Optimization of Direct Amplification Methods for DNA Samples from Common Pipe Bomb Substrates Using the GlobalFiler® Kit

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INTRODUCTION

Improvised explosive devices (IEDs) such as pipe bombs are often used to cause fear and devastation within communities. Several methods can be used to identify the manufacturers or those who have handled pipe bombs and other explosive devices, including fingerprint, toolmark, explosive residue, and DNA analyses [1].

When attempting to analyze DNA from recovered post-blast fragments, heat degradation, PCR inhibitors, and minute DNA quantities can make DNA typing extremely difficult. DNA degradation and inhibition can result in partial profiles, and amplifying minute quantities of DNA can cause stochastic effects. Effects such as peak height imbalance, allele and/or locus dropout, and failed amplification can render a profile uninterpretable and result in lost investigative leads [2]. Therefore, the efficiency of the method used for the initial collection of DNA from challenging items of evidence is important in order to maximize the amount of DNA available for downstream analysis.

The aim of this study was to optimize the recovery of mock touch DNA from common pipe bomb substrates by exploring two swab types (cotton and microFLOQ® direct) and alternate direct amplification methods.

SAMPLE PREP & COLLECTION

- An epithelial (buccal) cell suspension was created from a single male donor and diluted to approximately 6 cells/µL or 40 pg/µL
- Ten 5 µL replicate aliquots of cell suspension (≈ 200 pg) were placed onto each pipe bomb substrate: PVC pipe, galvanized steel pipe, electrical tape, copper wires
- Dried cell spots were swabbed with either cotton swabs (Puritan) with 2% SDS or microFLOQ® direct swabs (Copan Italia) with dH_2O for a approximately 30 s

RESULTS & DISCUSSION

CONTROLS

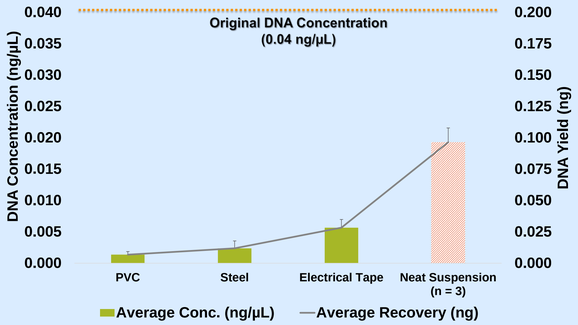

Figure 1. Average DNA concentration for extracted cotton controls (N = 10 per substrate) and neat cell suspension extracts (N = 3). Error bars represent standard deviation.

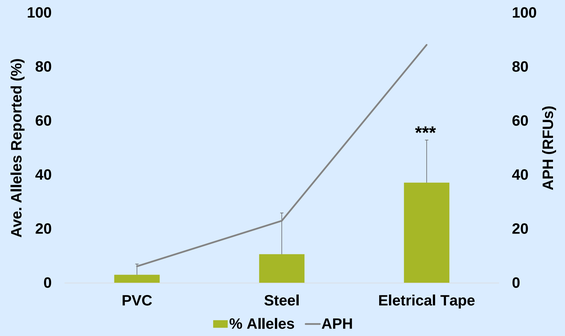

Figure 2. Average percent of alleles reported and average peak height for control extracts (N = 10) per substrate. Error bars represent standard deviation. Statistical significance determined with Welch t-tests.

ALTERNATE METHODS

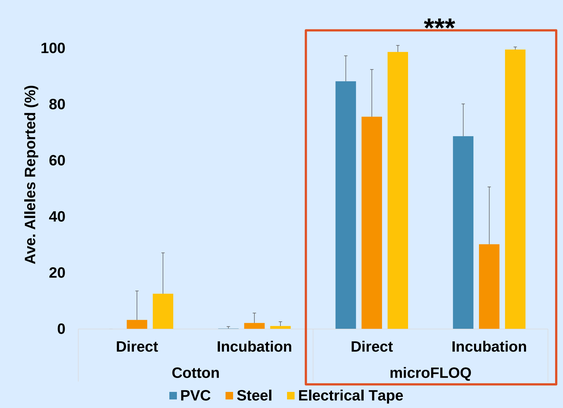

Figure 3. Comparison of direct amplification and incubation method. Error bars represent standard deviation. Significance determined by three-factor ANOVA.

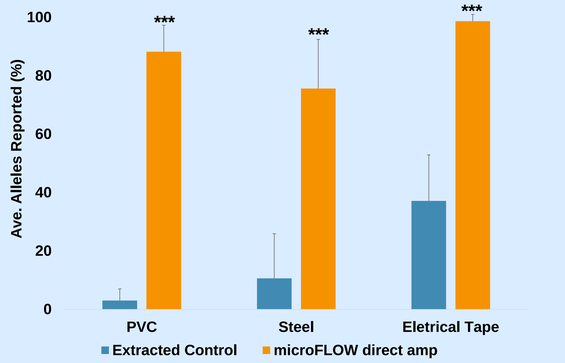

Figure 4. Average percent of alleles reported for extracted controls and microFLOQ® direct swabs with direct amplification. Error bars represent standard deviation. Significance determined by Welch t-test.

SAMPLE PROCESSING

Control (cotton only):
Swab extracted with PrepFiler *Express™* on Automate *Express™*

DNA Quantification:
QuantiFiler® Trio

STRs:
GlobalFiler®
(29 cycles)

Method 1:
Direct PCR

STRs:
GlobalFiler®
(29 cycles)

Method 2:
Elution/Pre-treatment

Incubation:
- **Cotton**: 400 µL TE 90 ˚C with 750 rpm shaking
- **microFLOQ**: 40 µL TE at RT (microFLOQ®) for 20 min

STRs:
GlobalFiler® (29 cycles)
15 µL of lysate into PCR

Separation and detection was performed on a 3500 Genetic Analyzer and analyzed using GeneMapper® ID-X and in-house excel sheets

Controls:
- More than half of the DNA was lost using traditional collection and automated extraction regardless of the substrate (Fig. 1)
- Electrical tape resulted in the highest percentage of reportable alleles compared to other substrates when using traditional collection and extraction methods ($p < 0.0001$) (Fig. 2)

Alternate Methods:
- microFLOQ® direct swabs resulted in more reported alleles on average compared to cotton swabs ($p < 0.0001$) (Fig. 3)
- Electrical tape resulted in the most reported alleles compared to other substrates (Fig 3)
- No significant difference in reported alleles between direct and incubation methods for microFLOQ® collection from electrical tape. However, the average peak height was significantly lower ($p < 0.001$) for the incubation method (data not shown)
- Direct amplification using microFLOQ® direct swabs was more successful than traditional methods for all substrates tested (Fig. 4)

Overall:
- Swabs taken from the copper wire samples were inhibited and failed to amplify
- Direct amplification using microFLOQ® swabs resulted in higher STR success with fewer processing steps and was particularly success with electrical tape

ACKNOWLEDGEMENTS

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[2] A. Berti, F. Barni, A. Virgili, C. Colozza, F. Maiorino, M. Tocca, The recovery of DNA profiles from saliva and touch evidences after postal bomb explosion, Forensic Sci. Int. Genet. Suppl. Ser. 3 (1) (2011) e471–e472.