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Reduction of PCR-amplifiable DNA by ethylene oxide treatment of forensic consumables

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ABSTRACT

A reliable method to provide molecular biology products free of contaminating DNA is of forensic interest. Ethylene oxide (EO) treatment has been demonstrated as an effective method in published studies. This study aimed to address some additional experiments that are closer to forensic practice. In the first part of this study, different consumables such as cotton swabs, latex gloves and micro test tubes were spiked with saliva, blood and skin cells to mimic a real-life contamination scenario. EO treatment was performed for a period of 3, 5, 7, and 10 h, respectively. For comparison, gamma and electron beam treatment was applied. In the second part of this study, a cell culture line (K562) was used to apply defined cell counts on cotton swabs followed by EO treatment for 3 and 5 h. After extraction of samples, the DNA content was quantified using a real-time PCR based system. STR analysis was performed using a latest generation STR kit to meet current sensitivity limits. A good correlation of real-time PCR results and STR results was observed. This work confirmed the findings of earlier studies showing that chemical EO treatment is much more successful in reducing the amount of PCR-amplifiable DNA than ionising radiation. Furthermore, the efficacy of EO treatment is affected by the nature of the samples. DNA in saliva was more susceptible to damage by EO gas than DNA in blood. Our results show, that accessibility of the sample to EO gas has a strong influence on the method's efficiency. While treatment of samples on cotton swabs packed into gas-permeable bags was very successful, samples inside a closed micro test tube were resistant to the same treatment conditions. Our work with defined K562 cell numbers and multi-copy quantitative PCR could show that a 5 h EO treatment results in a 10^5 fold reduction of PCR-amplifiable DNA. Corresponding STR-PCR results also show only sporadic allele calls in the Mini-loci range, providing a reliable interpretation of forensic analysis. Finally, we do recommend an EO treatment of forensic consumables and a multi-copy quantitative PCR approach to establish reliable treatment conditions.

1. Introduction

In recent years the presence of production-related contamination has alerted the forensic community. As early as 2004, the Forensic Science Service (FSS) communicated a substantial amount of contamination found in forensic consumables [1]. Since that time, an increase in case numbers as well as the increased sensitivity of a new generation of short-tandem-repeat (STR) kits and capillary electrophoresis instruments have contributed to a stronger focus on the issue of contamination.

DNA contamination can occur during fabrication of forensic consumables, at the crime scene while collecting evidence, and in the laboratories during sample preparation for forensic analysis. Contamination, regardless to the time of deposition on the evidence item, can lead to profound bias ultimately invalidating the results derived from the forensic process.

Methods like irradiation of consumables by ultraviolet light, gamma or electron beam or chemical treatment like ethylene oxide

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(EO) exposure are successfully used for sterilisation [2–4]. EO treatment has been tested repeatedly and was confirmed as suitable for treatment of forensic consumables to reduce contaminating DNA [2,3]. Shaw et al. [3] compared the aforementioned methods and reported that EO treatment appears to be the best method without affecting any downstream DNA analysis. Archer et al. [2] investigated the same methods and verified that EO treatment reduces detectable DNA levels more effectively.

Ethylene oxide (C_2H_4O) is a highly reactive cyclic ether and a colourless gas at room temperature. EO is so far routinely used to manufacture a large variety of sterile medical products. The mechanism of bactericidal action is alkylation of the amine groups of DNA causing mutations and strand breaks [4–6]. It has been shown that EO has a direct effect on DNA in vitro and in vivo and therefore a damaging of DNA to an extent that prevents amplification in a PCR reaction is possible [5,6]. The degree of microbial death and DNA damaging is a function of time of exposure to EO for a given EO concentration, humidity and temperature. EO treatment is a method especially suitable for terminal sterilisation, meaning the treatment is applied to a finished product placed into a sealed gas-permeable packaging that allows ingress and egress of EO and humidity but is microbially resistant [4].

EO treatment is carried out in a chamber in which the product is exposed to a combination of humidity, ethylene oxide gas, temperature and time. Vacuum cycles are used to drive humidity and ethylene oxide into boxed product [4]. Depending on the product and the sterilisation requirements, total cycle times can vary from some hours to several days [4]. The sterilisation process must be validated for every class of product [4].

Gamma and electron beam irradiation are terminal sterilisation methods widely used in the food and medical industry [7,8]. Gamma irradiation uses radiation generated from radioactive decay of a cobalt-60 source and the emitted gamma photons have a strong penetration capacity, allowing for batch sterilisation [4,9]. Electron beam treatment uses high-energy electrons from accelerators and products have to pass the electron beam for efficient sterilisation [4,11]. The radiation from both methods can either have a direct damaging effect on DNA or produce intracellular radicals that lead to death of bacteria [4,9,11].

It is crucial to remember that neither of these terminal treatments can remove contaminating DNA, only a damage of the possibly present DNA can be achieved. The extent of damage afflicted to the DNA might be sufficient to prevent a successful amplification of the DNA in a subsequent PCR reaction.

A high qualitative standard for manufactured items with minimised contamination is a prerequisite for a reliable forensic analysis. This qualitative standard must be cost effective and easily applicable. The aim of this study was to develop such a forensic/qualitative standard for molecular biology products.

The first part of this study focused on real-life contamination scenarios to test the limits of different EO treatment protocols. As contaminants we used body fluids due to their risk of being introduced by human interaction during manufacturing, packaging or sample collection and applied them to laboratory items with direct contact to evidence material.

In detail we addressed the contaminants saliva and blood in small amounts (2 μl) as well as skin cells and pure DNA. We applied them to carrier materials such as cotton swabs, latex gloves and (closed) micro test tubes. In the second part of this study we concentrated on quantification of amplifiable DNA after EO treatment. We used cell cultured cells (cell line K562) and applied defined numbers of cells onto cotton swabs. Additionally, cotton swabs were contaminated with different amounts of saliva. This part of the study was restricted to cotton swabs, which represent the major source for contamination known so far and were the most suitable carrier material for cell suspensions.

2. Materials and methods

2.1. Preparation of body fluid samples on different materials

In the first study 2 μl of saliva, 2 μl of blood and 5 μl of pure DNA (AmpFlSTR Control-DNA 007, 0.1 ng/μl) was deposited on latex gloves (Kimberly-Clark, Powder-Free Latex Exam Gloves, non-sterile), cotton swabs (wooden shafted, EO-pretreated, Heinz Herenz) and micro test tubes (Eppendorf, 1.5 ml safe-lock micro test tubes, PCR clean) to mimic contamination. Latex gloves were spiked on the inside of the glove and allowed to dry prior to treatment. The latex gloves were then reversed, tied, and placed in the middle of a glove box containing 100 gloves, or were sealed into gas-permeable, transparent bags (for subsequent gamma and electron-beam treatment only). Cotton swabs were spiked, allowed to dry and then sealed in gas-permeable bags. Micro test tubes were spiked on the inside bottom of the tube. The micro test tubes were closed after drying and then sealed into gas-permeable bags.

Contamination by skin cells was simulated by rubbing an area of both inner forearms of 2 cm^2 with a cotton swab. A person known to be a good shedder was chosen as a donor. Skin cells were applied only to cotton swabs and were sealed in gas-permeable bags.

The first study contained seven batches of samples. One batch remained untreated and was used as control. Four batches, each arranged similarly in one cardboard box, were prepared for ethylene oxide treatment according to the selected incubation time tables, one batch for gamma radiation and one for electron-beam treatment, whereupon the treatment proceeded directly in the gas-permeable, transparent bags. Each batch contained 4 replicates of every spiked consumable.

2.2. Preparation of samples with defined cell numbers

The second study was performed using culture cells K562 (DSMZ, Braunschweig, Germany). K562 is a continuously growing erythromyeloid human cell line which was cultured in a humidified incubator at 37 °C and 5% CO_2 in RPMI1640 medium (Biochrom, Berlin, Germany) supplemented with 10% FCS (PAA, Pasching, Austria), 100 U/ml penicillin, 100 μg/ml streptomycin (Biochrom), 2 mM L-glutamine (Biochrom), and 1 mM sodium pyruvate (Biochrom). Cells were washed and resuspended in saline (Braun, Melsungen, Germany) at a concentration of 1×10^6 cells/ml. Cell number was determined by light microscopy. Cells viability was 100% as assessed by trypan blue (Sigma Aldrich, Munich, Germany) exclusion. The cells were further diluted with saline to the following concentrations: 6×10^5/ml, 4×10^5/ml, 3×10^5/ml, 2×10^5/ml, 1×10^5/ml, 6×10^4/ml, 4×10^4/ml, 2×10^4/ml, 1×10^4/ml, 2×10^3/ml. 50 μl of each dilution of the cell suspensions was pipetted into seven wells of a 96 well round bottomed plate (Nunc, Roskilde, Denmark). The suspension in each well was absorbed with a fresh cotton swab (wooden shafted, EO-pretreated, Heinz Herenz), which was inserted in an upright position into the well. The cotton swab was twisted to and fro ten times between two fingers to ensure complete transfer of the samples, resulting in cell numbers from 50,000 to 100 cells on the swabs. Finally, the swab was placed vertically into a perforated plastic plate for 24 h at room temperature for drying. For the typing control 1×10^5 cells were resuspended in 50 μl saline and absorbed with a swab as described above. In addition, cotton swabs were contaminated with different amounts of saliva (1, 2, 4, 8 and 16 μl). After the spiking procedure all samples were sealed in gas-permeable, transparent bags. A set of all 10 specific cell concentrations and five different saliva volumes was prepared in triplicate for each exposure time tested. The sealed samples were packed in cardboard boxes and underwent EO treatment for 3 and 5 h respectively.