

# Comparison between Various DNA Sterilization Procedures Applied in Forensic Analysis

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## Research Article

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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.

**Methods and 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 minutes

## Introduction

Forensic casework subjected to DNA analysis is now common in crime laboratories and is used to make crucial decisions in intelligence and justice. Errors can occur, and they can have serious consequences such as DNA transfer and contamination [1]. DNA contamination is one of the most common causes of faults [2]. 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. 3) external contamination which happens between the DNA samples and the police force or crime scene experts or manufacturers of reagents or consumables [1]. 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 the upsurge number of forensic cases which request for DNA testing and an increase demand of DNA analyst's employment. 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, with 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 investigators [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 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 crime scene workers 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 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.

## Materials And Methods

### Experimental design

Different experiments were designed to assess 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 applied gDNA of ~ 20 ng/ul in clean surfaces. The DNA was dried and left for 15 minutes before any treatment. The collection of cells was done using cotton swabs (SceneSafe, UK). All research was performed in accordance with relevant guidelines/regulations.

- **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, then applied the 85% ethanol solution.

- **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 then applied the 85% ethanol solution and DNA-ExitusPlus IF then swabbed again.

- **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.

- **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 induced DNA on the tested surfaces, 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 induced DNA on the tested surfaces, 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 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 in the presence of an open tube.**

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 in the presence of an open tube 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.

Table 1

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

<b>1) Disinfecting the working area surfaces and instruments with 85% ethanol solution</b>			
<b>Sr. No</b>	<b>Experiment details</b>	<b>Amount of gDNA (ng/ul)</b>	
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
<b>2) Disinfecting the working area surfaces and instruments with 85% ethanol solution and DNA-ExitusPlus IF</b>			
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
<b>3) Disinfecting the PCR cabinets using different exposure time of UV light</b>			
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	
<b>4) Disinfecting the working area using different exposure time of DNA-ExitusPlus IF</b>			
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	
<b>5) Disinfecting the working area using different exposure time of bleach</b>			

**\*UD: Underdetermined**

<b>1) Disinfecting the working area surfaces and instruments with 85% ethanol solution</b>		
<b>18</b>	Induced contamination, Bleach, 0 min	0.0845
<b>19</b>	Post-cleaning, Bleach, 10 min	UD
<b>20</b>	Post-cleaning, Bleach, 15 min	0.0002
<b>21</b>	Post-cleaning, Bleach, 20 min	UD
<b>22</b>	Post-cleaning, Bleach, 25 min	UD
<b>23</b>	Post-cleaning, Bleach, 30 min	UD
<b>24</b>	Post-cleaning, Bleach, 35 min	0.0003
<b>6) Disinfecting the working area using different concentrations of ethanol solution</b>		
<b>25</b>	Induced contamination, Ethanol, 0%	0.0096
<b>26</b>	Post-cleaning, Ethanol, 70 %	0.0045
<b>27</b>	Post-cleaning, Ethanol, 75 %	0.0038
<b>28</b>	Post-cleaning, Ethanol, 80 %	0.0043
<b>29</b>	Post-cleaning, Ethanol, 85 %	0.0017
<b>7) DNA testing of gloves during work</b>		
<b>30</b>	Random swabbing gloves 1	0.0022
<b>31</b>	Random swabbing gloves 2	0.0033
<b>32</b>	Random swabbing gloves 3	0.0022
<b>8) Talking in the presence of an open tube.</b>		
<b>33</b>	Talking inside DNA tube, pre-PCR 1	UD
<b>34</b>	Talking inside DNA tube, pre-PCR 2	0.0002
<b>35</b>	Talking inside DNA tube, post-PCR 1	0.0006
<b>36</b>	Talking inside DNA tube, post-PCR 2	0.0002
<b>9) Presence of DNA in the air</b>		
<b>37</b>	Air swabbing, Staff office, CW lab	0.0045

**\*UD: Underdetermined**

1) Disinfecting the working area surfaces and instruments with 85% ethanol solution		
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
<b>*UD: Underdetermined</b>		

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.

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 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 seconds 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).

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.

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**.

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.

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.

## 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.

## **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.

### **Ethics approval**

All the experiments conducted under the approval of MOI, Bahrain

### **Consent to participate**

The consent to participate was obtained from MOI, Bahrain

### **Consent for publication**

The consent for publication was obtained from MOI, Bahrain

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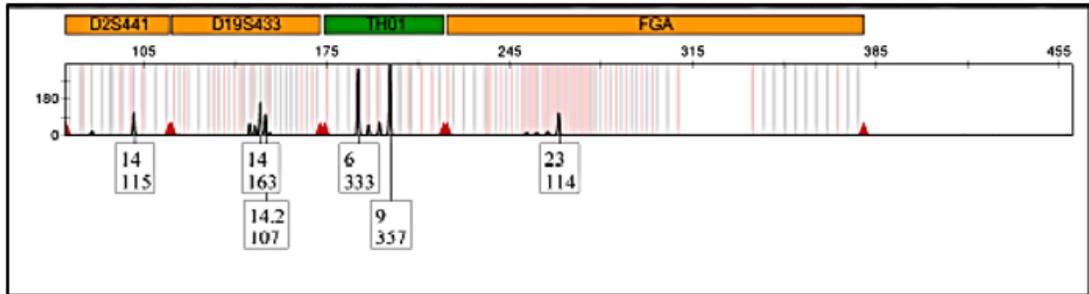
We would like to thank the authorities in our Forensic Science for allowing us to utilize the Forensic Science Laboratory. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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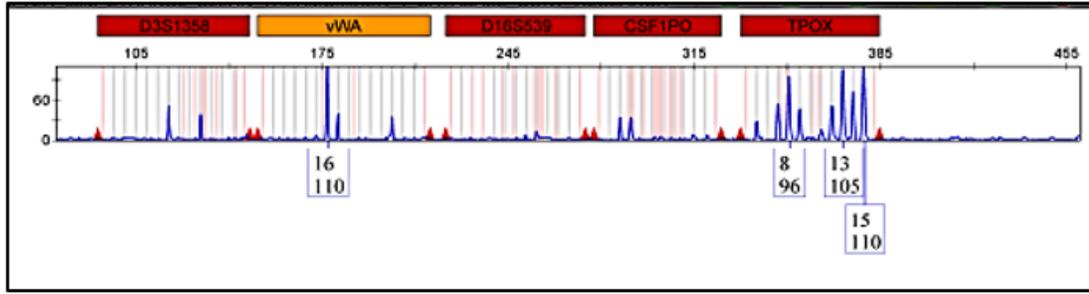
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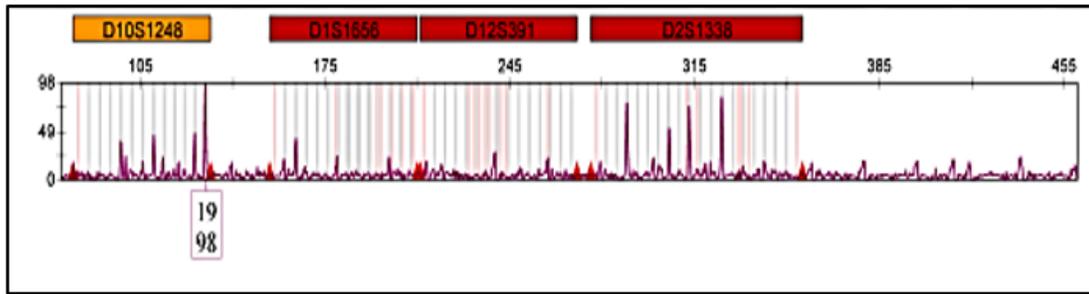
## Figures



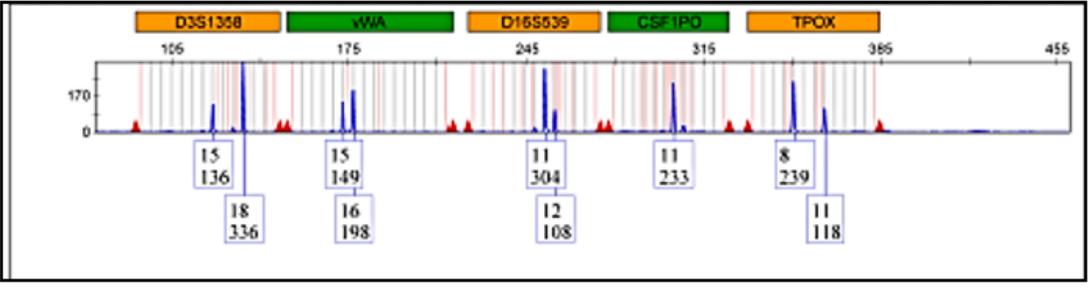
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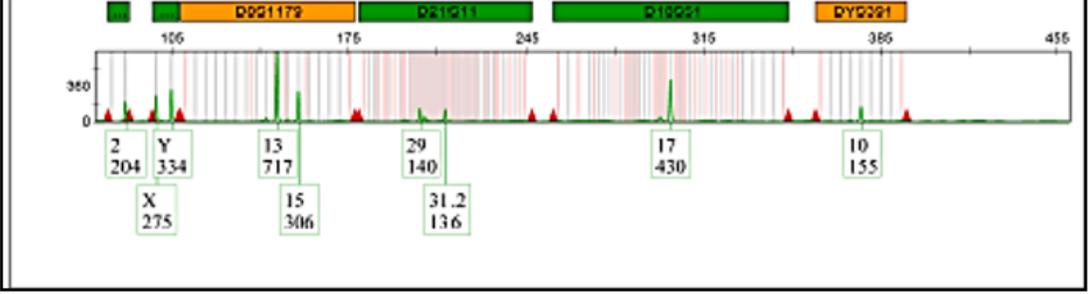
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**0.0003 ng/ul**



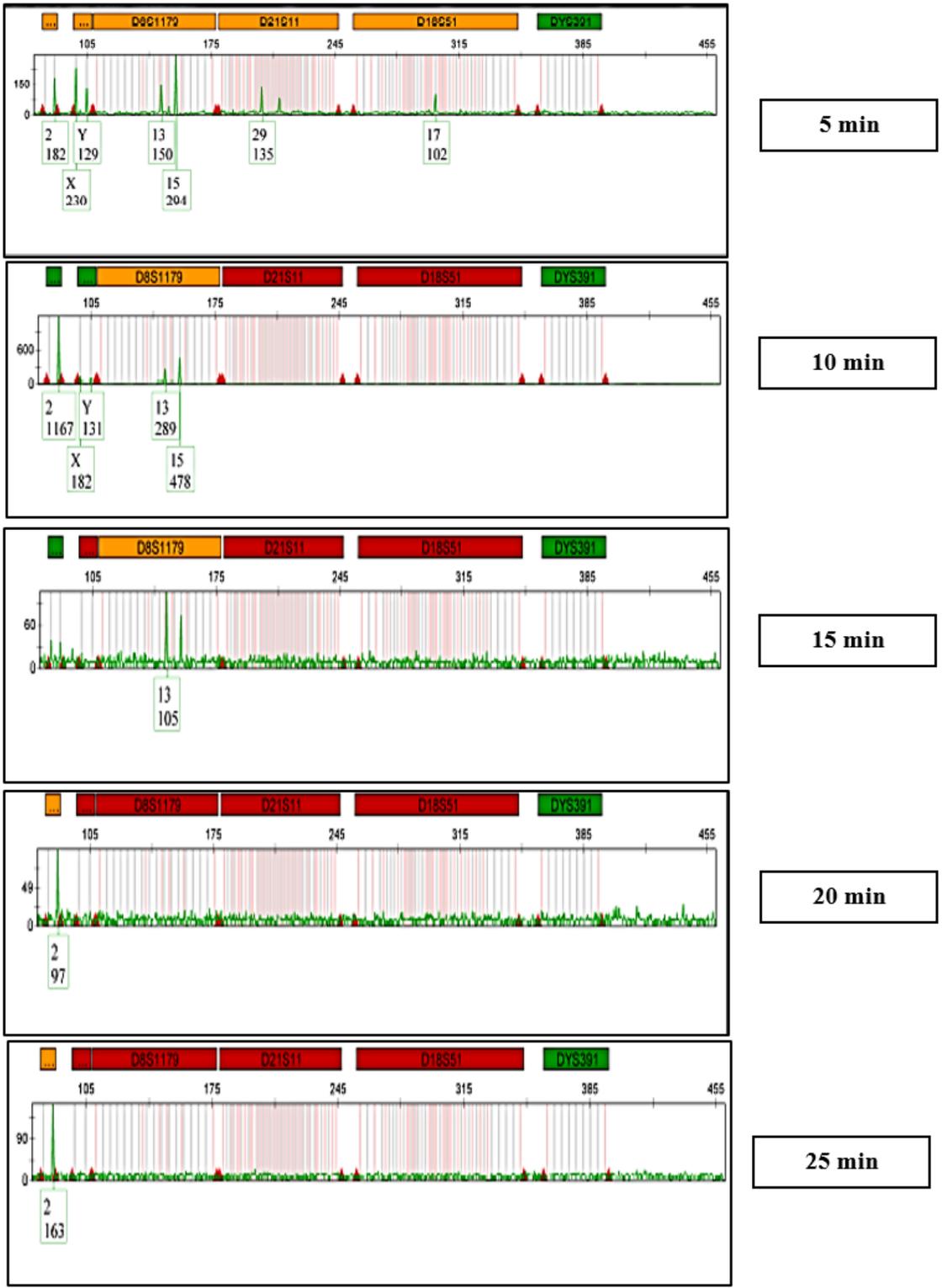
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**0.0017 ng/ul**

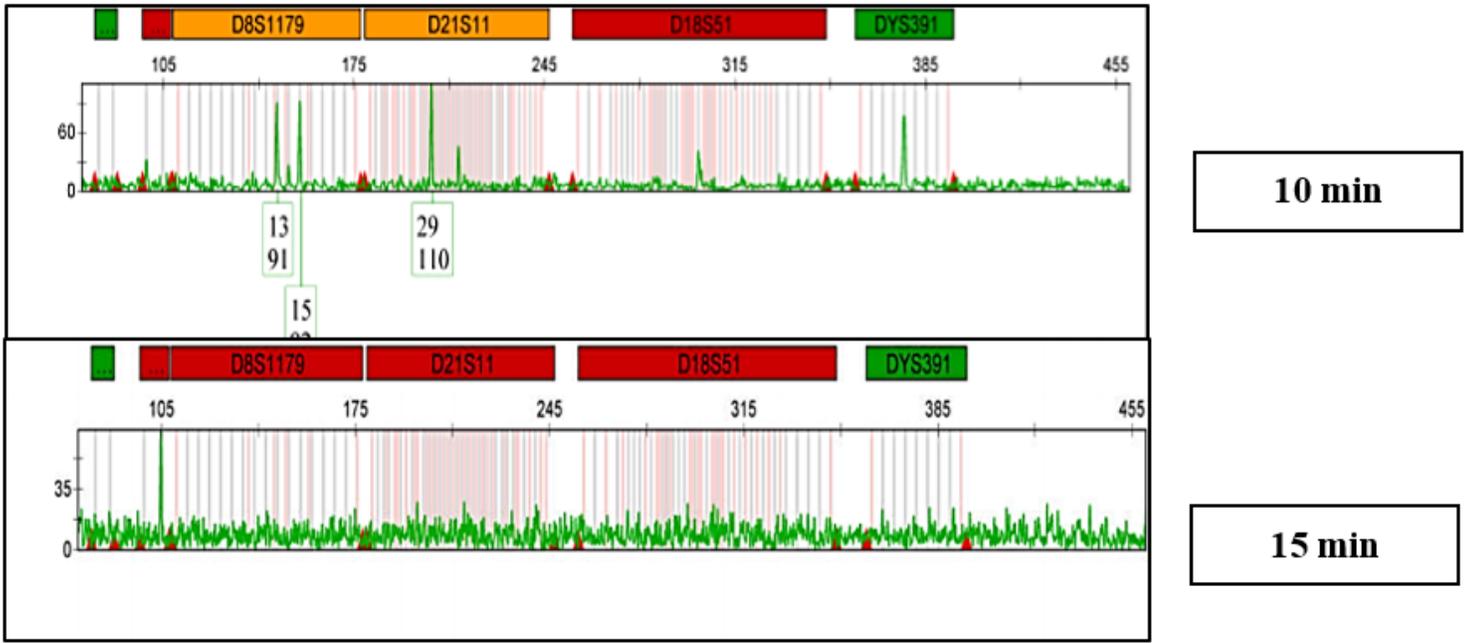
**Figure 1**

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



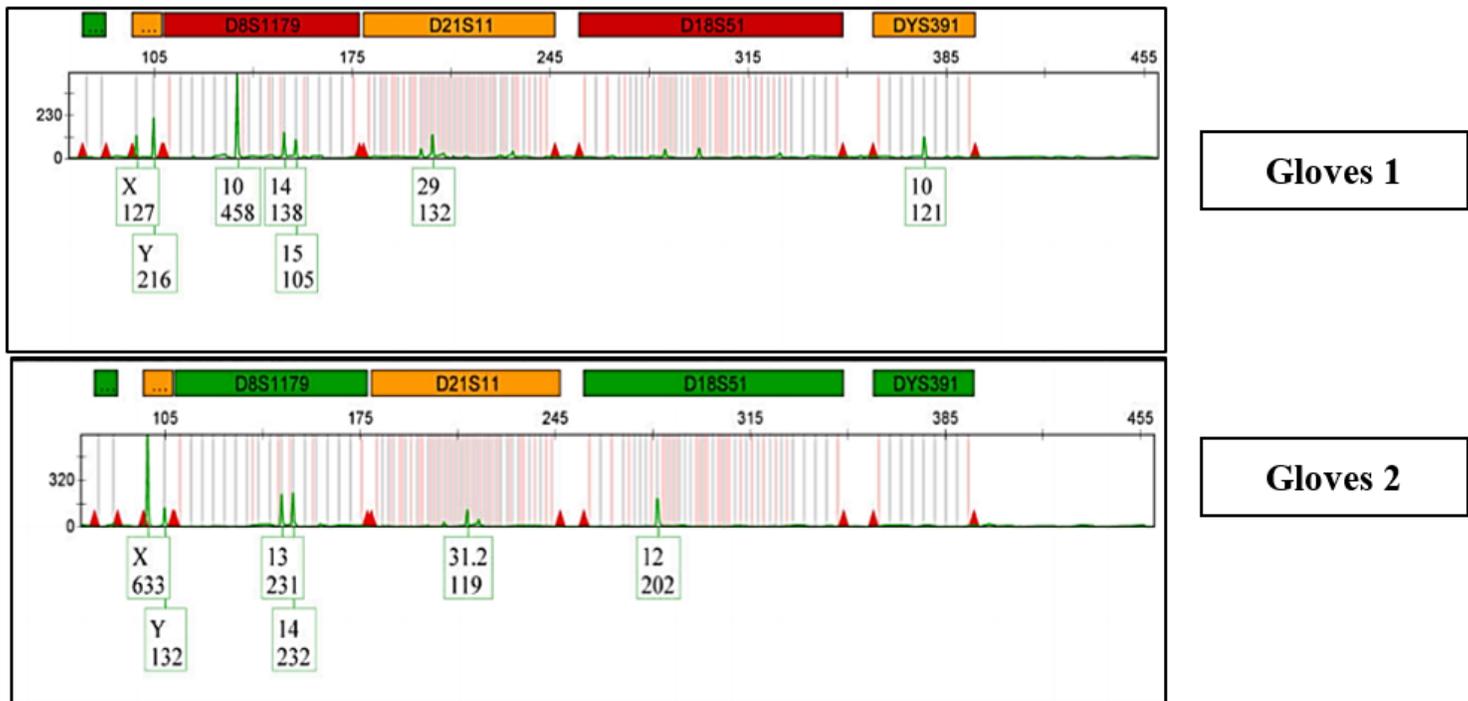
**Figure 2**

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



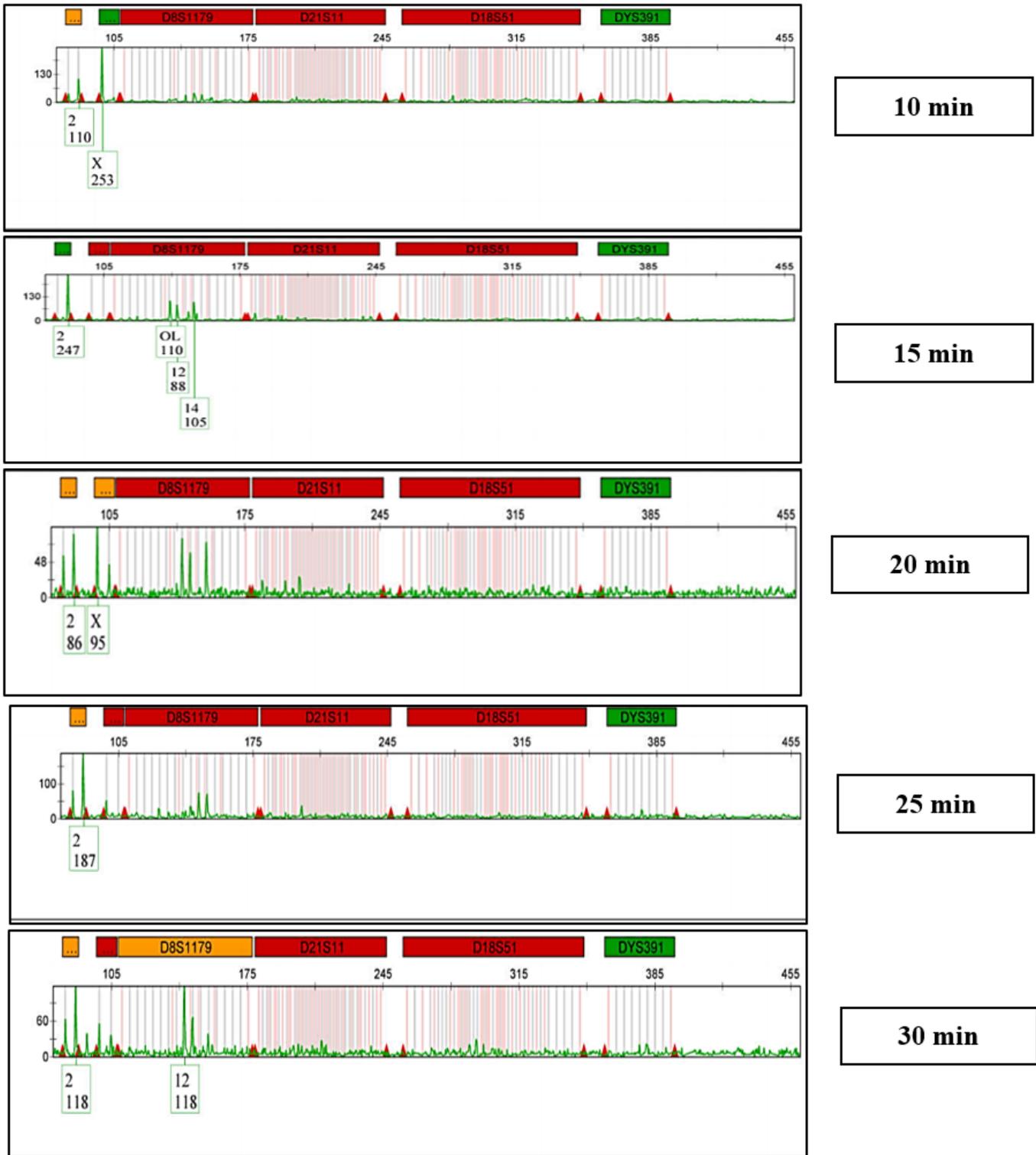
**Figure 3**

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



**Figure 4**

Random swabbing of equipped gloves during DNA testing



**Figure 5**

The effect of 10 % bleach in performing DNA sterilization