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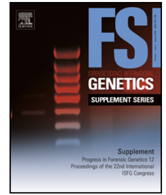

Potential degrading effect of sodium hypochlorite on exhibits containing DNA

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ABSTRACT

The use of a sodium hypochlorite solution as a cleaning reagent is common practice among many laboratories for contamination minimisation purposes. Whilst its effectiveness in the decontamination of tools and surfaces has been verified at specific concentrations, it has not yet been established whether any residual sodium hypochlorite potentially remaining on tools/surfaces following cleaning has a detrimental effect if direct contact is made with an exhibit containing DNA. To investigate the effect of residual hypochlorite, surfaces were treated with 10% hypochlorite (air-dried or wiped dry), 1% hypochlorite (air-dried or wiped dry), or 1% hypochlorite (wiped dry) followed by the application of water (wiped dry). Treated surfaces came into contact with surfaces carrying 200 ng of DNA within 100 μL, or 20 ng within 20 μL. To observe the potential degrading effects of sodium hypochlorite, the quantity and quality of DNA within DNA deposits following contact with treated and untreated surfaces were compared. Overall, no degrading effect on DNA quantity/quality was observed, with the exception of DNA deposits that came into contact with surfaces treated with 10% hypochlorite and air-dried. It is therefore recommended that surfaces cleaned with high concentrations of hypochlorite be wiped dry or rinsed with an appropriate agent (water) following application.

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1. Introduction

The presence of background DNA on various surfaces, tools and equipment within forensic laboratories has been documented and the risk of contamination occurring as a result of contact and subsequent transfer is recognised [1–3]. Research has also indicated that contamination events may take place as a result of practitioners failing to adhere to cleaning protocols [4], defective and/or redundant decontamination procedures [2,5,6], or through the presence of DNA on manufactured laboratory supplies, including unused laboratory gloves [4,7].

Considerable effort goes into minimising the risk of contamination, with the wearing of protective clothing and application of validated cleaning procedures forming the initial preventative measures. Protocols regarding such measures vary among laboratories, however one of the most commonly used reagents for the removal of DNA is sodium hypochlorite (NaClO or bleach). Whilst its effectiveness in the decontamination of surfaces has been verified at specific concentrations [3,6,8], to our knowledge it has not yet been established whether any residual sodium hypochlorite potentially remaining on tools and surfaces following cleaning has a detrimental effect if direct contact is made with an exhibit containing DNA. Here we investigate the detrimental effects of various commonly applied sodium hypochlorite treatments on deposits of DNA.

2. Materials and methods

2.1. DNA deposits contacting decontaminated glass plates

DNA-free glass plates (220 mm × 140 mm) were treated with 10% hypochlorite air-dried for 30 min ($n = 6$); 10% hypochlorite wiped dry ($n = 3$); 1% hypochlorite air-dried for 30 min ($n = 6$); or 1% hypochlorite (wiped dry) followed by the application of distilled (DI) water (wiped dry), as per our current laboratory procedure ($n = 6$). Additional glass plates remained untreated as controls for comparative purposes ($n = 4$). To simulate contact with an exhibit carrying DNA, moist swab tips (150C swabs, Copan) containing 200 ng of DNA (2 ng/μL, 2800 M, Promega), were swabbed across the entire surface of treated and untreated glass plates. To collect

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Table 1
Mean concentration, detectable alleles and average peak height (s.d.) obtained from DNA deposits following contact with treated surfaces.

Treated surface	Treatment	Replicates (n)	Concentration (ng/μL)	Alleles	Average peak height (RFU)
Glass plates	10% Hypochlorite (air-dried)	6	0.00 (0.00)	14 (21)[b]	409 (657)[b]
	10% Hypochlorite (wiped dry)[a]	3	0.92 (0.27)	42 (0)[c]	5969 (276)[c]
	1% Hypochlorite (air-dried)	6	1.81 (0.31)	42 (0)[c]	6149 (297)[c]
	1% Hypochlorite (water/wiped dry)	6	1.48 (0.33)	42 (0)[c]	5920 (295)[c]
	Untreated—controls	4	1.48 (0.19)	42 (0)[c]	4188 (882)[c]
Gloves	10% Hypochlorite (air-dried)	4	0.00 (0.00)	0 (0)	0 (0)
	10% Hypochlorite (wiped dry)	4	0.21 (0.03)	42 (0)[c]	4096 (691)[c]
	1% Hypochlorite (wiped dry)	4	0.15 (0.02)	42 (0)[c]	4453 (777)[c]
	Untreated—controls	4	0.20 (0.04)	42 (0)[c]	5084 (1482)[c]

[a] Data for this treatment group was excluded from statistical analysis due to the low number of replicates performed.
[b] Four of the six replicates produced no detectable alleles.
[c] Two replicates with the lowest concentration were amplified/profiled and are represented here.

DNA lost to the glass plate during swabbing, a second moist swab was applied and processed with the first.

2.2. Decontaminated gloves contacting DNA deposits

Mock exhibits comprised of designated 1.5×1.5 cm areas on DNA-free glass plates deposited with 20 ng of DNA (1 ng/μL, 2800 M, Promega) and dried at room temperature for 12 h prior to experimentation. Two nitrile gloves (InControl) were worn on each hand with various treatments applied to the outer surface including 10% hypochlorite air-dried for 5 min ($n = 4$), 10% hypochlorite wiped dry ($n = 4$), or 1% hypochlorite wiped dry ($n = 4$). Additional gloves remained untreated as controls for comparative purposes ($n = 4$). Pressure contact without friction was applied to the deposit with the index, middle and ring fingers of treated or untreated gloves for 5 s, with the outer pair of gloves replaced after each replicate. The sample was collected from the deposit area using wet/dry swabbing.

2.3. Treatment application

Hypochlorite (Rowe Scientific) and DI water were applied via spray bottle with 3 sprays per treatment application. Treatments were applied directly to the surface of glass plates. To treat gloves, hypochlorite was sprayed on the left gloved-hand and transferred to the right gloved-hand through wiping/clasping motions. Kimwipes™ were used as the wiping material.

Glass plates were cleaned with 1% hypochlorite (wiped dry) followed by multiple applications of water (wiped dry), prior to treatment application or DNA deposition. Negative control swabs taken from these plates displayed no detectable DNA ($n = 8$). Gloves were assumed clean prior to use, with four negative control swabs displaying no detectable DNA.

2.4. Sample processing and data analysis

Swab tips were removed within 1 h of sampling and stored for 5 days at $-20\,^{\circ}$C before DNA was extracted using the DNA IQ™ system (Promega) to a final volume of 50 μL and quantified with Quantifiler® (Life Technologies). Within each treatment group the two replicates yielding the lowest DNA concentration, or samples with no measurable DNA concentration, were amplified with PowerPlex® 21 (Promega, USA) and products were analysed on the 3500xl Genetic Analyser (Applied Biosystems). 0.5 ng or 15 μL (when $<$0.033 ng/μL) of template DNA was used during amplification. Genotyping was performed in GeneMapper® ID-X (Applied Biosystems) with a baseline threshold of 175 RFU. One-way ANOVA was performed in SPSS (v20.0, IBM).

3. Results and discussion

The swabbing of untreated glass plates with moist swabs containing DNA (controls) resulted in samples with an average concentration of 1.48 ng/μL and full profiles with peak heights averaging 4188 RFU (Table 1). Compared to controls, there was significant ($p < 0.001$) degradation in samples produced from swabs of glass plates treated with 10% hypochlorite and air-dried, with replicates displaying no measurable DNA concentration. While not all DNA was destroyed, with two replicates producing full profiles, substantial degradation is evident with the average peak height of these two samples decreasing to 1228 RFU; the remaining four replicates produced no detectable alleles. 10% hypochlorite appeared to be less degrading when excess had been removed with Kimwipes™, with full profiles obtained and an average peak height comparable to control samples. No degrading effect on DNA quantity/quality was observed with treatments of 1% hypochlorite.

Further investigations eliminating the moisture content of the deposit as a possible contributing factor to the degradation observed, by reactivating the dried/crystallised sodium hypochlorite, produced results that are consistent with initial findings; dried DNA deposits contacted by gloves treated with 10% hypochlorite and air-dried, indicated significant degradation ($p < 0.001$) with samples yielding no detectable DNA concentration; removal of excess hypochlorite (10%) decreased degradation; and no degrading effect was observed with treatments of 1% hypochlorite ($p > 0.05$).

It is recommended that surfaces decontaminated with high concentrations of sodium hypochlorite, which may come into contact with exhibits containing DNA, be wiped dry or rinsed with an appropriate agent following treatment application.

Conflict of interest

None.

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