other
General remarks?	Text answer

Table 2. (a) Nb of answers received to the questionnaire among years and according to the origin of the contaminant profile. (b) Frequency of contamination analyzed per year per 1'000 profiles sent to the Swiss DNA database.

(a)	2011	2012	2013	2014	2015	Total
Police officers	70	97	131	89	89	476
laboratory employees	11	9	9	12	7	62*
Others**	0	0	3	0	2	14*
Total	81	106	143	101	98	552

* No date available for the data of one lab; ** positive contrôle, unknown profiles, stain-stain, etc.

4

(b)	2011	2012	2013	2014	2015	Total
Police officers	9.2	9.9	12.3	8.5	9.3	9.9
laboratory employees	1.3	0.8	0.8	1.0	0.7	1.2*
Others**	0	0	0.3	0	0.2	0.2 *
Total	10.6	10.8	13.4	9.6	10.3	11.5

* No date available for the data of one lab; ** positive contrôle, unknown profiles, stain-stain, etc.

5

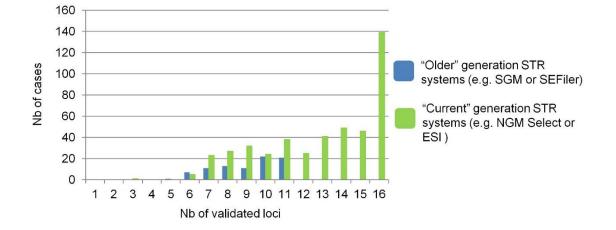
- **Table 3.** Distribution of contaminations involving police staffs, according to the place where it
- 3 occurred and the activity of the contaminant.

	Likely places of	_		
Activity of the contaminant	Crime scene	Transport/ storage	Examination room of the police	total
Involved in the collection of the stain	176	0	73	249
Involved in the collection or transport of the item	27	3	0	30
Present on the crime scene	23	-	-	23
Manipulated the item during storage	-	9	-	9
Photograph	1	-	2	3
Object stored on the desk	-	2	-	2
Total	227	14	75	316

Table 4. Explanations given about some contaminations and related potential of improvements.

Qualitative explanations	Potential of improvement of procedures		
(i) Direct contact with the item or with the surface to swab	 (i) Wear appropriate protective clothing (gloves, lab coats) to prevent any direct contact with any item or surface that might be swabbed 		
(ii) Potential contaminations of gloves in their package	(ii) Use individual gloves packaging		
(iii) Potential contaminations of gloves before the swabbing of a stain	(iii) Frequent glove changes		
(iv) Spitting or sneezing	(iv) Always wear face masks and minimize spoken interaction close to a piece of evidence		
 (v) Multiple handling of an object to look for other evidences (e.g. photographs, fingermarks) or difficulty to open a package 	 (v) Multiple handling should be avoided; sample DNA evidence first, share activities between different persons following good laboratory practices 		
(vi) Contamination of the swabbing kits after multiple use	(vi) Use individual swabbing kits, do not use swabs if they are out of their original package		
(vii) Transport or storage in open bags, storage on office desks.	(vii) Use only closed bags to transports or store an item. No storage on office desks.		
(viii) Potential DNA reservoirs in laboratories	(viii) Separate living environment (e.g. offices) from laboratories or examination rooms, restrict access to laboratory spaces, improve cleaning procedures of these places, environmental DNA monitoring programs		

1 Figure 1



1 Figure 2

