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ORIGINAL ARTICLE

Comparison of the effects of sterilisation techniques on subsequent DNA profiling

Kirsty Shaw · Ivana Sesardić · Nikki Bristol · Carole Ames · Kathryn Dagnall · Caryn Ellis · Fiona Whittaker · Barbara Daniel

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Abstract It is important that contamination from extraneous DNA should be minimised on items used at crime scenes and when dealing with exhibits within the laboratory. Four sterilisation techniques (UV, gamma and beta radiation and ethylene oxide treatment) were examined for their potential to degrade contaminating DNA to such an extent that subsequent DNA profiling was impossible. This work indicated that the most successful technique to reduce DNA contamination was ethylene oxide treatment. Of the radiation techniques tested in this study, gamma was the most successful at eradicating DNA and UV radiation was the least. None of the contaminated samples treated with ethylene oxide and subsequently subjected to DNA analysis met the DNA profile criteria necessary for acceptance on the UK National DNA Database. Contaminated cotton swabs and micro-centrifuge tubes treated with ethylene oxide showed a marked decrease in amplifiable DNA post-treatment. Ethylene oxide treatment to sterile swabs and tubes did not significantly affect subsequent DNA analysis.

K. Shaw · I. Sesardić · N. Bristol · B. Daniel
Department of Forensic Science and Drug Monitoring,
King's College London,
Franklin-Wilkins Building, 150 Stamford Street,
London, SE1 9NH, UK

C. Ellis · F. Whittaker
LGC Forensics,
Queens Road, Teddington,
Middlesex, TW11 0LY, UK

C. Ames (✉) · K. Dagnall
Directorate of Forensic Services,
Metropolitan Police Service, New Scotland Yard,
10 Broadway,
London, SW1H 0BG, UK
e-mail: carole.ames@met.police.uk

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Introduction

It is well-documented that DNA contamination on items used at both the crime scene and within the forensic laboratory can result in DNA profiles that may incorrectly implicate individuals or make evidence meaningless. Whilst mixtures of DNA profiles caused by exogenous DNA can be separated into major and minor components by quantification for both nuclear DNA [1] and mitochondrial DNA [2], contamination should be avoided if at all possible. Contamination can occur at the manufacturing stage of products as noted by Sullivan et al. [3]. At present, most items associated with the collection of DNA at the scene and the subsequent extraction and profiling are sterilised before use or assembled in a 'clean' room. However, 'clean' does not necessarily mean that an item is free from contamination with human DNA. A variety of sterilisation methods exist including ionising radiation (ultraviolet (UV), gamma, X-ray and beta) and chemical treatments (ethylene oxide and hydrogen peroxide). There is some discrepancy amongst published data as to how effective these methods are at removing contaminating DNA. In some instances, gamma radiation was shown to eliminate small amounts of genomic and plasmid DNA [4]. Withrow et al. [5] demonstrated that full mitochondrial and nuclear profiles were obtained after beta radiation doses of 29.3 and 51.6 kGy even though it was demonstrated that the radiation significantly reduced the total amount of DNA extracted and that some evidence of degradation was present. Work conducted by Castle et al. [6] corroborated this result and showed that the amount of DNA available

for profiling is reduced after beta radiation but that 16 STRs (including all 13 CODIS STRs) were detectable after treatment. Hall and Ballantyne [7] noted that it was possible to obtain full profiles from extracted DNA exposed to UV for 1 min but that no profile was obtainable after 16 min. This was in contrast to bloodstains where chromosomal packaging was still presumably intact. In this case, complete loss of DNA profiles did not occur until 102 h of exposure to UV radiation.

In this work, the effectiveness of eliminating amplifiable DNA using three irradiation techniques, UV, beta, gamma and treatment with ethylene oxide was compared. UV irradiation can be conducted within the laboratory. Sterilisation time is set by the user according to laboratory protocols. Beta and gamma irradiation is more hazardous and therefore is conducted by an accredited company. Again, sterilisation time is set by company guidelines. Ethylene oxide is a poisonous gas and therefore can only be used for sterilisation by approved companies. Items sterilised by the ethylene oxide must also go through a decontamination stage to ensure all residual gas is removed.

Materials and methods

Comparison of four sterilisation techniques

Saliva was obtained from a single volunteer and various volumes (1, 2, 5 and 10 μl) were spotted onto 2 surface types, porous (1.5×1.5 cm Calico material) and non-porous (6 cm petri dish). All conditions were repeated in triplicate for each of the 4 sterilisation techniques to give a total of 96 samples. Samples were then packaged in tamper evident bags. A negative control was included, which contained no saliva but underwent the sterilisation technique and a positive control, which contained saliva but did not undergo the sterilisation technique. Samples were subjected to sterilisation according to normal practice. UV sterilisation was conducted at LGC Forensics, Teddington, UK (254 nm at 12,000 mJ/cm^2/min) in a lab-based UV cross-linker for 10 min. Gamma (total dose of 56.4 kGy), beta (total dose of 50 kGy) and ethylene oxide (4 h exposure) sterilisation was conducted at Isotron, UK.

The non-porous samples were double swabbed using 5 mm^2 onserts of filter paper (Whatman) following the method of Sweet et al. [8]. The porous material was placed in 1.5 ml micro-centrifuge tubes. Extraction was conducted in sterile conditions to avoid recontamination of the items. DNA was extracted from the samples by the addition of 180 μl of 5% Chelex™ (Sigma) and 6 μl of 10 mg/ml proteinase K (Sigma). After an incubation of 10 min at 56°C, the samples were vortexed and then incubated for 8 min at 100°C. After centrifugation (15,000×g for 3 min), the supernatant was transferred to a Microcon™ spin column (Millipore, USA) and DNA was eluted according to the manufacturer's instructions. Sterile water (50 μl) was added to the DNA solution. The extracted DNA was amplified using the Amp*FL*STR SGM*Plus* kit (with 10 loci plus the sex marker amelogenin) for 28 cycles and profiled using the ABI 3100 Genetic Analyser according to the manufacturer's protocols (Applied Biosystems, Foster City, CA). This part of the research was carried out at LGC Forensics, Teddington, UK.

Further examination of ethylene oxide as a sterilisation technique

Saliva was obtained from 6 different volunteers and was similarly applied to the two surface types at 2, 5, 10 and 20 μl volumes. For 1 volunteer, a volume of 100 μl was also included. All conditions were repeated in triplicate to give a total of 150 samples. Samples were packaged in tamper-evident paper bags. Controls were also included as described above. Samples were sterilised with ethylene oxide at Isotron, UK. The exposure time to the ethylene oxide was 6 h.

Instead of filter onserts, the non-porous surface was double swabbed with taper tip swabs (Medical Wire and Equipment, UK). DNA extraction was conducted in a sterile flow cabinet to avoid re-contamination of sterilised materials. Due to the increase in material within the micro-centrifuge tube, the volume of Chelex™ and proteinase K was doubled to 360 and 12 μl, respectively. The rest of the extraction protocol was used for the comparison of the sterilisation techniques. The DNA extracts were quantified with real time PCR using Quantifiler™ Human DNA Quantification kit (Applied Biosystems, USA) according to the manufacturer's protocols. Subsequent DNA amplification was carried out for 28 cycles as before. DNA profiling was carried out using the ABI 310 Genetic Analyser according to the manufacturer's protocols (Applied Biosystems) at King's College, London.

To examine whether the ethylene oxide technique could be used on laboratory items, two types of swab (Greiner Labortechnik, DE and Eurotubo, IASA, ES) were contaminated with 100 μl saliva from 1 volunteer. The swab label was replaced with one of similar material and size. Three different volumes of saliva (2, 10 and 100 μl) were also added to 1.5 ml micro-centrifuge tubes. All conditions were conducted in triplicate and along with the negative controls sent to Isotron for ethylene oxide treatment as before. Positive controls for comparison were also set up but not sent for treatment. DNA was extracted using QIAGEN spin columns according to the manufacturer's protocol and eluted in 100 μl ddH_2O.

To assess whether the ethylene oxide treatment affects subsequent DNA analysis, unused swabs and 1.5 ml tubes

were also sent for treatment. After sterilisation, 50 μl of saliva was added to 3 tubes or swabbed from a tile using 1 of the treated swabs. The DNA was extracted using QIAGEN spin columns as before. Negative controls (50 μl ddH_2O) were also included.

DNA extracts from swabs and tubes were quantified with real time PCR using Quantifiler™ Human DNA Quantification kit (Applied Biosystems, USA) according to the manufacturer's protocols. DNA extracts were amplified and profiled as before.

Results and discussion

Comparison of four sterilisation techniques

Figure 1 illustrates the proportion of different DNA profile types produced after the different sterilisation techniques. The full and partial profiles obtained matched the profiles from the respective volunteers. No allelic drop-in was observed in the sample profiles, indicating that post treatment contamination had not occurred. It is clear from this study that UV radiation, using the parameters specified here, does not degrade amplifiable DNA sufficiently, as full profiles were obtained from all samples. Profiles were obtained in all samples after gamma radiation, although 30% of these were poor partial profiles, classified as being unloadable on the UK National DNA Database (NDNAD; entry criteria to the database are profiles that consist of allele designations at four or more specified loci). After beta radiation, whilst 3% of the samples produced no profile, the remainder were of sufficient quality for loading onto the NDNAD; and of the 24 samples, 70% were full profiles. Of the 24 samples treated with ethylene oxide, no profiles of sufficient quality for loading onto the NDNAD were obtained and 13% of the samples failed to produce any profile at all.

Figure 1 shows the combined data for all volumes and surfaces. For beta and gamma irradiation, the extent of DNA degradation was more successful with DNA sample volumes of 1 or 2 μl. However, after ethylene oxide treatment the number of peaks present in a profile ranged between 0 and 3 out of 22 for all volumes, which indicates that the effectiveness of ethylene oxide is not volume dependent.

The total DNA profile peak areas obtained after the treatments and for the different volumes of saliva were compared statistically. There was a significant difference between the total DNA profile peak area of the samples that were either irradiated or treated with ethylene oxide and control samples (ANOVA $F=44.2$, $p<0.01$). This indicates that even though UV radiation treatment did not reduce the number of peaks within the DNA profiles, it did reduce the total peak area significantly.

The results obtained for the different sample volumes were significantly different from each other ($F=8.263$, $p<0.01$) but the ANOVA indicated that there was no correlation between the volume of saliva and the sterilisation treatment ($F=1.452$, $p=0.160$). As might be expected, the 1 μl volume samples had significantly lower total DNA profile peak areas than 10 μl for each method of sterilisation.

Further examination of ethylene oxide as a sterilisation technique

After the first results, it was decided to examine the use of ethylene oxide further. More volunteers were included in this study and whilst it should be noted that none of the saliva samples contained on average as much DNA as the first volunteer's saliva sample, they do represent a range of salivary DNA concentrations.

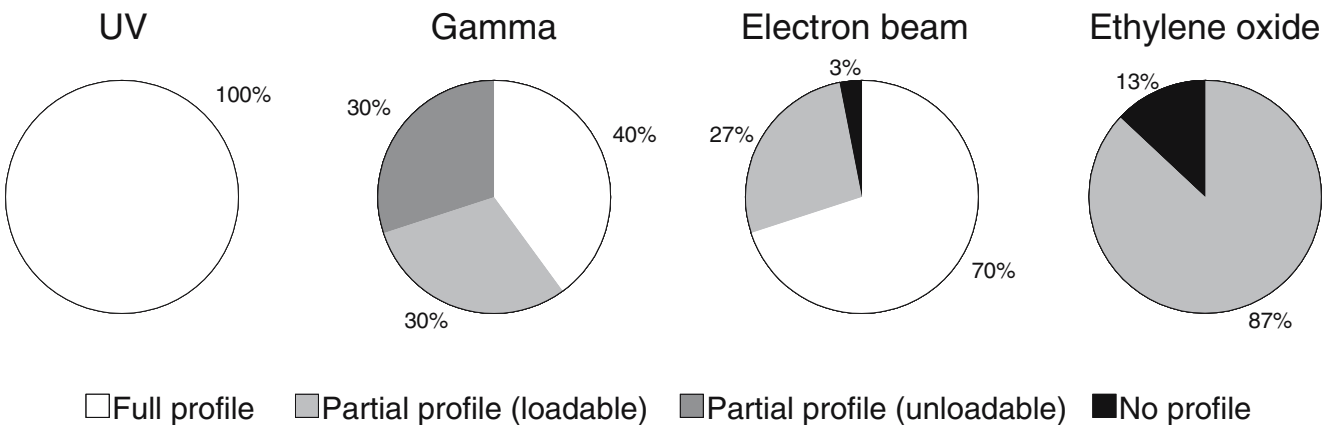

Fig. 1 Proportion of samples for each classification based on the sterilisation treatment applied. Profiles on both porous and non-porous surfaces are included here. Loadable profiles are those that contain a minimum of complete designations at four specified loci and a match probability of less than one in a million

There was no significant difference in DNA concentrations from porous vs non-porous surfaces (F=1.80, p=0.18) after sterilisation, thus data from both surface types were pooled. This indicates that the surface does not affect the sterilisation and recovery of DNA.

Figure 2 illustrates the mean DNA concentrations of ethylene oxide-treated and control saliva samples. Ethylene oxide treatment significantly decreased DNA levels compared to those of the control samples (F=15.84, p<0.01). After ethylene oxide treatment, all samples were found to have DNA concentrations below that of the lowest Quantifiler™ quantification standard (0.023 ng/μl) regardless of the initial volume of saliva. All samples were below the optimal DNA concentration required for effective profiling (0.05–0.1 ng/μl).

Of the total ethylene oxide-treated samples, 23% were not detectable by Quantifiler™ and thus, not considered further as they were assumed to not contain enough DNA to render a DNA profile. Of the samples that were profiled, 49% provided no profile and 51% provided unloadable partial profiles with a maximum number of 4 out of 22 alleles noted in a profile. Half of the unloadable partial profiles contained the sex marker only and provided no extra discriminatory information. The remaining unloadable partial profiles showed only the smaller molecular weight loci; most were found to be below 200 base pairs in length. This indicates that ethylene oxide is degrading the contaminating DNA so that only the shorter loci can be amplified.

The average decrease in the amount of DNA on both types of cotton swabs was 99.98% after ethylene oxide sterilisation when compared with the average positive control values (Table 1). Table 1 also indicates that after ethylene oxide sterilisation, the amount of amplifiable DNA present was between 0% and 1.39% of the respective positive control for the contaminated micro-centrifuge tubes. It should be noted that the concentration of DNA extracted from 100 μl of contaminating saliva on one of the swabs and tubes is at the lower threshold for successful SGMplus profiling (0.05 ng/μl). This indicates a possible upper threshold of contamination that ethylene oxide sterilisation can remove. However, this contaminating volume is considerably higher than an airborne contaminant and would be stopped by the appropriate protective equipment.

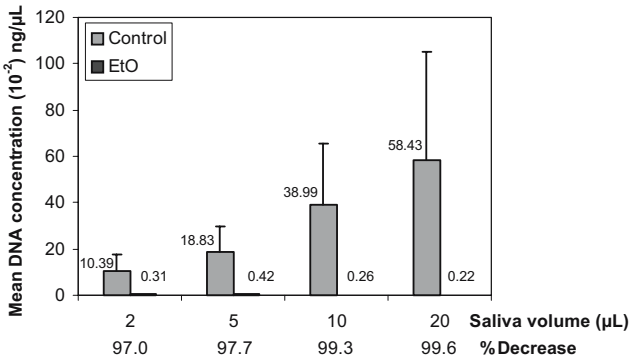

Fig. 2 Mean DNA concentration (10^{-2}) in ng/μl extracted from the untreated control and ethylene oxide-treated saliva samples of varying volumes. The percentage decrease in DNA concentration after ethylene oxide treatment compared to the untreated control is shown *below* the graph. *Error bars* indicate standard deviation

Table 1 The average amount of DNA (ng) extracted from two types of swabs and 1.5 ml micro-centrifuge tubes contaminated with saliva with and without ethylene oxide sterilisation

Material	Average amount of DNA (ng)		
	Control	Ethylene oxide sterilisation	% Difference
Greiner swab+100 μl saliva	247.00	0.05	0.02
Eurotubo swab+100 μl saliva	139.00	0.02	0.02
1.5 ml tube+2 μl saliva	0.31	0.00	0.00
1.5 ml tube+10 μl saliva	1.44	0.02	1.39
1.5 ml tube+100 μl saliva	29.87	0.05	0.17

There was no significant difference in the quantity of DNA extracted between uncontaminated ethylene oxide sterilised swabs and tubes and those that hadn't been sterilised (ANOVA; swabs F=0.119, p=0.747; tubes F=3.580, p=0.131). All swab and tube extracts produced full DNA profiles.

Conclusion

This study demonstrated that not all conventional irradiation techniques result in the complete removal of amplifiable DNA. The advantage of UV radiation is that it can be conducted within the laboratory. However, after UV radiation treatment, using the parameters specified in this study, full profiles were produced from all samples, indicating the limitations of the technique for sterilisation. However, Tamariz et al. [9] have recently published certain parameters that can improve UV sterilisation including distance of items to the UV bulb, increased exposure time and the use of aluminium foil.

Beta and gamma, under the conditions of this study, have the ability to reduce the number of full profiles but not consistently.

This study indicates that ethylene oxide is capable of reducing the DNA contamination so that loadable DNA profiles are not possible after sterilisation. This reduction in contaminating DNA was also evident with sealed cotton swabs and closed micro-centrifuge tubes, indicating that forensic consumables could be sterilised using this method. Ethylene oxide gas treatment is not corrosive to metal and

thus, can be also used to clean plastic and metal laboratory equipment. Preliminary tests conducted in this study show that ethylene oxide does not affect any downstream DNA analysis.

The disadvantage of using ethylene oxide is that items must be sent away for treatment over several days. This means that large fixed items such as laboratory bench surfaces cannot be sterilised in this manner. However, this study showed that ethylene oxide appears to be the best sterilisation method, of those tested, for smaller items. Therefore, it is recommended that ethylene oxide be utilised to significantly reduce the amount of amplifiable DNA on items for forensic use. The authors intend to work further with Isotron to examine the different cycling parameters of the ethylene oxide sterilisation treatment further, to indicate the optimal conditions for DNA decontamination and validate this process.

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