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Comparison between various DNA sterilization procedures applied in forensic analysis

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Abstract

Background: 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.

Results: 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 their ability to efficiently eliminate DNA in a sample. Inactivation methods included 10% bleach, ethanol, UV light, and DNA-ExitusPlus IF. Exposure to the different inactivation protocols for varying periods of time was performed in two lab settings: low template DNA and DNA database labs. Surfaces were swabbed and any adhering DNA was quantified using HID real-time PCR. Results were detected using HID Real-Time PCR Analysis Software v1.2 and GeneMapper ID-X Software v1.4.

Conclusions: 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 the incubation time increased from 10 to 15 min.

Keywords: DNA decontamination, DNA-ExitusPlus IF, Contamination, Sterilization, Forensic analysis, DNA evidence

Background

Forensic casework subjected to DNA analysis is now common in crime laboratories and is used to make crucial decisions in intelligence and justice. Errors such as DNA transfer and contamination may occur, and they can have serious consequences (Kloosterman et al. 2014). DNA contamination is one of the most common causes of faults (Basset and Castella 2019). Three types of DNA contamination can occur: (1) internal contamination between the samples and the DNA analysts, (2) cross-contamination between evidence of same case or different cases, and (3) external contamination which happens between the DNA samples and the police force or crime scene experts or manufacturers of reagents or consumables (Kloosterman et al. 2014). Sometimes 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. There is an increase amount of DNA contamination due to human errors and unawareness of investigators during handling of samples. 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 (Westen et al. 2009; Ballantyne et al. 2013). The police contamination can mask the true match in the DNA evidence thus causing loss of significant leads (Basset and Castella 2019; Lapointe et al. 2015). An inconsistency in

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forensic DNA analysis can lead to poor investigative or legal decisions with far-reaching implications, with the arrest of innocent suspects, the exoneration of convicted suspects, or the failure to identify criminals (Possley et al. 2004). 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 (Lapworth and Teal 1994). Also, it is always mandatory to sustain the trust and good reputation of forensic parties such as the crime labs and crime scenes investigators (Basset and Castella 2019). Several procedures have recently been described to minimize the incidence of DNA contamination at the crime scene and in the laboratory (Ballantyne et al. 2013; Oorschot RAv, Found B, Ballantyne KN. 2015; Fonneløp et al. 2016). Some of these procedures are well-suited to the laboratory setting. These include (1) staff awareness about contamination; (2) the proper use of personal protective equipment (PPE); (3) limiting access to the laboratory working area; (4) effective cleaning and sterilization of all equipment and laboratory zones (Arena 2010); (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 (Ballantyne et al. 2013; Oorschot RAv, Found B, Ballantyne KN. 2015; Fonneløp et al. 2016). Understanding the causes of contamination events provides the most insight into preventing them and preserving the integrity of forensic evidence (Balk 2015). Previously, different DNA sterilization was explained, such as UV irradiation. However, with the use of the PCR technique, which has become a powerful and very sensitive tool in a wide range of research, false-positive PCR results due to various types of contamination are easily detected (Preuße-Prange et al. 2009).

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/DNA analysts and the DNA evidence. In some cases, we have used commercially available decontamination products but have modified manufacturer's recommended protocols to give maximum effectiveness in eliminating unwanted DNA. DNA sterilization methods tested included exposure of items harboring DNA to various concentrations of ethanol, exposure for varying periods to ultraviolet light (UV) light, exposure to 10% (v/v) bleach (hypochlorite), and exposure to the commercially available product 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.

Methods

Experimental design

Different experiments were designed to assess DNA sterilization methods such as decontamination using 10% bleach solution, ethanol solution, and 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) as shown in Table 1. In this study, we tested various approaches to remove DNA from hard laboratory surfaces and instruments. We applied gDNA of ~ 20 ng/ul in clean surfaces as a control to show the effectiveness of the sterilization procedures. The DNA was dried and left for 15 min before any treatment. The collection of cells was done using duplicate cotton swabs (SceneSafe, UK). All research was performed in accordance with relevant guidelines/regulations as per for standard operating procedures (SOPs). The research ethics and consent were provided from the Ministry of Interior (MOI).

Applying 85% ethanol solution to disinfect the working area surfaces and instruments

Applying 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 first pre-swabbed as a control, then applied the 85% ethanol solution to check the effectiveness.

Applying 85% ethanol solution and DNA-ExitusPlus IF to disinfect working area surfaces and instruments

Applying 85% ethanol solution and DNA-ExitusPlus IF (PanReac AppliChem, Germany) to sanitize surfaces of working areas such as DNA extraction benches and PCR cabinets in both CW and DB labs. Each instrument was first pre-swabbed as control then applied the 85% ethanol solution and DNA-ExitusPlus IF then swabbed again to check the effectiveness

Applying different exposure time of UV light to disinfect PCR cabinets

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. The first DNA sample was applied as a control, then each part was swabbed in different time intervals to test the effectiveness of UV light.

Table 1 The experimental design

Sr. No	Experiments	Details
1	Disinfecting the working area surfaces and instruments with 85% ethanol solution	This experiment was done to show the effectiveness of applying 85% ethanol solution to surfaces. Method: This is a screening test; therefore, all the surfaces were swabbed for touch DNA, then 85% ethanol solution was applied. Finally, the surfaces were swabbed. Number of samples collected: a total of 12 replicate cotton swabs for pre- and post-cleaning of the thermomixer surfaces.
2	Disinfecting the working area surfaces and instruments with 85% ethanol solution and DNA-ExitusPlus IF	This experiment was done to show the effectiveness of applying both 85% ethanol solution and DNA-ExitusPlus IF to surfaces. Method: This is a screening test; therefore, all the surfaces were swabbed for touch DNA, then both 85% ethanol solution and DNA-ExitusPlus IF were applied. Finally, the surfaces were swabbed. A number of samples collected: a total of 24 replicate cotton swabs for pre- and post-cleaning of different surfaces of benches, cabinets, drawers, and tools.
3	Disinfecting the PCR cabinets using different exposure time of UV light	This experiment was done to show the effectiveness of using different exposure times of UV irradiation to samples. Method: Here, we have applied saliva (~ 20 ng/ul) in different surfaces and then we have swabbed each spot in different timings. Number of samples collected: a total of 12 replicate cotton swabs for each time exposure on the working area of PCR cabinets.
4	Disinfecting the working area using different exposure time of DNA-ExitusPlus IF	This experiment was done to show the effectiveness of using different exposure time of DNA-ExitusPlus IF to samples. Method: Here, we have applied saliva (~ 20 ng/ul) in different surfaces and then we have swabbed each spot in different timings. Number of samples collected: a total of 6 replicate cotton swabs for each time exposure in DNA collection benches.
5	Disinfecting the working area using different exposure time of bleach	This experiment was done to show the effectiveness of using different exposure times of bleach to samples. Method: Here, we have applied first saliva (~ 20 ng/ul) in different surfaces and then we have swabbed each spot in different timings. Number of samples collected: a total of 14 replicate cotton swabs for each time exposure in DNA collection benches.
6	Disinfecting the working area using different concentrations of ethanol solution	This experiment was done to show the effectiveness of using different concentrations of ethanol solution to samples. Method: Here, we have applied saliva (~ 20 ng/ul) in different surfaces and then we have swabbed each spot in different concentrations. Number of samples collected: a total of 10 replicate cotton swabs for each concentration in DNA collection benches.
7	DNA testing of gloves during work	This experiment was designed to check the possibility of DNA transfer using gloves during work. Method: This is a screening test, in which different used gloves were swabbed during their lab work. Number of samples collected: a total of 6 replicate cotton swabs for each glove
8	Talking in the presence of an open tube.	This experiment was done to detect any possibilities for DNA contamination in pre- and post-PCR working area. Method: tubes containing DNA extract was used to talk while the tubes were open, in pre- and post-PCR procedures without wearing masks. Number of samples collected: a total of 8 replicate tubes for each experiment.
9	Presence of DNA in the air	In this experiment, it was designed to check if the air contains any DNA. Method: swabs were taken from air by moving the air around different locations. Number of samples collected: a total of 10 replicate cotton swabs for each location.

Applying different exposure time of DNA-ExitusPlus IF to disinfect working area

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 DNA on the tested surfaces and swabbed part of DNA as a control, then sprayed the solution then waited for the studied time then swabbed again to check for efficiency.

Applying different exposure time of bleach to disinfect working area

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 DNA on the tested surfaces and swabbed part of DNA as a control, then sprayed the solution then waited for the studied time then swabbed again to check for efficiency.

Applying different concentrations of ethanol solution to disinfect working area

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 DNA on the tested surfaces and swabbed part of the DNA as a control, then sprayed the ethanol solution using the abovementioned concentrations and waited for 10 min and then swabbed again each part to check for efficiency of using different ethanol concentration

DNA testing of gloves during work

Random swabbing was done during DNA testing for different DNA experts to show the possibility of DNA transfer during work. Each of the three gloves was swabbed using duplicate cotton swabs.

Talking in the presence of an open tube

We have talked and coughed inside DNA test tubes—without wearing masks—prior to proceed for pre and post PCR amplification to study the effect of DNA contamination from unprocessed DNA such as the saliva. The procedure was done in pre- and post-PCR cabinets. The time of exposure was 10 s for each tube.

Presence of DNA in the air

Random swabbing was done in the air to check for the presence of DNA in the air such as saliva particles. Different areas were swabbed using duplicate cotton swabs such as working areas, PCR cabinets, and offices for CW and DB labs. The time of exposure was 10 s for each area.

Table 1 summarizes the methods applied to show the effectiveness of DNA sterilization and the presence of different DNA contamination in laboratory.

DNA processing

Genomic DNA (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 in 50 µl elution volume (Davis et al. 2012). In each procedure, unused cotton swab was extracted as a negative control and another buccal swab from a DNA analyst was used as a positive control.

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 (Holt et al. 2016). The kit can reproducibly quantify 5 pg/µL of human genomic DNA in a sample and can quantify DNA concentrations from 0.005 to >50 ng/uL. About 1.2 ng of the extracted DNA (15 µl) was amplified using GlobalFiler PCR Amplification Kit in total of 25 µl reaction volume (Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to manufacturer's recommendation (Ludeman et al. 2018).

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 (Ludeman et al. 2018).

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 (Al-Snan et al. 2019).

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 2, the detection of gDNA in several experiments conducted with *R*-squared (R^2) values equal to 0.998 and 1.0 defining perfect predictive accuracy. 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 cabinet 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 2) as the 10% bleach may possibly interfere with the proper interpretation of results. Therefore, STR profiles were required to give reliable results.

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 min 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 2); 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 in the presence of an open tube 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 waving a clean swab in the air for 30 s to show the presence of DNA in different premises. As shown in Table 2, 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), and 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 Fig. 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).

Regarding different time exposure to the UV light, we have studied different exposures in terms of 5 to 25 min using 5-min time intervals. As shown in Fig. 2, the DNA quantity is declining by increasing the time exposure with the UV light. Yet in 25 min 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.

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

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 Fig. 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.

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

Sr. No	Experiment details	Amount of gDNA (ng/ul) (mean)		**Ct values (mean)
1) **Disinfecting the working area surfaces and instruments with 85% ethanol solution**				
1	Thermomixers (1), DNA CW lab	Precleaning: 0.002	Post-cleaning: 0.0004	31.5, 33.8
2	Thermomixers (2), DNA CW lab	Precleaning: 0.0021	Post-cleaning: 0.0022	31.4, 31.3
3	Thermomixers (3), DNA DB lab	Precleaning: 0.0015	Post-cleaning: 0.0023	31.9, 31.2
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		33.39, 30.2, 30.76
5	PCR cabinet, DNA CW lab	Precleaning: 0.0041, post-cleaning ethanol: 0.0003, post-cleaning DNA-exitus: 0.0003		30.54, 34.28, 34.34
6	PCR cabinet, DNA DB lab	Precleaning: 0.0011	Post-cleaning DNA-exitus: *UD	32.34, UD
7	PPE drawer, DNA DB lab	Precleaning: 0.0013	Post-cleaning ethanol: 0.0006	33.11, 32.14
8	1000ul pipettes, DNA CW lab	Precleaning: 0.061	Post-cleaning ethanol: 0.0004	3.74, 26
3) **Disinfecting the PCR cabinets using different exposure times of UV light**				
9	Induced contamination, UV light, 0 min, control	0.0081		33.54
10	Exposure UV light, 5 min	0.0045		34.36
11	Exposure UV light, 10 min	0.0063		33.89
12	Exposure UV light, 15 min	0.0009		36.65
13	Exposure UV light, 20 min	0.0024		35.32
14	Exposure UV light, 25 min	0.0018		35.61
4) **Disinfecting the working area using different exposure times of DNA-ExitusPlus IF**				
15	Induced contamination, DNA-Exitus, 0 min, control	0.0507		35.41
16	Post-cleaning, DNA-Exitus, 10 min	0.0011		34.41
17	Post-cleaning, DNA-Exitus, 15 min	UD		36.35
5) **Disinfecting the working area using different exposure times of bleach**				
18	Induced contamination, bleach, 0 min, control	0.0845		33.12
19	Post-cleaning, bleach, 10 min	UD		UD
20	Post-cleaning, bleach, 15 min	0.0002		34.41
21	Post-cleaning, bleach, 20 min	UD		UD
22	Post-cleaning, bleach, 25 min	UD		UD
23	Post-cleaning, bleach, 30 min	UD		UD
24	Post-cleaning, bleach, 35 min	0.0003		36.35
6) **Disinfecting the working area using different concentrations of ethanol solution**				
25	Induced contamination, ethanol, 0%, control	0.0096		27.05
26	Post-cleaning, ethanol, 70%	0.0045		30.4
27	Post-cleaning, ethanol, 75%	0.0038		30.63
28	Post-cleaning, ethanol, 80%	0.0043		30.46
29	Post-cleaning, ethanol, 85%	0.0017		30.17
7) **DNA testing of gloves during work**				
30	Random swabbing glove 1	0.0022		31.36
31	Random swabbing glove 2	0.0033		30.82
32	Random swabbing glove 3	0.0022		31.38
8) **Talking in the presence of an open tube**				
33	Talking inside DNA tube, pre-PCR 1	UD		UD
34	Talking inside DNA tube, pre-PCR 2	0.0002		34.91
35	Talking inside DNA tube, post-PCR 1	0.0006		33.27
36	Talking inside DNA tube, post-PCR 2	0.0002		34.51
9) **Presence of DNA in the air**				
37	Air swabbing, staff office, CW lab	0.0045	30.38	
38	Air swabbing, DNA extraction room, CW lab	0.0003	34.34	
39	Air swabbing, staff office, DB lab	0.0012	32.21	
40	Air swabbing, PCR cabinet, CW lab	UD	UD	
41	Air swabbing, PCR cabinet, DB lab	UD	UD	

**UD* Underdetermined, ***Ct values* Cycle Threshold.

R^2 values: 0.998 and 1.0

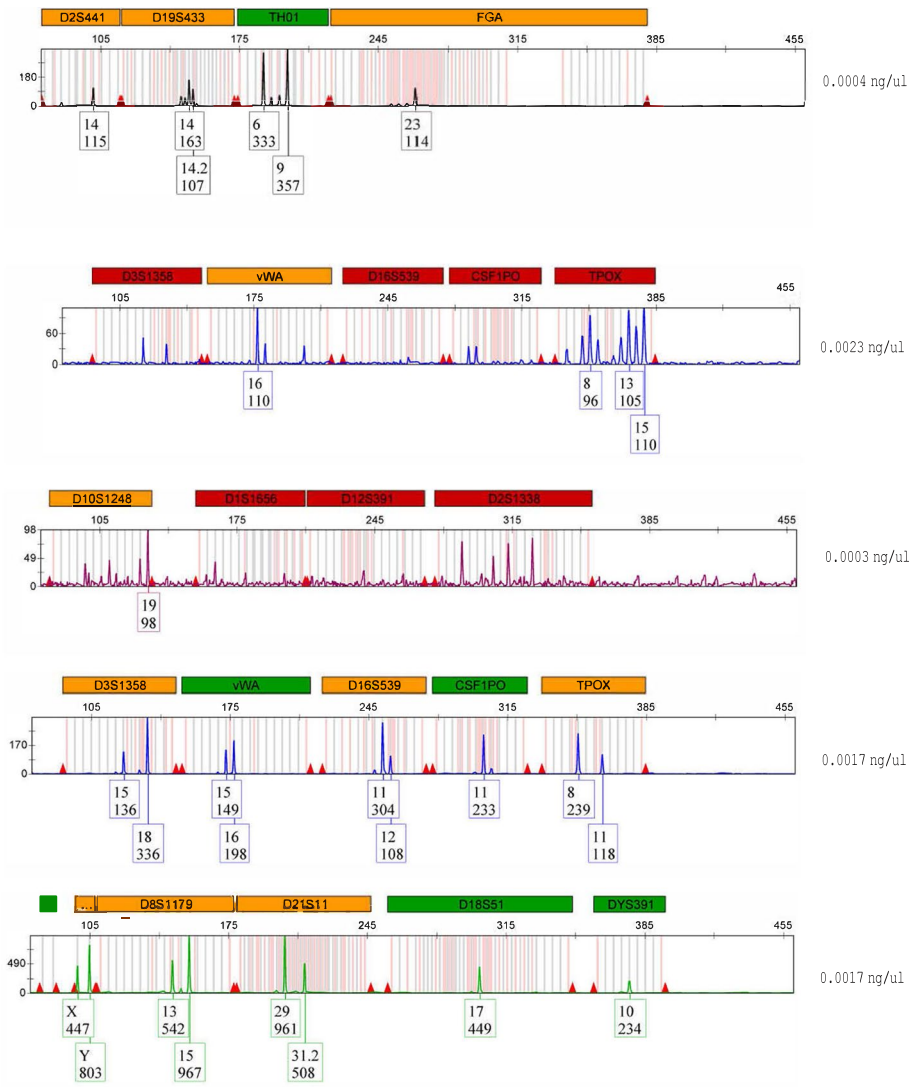

Fig. 1 STR profiles generated from post-sterilization with different concentrations of ethanol

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-min time intervals. All the STR profiles results displayed different loci after disinfection. As shown in Fig. 5, 10% bleach was not sufficient for proper DNA disinfection.

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, and biotechnology. 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 (Gefrides et al. 2010). Inclusion of extraction and amplification negative controls is one of the methods conducted to investigate the presence of cross-contamination/consumable contamination in the DNA testing. Crime scene examiners as well as forensic analysts must equip PPE, i.e., masks, sterile suits, hair cap, gloves,

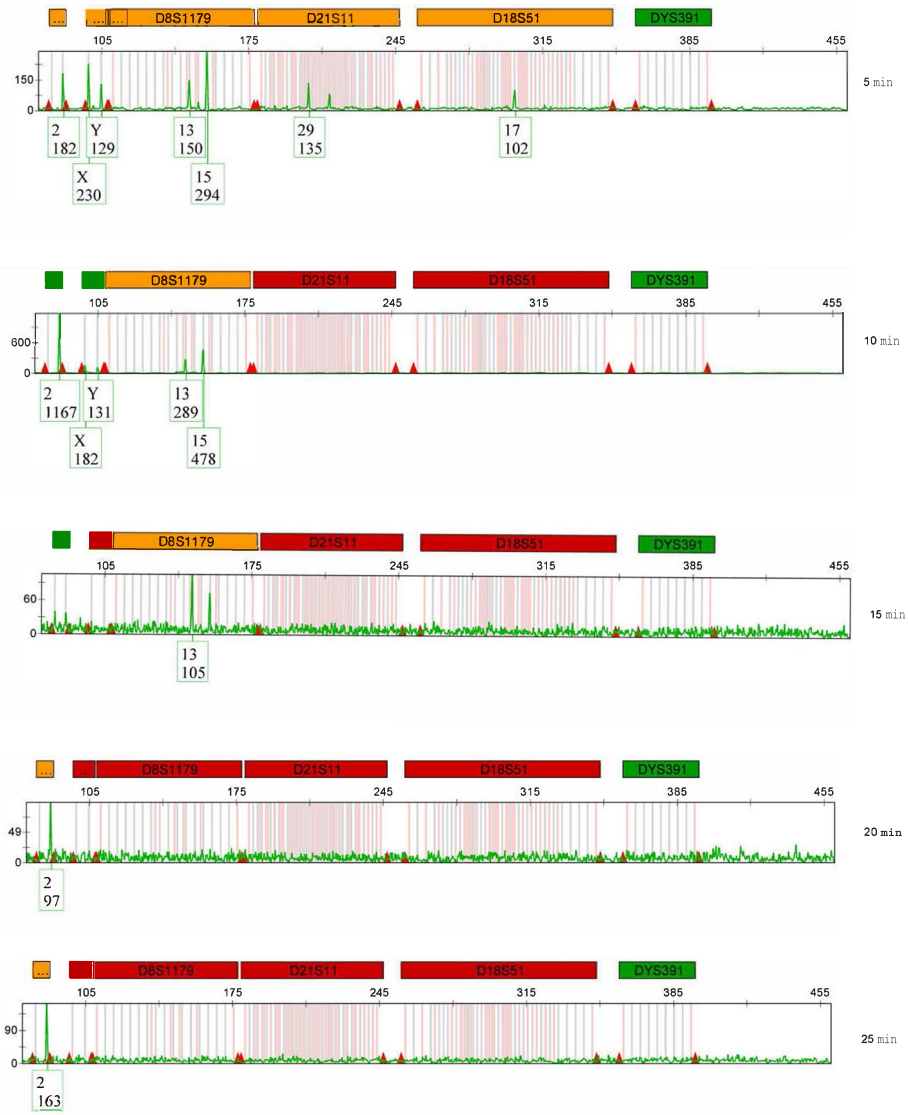

Fig. 2 The effect of UV light in DNA sterilization using different time exposure

as it greatly protects the evidence from contamination (Rutty et al. 2003). 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.

Using HID Real-Time PCR Analysis Software v1.2 in 7500 Real-Time PCR System (Thermo Fisher Scientific, Inc., Waltham, MA, USA), R^2 values obtained indicating perfect predictive accuracy of the results offering good confidence of interpretation. A cycle threshold (Ct value) is defined as the number of amplification cycles required to reach a fixed background level of fluorescence at which the diagnostic result of the real-time PCR changes from negative (not detectable) to positive - (detectable). The amount of DNA obtained in parallel with Ct values (mean) refer to the level of DNA inhibition, higher Ct values, and more PCR cycles is required to perform which lead to presence of DNA inhibition (Sidstedt et al. 2020). For example, in UV light exposure after 15 min, it gave Ct values of approximately ~ 35, which indicates the high level of DNA degradation from the UV light. Similarly with DNAexitus method, Ct values were above 34 in all the time exposures as it was more effective in sterilization surfaces. However, even with the Ct values, the STR profiles must be assessed to have a better finding of the results.

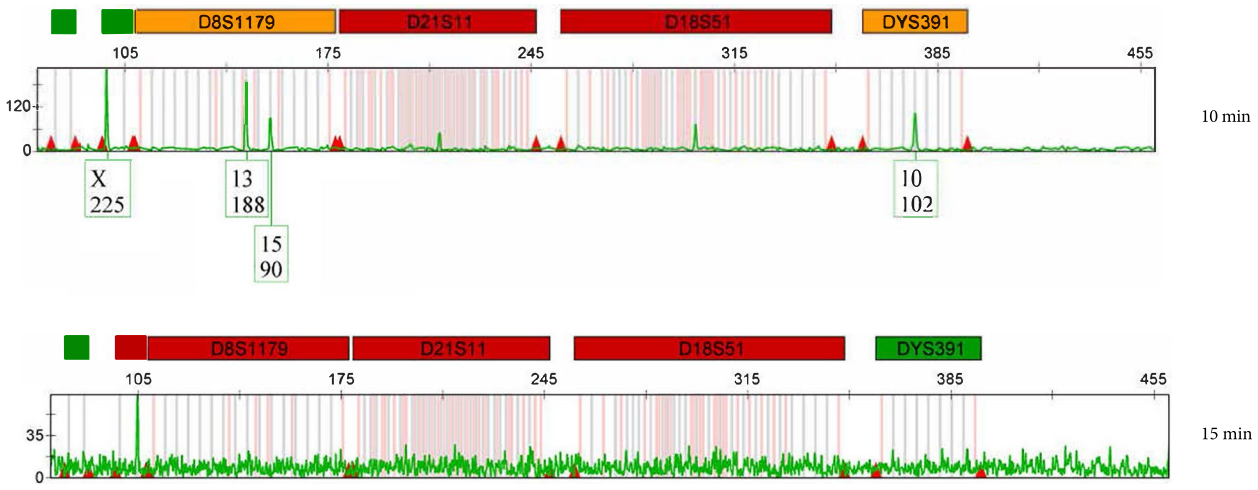

Fig. 3 The effect of DNA-ExitusPlus IF in DNA sterilization using different time intervals

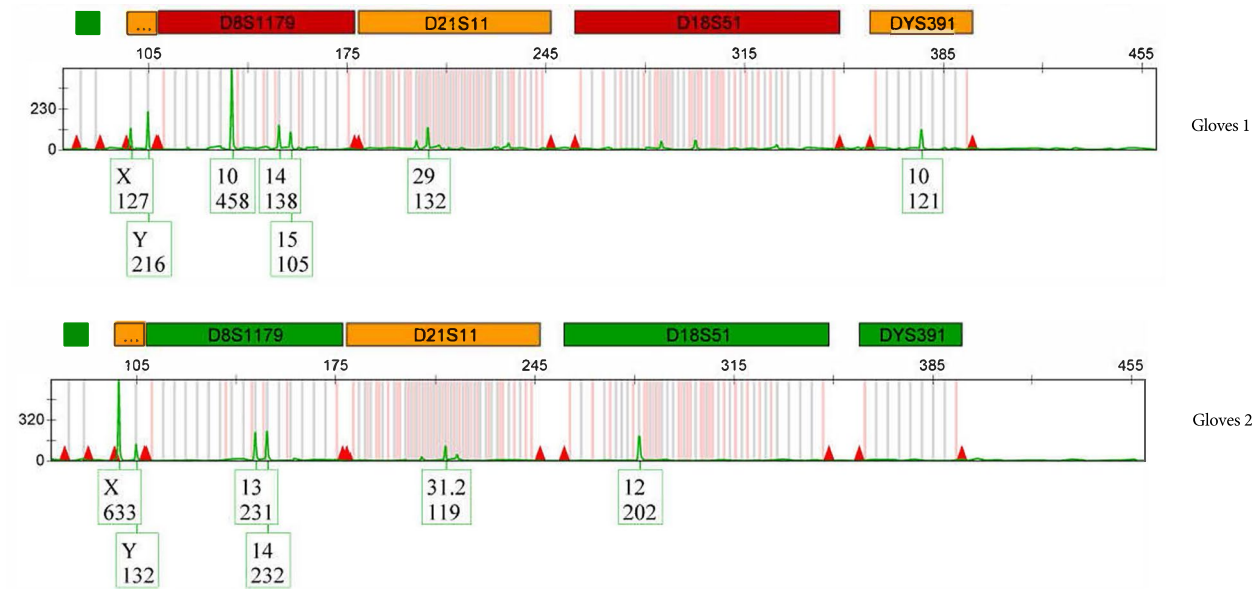

Fig. 4 Random swabbing of equipped gloves during DNA testing

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 2, 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 min instead of 10 min on the surfaces/instruments before wiping (Arena 2010). 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 (Kampmann et al. 2017; Ballantyne et al. 2015), 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.

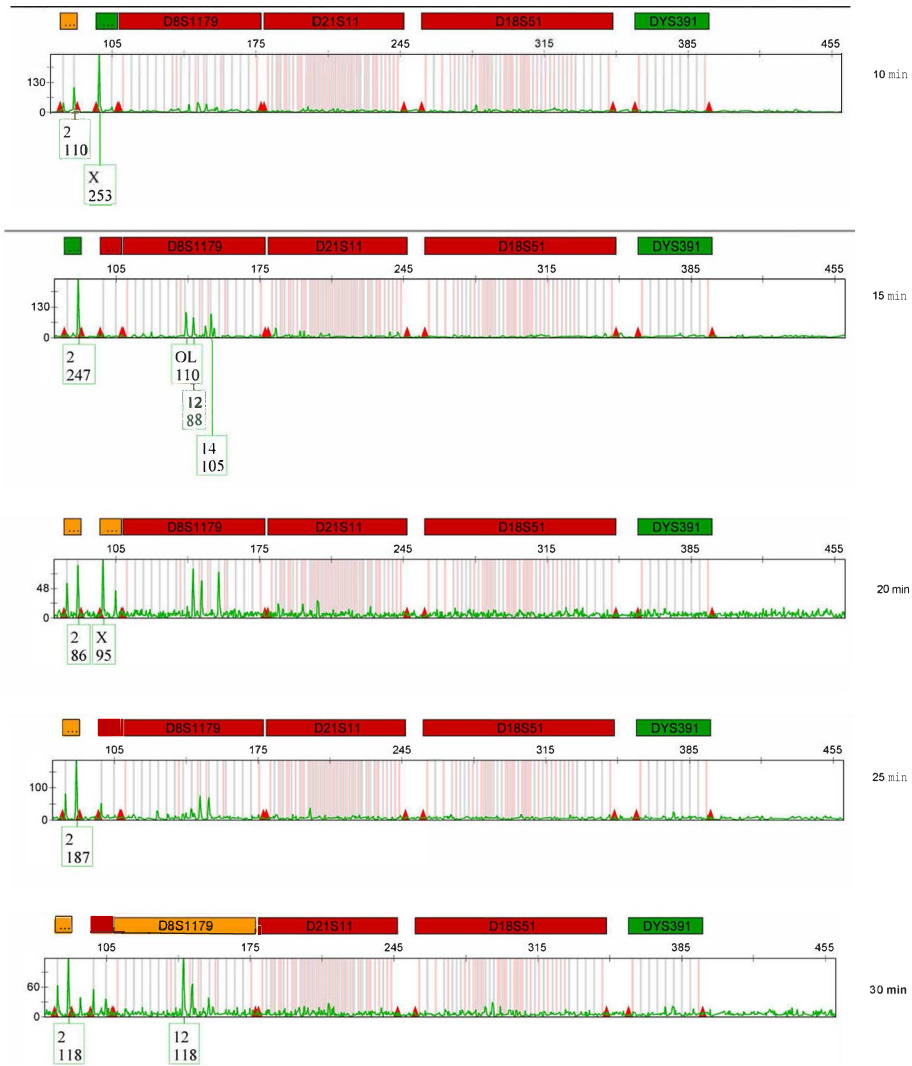

Fig. 5 The effect of 10% bleach in performing DNA sterilization

Conclusions

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.

Abbreviations

CW: Casework lab (low DNA lab); Ct: Cycle threshold; DB: Database lab (reference DNA lab); gDNA: Genomic DNA; HID: Human identification; Min: Minutes; MOI: Ministry of interior; PPE: Personal protective equipment; R2: R-squared; SOPs: Standard operating procedures; STR: Short tandem repeats; UV: Ultraviolet.

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Authors' contributions

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. The authors have read and approved the manuscript.

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

Declarations

Ethics approval and consent to participate
All the experiments conducted under the approval of MOI, Bahrain. The consent to participate was obtained from MOI, Bahrain. The reference number is ***not applicable.***

Consent for publication
Written informed consent to publish this information was obtained from study participant's next of kin and/or parent/legal guardian which will be available upon request. The consent for publication was obtained from MOI, Bahrain.

Competing interests
The authors declare that they have no competing interests.

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