Journal of Forensic Research
ISSN: 2157-7145

Vandewoestyne et al. J Forensic Res 2011, S2
DOI: 10.4172/2157-7145.S2-001

Special Issue Open Access

Sources of DNA Contamination and Decontamination Procedures in the Forensic Laboratory

Mado Vandewoestyne, David Van Hoofstat, Sabine De Groote, Nicky Van Thuyne, Saskia Haerinck, Filip Van Nieuwerburgh, and Dieter Deforce*

Laboratory of Pharmaceutical Biotechnology, Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium

Abstract

The sensitivity of forensic DNA typing techniques can cause problems when evidence samples are inadvertently contaminated with DNA from another source. Therefore, precautions need to be taken to minimize the risk of contamination. In this study, laboratory air and surfaces, tools and equipment were evaluated as potential sources of contaminating DNA. Subsequently, two decontamination procedures, i.e. the conventionally used sodium hypochlorite and the commercially available DNA decontamination solution DNA *ZAP*™ (Applied Biosystems), were compared for their use in removing potentially contaminating DNA from the laboratory working environment.

From our results, it can be concluded that air is unlikely to be the source of observed DNA contamination in the laboratory whereas DNA accumulating on surfaces, tools and equipment within the laboratory environment may potentially be transferred to evidence samples. DNA *ZAP*™ outperformed the conventionally used sodium hypochlorite decontamination procedure. Stringent preventive measures and decontamination of equipment and laboratory surfaces is important to avoid secondary transfer of this contaminating DNA to evidence samples.

Keywords: Contamination; Decontamination; DNA; Polymerase chain reaction; Short tandem repeat analysis

Abbreviations: DNA: Deoxyribonucleic Acid; LT: Low template; PCR: Polymerase Chain Reaction; RFU: Relative Fluorescence Units; STR: Short Tandem Repeat; UV: Ultraviolet

Introduction

It is well known that forensic DNA typing by short tandem repeat (STR) analysis has a risk of being affected by low levels of contamination [1]. Contamination is defined as the inadvertent addition of an individual's DNA during or after collection of the evidence sample [2] and may thus occur both at the crime scene and in the laboratory. Especially low template (LT) DNA analysis, i.e. the analysis of less than ~100pg input DNA [3], suffers from amplification of alleles not associated with the crime stain [4] while in samples with high amounts of input DNA low levels of contamination can remain undetected.

In forensic laboratories, precautions are taken to minimize the risk of contamination disposable tools are used to avoid transfer of DNA from one piece of evidence to another, pre- and post-polymerase chain reaction (PCR) products are kept physically separated, evidence and reference samples are processed separately, DNA-free consumables and negative controls are used at every stage of the analysis, etcetera [2,5]. However, even when stringent precautions are taken, contamination cannot be ruled out completely.

Contamination in the forensic laboratory can be caused both by primary transfer (i.e. direct transfer of contaminating DNA from an analyst or other person in the laboratory to the sample) and secondary transfer (i.e. transfer of contaminating DNA to an object used in the laboratory and subsequently from this object to the sample). It has been shown that cellular, purified and amplified DNA can accumulate on surfaces, tools and equipment within the laboratory environment and may potentially be transferred to evidence samples [6]. Contaminated equipment may thus act as a vector for transfer of DNA and DNA containing material.

As contamination is a critical issue in the analysis and interpretation of trace DNA, Van Oorschot et al. emphasizes the importance of frequent and thorough cleaning of work areas [5] and routine decontamination of all fixed and non fixed equipment [6]. In most laboratories, sodium hypochlorite (NaOCl or bleach) is used as a decontaminating solution [6-8]. As an alternative for the conventionally used sodium hypochlorite, different commercial decontamination solutions are available. To our knowledge, the decontaminating potential of sodium hypochlorite versus a commercially available decontamination solution has not yet been studied.

In this study, laboratory air and surfaces, tools and equipment are evaluated as potential sources of contaminating DNA in a pre-PCR environment. Subsequently, two decontamination procedures, i.e. the conventionally used sodium hypochlorite and the commercially available DNA decontamination solution DNA *ZAP*™ (Applied Biosystems, Carlsbad, California, USA), are compared for the removal of contaminating DNA in the laboratory working environment.

Materials and Methods

Air sampling

Air sampling was performed in 3 different locations: inside a laminar flow cabinet (CB1804, Clan LAF, TCPS Laboratories, Werchter, Belgium), on a bench in the pre-PCR laboratory where mouth masks, gloves and lab coats are worn and on a desk in an open office shared with 9 people. Every location was sampled twice. Air was collected using a MAS-100 *Eco* impaction sampler operating at a flow rate of

***Corresponding author:** Prof. Dieter Deforce, Faculty of Pharmaceutical Sciences, Laboratory of Pharmaceutical Biotechnology, Ghent University, Harelbekestraat, Ghent, Belgium, Tel: +32 (0)9 264 80 52; Fax: +32(0)9 220 66 88; E-mail: Dieter.Deforce@Ugent.be

Accepted July 21, 2011; **Published** July 28, 2011

Citation: Vandewoestyne M, Van Hoofstat D, De Groote S, Van Thuyne N, Haerinck S, et al. (2011) Sources of DNA Contamination and Decontamination Procedures in the Forensic Laboratory. J Forensic Res S2:001. doi:10.4172/2157-7145.S2-001

100 L/min (Merck, Whitehouse Station, NJ). In the MAS-100 *Eco*, air is aspirated through a sterile perforated lid and particles present in the air are impacted on a solid material of choice [9]. For every sample, 10000 L of air was impacted on a sterile gauze (Stella 1, Lohmann & Rauscher International, Rengsdorf, Germany) humidified with pure water (MilliQ, Millipore, Billerica, MA, USA), which was subsequently subjected to DNA extraction.

Sample preparation

Samples were taken from 19 different surfaces/equipment using sterile cotton swabs (Greiner Bio-One, Wemmel, Belgium) before any decontamination procedure was applied.

Subsequently, samples were taken from 8 of the surfaces/equipment that showed the highest numbers of contaminating alleles, before and after decontamination with one of both decontamination procedures.

Decontamination procedures

Conventional sodium hypochlorite procedure (NaOCl or bleach): A 5% sodium hypochlorite solution (Forever Products, Courcelles, Belgium) was sprayed on the surfaces and equipment to be decontaminated. Subsequently, a 70% ethanol solution (Merck, Darmstadt, Germany) was applied over the sodium hypochlorite solution and wiped off with disposable paper towels.

DNA ZAP™ procedure

DNA *ZAP*™ solution 1 was sprayed on the surfaces and equipment to be decontaminated. DNA *ZAP*™ solution 2 was immediately applied over solution 1. Subsequently, the surfaces and equipment were thoroughly rinsed with distilled water to remove degraded nucleic acids and DNA *ZAP*™ residue.

DNA extraction

From all samples, DNA was extracted using a slightly modified Chelex® extraction method [10]. In a first step, the 3 top layers of the sterile gauze from the air samples and the cotton heads of the surface swabs were removed with a sterile scalpel and incubated in 1ml sterile water for 30 minutes at room temperature. After incubation, gauze or cotton heads were removed and samples were centrifuged at 14000 rpm for 5 minutes. Subsequently, supernatant was removed and the pellet was resuspended in 200µl 5% Chelex® solution (Chelex®100 resin, Bio-Rad, Hercules, CA, USA). The samples were incubated at 56°C for 30 minutes and subsequently put in boiling water for 8 minutes. After centrifugation at 14000rpm for 3 minutes, 30µl of the supernatant was used for PCR.

Amplification and detection

A multiplex of 4 STR loci (CD4, TH01, D21S11 and SE33) was used as described earlier [11] with slight modifications. In short, 1.3 units of Hotstar Taq DNA polymerase (Qiagen, Venlo, The Netherlands) was used and the samples were amplified on an Applied Biosystems GeneAmp 9700 60 Well thermal cycler. Amplification parameters were: preincubation at 95°C for 15 minutes, followed by 33 cycles of denaturation for 60 seconds at 94°C, annealing for 60 seconds at 58°C and extension for 80 seconds at 72°C. This was followed by a final elongation step of 10 minutes at 72°C. At the end of the PCR reaction the temperature was kept at 4°C.

After PCR, the amplified fragments were separated and analyzed by capillary electrophoresis using an ABI 310 Genetic Analyzer (Applied Biosystems).

Results and Discussion

In a first part of this study, laboratory air and surfaces, tools and equipment were evaluated as potential sources of contaminating DNA. Air sampling was performed twice at 3 different locations with presumably increasing levels of contaminating potential: inside a laminar flow cabinet, on a bench in the pre-PCR laboratory where mouth masks, hats, gloves and lab coats are worn and on a desk in an open office, shared with 9 people, where no safety measurements were taken. None of these six air samples showed contaminating alleles, hence it can be concluded that air is unlikely to be the source of observed DNA contamination in a forensic laboratory. Our data are supported by the study of Witt et al. [12], who showed that air is not the source of most of the reported contaminations in real-time PCR analyses.

In a second part of this study, laboratory surfaces, tools and equipment, present in a pre-PCR laboratory where mouth masks, hats, gloves and lab coats are worn in order to prevent contamination, were analyzed for the presence of contaminating human DNA. The results of this analysis are shown in Table 1. In our laboratory, a cut off of 100 Relative Fluorescence Units (RFU) is used as a threshold for detection of a locus. As the choice of this cut off value is arbitrary and may differ from one laboratory to another, alleles with an RFU ≥ 50 are also mentioned in Table 1. Eleven samples out of 19 showed at least one contaminating allele with an RFU ≥ 100 while 13 samples had one or more alleles with an RFU ≥ 50. All detected alleles could be attributed to laboratory staff. It is clear that these surfaces and equipment are frequently contaminated with DNA and/or DNA containing cells and could act as vectors for secondary transfer of contaminating DNA. Hence, stringent cleaning procedures and operating procedures are mandatory. The samples that did not contain contaminating alleles were the outside surface of a recently autoclaved container, the front and working surface of a laminar flow cabinet and 3 samples taken inside a pipetting liquid handler equipped with a DNA inactivating ultraviolet (UV) lamp.

Two decontamination procedures were compared for their ability to remove contaminating DNA and DNA containing material from surfaces and equipment. The results of this comparison are shown in Tables 2 and 3. These experiments were performed on the 8 surfaces/tools that showed the highest numbers of contaminating alleles in Table 1. Using a Wilcoxon Signed Ranks test in SPSS (non-parametric test for paired samples, 1-tailed) no statistically significant difference was seen in the detected number of contaminating alleles in both groups of surfaces/tools before decontamination (p-value for RFU≥100: 0.203; p-value for RFU≥50: 0.469). Both decontamination procedures performed well in removing the contaminating DNA and/or DNA containing material: the p-value for the conventional sodium hypochlorite decontamination procedure was 0.016 for RFU≥100 and 0.008 for RFU≥50, whereas the p-value for the DNA *ZAP*™ decontamination procedure was 0.004 for RFU≥100 as well as for RFU≥50.

Taking only alleles with an RFU≥100 into account, the DNA *ZAP*™ procedure performed significantly better than the conventional sodium hypochlorite procedure, with a p-value of 0.031. Although incomplete decontamination is achieved using sodium hypochlorite, it is widely used in forensic laboratories. The main reason for this is the very low purchase cost of sodium hypochlorite. Using DNA *ZAP*™, more thorough decontamination was achieved and no corrosion was observed, whereas sodium hypochlorite may lead to corrosion of some surfaces.

It can be concluded that complete decontamination of equipment and surfaces from DNA and DNA containing biological material is important in forensic DNA laboratories in order to avoid secondary transfer of this contaminating DNA to evidence samples. Therefore, it is recommended to regularly perform a stringent decontamination of laboratory surfaces and equipment and to check surfaces after decontamination to guarantee the effectiveness of the decontamination procedure. Also, prevention of secondary transfer from potentially contaminated objects and surfaces is of particular importance, e.g. by frequent renewal of gloves. The emphasis of contamination prevention procedures should not lie on filtering laboratory air, as air is unlikely to be the source of DNA contamination, but on proper decontamination procedures and appropriate laboratory operating procedures aiming at avoiding secondary transfer.

Surface/equipment	Number of alleles detected	
	RFU≥100	RFU≥50
Drawer of laboratory cupboard (outside surface)	10	17
Laboratory bench	12	19
Centrifuge used for reference samples (outside surface)	1	5
Centrifuge used for evidence samples (outside surface)	1	1
Container with autoclaved tubes (outside surface)	0	1
Pipetholder in laminar flow cabinet	0	1
On/off button laminar flow cabinet	7	9
Container with autoclaved filtertips	0	0
Handle laboratory freezer	7	13
Handle laboratory fridge	2	7
Box containing centrifugal filter devices (outside surface)	1	5
Rack for tubes (empty)	14	18
Vortex	3	7
Electronic pipette	5	10
Outside laminar flow cabinet (front side)	0	0
Inside laminar flow cabinet (bottom)	0	0
Inside pipetting liquid handler with UV lamp (left side)	0	0
Inside pipetting liquid handler with UV lamp (right side)	0	0
Inside pipetting liquid handler with UV lamp (bottom)	0	0

Table 1: Numbers of alleles detected on surfaces and equipment (before decontamination procedure).

Surface/equipment	Number of alleles detected			
	Before decontamination		After decontamination	
	RFU≥100	RFU≥50	RFU≥100	RFU≥50
Drawer of laboratory cupboard (outside surface)	8	10	2	6
Laboratory bench	10	15	0	0
On/off button laminar flow cabinet	6	12	0	2
Handle laboratory freezer	1	3	1	1
Handle laboratory fridge	2	4	1	1
Rack for tubes (empty)	5	13	1	5
Vortex	1	5	0	0
Electronic pipette	1	1	1	1

Table 2: Numbers of alleles detected on surfaces and equipment before and after decontamination with sodium hypochlorite.

Surface/equipment	Number of alleles detected			
	Before decontamination		After decontamination	
	RFU≥100	RFU≥50	RFU≥100	RFU≥50
Drawer of laboratory cupboard (outside surface)	3	6	0	0
Laboratory bench	7	9	0	1
On/off button laminar flow cabinet	6	6	0	0
Handle laboratory freezer	8	13	0	0
Handle laboratory fridge	6	8	0	3
Rack for tubes (empty)	10	11	0	0
Vortex	2	9	0	1
Electronic pipette	3	5	0	0

Table 3: Numbers of alleles detected on surfaces and equipment before and after decontamination with DNA *ZAP*™ (Applied Biosystems).

Acknowledgements

The authors would like to thank Sylvie Decraene, Evelien Dierick, Delphine Labie, Petra Paelinck, Sofie Rogge and Eveline Vanden Eynde for excellent technical support. The Research was funded by a PhD grant from the Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen) to Mado Vandewoestyne.

References

1. Scherczinger CA, Ladd C, Bourke MT, Adamowicz MS, Johannes PM, et al. (1999) A systematic analysis of PCR contamination. J Forensic Sci 44: 1042-1045.
2. Rudin N, Inman K (2002) An Introduction to Forensic DNA Analysis. (2nd edition).
3. Gill P, Whitaker J, Flaxman C, Brown N, Buckleton J (2000) An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA. Forensic Sci Int 112: 17-40.
4. Gill P (2001) Application of low copy number DNA profiling. Croat Med J 42: 229-232.
5. van Oorschot RA, Ballantyne KN, Mitchell RJ (2010) Forensic trace DNA: a review. Investig genet 1: 14.
6. Poy AL, van Oorschot RA (2006) Trace DNA Presence , Origin , and Transfer within a Forensic Biology Laboratory and its Potential Effect on Casework. Journal of Forensic Identification 56: 558-576.
7. Kemp BM, Smith DG (2005) Use of bleach to eliminate contaminating DNA from the surface of bones and teeth. Forensic Sci Int 154: 53-61.
8. Prince AM, Andrus L (1992) PCR: how to kill unwanted DNA. BioTechniques 12: 358-360.
9. Vanhee LME, Nelis HJ, Coenye T (2009) Rapid detection and quantification of Aspergillus fumigatus in environmental air samples using solid-phase cytometry. Environ Sci Technol 43: 3233-3239.
10. Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques 10: 506-513.
11. Van Hoofstat DE, Deforce DL, Millecamps RE, Brochez VM, Van Geldre EG, et al. (1998) Population genetic study of four short tandem repeat loci in the Belgian population, using capillary electrophoresis. Electrophoresis 19: 719-722.
12. Witt N, Rodger G, Vandesompele J, Benes V, Zumla A, et al. (2009) An assessment of air as a source of DNA contamination encountered when performing PCR. J Biomol Tech 20: 236-240.