J Forensic Sci, May 2004, Vol. 49, No. 3
Paper ID JFS2003127
Available online at: www.astm.org

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Using STR Analysis to Detect Human DNA from Exploded Pipe Bomb Devices*

ABSTRACT: This study investigated the possibility of recovering a bomb assembler's DNA from an exploded pipe bomb device. Metal and polyvinyl chloride (PVC) pipes were examined to determine if one surface type would allow better DNA recovery than the other. Ten subjects each handled components of one metal and one PVC pipe bomb. The bombs were exploded, the fragments were collected and swabbed using the double swab technique, and the samples were extracted, quantified, amplified, and genotyped using polymerase chain reaction/short tandem repeat (PCR/STR). Of the 20 bombs handled by the subjects, four bombs gave reportable results that matched the subject's known DNA profiles. An additional eight profiles, also matching the subject's known DNA profiles, were generated but were below the reportable threshold. There was no difference in the success rate of obtaining DNA profiles related to the use of either PVC or metal in the manufacture of the pipe bomb. The variables that appeared to have the greatest influence on the success of generating a DNA profile were the amount of fragmentation and subsequent recovery of the bomb fragments. It is suspected that successful DNA profiling could also be dependent upon the bomb assembler's propensity to slough skin cells on objects they handled.

KEYWORDS: forensic science, STR, DNA, pipe bombs, explosives

Terrorism is a very real worldwide problem today. Recent events have taught us that terrorists can strike anywhere at anytime. Explosives are used in more than 70% of terrorist attacks, and although large-scale attacks usually involve more sophisticated incendiary devices, pipe bombs account for 31% of all improvised explosive devices (1).

A pipe bomb is a fairly simple device. It is literally a length of pipe that is capped on both ends and filled with an explosive (2). They come in many shapes and sizes, and can be composed of metal, plastic, or some related material. The inside can be filled with a low explosive (like smokeless powder) or a high explosive such as dynamite. The major difference between the two types lies in the speed of the explosion. High explosives detonate. A detonation causes the solid material to instantaneously change to a gas accompanied by high heat and a pressure shock wave (1). Detonation temperatures with high explosives can reach 5500 K (approximately 5227°C) (3). Low explosives, like smokeless powder, deflagrate (1). Deflagration is a rapid burn that occurs slower than the speed of sound where the solid material changes to a gas relatively slowly. A database on gunpowder revealed that the explosion temperature for confined smokeless powder reaches only between 2500 and 3000°C (4).

Statistically speaking, the most commonly encountered explosive device in the United States is the pipe bomb (5). In the 1999 Columbine High School shooting, several unexploded pipe bombs were discovered. Pipe bombs, or similar explosives, were also utilized in the Olympic Park bombing in Georgia in 1996, the bombing of IRS buildings by Dean Hicks in 1991, and the 17-year reign of terror by the Unibomber (1,2,5,6). Unlike shootings, those who set off bombs are often not present when the device explodes, thus making it difficult to associate the person with the crime (7). This study was designed to examine the feasibility of obtaining sufficient human DNA from exploded bomb fragments to yield interpretable DNA profiles. Such profiles would be valuable assets in identifying those individuals who may have handled the bomb prior to deflagration.

In their 1997 article, Van Oorschot and Jones first demonstrated that one's genetic profile could be obtained from swabs taken from a person's hand, or from objects a person touched (8). Numerous researchers have since explored a host of variables that may affect the deposition, recovery, and subsequent DNA profiling of sloughed skin cells on objects (9–11). In this study, subject-handled metal and PVC pipe bombs were subjected to low explosions (using smokeless powder) to determine what effect, if any, an explosion has on the successful recovery of human DNA.

Materials and Methods

Decontamination and Sample Collection

Twenty-seven 1-in.-diameter (2.54 cm) pipe bombs were assembled for the present study (13 were made with PVC, and 14 with galvanized steel). All the pipes and caps were purchased at a local hardware store, decontaminated with a 10% bleach solution, and exposed to ultraviolet (UV) light to remove any preexisting DNA prior to being assembled. Each of ten Caucasian volunteers was then given the decontaminated components for one metal and one PVC bomb and asked to handle the pipe, caps, and fuse for approximately ten seconds each. These components would later be

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* Thesis project supported by Michigan State University and the Michigan State Police Crime Laboratory, Northville, MI, and was presented as a poster at the Annual American Academy of Forensic Sciences meeting in Chicago, IL, 20 February 2003; also presented orally at the Annual Midwestern Association of Forensic Scientists meeting in Milwaukee, WI, 20 September 2002.
Received 8 April 2003; and in revised form 18 Aug. 2003, 10 Jan. 2004; accepted 10 Jan. 2004; published 7 April 2004.

transferred to the Michigan State Police Bomb Squad, who assembled these bombs and added gunpowder to them. Latex gloves were worn by the staff transferring the bomb components and by officers assembling the components.

The components for the remaining three PVC bombs and three of the four remaining steel bombs were not handled by the volunteers, but served as negative controls to ensure the decontamination procedure was working properly, and to ensure contamination was not occurring during collection or DNA processing. The remaining galvanized steel pipe bomb was handled by a randomly selected volunteer and was assembled as described above, but was not exploded. This bomb served as a positive control to ensure skin cells were being deposited as the subjects handled the bombs.

Bomb Assembly and Deflagration

For safety reasons, the assembled bombs were transported to the demolition site (a rock quarry) near Ann Arbor, Michigan, prior to adding gunpowder. At the quarry, two Michigan State Police bomb squad members wearing latex gloves added gunpowder to the assembled bombs one bomb pair at a time. The smokeless powder (IMR powder SR 4756 by Dupont) was funneled in and the final cap was secured. Two small holes were dug at the blast site, each approximately 6–12 in. (~15–30 cm) deep and 6 in. in diameter. The metal and PVC bombs were placed in separate holes with the fuse end up. A large, heavy rock was placed on top of each hole to keep the fragments contained within each hole. This was done for two reasons: first, to make the fragments easier to find, and second, to ensure that cross contamination of pieces from different bombs would not occur. In some instances, fragments did escape the holes but were not collected for analysis to avoid possible cross contamination between bombs. Once deflagrated, the fragments from each hole were collected by two investigators wearing gloves. The fragments were placed in separate paper bags and the bags were sealed. The above procedure was performed a total of 13 times, once for each bomb pair.

Laboratory Sample Collection

In order to document the relative degree of fragmentation and the variation in amount of fragments recovered, each bomb was photographed at the lab prior to swabbing. The fragments were swabbed using the double swab technique as described by Sweet (12). Two swabs were used per bomb, and both were cut up and placed in the same extraction tube. Since the fragments were coated in soil from being buried in the ground, this technique was repeated over as many fragments as possible until the amount of soil became so great that the swabs were saturated.

Prior to exploding the pipe bombs, the components from all three pairs of negative control bombs were swabbed (also using the double swab technique) to ensure the decontamination procedure worked properly. Likewise, a positive control was required to demonstrate that skin cells were being transferred to the pipe bombs when handled by the subjects. This bomb was swabbed after handling (and not exploded) using the double swab technique.

DNA Extraction and Quantitation

Once dry, the tips of the swabs were removed, cut up, and placed in extraction tubes and incubated at 56°C for 3 h in 600 μL of stain extraction buffer (Tris/EDTA/NaCl/SDS) and 30 μL Proteinase K. The swabs were transferred to a spin basket and centrifuged at 10,000 rpm for 5 min. An organic extraction was performed as described by Budowle (13). DNA was concentrated using Amicon Centricon®-100 concentrators (Amicon/Millipore Corp., Bedford, MA). Each extract was recovered in 80–140 μL of TE^{-4}.

Using 4 μL of each extract, the total amount of DNA was quantified by a yield gel. Human DNA was quantified by a QuantiBlot Kit (PE/Applied Biosystems, Foster City, CA) using 5 μL of each extract in 150 μL of spotting solution (0.4N NaOH, 25 mM EDTA, 0.00008% Bromothymol Blue).

DNA Amplification

The D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, and D7S820 loci were amplified using the PE Applied Biosystems AmpF*l*STR® Profiler Plus™ PCR Amplification Kit (PE/Applied Biosystems, Foster City, CA). A 25 μL reaction volume was used consisting of 15 μL master mix and 10 μL input DNA. The master mix "stock" was made according to Perkin-Elmer's recommendations: 10.5 μL reaction mix, 5.5 μL of fluorescently tagged primer sets and 0.5 μL Taq per sample, of which 15 μL was used in the reaction volume. Thermal cycling was performed using the PE Applied Biosystems GeneAmp™ 2400 PCR Instrument System (Foster City, CA) with the following parameters: 95°C for 11 min; 94°C for 1 min, 59°C for 1 min, 72°C 1 min (28 cycles); 60°C for 45 min; 25°C on hold until removed.

Initially, 10 μL of unconcentrated extract was removed from each sample (from the total volume of 80–140 μL) and amplified. Subsequently, the remaining extract was concentrated using Microcons® according to manufacturer directions. Each sample extract was reduced from 80–140 μL down to a volume of 10 μL. All 10 μL of this concentrated extract were then amplified for each sample.

Capillary Electrophoresis

Aliquots (3 μL) from each of the amplified samples were combined with 25 μL of master mix (24 μL deionized formamide, 1 μL GeneScan ROX-500 internal size standard) prior to capillary electrophoresis with the ABI Prism 310 Genetic Analyzer™. After denaturation of samples at 95°C for 3 min, they were snap cooled in a bench top cooler (from −20°C freezer) for 3 min. The samples were run using the following parameters: GS STR POP4F, 5-sec injection, 15.0 kV, 60.0°C, 24 min (run time per sample). After the matrix was applied, allele sizes were estimated using the local Southern method from GeneScan™ 2.0.2 and Genotyper™ 2.1 Analysis software (PE Applied Biosystems, Foster City, CA).

The authors provide a modified list of the guidelines used in their laboratory for STR analysis. Only those guidelines relevant to these samples are listed below (14,15):

1. Alleles of a genetic profile with an RFU value of 150–4500 were declared "reportable."
2. Alleles of a genetic profile with an RFU value of 50–149 were declared "active." Active alleles are not reportable and may not be used in statistical calculations, but may be used for exclusions.
3. Alleles of a genetic profile with an RFU value below 50 were declared "undetectable." Undetectable alleles were not genotyped.
4. Validation by the Michigan State Police Crime Laboratory determined the heterozygote peak ratio to be $\geq$70%. If less than 70%, a heterozygote imbalance exists and must be interpreted with caution.

TABLE 1—*Quantitation of recovered DNA.*

Quantity (ng/μL)*	Negative Control Bomb Samples (Pre-deflagration)†	Negative Control Bomb Samples (Post-deflagration)†	Positive Control Bomb Samples†	Subject Handled Bombs†
2.0	...	...	...	...
0.25	...	...	...	...
0.03125	...	...	2	...
<0.03125	...	...	...	8
≪0.03125	...	3	...	5
ND	6	3	...	7

* Each sample volume ranged from approximately 80 to 140 μL.
† The number presented in each cell is the number of bomb samples having the indicated range of DNA on the Slot Blot.
ND = not detected.

5. For the purposes of this study, only 10 of the 13 core STR loci were examined. A full reportable genetic profile was declared if all the loci examined (10 loci) had alleles with RFU values between 150 and 4500, and heterozygote peak ratios were acceptable.
6. A partial reportable profile was declared when at least three of the STR loci had alleles with RFU values between 150 and 4500 and heterozygote peak ratios were acceptable.
7. An active profile was declared when one or more loci exhibited alleles with an RFU value between 50 and 150, and did not meet the guidelines for a full or partial profile type. Also included were heterozygous loci that showed extreme imbalances and were thus not reportable and/or one of the alleles was reportable and the other allele was active.
8. The lab employed a detection threshold cutoff of 50 RFU. If all the alleles were below the threshold, an undetectable profile was declared.

Results and Discussion

DNA Recovery

Table 1 shows the amount of DNA recovered on the QuantiBlot for the subject handled bomb samples, as well as all of the positive and negative control bombs. While the positive controls were at or above the lowest quantitation standard, all 20 of the subject-handled bombs were below the lowest quantitation standard.

Profiling Success: Unconcentrated versus Concentrated DNA

Amplifying unconcentrated DNA from the 20 subject handled bombs resulted in one partial profile, six active profiles, and 13 undetectable profiles. The number of alleles present in a profile and their respective peak heights reflect the limited amount of DNA that was added to the PCR reaction. Concentrating the DNA extract prior to amplification resulted in one full reportable profile, two partial profiles, five active profiles and twelve undetectable profiles. Thus, concentrating the extract did dramatically improve the signal for the majority of the samples. However, five samples actually decreased in signal intensity upon concentrating the DNA extract. This phenomenon may be attributed to sampling error, inhibition, or a combination of both factors.

The data from the unconcentrated and concentrated extracts were then combined and the most "active" profile for each bomb sample was reported. In combining the data, there was a total of one full profile, three partial profiles, eight active profiles, and eight undetectable profiles (Table 2). The electropherograms corresponding to the combined data were used throughout the remainder of the study.

TABLE 2—*Comparison of unconcentrated versus concentrated DNA extract with regard to DNA profiling success.*

Profile Type	Unconcentrated DNA	Concentrated DNA	Combined (Overall Results)
Full	0	1	1
Partial	1	2	3
Active	6	5	8
Undetectable	13	12	8

Profiling Success: Fragmentation Patterns

The exploded bombs were placed in one of three categories based on the degree of fragmentation and recovery. Category 1 was for bombs where the majority of the device was intact and thus the majority of the device was recovered, Category 2 was for highly fragmented bombs where many pieces were recovered, and Category 3 was for highly fragmented bombs with very few pieces recovered. Of the subject-handled bombs, there were seven bombs in Category 1, eleven bombs in Category 2, and three bombs were in Category 3. This information was then compared with the type of DNA profile generated from the recovered explosive devices.

In combining the data from the 310 runs (unconcentrated and concentrated DNA extracts), Category 1 bombs yielded 14% full profiles, 43% partial profiles, and 43% active profiles. There were no undetectable profiles in Category 1 bombs. Category 2 bombs yielded 45% active and 55% undetectable profiles. There were no full or partial profiles in Category 2. Category 3 bombs yielded 100% undetectable profiles. Not surprisingly, these results suggest that the success of generating a DNA profile from the bomb manufacturer depends upon the amount of bomb fragments recovered.

Profiling Success: Metal versus PVC Bombs

In addition to evaluating the effects of fragmentation on profiling success, the effect of metal versus PVC on profiling success was also evaluated. Initially, metal bombs were expected to give inferior results with respect to the PVC bombs. It was hypothesized that the DNA may not survive the heat generated by the explosion, which would be exacerbated by the thermal conductivity of metal, which could cause further degradation of the DNA. It was postulated that, with plastic, there may be a better chance for the DNA to remain intact, as plastic does not conduct heat well. Consideration was also given as to how the two materials fragment upon an explosion. Galvanized steel is much more durable than the more brittle, lightweight PVC bomb. While PVC bombs would have a tendency

to fragment into small pieces, galvanized steel bombs would tend to stay in larger but more mangled or twisted pieces. Since the amount of fragmentation and recovery is critical to generating DNA profiles, the metal bombs may have an advantage in that they tend to fragment less than the PVC bombs. However, the data show that metal and PVC bombs had a similar success rate for DNA profile recovery (personal observations).

Profiling Success: Issues Encountered

In all cases where a profile was generated (one full, three partial, and eight active), the alleles that were genotyped matched the known profile of the subject who handled that particular bomb. Of the eight active profiles, three had additional alleles at some loci. However, in all three cases, the alleles could be traced to an investigator who assisted in assembling bombs and collecting bomb fragments.

Of the three negative control bombs which produced $\ll$0.03125 ng DNA, one bomb produced an undetectable profile. The other two bombs each exhibited one active allele. One of these bombs exhibited a 21 allele at vWA with a signal intensity of 54 RFUs, and the other bomb had an X allele at Amelogenin with a signal intensity of 56 RFUs. Note these two alleles are only 4 and 6 units, respectively, above the 50 RFU detection limit for the instrument. While these two alleles are consistent with one of the investigator's profiles, the fact that there are only two alleles at such a low level makes it difficult to ascertain their true origin. This demonstrates the need for having succinct and reasonable threshold criteria for defining reportable profiles.

Conclusion

The purpose of this research was to determine whether it is possible to obtain usable genetic profiles from exploded pipe bomb devices. The results verify that recovering DNA from the individual who handled the bomb prior to the explosion was successful in several instances. Of the 20 bombs handled by subjects, four bombs gave reportable results that matched the subject's known DNA profiles. An additional eight active profiles, also matching the known DNA profiles of the subjects, were generated but were below the reportable threshold.

Successful DNA recovery is dependent upon several factors. First, the majority of the pipe bomb must be recovered. The more fragments recovered, the more surface area that can be swabbed for sloughed skin cells. Second, there is no evidence to suggest that the success of recovering DNA from a bomb is related to whether the bomb is made of metal or PVC. Third, successful DNA recovery may be dependent upon the "bomb assembler's" propensity to slough skin cells on objects he/she handles (16). Even if enough pieces from the bomb are collected and analyzed properly, it is possible that a DNA profile may not be obtained. This could be due to the perpetrator wearing gloves or that he/she may not have shed enough skin cells on the bomb.

Acknowledgment

The authors wish to thank Dan Gandor of the Illinois State Police, Joliet Forensic Science Laboratory, for his assistance in editing the manuscript and his scientific advice.

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