Comparison between various DNA sterilization procedures applied in forensic analysis

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Highlights

- **In this paper, we have conducted different DNA decontamination methods by in instruments, PCR cabinets, offices, gloves…etc.**
- **Several DNA decontamination procedures such as DNA-ExitusPlus IF, ethanol, bleach and UV light were studied to show the most suitable method for consideration in forensics.**
- **DNA swabbing was done for air to show the presence of DNA.**
- **Randomly swabbing was done during work in different DNA forensic laboratories.**
- **Modified protocols and instructions were established for police, crime scene and forensic laboratory experts to minimize DNA contamination on crucial evidence.**

Abstract

The advanced sensitive STR kits applied in forensic DNA typing techniques can cause challenging issues when evidence samples are contaminated with minute quantities of DNA from another source such as forensic analysts or crime scene examiners. In this study, laboratory air and surfaces, gloves, tools, and equipment were evaluated as potential sources of contaminating DNA. Different sterilization methods were tested for efficiency such as 10% bleach, ethanol, UV light and DNA-ExitusPlus IF by using several time intervals exposures and concentrations in two different lab settings: low template DNA and DNA database labs. Swabbing was done and proceeded for DNA typing using magnetic beads principle. Results were detected using HID Real-Time PCR Analysis Software v1.2 and GeneMapper ID-X Software v1.4. It was concluded that most of the DNA decontamination methods are not suitable for highly sensitive and precision STR kits such as GlobalFiler PCR Amplification Kit. The most suitable tested method was using DNA-ExitusPlus IF with increase of incubating time to 15 minutes instead of 10 minutes.

Keywords: DNA decontamination; DNA-ExitusPlus IF; contamination; Sterilization; Forensic analysis; DNA evidence

Introduction

Forensic DNA casework is now considered as one of the most common forms of forensic evidence, and it is used to make crucial decisions in intelligence and justice. However, errors can happen, and they can have serious consequences such as DNA transfer and contamination **(1).** DNA contamination is one of the most common causes of forensic genetics faults **(2)**. There are three types of DNA contamination can occur: internal contamination between the samples and the DNA analysts, cross-contamination between evidence of same case or different cases. last is the external contamination which happens between the DNA samples and the police force or crime scene experts or manufacturers of reagents or consumables **(1)**. There are many cases when it is difficult to consider and interpret the police DNA match if it is a true match or a contamination, which deteriorates the judicial evidence. A near match/non-match error is defined as an event that has the potential to lead to the reporting of a wrongful match/non-match. As it is expected to have an increase in the amount of DNA contamination due to the upsurge number of forensic cases which request for DNA testing and thus an increase demand of DNA analyst's employment. Moreover, there is an increase number of DNA profiles leading to the growth of DNA database (including casework or elimination DNA database). Furthermore, with the current sensitivity of profiling STR kits, preventing background DNA and contamination events from police or experts analyzing crime scene samples is becoming more challenging **(3, 4).** The police contamination can mask the true match in the DNA evidence thus causing loss of significant leads **(2, 5).** An inconsistency in forensic DNA analysis can lead to poor investigative or legal decisions with far-reaching implications, such as the arrest of innocent suspects, the exoneration of convicted suspects, or the failure to identify criminals **(6).** Defining, recording, and reporting error rates have long been considered beneficial in other scientific fields, which have emphasized the need to establish protocols and guidelines to improve and develop good practices for crime scene and forensic laboratory experts **(7).** Also, it is always mandatory to sustain the trust and good reputation of forensic parties such as the Crime labs and crime scenes **(2)**. Several procedures have recently been described to minimize the incidence of DNA contamination at the crime scene and in the laboratory **(4, 8, 9)** . Some of these procedures are well-suited to the laboratory setting. These include 1) staff awareness education about contamination, 2) the proper use of protective clothing (PPE), 3) limiting access to the laboratory working area; 4) effective cleaning and sterilization of all equipment and laboratory zones **(10)**; 5) physical separations between offices, laboratories, or storage facilities to reduce DNA contamination; and 6) the distribution of specific activities (e.g., trace collection) among different people to disrupt contamination chains **(4, 8, 9).** In this paper, we have compared various DNA decontamination techniques which are applied in international crime labs and crime scenes worldwide. We identified the most suitable DNA sterilization method to ensure minimum level of DNA transfer or cross contamination between the police workers and the DNA evidence. By applying different procedures, we have modified manufactures' protocols to give maximum results. Several criteria were tested such as various concentrations of alcohol sterilization as well as different exposure time of DNA evidence to several materials such as UV light, commercially available solution such as DNA-ExitusPlus IF (PanReac AppliChem, Germany) and 10% (v/v) Clorox bleach (equivalent to ~0.55%, w/v, solution of sodium

hypochlorite) to display the results and to elucidate the DNA transfer incidence by the police force.

Materials and methods

Designing the experiments

Different experiments were designed to assess several DNA sterilization methods such as decontamination using 10% bleach solution, ethanol solution, DNA-ExitusPlus IF (PanReac AppliChem, Germany) in two different DNA laboratory settings, i.e low template DNA lab and reference DNA lab (abbreviated as CW and DB respectively). In this study, we tested various approaches to remove DNA from hard laboratory surfaces and instruments. We contaminated clean surfaces with buccal swabs, with gDNA of ~ 20 ng/ul. The DNA was dried and left for 15 minutes before any treatment. Swabbing was done using cotton swabs (SceneSafe, UK). All research was performed in accordance with relevant guidelines/regulations.

- **Disinfecting the working area surfaces and instruments with 85% ethanol solution**

Using conventional method, i.e 85% ethanol solution to sterilize surface of widely used instruments and working areas such as thermomixers (Eppendorf, Germany), drawers and pipettors in both CW and DB labs. Each instrument was pre-swabbed then applied the 85% ethanol solution.

- **Disinfecting the working area surfaces and instruments with 85% ethanol solution and DNA-ExitusPlus IF**

By comparing conventional and commercially available methods, i.e 85% ethanol solution and DNA-ExitusPlus IF (PanReac AppliChem, Germany) to sterilize surfaces of mostly contaminated working areas such as DNA extraction benches and PCR cabinets in both CW and DB labs. Each instrument was pre-swabbed then applied the 85% ethanol solution and DNA-ExitusPlus IF.

- **Disinfecting the PCR cabinets using different exposure time of UV light**

By applying different exposure time of UV light to decontaminate the PCR cabinets using the following time intervals: 5 min, 10 min, 15 min, 20 min and 25 min. To ensure proper decontamination, we have applied induced DNA on the tested surfaces.

- **Disinfecting the working area using different exposure time of DNA-ExitusPlus IF**

By applying different exposure time of DNA-ExitusPlus IF (PanReac AppliChem, Germany) to decontaminate the working area using the following time intervals: 10 min and 15 min. To ensure proper decontamination, we have applied induced DNA on the tested surfaces, sprayed the solution then waited for the studied time then swabbed again to check for efficiency.

- **Disinfecting the working area using different exposure time of bleach**

By applying different exposure time of 10% bleach solution (commercially available) to decontaminate the working area using the following time intervals: 10 min, 15 min, 20 min, 25 min, 30 min and 35 min. To ensure proper decontamination, we have applied induced DNA on the tested surfaces, sprayed the solution then waited for the studied time then swabbed again to check for efficiency.

- **Disinfecting the working area using different concentrations of ethanol solution**

By applying different concentrations of ethanol solution to decontaminate the working area using the following concentrations: 70%, 75%, 80% and 85%. To ensure proper decontamination, we have applied induced DNA on the tested surfaces, sprayed the ethanol solution using the abovementioned concentrations and waited for 10 minutes then swabbed again to check for efficiency.

- **DNA testing of gloves during work**

Random swabbing was done during DNA testing for different DNA experts.

- **Talking inside DNA test tubes**

We have talked and coughed inside DNA test tubes prior to proceed for pre and post PCR amplification to study the effect of DNA contamination from unprocessed DNA such as saliva.

- **Presence of DNA in the air**

Random swabbing was done in the air to check for the presence of DNA, i.e working areas, PCR cabinets and offices for CW and DB labs.

DNA processing

Genomic DNAs (gDNA) were extracted from the collected cotton swabs samples (SceneSafe, UK) using AutoMate Express DNA Extraction System (Thermo fisher Scientific, Inc., Waltham, MA, USA) following magnetic beads principle **(11).** Subsequently the extracted DNAs were quantified using Quantifiler HP DNA Quantification Kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA) in the 7500 Real-Time PCR System (Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to manufacturer's recommendation **(12)**. About 1.2 ng of the extracted DNA was amplified using GlobalFiler PCR Amplification Kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to manufacturer's recommendation **(13).** A total of 24 loci were amplified, including 21 autosomal STR loci and three gender determination loci in 29 cycles *via* MicroAmp Optical 96-Well Reaction Plate (Thermo Fisher Scientific Company, Carlsbad, USA) along with the Previously genotyped male control (provided with the kit) and low TE buffer as a negative control using 96-Veriti thermal cycler (Thermo Fisher Scientific Company, Carlsbad, USA). The PCR products (1µl) were separated by capillary electrophoresis in an ABI 3500xl Genetic Analyzer (Thermo Fisher Scientific Company, Carlsbad, USA) with reference to the LIZ600 size standard v2 (Thermo Fisher Scientific, Inc., Waltham, MA, USA) in

total of 10 µl master mix consisting of LIZ600 size standard and Hi-Di formamide (Thermo Fisher Scientific, Inc., Waltham, MA, USA). GeneMapper ID-X Software v1.4 (Thermo Fisher Scientific, Inc., Waltham, MA, USA) was used for genotype assignment **(13)**. DNA typing and assignment of nomenclature were based on the ISFG recommendations.

Analysis

The results from the 7500 Real-Time PCR System (Thermo Fisher Scientific, Inc., Waltham, MA, USA) were detected using the HID Real-Time PCR Analysis Software v1.2. All the results were input in a table format. Additionally, the STR profiles were analyzed and interpreted using GeneMapper ID-X Software v1.4 (Thermo Fisher Scientific, Inc., Waltham, MA, USA) by direct counting the number of loci/ peaks found in the STR profiles and inserted in the table format. RFU for reference samples were done using in house validation for the GlobalFiler Amplification Kit to differentiate between the stochastic threshold and possible allele drop out **(14)**.

Results

DNA Quantification

The results obtained from the HID Real-Time PCR Analysis Software v1.2 displayed the amount of DNA using the small autosomal (SA) human target available in the Quantifiler HP DNA Quantification Kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA). SA consists of relatively short amplicons (75 to 80 bases) to improve the detection of degraded gDNA. As shown in **table 1**, the detection of gDNA in several experiments conducted and labeled as followed: 1) Disinfecting the working area surfaces and instruments with 85% ethanol solution, 2) Disinfecting the working area surfaces and instruments with 85% ethanol solution and DNA-ExitusPlus IF, 3) Disinfecting the PCR cabinets using different exposure time of UV light 4) Disinfecting the working area using different exposure time of DNA-ExitusPlus IF, 5) Disinfecting the working area using different exposure time of bleach 6) Disinfecting the working area using different concentrations of ethanol solution, 7) DNA testing of gloves during work, 8) Talking inside DNA test tubes and 9) Presence of DNA in the air. Overall, it is evident that applying ethanol sterilization to the working benches and instruments did decontaminate the presence of gDNA but did not sterilize to the optimal level. However, there was some remains of gDNA found on the instruments and benches. With examining different concentration of ethanol disinfection used, it was clearly concluded that 85% ethanol was the best for sterilization. Yet, using exclusively 85% ethanol solution for sterilization did not completely sterilize the working area. Secondly, when using UV light for PCR cabinets decontamination, different time exposure was conducted to the induced contamination to show if 15 min exposure was the optimum as recognized in international instructions for many user guides. Nevertheless, it was found out that exposing the gDNA to the UV light even after 25 min did not totally sterilize the PCR cabinets from the presence of gDNA, particularly when amplifying the DNA using overly sensitive amplification kits such as GlobalFiler PCR Amplification Kit. Therefore, using solely UV light to decontaminate the PCR cabinets was not sufficient for any DNA testing labs. Subsequently, various time exposure of 10% bleach solution was tested to show the ideal time for gDNA

sterilization using the bleach. It was noticed that most of the used timings were undetermined **(Table 1)** as the 10% bleach may possibly interfere with the proper interpretation of results. Therefore, STR profiles were required to give reliable results.

Table 1 The detection of gDNA from different experiments obtained through HID Real-Time PCR Analysis Software v1.2.

1) Disinfecting the working area surfaces and instruments with 85% ethanol solution			
Sr. No	**Experiment details**	**Amount of gDNA (ng/ul)**	
1	Thermomixers (1), DNA CW lab	Precleaning: 0.002	post-cleaning: 0.0004
2	Thermomixers (2), DNA CW lab	Precleaning:0.0021	post-cleaning: 0.0022
3	Thermomixers (3), DNA DB lab	Precleaning:0.0015	post-cleaning: 0.0023
2) Disinfecting the working area surfaces and instruments with 85% ethanol solution and DNA-ExitusPlus IF			
4	Bench inside the DNA extraction room, DNA CW lab	Precleaning: 0.0052 Post-cleaning ethanol: 0.0035 Post-cleaning DNA-exitus: 0.0005	
5	PCR cabinet, DNA CW lab	Precleaning: 0.0041 Post-cleaning ethanol: 0.0003 Post-cleaning DNA-exitus: 0.0003	
6	PCR cabinet, DNA DB lab	Precleaning: 0.0011	post-cleaning DNA-exitus: UD
7	PPE Drawer, DNA DB lab	Precleaning: 0.0013	post-cleaning Ethanol: 0.0006
8	1000ul Pipettes, DNA CW lab	Precleaning: 0.061	post-cleaning Ethanol: 0.0004
3) Disinfecting the PCR cabinets using different exposure time of UV light			
9	Induced contamination, UV light, 0 min	0.0081	
10	Exposure UV light, 5 min	0.0045	
11	Exposure UV light, 10 min	0.0063	
12	Exposure UV light, 15 min	0.0009	
13	Exposure UV light, 20 min	0.0024	
14	Exposure UV light, 25 min	0.0018	
4) Disinfecting the working area using different exposure time of DNA-ExitusPlus IF			
15	Induced contamination, DNA-Exitus, 0 min	0.0507	
16	Post-cleaning, DNA-Exitus, 10 min	0.0011	
17	Post-cleaning, DNA-Exitus, 15 min	UD	
5) Disinfecting the working area using different exposure time of bleach			
18	Induced contamination, Bleach, 0 min	0.0845	
19	Post-cleaning, Bleach, 10 min	UD	
20	Post-cleaning, Bleach, 15 min	0.0002	
21	Post-cleaning, Bleach, 20 min	UD	
22	Post-cleaning, Bleach, 25 min	UD	
23	Post-cleaning, Bleach, 30 min	UD	
24	Post-cleaning, Bleach, 35 min	0.0003	
6) Disinfecting the working area using different concentrations of ethanol solution			
25	Induced contamination, Ethanol, 0%	0.0096	
26	Post-cleaning, Ethanol, 70 %	0.0045	
27	Post-cleaning, Ethanol, 75 %	0.0038	
28	Post-cleaning, Ethanol, 80 %	0.0043	
29	Post-cleaning, Ethanol, 85 %	0.0017	
7) DNA testing of gloves during work			
30	Random swabbing gloves 1	0.0022	
31	Random swabbing gloves 2	0.0033	
32	Random swabbing gloves 3	0.0022	
8) Talking inside DNA test tubes			
33	Talking inside DNA tube, pre-PCR 1	UD	
34	Talking inside DNA tube, pre-PCR 2	0.0002	
35	Talking inside DNA tube, post-PCR 1	0.0006	
36	Talking inside DNA tube, post-PCR 2	0.0002	
9) Presence of DNA in the air			
37	Air swabbing, Staff office, CW lab	0.0045	
38	Air swabbing, DNA extraction room, CW lab	0.0003	
39	Air swabbing, Staff office, DB lab	0.0012	
40	Air swabbing, PCR cabinet, CW lab	UD	
41	Air swabbing, PCR cabinet, DB lab	UD	

***UD: Underdetermined**

DNA-ExitusPlus IF (PanReac AppliChem, Germany) was used additionally to test its effectiveness for DNA sterilization in the forensic laboratory. The recommended time exposure of the solution is 10 min based on the user guide. However, we have noticed that increasing the time expose of gDNA with the solution up to 15 minutes gave the optimal DNA sterilization results. Further, DNA-ExitusPlus IF sterilization was combined along with 85% ethanol to illustrate if the combination of the treatments may possibly increase the level of sterilization. In some cases, the gDNA detection was less after the application of DNA-ExitusPlus IF **(table 1)**, in other cases, it remained the same. Overall, the DNA-ExitusPlus IF showed the most effective method in DNA sterilization and decontamination of working area, instruments, and tools.

In second part of the experiments, we have examined the presence of DNA on the gloves while DNA analysts performed DNA testing.

All the random swabbing of the equipped gloves gave detection of gDNA on the exterior surfaces of the gloves, which gave an ideal justification for having DNA transfer and cross contamination between forensic cases.

The DNA analysts were using 85% ethanol to wipe the worn gloves during work instead of replacing the gloves with new ones. Additionally, we have demonstrated if talking inside the DNA tubes may possibly cause DNA contamination in two steps: pre and post PCR steps. However, all the tested samples gave negative detection of gDNA (~0.0002 ng/ul), which clarifies that it was nearly impossible to cause DNA transfer to the extracted DNA tubes and it was safe to perform DNA amplification and detection steps without the need to use masks to protect the samples from contamination.

Finally, we have performed random swabbing of air to show the presence of DNA in different premises. As shown in table 1, there was variations of gDNA detection, with maximum results in the staff office room in the CW DNA lab with the value of ~ 0.0045 ng/ul (the office was crowded with more than six DNA analysts in 3 x 3 m room), it was clear that there are very few gDNA detection in the air.

STR profiles

To assist the results obtained from the HID Real-Time PCR Analysis Software v1.2, STR profiles were generated for all the samples to study the consistency of the outcomes. Most of the STR profiles obtained after disinfection with the ethanol - regardless of its concentration - retained some of the loci. As shown in **figure 1**, some of the different loci found which may possibly cause contamination to the original DNA found in the forensic cases. The rest of loci were excluded in the figure as they were blank (for the sake of spacing).

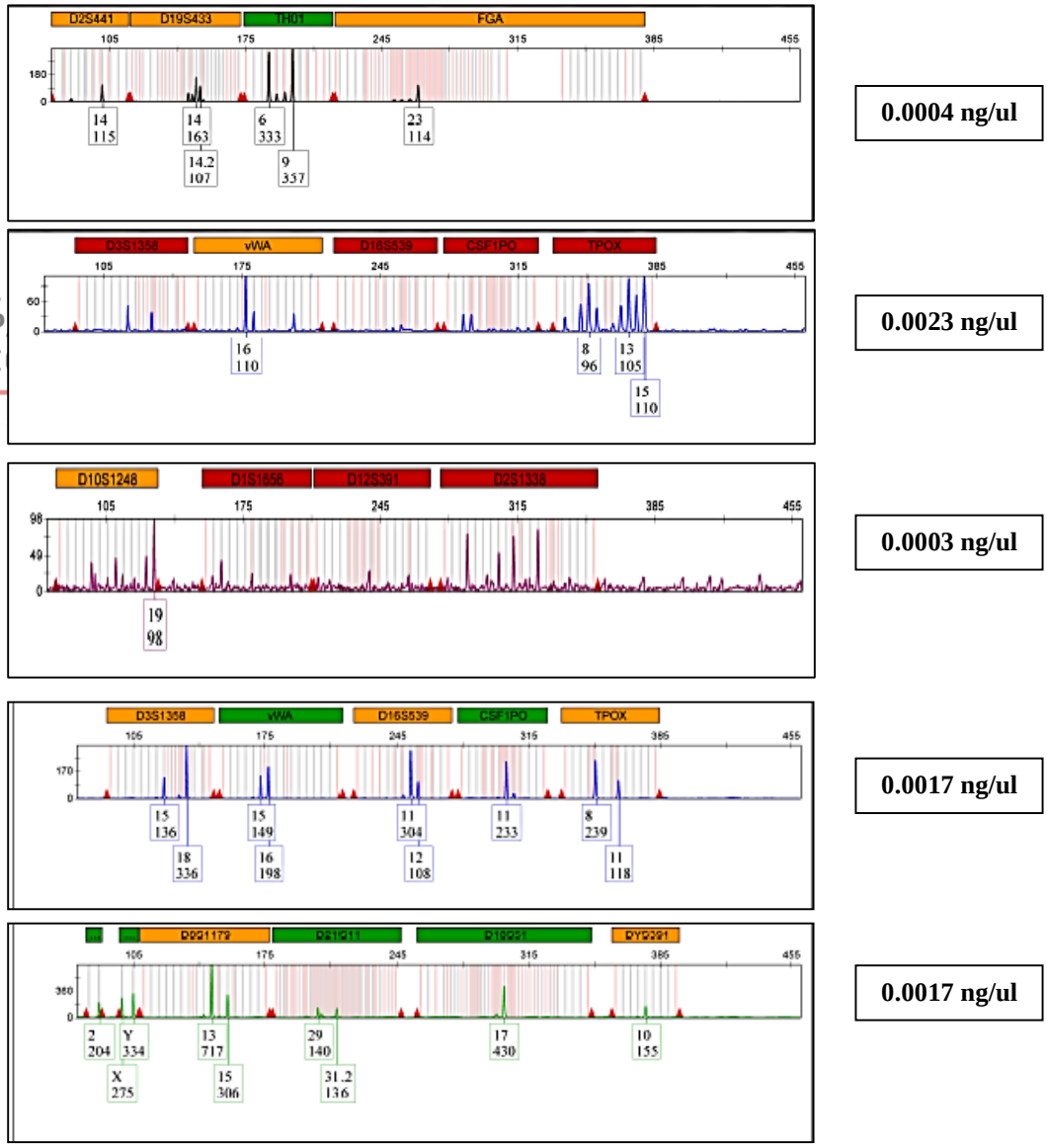

Figure 1 STR profiles generated from post-sterilization with different concentrations of ethanol

Regarding different time exposure to the UV light, we have studied different exposures in terms of 5 minutes to 25 minutes using 5 minutes time intervals. As shown in **figure 2,** the DNA quantity is declining by increasing the time exposure with the UV light. Yet in 25 minutes still the Y indel locus was observed (1 insertion/deletion polymorphic marker on the Y chromosome), which might interfere with the original DNA found in evidence.

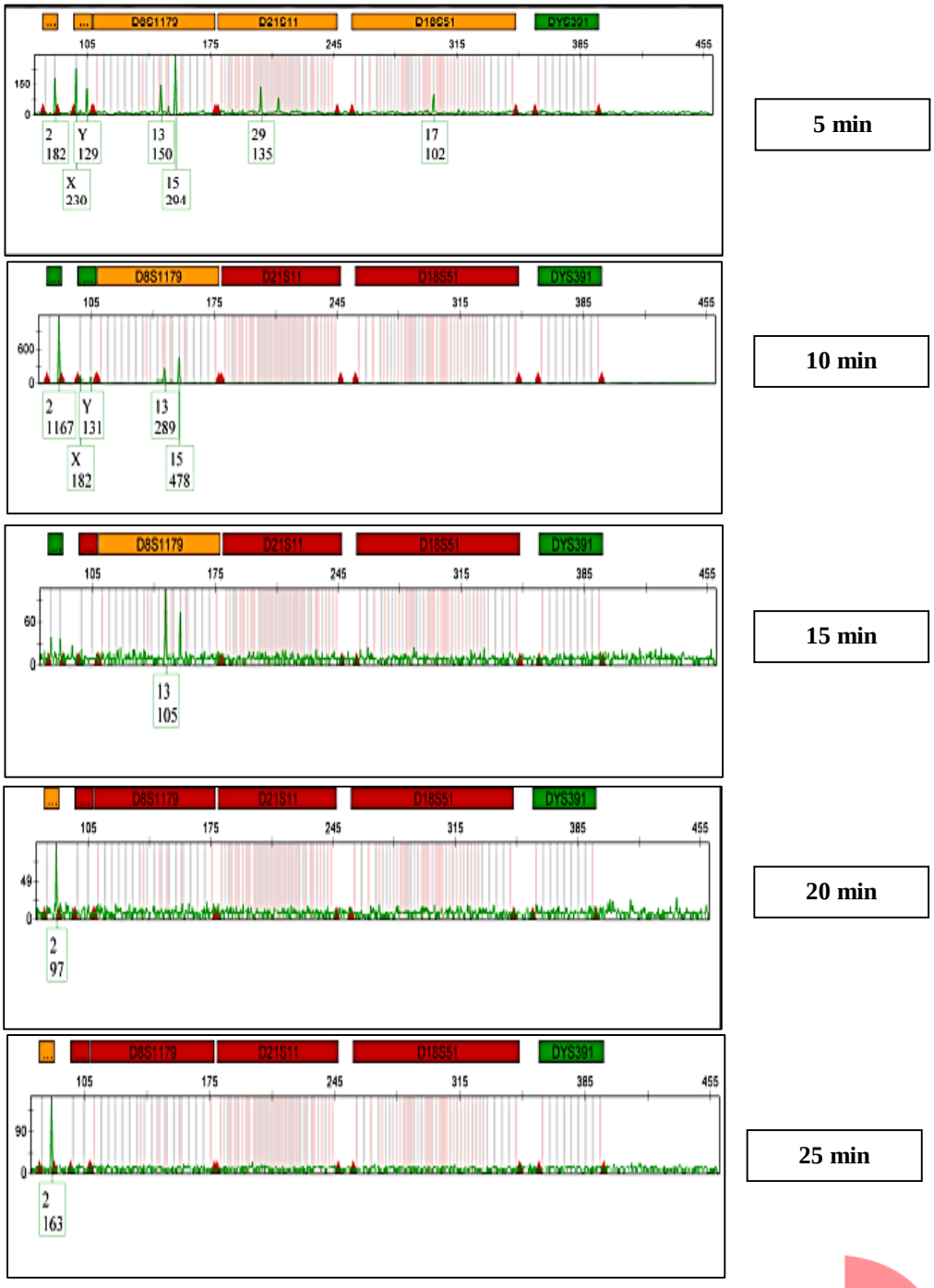

Figure 2 The effect of UV light in DNA sterilization using different time exposure

Additionally**,** we have tested the DNA-ExitusPlus IF to decontaminate the instruments and working areas in two different timings (10 and 15 minutes). It was recommended by the manufacturer to use 10 minutes to perform DNA sterilization. Yet, 10 minutes was not sufficient to have proper disinfection. The optimal timing was 15 minutes as shown in **figure 3.**

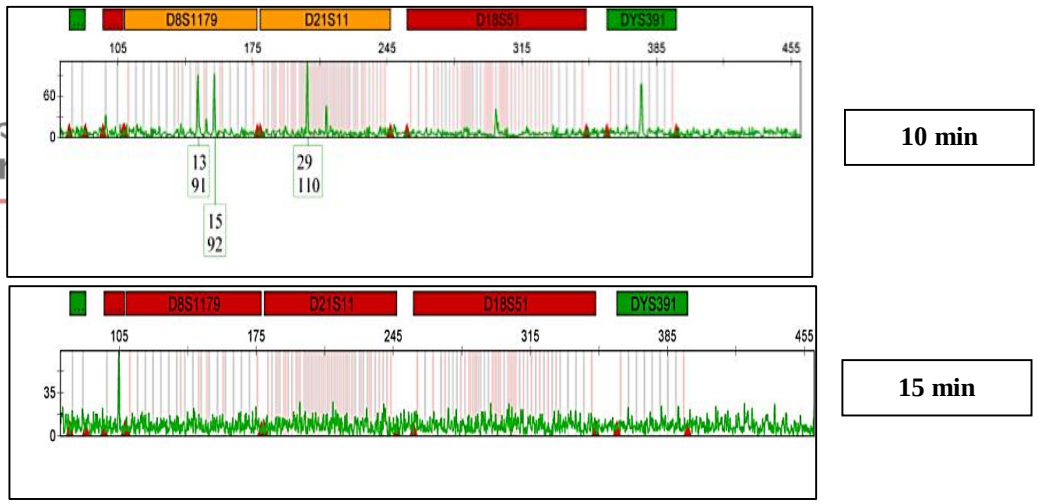

Figure 3 The effect of DNA-ExitusPlus IF in DNA sterilization using different time intervals

Furthermore, we have tested different equipped nitrile gloves during DNA testing from three random DNA analysts. All the worn gloves gave DNA profiles when swabbed. In **figure 4**, the generated STR profiles from different gloves which emphasized on the importance of replacing gloves with new pair instead of spraying ethanol to the gloves.

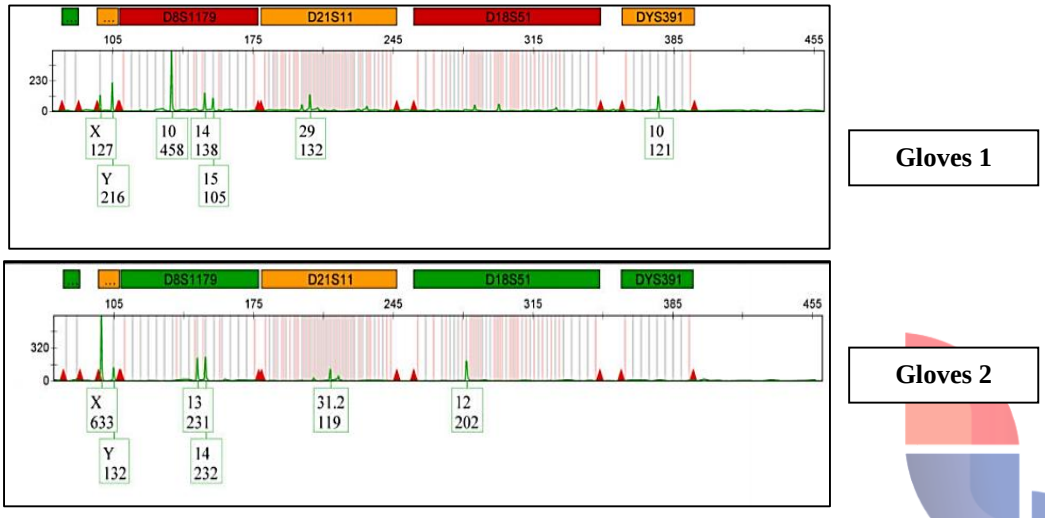

Figure 4 Random swabbing of equipped gloves during DNA testing

Moreover, we have tested the effect of 10% bleach in DNA sterilization as it is widely used in DNA testing labs. Different time exposures were investigated in 5 minutes time intervals. All the STR profiles results displayed different loci after disinfection. As shown in **figure 5,** 10% bleach was not sufficient for proper DNA disinfection.

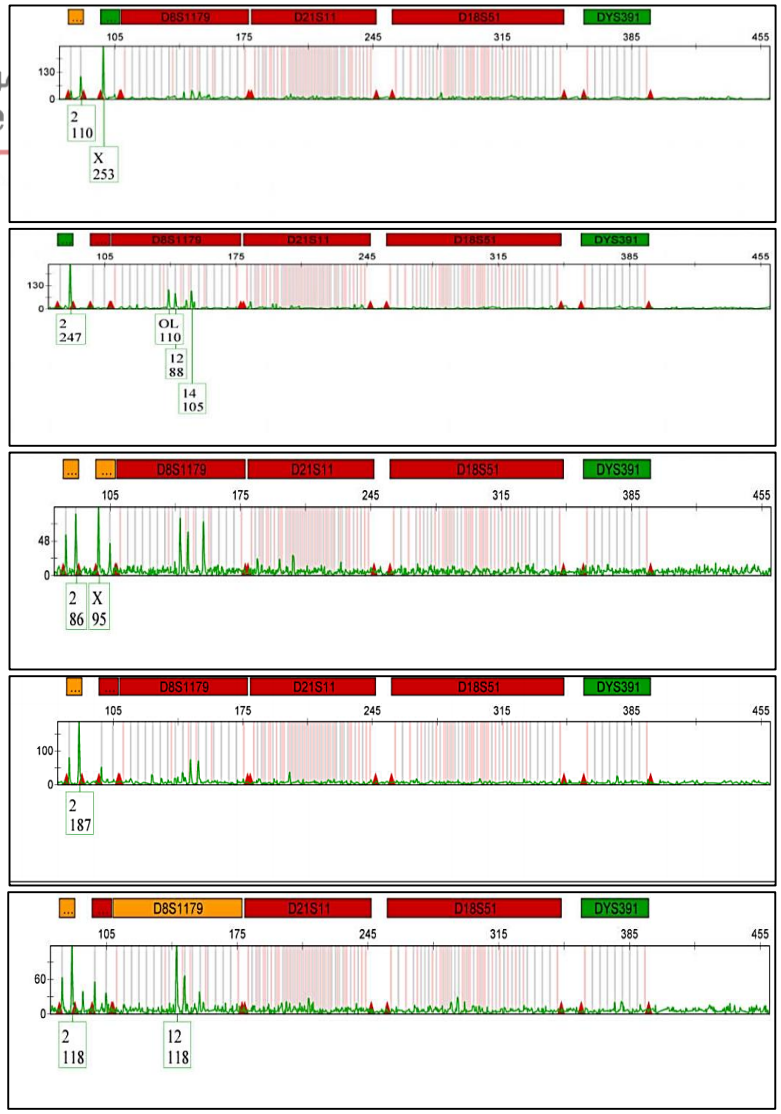

Figure 5 The effect of 10 % bleach in performing DNA sterilization

Discussion

There are different DNA sterilization methods, mainly the 85% ethanol, 10 % bleach, UV light and commercially available spray bottles such as DNA-ExitusPlus IF. Each of these methods is extensively used in many of the international forensic labs and crime scenes divisions to ensure proper decontamination of the premises and instruments prior to evidence examination. In this paper, we have investigated the most common DNA decontamination methods which can be applied in different fields such as forensics and law enforcements, medical, biotechnology…etc. DNA Contamination is sporadic, which is difficult to detect and more challenging to interpret the results. Although elimination database is a good method to identify the source of contamination, it is better to avoid contamination preceding to DNA typing than to identify it after samples are processed **(15).** Inclusion of extraction and amplification negative controls is one of the methods conducted to investigate the presence of cross contamination/ consumables contamination in the DNA testing. Crime scene examiners as well as forensic analysts must equip PPE, i.e masks, sterile suits, hair cap, gloves…etc., as it greatly protects the evidence from contamination **(16)**. Also, gloves must be replaced with new pair instead of spraying or wiping the gloves with 85% ethanol as it is not sufficient to have a suitable decontamination.

In conjunction with innovative DNA technologies, DNA amplification has an increased sensitivity that even the lowest amount of gDNA (~0.0002 ng/ul) can be amplified. Thus, DNA sterilization methods must be an ideal solution to the advanced STR kits. As shown in **table 1,** the decontamination with 85% ethanol, 10% bleach and UV light were inadequate to have a proper sterilization. The most suitable method was using DNA-ExitusPlus IF and to incubate for 15 minutes instead of 10 minutes on the surfaces/ instruments before wiping **(10)**. This method was more accurate to be used when applying sensitive amplification kits such as GlobalFiler PCR Amplification Kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Although some papers concluded to use hypochlorite as superior solution to clean laboratory surfaces **(17, 18)**, in this study we have proved that hypochlorite might not be sufficient to completely decontaminate all of the loci such as Y indel locus found in the GlobalFiler PCR Amplification Kit which might interferes with STR interpretation.

Conclusion

In this paper, we have investigated different sources of DNA contamination in air, laboratory surfaces, gloves, and tools. Different DNA sterilization methods were applied to test the efficiency using sensitive STR kits, i.e GlobalFiler PCR Amplification Kit. Results showed the insufficiency of the current methods to perform complete decontamination procedures. Modified protocols were suggested for some procedures such as using DNA-ExitusPlus IF.

Acknowledgments

We would like to thank the authorities in our Forensic Science for allowing us to utilize the Forensic Science Laboratory. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declarations

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of Interests

The authors declare that they have no conflict of interest**.**

Availability of data and material

The datasets generated during and/or analyzed during the current study are available within the text.

Code availability

All the software applications are mentioned within the text.

Author Contributions Statement

Conceptualization: N.R.A; Investigation: N.R.A, N.M.A; Formal analysis: N.R.A, N.M.A; Writing - original draft preparation: N.R.A; Writing review and editing: N.R.A, N.M.A; Visualization: N.R.A, N.M.A; Supervision: N.R.A.

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