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Authors: Basset P, Castella V

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POSITIVE IMPACT OF DNA CONTAMINATION MINIMIZATION PROCEDURES TAKEN WITHIN THE LABORATORY.

Patrick BASSET [a]* and Vincent CASTELLA [a #]

[a] Unité de Génétique Forensique, Centre Universitaire Romand de Médecine Légale, Centre Hospitalier Universitaire Vaudois et Université de Lausanne, Ch. de la Vulliette 4, 1000 Lausanne, Switzerland

* Corresponding author, email address: Patrick.Basset@chuv.ch

Email address: Vincent.Castella@chuv.ch

Highlights

- A study of the impact of DNA contamination minimization procedures
- Contamination minimization procedures significantly decrease contaminations
- Minimization procedures should be implemented all along the chain of analysis

ABSTRACT

DNA contamination incidents are one of the most frequent sources of error in forensic genetics and can have serious consequences. It is therefore essential to take measures to prevent these events and to monitor the real impact of contamination minimization procedures. In this study, we review and compare the number of contamination events detected on trace samples analyzed by the Forensic Genetic Unit (FGU) of the University Center of Legal Medicine in Switzerland before and after the implementation of new contamination minimization procedures. Interestingly, the number of contamination events by laboratory staff was significantly reduced by more than 70% after the implementation of the procedures. However, no significant change was observed for contamination events by police collaborators. This difference is likely to be explained by the differential impact of procedures taken in the laboratory and on crime scene. It suggests that the reduction observed for laboratory contamination incidents is due to the new procedures taken. In conclusion, our study highlights that taking appropriate measures is efficient and can reduce the number of contamination incidents. However, it is important that such contamination minimization procedures be implemented all along the chain of analysis of a stain (i.e. from crime scene to the laboratory).

KEYWORDS: Forensic DNA analysis; DNA contamination; Transfer; Recommendations.

1. INTRODUCTION

With the current sensitivity of profiling STR kits, it becomes more and more difficult to prevent background DNA and contamination events from persons collecting or analyzing crime scene samples [1, 2]. While one cannot control background, one can limit contamination. These contamination events represent one of the most frequent sources of error in forensic genetics and can have serious consequences for the judicial system [2]. First, the unwanted profile might mask the DNA profile of a crime stain and prevent the use of relevant profiles [3]. Second, as long as it is unidentified, an irrelevant profile might create erroneous investigative leads. This increases the risk of wrongfully discarding correct investigative leads and might have costly consequences (e.g. an increase of resources needed to process comparisons, delay in the process of other cases) [3]. Most importantly, if contamination incidents are not detected early enough, they can generate miscarriage of justice, lead to mistrust and damage the reputation of forensic actors (i.e., police services or genetic laboratories) [2]. For these reasons, it is necessary to take all the possible actions to prevent these events as much as possible.

Several procedures have recently been proposed to reduce the risk of DNA contamination incidents on both the crime scene and in the laboratory [1, 4-9]. Among these procedures, several are particularly adapted to the laboratory environment. These include (i) the awareness education of the staff about transfer mechanisms, (ii) the correct use of protective clothing (e.g. gloves, facial masks, lab coats) and their frequent change, (iii) the restriction of access to laboratory space, (iv) the efficient cleaning and decontamination of all equipment and laboratory zones, (v) the physical separations between living environments, laboratories or storage facilities to reduce DNA reservoirs, (vi) the split of specific activities (e.g. collection of traces) among different persons to interrupt chains of contamination. While some of these procedures can easily be adopted within any forensic DNA laboratory (e.g. awareness education of the staff, efficient use of protective clothing), other procedures might be more difficult to set up,

costly and/or time consuming (e.g. change the laboratory design, dedicate two or more persons to specific activities). Although many of the contamination minimization procedures are adapted from standard good laboratory practices, the real impact of such procedures on the contamination level have been rarely tested.

The Forensic Genetic Unit (FGU) of the University Center of Legal Medicine is an ISO 17025 accredited forensic DNA laboratory located in Switzerland. The FGU laboratory processes approximately 12'000 traces per year mainly for the six French-speaking police services of Switzerland. Both the profiles of one contributor (single or major contributor) and mixtures of two contributors can be sent to the Swiss national DNA database if at least six, respectively eight, loci have been validated. In contrast, mixtures of more than two contributors and minor components of mixtures cannot be sent to the database [5]. For improvement purposes, the laboratory records all the contamination incidents of trace samples analyzed within the laboratory. These contamination incidents are generally detected through the use of the staff index of the Swiss database, through controls in a local laboratory staff database or after controls requested by the police [5]. Potential matches with staff profiles are reported by the Swiss database when at least 5 loci, respectively 7 loci, are shared in cases of profiles appearing as single source or as mixtures of two persons. For controls in the local laboratory staff database, potential matches are reported when more than 75% of the profile is shared. Each potential contamination incident is then checked on a case-to-case basis. In 2015, the FGU laboratory moved to a new building. Although the FGU laboratory was already taking numerous precautions to minimize contamination incidents (Table 1), this move was an opportunity to review and improve contamination minimization procedures within the laboratory. In this study, we review and compare the number of contamination detected before and after taking the additional measures. Our goal was to check if the additional procedures taken in our laboratory to minimize DNA contamination incidents (e.g., separation between living and

laboratory zones, systematic wear of lab coats in laboratory zones, different operators to collect traces) had a significant impact on the number of contamination incidents detected on DNA traces analyzed in the laboratory.

2. MATERIAL AND METHODS

2.1 Situation before 2015

Before the move into a new building, the laboratory already had several procedures to minimize DNA contamination incidents (Table 1a). These included the systematic wear of lab coats within the laboratory, the systematic wear of gloves and masks when handling evidences, the systematic cleaning of laboratory surfaces (e.g. bench, hoods) and instruments (e.g. scissors, tweezers) potentially in contact with the evidence as well as a physical separation between the process of traces and that from persons and pre- and post-PCR laboratory zones. In addition, the DNA profiles of all the laboratory staff were stored in the staff index of the Swiss national database. Finally, contaminations events were discussed within meetings to understand the potential causes of contamination incidents and increase the staff awareness of biological transfer mechanisms. However, because of space constraints, it was not possible to apply a clear separation between living and laboratory environments and therefore, the offices of the laboratory technicians were located within the different laboratory zones.

2.2 Situation after 2015

At the beginning of 2015, the laboratory moved to a new building with larger laboratory spaces allowing the improvement of the laboratory organization and in particular the contamination minimization procedures (Table 1b). For example, technician offices were in separate rooms and there was a clear distinction between living and laboratory environments. Additionally, to reduce as much as possible the level of background DNA within the laboratory, it was decided to systematically use different disposable lab coats according to the zone of the laboratory, to change these lab coats regularly (generally daily) and to request the personal not to wear these lab coats in living environments (e.g. offices, cafeteria). It was also requested that personal objects (such as phones or pens) did not enter the laboratory zones. This move was also an

opportunity to review and improve other contamination prevention measures. For example, to increase the potential frequency of glove change, it was decided to use a double gloving policy when manipulating traces during the extraction process. To interrupt the possible chains of contamination, it was also decided to split whenever possible the stain collection among different persons so that the person in contact with the swab never touched other surfaces potentially with background DNA such as evidence packaging, camera or computer keyboards. Finally, at the same period, DNA automated extraction systems were introduced for most standard traces. These systems allowed reducing the manual handling of traces therefore also potentially reducing opportunity for transfers.

Number of contamination detected

All the contamination events observed in the FGU laboratory between 2012 and 2017 (i.e. three years before and three years after the implementation of the additional contamination minimization procedures) are included in the study. As minimization procedures likely had different impacts on contamination incidents by laboratory staff or police collaborators, contamination incidents were also sorted according to the origin of the contaminant profile. Finally, to address the impact of the additional procedures, the frequency of contamination incidents (number of contamination incidents per number of traces analyzed) between 2012 and 2014 (before the additional procedures) was compared with the same frequency between 2015 and 2017 (after the additional procedures) with Fisher's Exact tests.

RESULTS AND DISCUSSION

A total of 260 contaminated traces have been detected between 2012 and 2017 at the FGU laboratory (Table 2). As expected most of these (i.e. 247/260) were described as touch DNA and the concentration of DNA recovered was generally very low (often below 20pg/ul) [5]. Contaminated traces represent a proportion of ~0.3% (i.e. 260 / 77'962) of the traces analyzed during the same period and a proportion of ~1% (i.e. 260 / 26'876) of the profiles sent to the Swiss DNA database. These frequencies are similar to the frequencies (between 0.1 – 1%) reported in other studies on contamination events [3-5, 10]. However, it likely represents an underestimation as most contamination incidents in Switzerland are detected by the staff index of the national database and only ~34% of the traces analyzed at the FGU laboratory are sent to the Swiss database [5]. Very partial DNA profiles and/or mixed DNA profiles with more than two persons cannot be sent to the database, but they can still be compared manually with other DNA profiles following a request from the police services. These DNA profiles represent about 10% of the traces analyzed and they do not generally undergo contamination check. Moreover, not all the persons in contact with crime scene traces have their DNA profile in the staff index database although this parameter is crucial to have an efficient detection of contamination incidents [3]. The proportion of 0.3% is therefore non-negligible and highlights the importance of improving procedures to minimize contamination incidents.

Overall the number of contamination incidents detected during the three years after the implementation of additional contamination minimization procedures was lower than during the three years before these additional procedures (110 vs 150) suggesting an overall slight effect of these procedures (Table 2; $P > 0.05$, Fisher's Exact test). Several factors such as the sensitivity of the STR profiling kits [1, 4], characteristics of the sample (touch DNA vs other secretions), amount of DNA characterizing the sample [3, 5], education of the staff or the effort made to look for contamination incidents (e.g. number of relevant profiles in the staff-index

database) [3] could also influence the detection of contamination incidents. However, none of these factors seem to have changed significantly between the different periods of our study.

Contamination can occur at different steps along the chain of analysis of a stain; from the collection on crime scene or in the examination room, to the transport or the storage, up to the DNA analysis in the forensic genetic laboratory. Therefore, according to where the contamination minimization procedures are taken, their impact will be different according to the origin of the contaminant profile. For example, in cases of contamination by police collaborators, the transfer of the contaminant DNA most likely occur on crime scene [5, 10]. It is therefore unlikely that procedures taken at the laboratory level can prevent this type of contamination. The procedures reported here were only taken in the laboratory and we have not been informed of particular contamination minimization procedures taken by the police during the study period. Hence, only contamination incidents occurring in the laboratory are expected to be affected by the measures taken in our study. Interestingly, the number of contamination events by laboratory staff was reduced by more than 70% after the implementation of the procedures (17 for 2012 – 2014 vs 4 for 2015 - 2017; Table 2) and the difference between the two periods was significant ($P < 0.01$, Fisher's Exact test). In contrast, during the same period, the number of contamination incidents by police collaborators was only slightly reduced (129 for 2012 – 2014 vs 102 for 2015 - 2017; $P > 0.05$, Fisher's Exact test) suggesting that the reduction observed for laboratory contamination incidents is actually mostly explained by the additional procedures taken in the laboratory.

With our data, it is not possible to make the distinction between the effects of the different measures that were taken. The physical separation between living environment and laboratory spaces, as well as the use of dedicated disposable lab coats were designed specifically to reduce background DNA reservoirs within laboratory spaces. Such background DNA reservoirs have been proposed as an explanation for the high proportion of indirect contamination

incidents in the laboratory environment [5, 11]. In contrast, the split between different operators as well as the double gloving were designed to interrupt the potential chains of contamination. This could affect both direct and indirect contamination incidents. During the study period, more than 60% (13/21) of the contamination incidents by laboratory staff could not be explained by a direct contact with the item and/or some behaviors such as speaking, sneezing or coughing near that item [12]. These contamination incidents can only be explained by one or more transfers involving unknown vectors and are thus considered as indirect. This value is higher than in recent studies, which reported possible indirect contamination incidents in about 35% of the cases [4, 11]. The relative frequency of indirect laboratory contamination incidents is similar before (10/17) and after (3/4) the additional procedures suggesting that background DNA is still present in the laboratory. To check for the presence of background DNA, it is recommended to regularly perform environmental DNA monitoring screening [1, 8, 11]. Environmental screening was performed occasionally in our laboratory and it confirmed the presence of background DNA on various laboratory surfaces and instrument. Thus, a systematic and regular screening is currently under evaluation to identify more thoroughly possible DNA reservoirs and further improve laboratory cleaning strategies.

Another factor that could explain the reduction of contamination events at the laboratory level is the introduction in 2015 in the laboratory of DNA automated extraction systems (AutoLys and STARlet from Hamilton robotics) for most standard traces. In contrast to manual extraction, this type of system requires less handling of the sample by the laboratory technicians, reduces the number of manual steps and therefore likely reduces the number of possibility for DNA transfer. The potential positive impact of automation is illustrated by the fact that only 1 of the 4 (25%) contamination events from laboratory staff (which most likely occurred during the extraction process) recorded since 2015 was extracted using the automated system although the proportion of traces extracted by this system was approximately 65%. In contrast, for

contamination by police collaborators (which most likely occurred on crime scene), the proportion of contaminated stain extracted by the automated system was of 59% (i.e. close to the expected number of 65% if the extraction method has no impact). However, because of the small number of laboratory contamination since 2015, further studies are necessary to determine whether automation can help to significantly reduce the number of contamination events.

In addition to practical measures, information about contamination risks is crucial to increase staff awareness about the potential consequences of these contamination incidents and about the potential biological transfer mechanisms [3]. The implementation of the additional procedures that have been taken in the laboratory undoubtedly participated to this information and likely increased the level of implication of laboratory staff in contamination prevention. This effect is likely illustrated by the fact that 3 of the 4 laboratory contamination incidents after the implementation of the new procedures occurred in 2015, i.e., shortly after the implementation of these procedures.

Finally, most of the contamination incidents were associated to police collaborators (89%; N= 231), whereas 8% (N = 21) were associated to laboratory staff and 3% (N = 8) were associated to other type of profiles such as positive controls, unknown profile or stain-stain contamination incidents (Table 2). This breakdown is similar to what was already observed in a previous study [5] and highlights the need for also taking contamination minimization procedures within the police. A study about DNA contamination incidents in Switzerland was recently conducted in collaboration with the different police services of Switzerland [5]. Several procedures to minimize contamination incidents of police collaborators were proposed but this study is too recent to know if the additional contamination prevention measures were taken in the police services and to address their potential impact on the number of contamination incidents from police collaborators.

In conclusion, our study shows the improvement of contamination prevention in our laboratory with relatively simple procedures reduced the number of contamination incidents from laboratory staff by more than 70%. This highlights that although it is probably impossible to fully eliminate contamination incidents, taking appropriate measures is efficient and can significantly decrease the risk of contamination. Finally, as expected, the number of contamination from police collaborators was not significantly reduced showing that contamination minimization procedures should be implemented all along the chain of analysis of a stain (i.e. from crime scene to the laboratory) to have a global effect on the frequency of contamination incidents.

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Table 1. Contamination minimization procedures taken at the FGU laboratory before 2015 (a) and additional procedures taken after the move of the laboratory beginning of 2015 (b).

(a) Before 2015

Contamination minimization procedures
- Awareness training of the staff
- DNA profiles of the laboratory staff loaded in the Swiss staff-index database
- Systematic wear of lab coats within the laboratory
- Systematic wear of gloves and masks when handling pieces of evidence
- Systematic handling of evidence under hoods or dedicated bench
- Separation between traces and persons laboratories
- Separation between rooms dedicated to DNA extraction, PCR set-up and PCR amplification
- Bench, hoods and other surfaces cleaned before and after every manipulations by DNA degrading reagents
- Laboratory instruments (e.g., scissors, tweezers) cleaned and sterilized before and after each use

(b) After 2015

Additional procedures
- Physical separation between living environment (e.g., offices) and laboratory zones; personal objects (e.g., phones, pens, etc.) must not enter the laboratory zones
- Double gloving to allow frequent glove change
- Systematic use of different disposable lab coats within the different rooms of the laboratory; lab coats generally changed daily
- Stain collection split among different operators so that the person in contact with the swab does not touch other surfaces (e.g., camera, evidence packaging)
- Automation of the DNA extraction for standard traces

Table 2. Number of traces analyzed by the FGU laboratory as well as the number of detected contaminated traces according to the origin of the contaminant profile.

			Number of detected contamination incidents			
Year	Total number of traces analyzed	Number of profiles sent to the Swiss DNA database	Laboratory staff	Police collaborators	Others *	Total
2012	12814	4964	2	38	1	41
2013	14828	5138	8	57	3	68
2014	13133	4897	7	34	0	41
Total 2012-2014	**40775**	**14999**	**17**	**129**	**4**	**150**
2015	12504	4252	3	32	2	37
2016	12697	4000	1	34	2	37
2017	11986	3600	0	36	0	36
Total 2015-2017	**37187**	**11852**	**4**	**102**	**4**	**110**

* positive controls, unknown profiles, stain-stain, etc.