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Author Manuscript

Faculty of Biology and Medicine Publication

This paper has been peer-reviewed but does not include the final publisher proof-corrections or journal pagination.

Published in final edited form as:

Title: Positive impact of DNA contamination minimization procedures taken within the laboratory.

Authors: Basset P, Castella V

Journal: Forensic science international. Genetics

Year: 2019 Jan

Issue: 38

Pages: 232-235

DOI: [10.1016/j.fsigen.2018.11.013](https://doi.org/10.1016/j.fsigen.2018.11.013)

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

3

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15 **Highlights**

- 16
- 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
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1 **ABSTRACT**

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

17

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

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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,

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

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

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

1 **2. MATERIAL AND METHODS**

2 *2.1 Situation before 2015*

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

15 *2.2 Situation after 2015*

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

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

10 *Number of contamination detected*

11 All the contamination events observed in the FGU laboratory between 2012 and 2017 (i.e. three
12 years before and three years after the implementation of the additional contamination
13 minimization procedures) are included in the study. As minimization procedures likely had
14 different impacts on contamination incidents by laboratory staff or police collaborators,
15 contamination incidents were also sorted according to the origin of the contaminant profile.
16 Finally, to address the impact of the additional procedures, the frequency of contamination
17 incidents (number of contamination incidents per number of traces analyzed) between 2012 and
18 2014 (before the additional procedures) was compared with the same frequency between 2015
19 and 2017 (after the additional procedures) with Fisher's Exact tests.

20

1 RESULTS AND DISCUSSION

2 A total of 260 contaminated traces have been detected between 2012 and 2017 at the FGU
3 laboratory (Table 2). As expected most of these (i.e. 247/260) were described as touch DNA
4 and the concentration of DNA recovered was generally very low (often below 20pg/ul) [5].
5 Contaminated traces represent a proportion of ~0.3% (i.e. 260 / 77'962) of the traces analyzed
6 during the same period and a proportion of ~1% (i.e. 260 / 26'876) of the profiles sent to the
7 Swiss DNA database. These frequencies are similar to the frequencies (between 0.1 – 1%)
8 reported in other studies on contamination events [3-5, 10]. However, it likely represents an
9 underestimation as most contamination incidents in Switzerland are detected by the staff index
10 of the national database and only ~34% of the traces analyzed at the FGU laboratory are sent
11 to the Swiss database [5]. Very partial DNA profiles and/or mixed DNA profiles with more than
12 two persons cannot be sent to the database, but they can still be compared manually with other
13 DNA profiles following a request from the police services. These DNA profiles represent about
14 10% of the traces analyzed and they do not generally undergo contamination check. Moreover,
15 not all the persons in contact with crime scene traces have their DNA profile in the staff index
16 database although this parameter is crucial to have an efficient detection of contamination
17 incidents [3]. The proportion of 0.3% is therefore non-negligible and highlights the importance of
18 improving procedures to minimize contamination incidents.

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

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

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

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

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

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

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

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

15 Finally, most of the contamination incidents were associated to police collaborators (89%; N=
16 231), whereas 8% (N = 21) were associated to laboratory staff and 3% (N = 8) were associated
17 to other type of profiles such as positive controls, unknown profile or stain-stain contamination
18 incidents (Table 2). This breakdown is similar to what was already observed in a previous study
19 [5] and highlights the need for also taking contamination minimization procedures within the
20 police. A study about DNA contamination incidents in Switzerland was recently conducted in
21 collaboration with the different police services of Switzerland [5]. Several procedures to
22 minimize contamination incidents of police collaborators were proposed but this study is too
23 recent to know if the additional contamination prevention measures were taken in the police
24 services and to address their potential impact on the number of contamination incidents from
25 police collaborators.

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

9

10 **ACKNOWLEDGEMENTS**

11 We would like to thank warmly all the FGU laboratory staff for their effort to minimize
12 contamination incidents. We also would like to thank Tacha Hicks for helpful comments on the
13 manuscript. This research did not receive any specific grant from funding agencies in the public,
14 commercial, or not-for-profit sectors.

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8

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

3 **(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
-

4

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

3

Year	Total number of traces analyzed	Number of profiles sent to the Swiss DNA database	Number of detected contamination incidents			
			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

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