ANALYSIS OF DNA OBTAINED FROM WIRELESS ELECTRONIC TRIGGERING MECHANISMS USED WITH IMPROVISED EXPLOSIVE DEVICES

By

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ABSTRACT

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In recent years IEDs have been used both domestically and internationally for unconventional warfare and terrorism. Some of the more advanced IEDs use a wireless triggering mechanism typically composed of a cell phone, two-way radio, or other small electronic device that can receive a signal from great distances. In past research the feasibility of obtaining a genetic profile directly from the explosive of an IED following handling and deflagration was examined. Due to the poor state of DNA in shed skin cells along with extreme temperatures of the deflagration, only highly degraded DNA is generally recovered from the resultant bomb fragments, decreasing the chance of obtaining a genetic profile of the assembler. Focusing on the trigger mechanism instead of the explosive may result in increased potential for obtaining a complete genetic profile, mainly from longer handling during assembly and its distance from the deflagration. In this study participants were asked to handle components of a mock electronic trigger. Steel or PVC pipes were filled with smokeless powder and affixed to the trigger, then detonated by fuse. Pieces of the mechanism were collected and DNA was isolated from the individual components, quantified, and analyzed using miniSTRs. Allele assignments were made blind before comparison to references. Results indicate that the success in identifying an individual who handled the IED by analyzing DNA from the triggering mechanism is higher than identification using the explosive device fragments.

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I am very grateful for the help of Lt. Shawn Stallworth and Sgt. Timothy Ketvirtis of the Michigan State Police Bomb Squad who coordinated the deflagrations and the Lansing Fire Department for providing a safe facility. If it wasn't for them this research would have never taken place. I also extend my gratitude to the volunteers who showed an interest in science and were patient enough to spend some of their time after class to handle all of the IED components, to Kamila Gomez who helped facilitate this process, and to my other classmates who have helped me out along the way.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Improvised explosive devices (IEDs) have been used extensively during Operation Iraqi Freedom and Operation Enduring Freedom, killing thousands of people and wounding even more (Department of Defense personnel and military casualty statistics 2011). Coalition forces and civilian infrastructure including police stations, markets, and mosques have been successfully attacked using IEDs, mainly because they are ideal weapons for an ambush (Wilson 2007). IEDs are easily concealed, use multiple methods of detonation, and are relatively hard to counter or defend against. Further, the components that make up an IED are often cheap and easy to obtain, and their employment tactics and procedures are very flexible. For many of these same reasons, IEDs pose a serious threat in the United States. In addition, the risk of IED attacks in America is continuing to increase due to several factors including involvement in the global war on terrorism, individuals capable of carrying out IED attacks are already living in the United States, the vulnerability of many targets, and the ease in which bomb designs and tactics can be spread over the internet, making it simple for almost anyone to learn how to make and effectively use an IED in an attack (Broun et al. 2009). Supervisory Special Agent Barbara Martinez, a senior official at the FBI's Critical Incident Response Group, said that "Terrorists' use of IEDs cannot be extrapolated into anything other than a major threat to this country," (Hsu and Sheridan 2007).

According to the Department of Defense Dictionary of Military and Associated Terms (2001), an IED is "a device placed or fabricated in an improvised manner incorporating destructive, lethal, noxious, pyrotechnic, or incendiary chemicals and

designed to destroy, incapacitate, harass, or distract. It may incorporate military stores, but is normally devised from nonmilitary components." The basic components of an IED are an initiation system, explosive fill, detonator, possibly a power supply for the detonator, and a container or other means of concealment (Globalsecurity.org 2005). There are almost limitless means by which to deploy an IED, with three main methods of detonation: 1) time delay, which is provided by mechanical or electronic timers, fuses, or chemicals, 2) action, which is a mechanical system that can be initiated by pushing, pulling, or applying pressure, and 3) command, by either an electrical or wireless signal (Bolz et al. 2005). The advantage of command detonation is that the attack can take place at an opportune moment when the amount of damage can be maximized; however, there must be line-of-site with the target in order to coordinate the attack, which may put the triggerman in a vulnerable position. Modern electronics such as long range cordless phones, key fobs, cell phones, and two way radios have helped to alleviate this drawback by allowing a greater stand-off distance from the IED, which is why these types of triggers are being used more often (Bolz et al. 2005).

With the great diversity in IED types and tactics comes an increasingly difficult task of defeating attacks. Countermeasures currently in place to help mitigate the threat of an IED attack in Iraq and Afghanistan are electronic jammers, X-ray equipment, radar systems, robotic explosive ordnance disposal equipment, and armor for vehicles and personnel (Wilson 2006). These methods have been engineered to protect personnel overseas and can be modified for domestic use as well; however, even with cutting edge research and development in IED defense, there is no way security forces can eliminate attacks completely. Further action needs to be taken to ensure the public's safety from

the IED threat. These actions are outlined in the Homeland Security Presidential Directive/HSPD-19 issued on February 12, 2007 which states:

> It is the policy of the United States to counter the threat of explosive attacks aggressively by coordinating Federal, State, local, territorial, and tribal government efforts and collaborating with the owners and operators of critical infrastructure and key resources to deter, prevent, detect, protect against, and respond to explosive attacks, including the following:
>
> (a) applying techniques of psychological and behavioral sciences in the analysis of potential threats of explosive attack;
>
> (b) using the most effective technologies, capabilities, and explosives search procedures, and applications thereof, to detect, locate, and render safe explosives before they detonate or function as part of an explosive attack, including detection of explosive materials and precursor chemicals used to make improvised explosive or incendiary mixtures;
>
> (c) applying all appropriate resources to pre-blast or pre-functioning search and explosives render-safe procedures, and to post-blast or post-functioning investigatory and search activities, in order to detect secondary and tertiary explosives and for the purposes of attribution;
>
> (d) employing effective capabilities, technologies, and methodologies, including blast mitigation techniques, to mitigate or neutralize the physical effects of an explosive attack on human life, critical infrastructure, and key resources; and
>
> (e) clarifying specific roles and responsibilities of agencies and heads of agencies through all phases of incident management from prevention and protection through response and recovery.

As stated in the directive, it is important to continue developing methods of determining who is responsible for an attack. Current procedures of analyzing IED evidence include class characterization of the IED components such as size, weight, materials, methods of assembly, etc., trace analysis of hairs, fibers, or any other microscopic evidence, and chemical tests of the explosive charge. Fingerprints are generally the only

individualizing evidence collected; however, they may be unrecoverable after deflagration (Shachtman 2007).

DNA as evidence in IED investigations

Van Oorshot and Jones (1997) showed that accurate genetic profiles can be obtained from shed epithelial cells left behind on objects that have been handled, which is referred to as "touch DNA". The likelihood of getting a complete profile depends on the quantity and quality of DNA collected. Some factors that seem to affect DNA deposition are the amount of handling, time since last hand wash, and the substrate handled (Kisilevsky and Wickenheiser 1999; Phipps and Petricevic 2007). The techniques used to obtain touch DNA are also important. The double swab technique (Sweet et al. 1997) may offer advantages over traditional swabbing methods where only one swab is used (Pang and Cheung 2007). Cotton swabs are also recommended over synthetic because they are hydrophilic and have an irregular surface which can increase the tendency of cells to adhere to their surface (Jobin and DeGouffe 1999 as cited by Wickenheiser 2002).

Currently the use of DNA in IED investigations is limited. For regular use, and to establish much needed standards of operation, research must be conducted on the collection and analysis of DNA from deflagrated IEDs. Research involving the analysis of DNA obtained from exploded IEDs began with a study by Esslinger et al. (2004) wherein conventional short tandem repeat (STR) analysis was used for DNA isolated from steel or PVC pipe bombs following deflagration. The success of determining the handler is this study was minimal, where a profile with both alleles present at all 10 loci

using AmpFlSTR Profiler Plus (Applied Biosystems, Foster City, CA) was obtained for only 1 of 20 bombs, likely due to the limited amount and condition of DNA. Work was continued by Foran et al. (2009) who analyzed the hypervariable regions of mitochondrial DNA (mtDNA) collected from deflagrated pipe bombs. Eighteen of 38 bombs were correctly assigned to their respective handler. However, mtDNA analysis is not individualizing. Further work by Kremer (2008) involved the analysis of DNA obtained from deflagrated pipe bombs using two sets of miniSTRs, which offer advantages over conventional STRs when dealing with low amounts of degraded DNA (see below), together with the analysis of mtDNA. Combining the two techniques, the handler of 8 bombs was determined out of a set of 34, while 9 were assigned to a subset of handlers.

Since there are limitations in analyzing DNA from the explosive charge, focusing on other components of IEDs may be the key to increasing success in determining the handler's profile. In a study by Hoffmann et al. (2011), backpacks, a common container for IEDs, were given to individuals who used them for 11 days. A steel or PVC pipe bomb was then placed inside each backpack. After deflagration of the IED, the remnants of the backpack were analyzed using an AmpFlSTR Minifiler PCR Amplification Kit. Genetic profiles from different areas of the backpacks were combined to create a single consensus profile, which was then compared to reference samples to assess profile accuracy. As a result, a full profile of the handler was obtained from every backpack except at a single locus, providing a feasible method to analyze deflagrated IEDs. However, due to variability in IED design, where a container may not be used, and since the handler of the backpack may not be the same individual who was responsible for the

IED's construction, other methods for collecting/analyzing DNA from deflagrated IEDs must be explored.

Low copy number and degraded DNA

In traditional STR analysis 1 ng of nuclear DNA is recommended (Applied Biosystems 2001); however, it is unlikely that that amount will be obtained from touch DNA where only a limited number of shed epithelial cells remain. Using current polymerase chain reaction (PCR) techniques a genetic profile can sometimes be obtained from 100 pg or less of DNA, which is referred to as low copy number (LCN) (Gill et al. 2000). In a single human cell there is around 5 pg of DNA, therefore as few as 20 cells worth is used in LCN analysis. There are, however, several difficulties in analyzing LCN DNA such as stutter, unbalanced peak heights, drop-out (missing alleles) due to stochastic sampling effects, drop-in, and higher susceptibility to contamination (Balding and Buckleton 2009). Approaches to help alleviate these problems include reducing the total PCR volume, using filtration of the PCR product to remove ions that compete with the DNA during injection into the capillary, using low conductivity formamide with increased PCR product added, and increasing injection time (Budowle et al. 2001). Increasing the PCR cycle number has also been mentioned (van Hoofstat et al. 1998; Gill 2001) but was cautioned against by Budowle et al. (2001) because of the risk of over amplifying exogenous DNA. Gill et al. (2000) recommended analyzing LCN DNA by amplifying in replicates and only calling alleles that are present more than once.

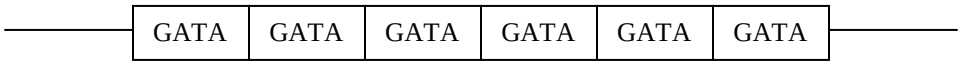
The quality of the DNA from deflagrated IEDs can also be problematic. The DNA obtained from epithelial cells found on the surface of the IED has likely been

subjected to environmental stresses such as direct sunlight, moisture, and bacterial and fungal contamination, which can cause DNA degradation (Pang and Cheung 2007). In addition, the DNA is exposed to intense heat and pressure from deflagration of the IED. In a study by Threadgold and Brown (2003) DNA from wheat seeds degraded at temperatures above 200ºC. The temperature DNA is subjected to during deflagration is not known but is undoubtedly far greater than this.

miniSTR analysis

STRs are made up of short stretches of DNA (between 2 and 6 base pairs (bp) in length) that are repeated in tandem and exhibit variation in length (based on the number of repeats) among individuals (Figure 1). Many of these regions found throughout the human genome can be utilized for analysis of DNA obtained during criminal investigations. The STR analysis starts with a PCR that is used to make many copies of the DNA. Fluorescently labeled primers are used in the PCR to target STR loci where amplification of the DNA occurs. The amplified DNA is then separated by size using capillary electrophoresis and the fragments are detected based on their fluorescent primers. By using a size standard, which is composed of many DNA fragments of known size, and an allelic ladder, which contains all of the common alleles, it is possible to determine the number of repeat units at each STR locus of the DNA obtained from evidence or reference samples, thereby constructing a genetic profile for the contributing individual.

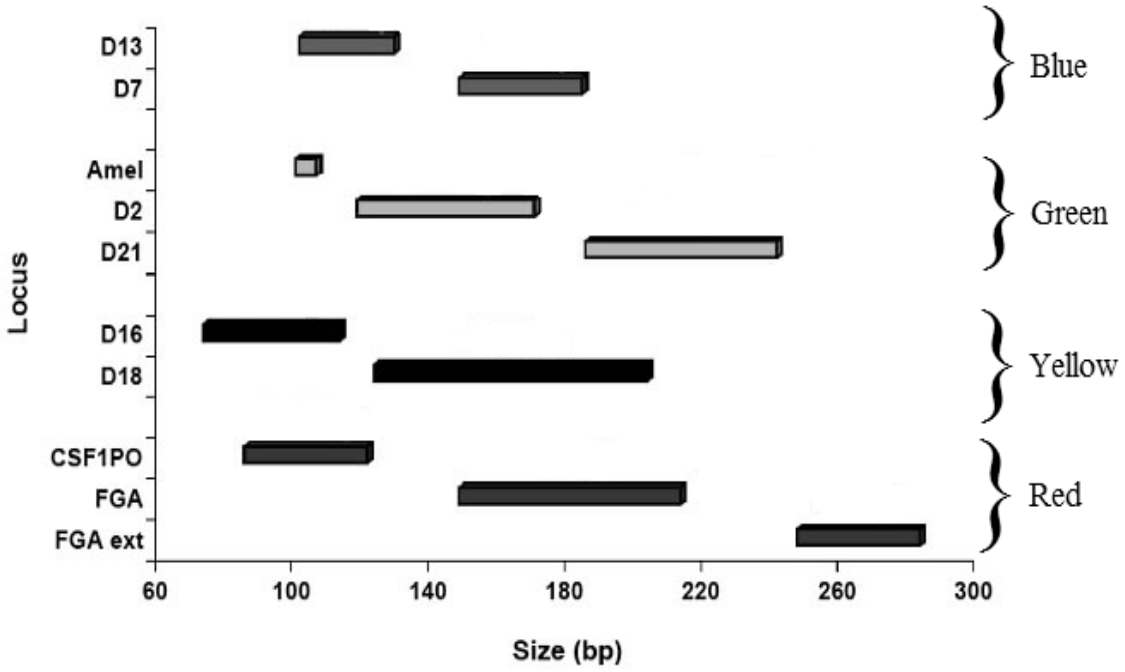
Figure 1. Example of an STR



An STR is made up of a short stretch of DNA that is repeated. Shown is an STR (GATA) that has six repeats.

Conventional STR kits such as AmpFlSTR Identifiler PCR Amplification Kit by Applied Biosystems or PowerPlex 16 System by Promega work well when analyzing higher quality DNA; however, when the DNA is degraded, some of the larger loci may drop-out resulting in a partial profile. By moving the primers to a location that closely flanks the repeat region, resulting in an overall decrease in amplicon size (known as miniSTRs), low quantities of highly degraded DNA can be more successfully typed (Butler et al. 2003). In 2007 Applied Biosystems released the AmpFlSTR Minifiler PCR Amplification Kit (Minifiler) which, in one PCR reaction, amplifies eight of the larger loci included in the Identifiler kit (D13S317, D7S820, D2S1338, D21S11, D16S539, D18S51, CSF1PO and FGA) along with the sex determining marker amelogenin (Applied Biosystems 2007). The kit was designed for genotyping degraded DNA that may or may not contain PCR inhibitors, with amplicons ranging from 70 to 283 bp in length (Figure 2). In a validation study by Mulero et al. (2008), results using Minifiler showed performance improvements over Identifiler in the analysis of degraded DNA and samples containing PCR inhibitors. Given these advantages, Minifiler may be better suited for amplifying DNA recovered from deflagrated IEDs, which is likely to be highly degraded.

Figure 2. Loci targeted by Minifiler



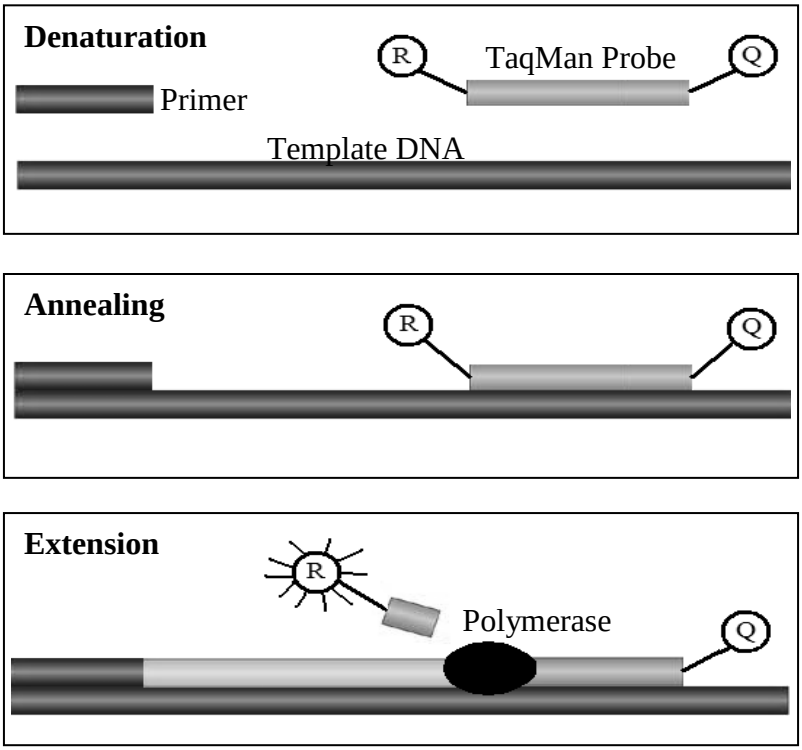
The size ranges of all 10 loci amplified by the Minifiler multiplex are shown with their corresponding dye color. (Product description, AmpFlSTR Minifiler PCR Amplification Kit, Applied Biosystems)

Quantification and detection of PCR inhibitors

As a quality control requirement for casework samples, any PCR-based assay must be preceded by quantification of nuclear DNA (nDNA) (DNA Advisory Board 2000). In 2006 Applied Biosystems released the Quantifiler Human DNA Quantification Kit (Quantifiler) which is a real time PCR quantification assay that is capable of detecting well below 100 pg of DNA (Green et al. 2005). The primers target the human telomerase reverse transcriptase locus located on chromosome 5, resulting in a 62 bp amplicon (Applied Biosystems 2006). In order to detect amplification in real time, a TaqMan probe is used (Figure 3). It is sequence specific and incorporates a fluorescent dye and a quencher that suppresses detection of the dye when the probe is intact. During the

annealing step of PCR the probe binds to a specific sequence within the amplicon. Taq polymerase then degrades the probe during extension which causes a release of the quencher from the dye. Once the dye has been separated from the quencher it can be detected. With each additional cycle of PCR there is an exponential buildup of detectable dye. Eight DNA standards of known concentrations are analyzed in the same manner to generate a standard curve to which the dye intensity of the unknown sample is compared to determine its DNA concentration.

Figure 3. Detection of the TaqMan probe



Shown are the three steps of real time PCR. The encircled "R" and "Q" represent the reporter and quencher of the TaqMan probe. During the extension phase, polymerase degrades the probe which releases the reporter from its quencher, resulting in fluorescence/detection.

A beneficial aspect of Quantifiler is the ability to detect PCR inhibition using its internal PCR control (IPC). The IPC consists of synthetic DNA with primers and a TaqMan probe that are specific to it. Since the concentration of synthetic DNA is constant, it should amplify to the same degree in all samples. Inconsistencies in the amplification rate of an unknown sample's IPC as compared to the standard's IPC indicate the presence of PCR inhibitors within the unknown. Though undocumented, it is likely that there are many potential inhibitors of PCR associated with residue from the deflagrated bombs or other components or chemicals that make up the IED, which could co-extract with the DNA. Methods used to overcome inhibition include chemical additives such as bovine serum albumin (BSA) (Comey et al. 1994) or betaine (Al-Soud and Rådström 2000), decreasing sample DNA volume to dilute inhibitors, increasing the amount of Taq polymerase (Bessetti 2007), using different types of polymerase (Eilert and Foran 2009), or separating the DNA from the inhibitors using filtration (Comey et al. 1994).

Research objectives

The focus of this study was to determine if the success in identifying the handler of an IED after deflagration can be increased by analyzing other components of an IED, such as electronic triggering mechanisms. Focusing on the triggering device may result in a greater potential of obtaining a complete genetic profile, for a variety of reasons. First, an electronic triggering device incorporates multiple components that require assembly and thus longer handling, potentially resulting in a greater accumulation of touch DNA. Second, DNA on the triggering mechanism may not experience the same

heat as the explosive charge, and therefore is less degraded. Third, the triggering mechanism may only separate into its individual components (wireless device, battery, circuit board, etc.), instead of fragmenting into many small pieces as does an explosive charge casing, making their recovery much easier. Fourth, depending on how the triggering mechanism is attached to the detonator of the IED, there can be substantial separation or obstacles between the two, resulting in decreased damage during the blast.

Participants in this study were asked to mock assemble an electronic IED triggering mechanism. Pipes were filled with smokeless powder and affixed to the mock triggers, then deflagrated by fuse in a controlled environment, after which all pieces of the mechanism were collected and swabbed. Following extraction, the DNA was quantified and analyzed using miniSTRs. Allele assignments were made blind before comparison to reference samples from the volunteers to determine the frequency of which the handler's profile could be accurately obtained.

MATERIALS AND METHODS

Collection and cleaning of mock trigger components

Equipment for 18 mock triggering mechanisms included ten used cell phones (various models), eight two way radios (approximately 3 x 5 inches), circuit boards from laptop computers (approximately 3 x 4 inches), AA batteries, wire (single, coated, approximately eight inches in length), clamps (PO-35 or HI-988, Bulldog Hardware, Memphis, TN), Velcro, screws (#6, 1/2 inch, Alma Bolt Company, Alma, MI), and 3/8 inch plywood (cut to approximately 11 1/2 x 6 1/4 inches). All components were soaked for one hour in a 10% bleach solution, scrubbed thoroughly on all surfaces, and rinsed with de-ionized water (except the battery, which was only scrubbed, and the Velcro, which was unhandled out of the packaging). They were then placed in a Spectrolinker XL-1500 UV Crosslinker (Spectronics Corporation, Westbury, NY) for 15 minutes each side (approximately 7.5 J/cm^2).

To ensure the donated phones were free of DNA after being cleaned, three were separately swabbed front and back. A double swab technique (Sweet et al. 1997) was used, where one swab (25-806 2PC, Puritan Medical Products Co. LLC, Guilford, ME), after being UV irradiated for five minutes, was moistened with 150 µl of digestion buffer (10 mM Tris, 20 mM EDTA, 0.1% SDS, pH 7.5) then passed over the targeted surface, immediately followed by a dry swab. The swab sets from the three phones (along with a clean swab set that served as a substrate blank) were placed separately into 1.5 ml microcentrifuge tubes with 350 µl of digestion buffer and 6 µl of proteinase K (20 mg/ml) and incubated overnight at 55^{o}C. The swabs were then removed and placed into

spin baskets with 2 ml collection tubes and centrifuged for 1 minute at 13,000 rpm and discarded. The extracted liquid was pipetted back into the original tubes. Five hundred microliters of phenol were added followed by vortexing and centrifugation at 13,000 rpm for 6 minutes. The aqueous layers were pipetted into clean (autoclaved and UV irradiated) 1.5 ml microcentrifuge tubes followed by the addition of 500 µl of chloroform. The tubes were vortexed and centrifuged at 13,000 rpm for 6 minutes. The aqueous layers were pipetted into Microcon YM-30 spin columns (Millipore Corporation, Billerica, MA), and 100 µl of TE (10 mM Tris, 1 mM EDTA, pH 7.5) was added. The columns were centrifuged at 14,000 x g for 12 minutes. The flowthrough was discarded and 200 µl TE was added to the samples, followed by centrifugation at 14,000 x g for 8 minutes. Twenty microliters of TE was added and left for 5 minutes. The columns were then inverted into clean tubes and centrifuged at 1,000 x g for 3 minutes. The extracts were amplified using primers specific to human amelogenin (Kiley 2009), 0.1 µl AmpliTaq Gold DNA polymerase with 1 µl buffer (Applied Biosystems), 200 µM dNTPs, 2.5 mM $MgCl_2$, and 4 µl water. Parameters for thermal cycling included a 94ºC hold for 12 minutes, 38 cycles of 94ºC for 30 seconds, 58ºC for 30 seconds, and 72ºC for one minute, and a 72ºC hold for two minutes. Post amplification products were electrophoresed on a 1.5 % agarose gel.

Preparation of pipe bombs and containers

Nine sections of PVC and galvanized steel pipe (one foot in length, one inch in diameter), with two end caps for each pipe, were obtained for use as explosive casings. Nine small cardboard boxes and backpacks (LEED'S, Pittsburgh, PA) were obtained for

IED containers. A 1/4 inch hole was drilled into the center of one of the end caps for each pipe. The pipes and end caps were cleaned by soaking in a 10% bleach solution for one hour, rinsed with de-ionized water and UV irradiated for five minutes on each side. The outside of the pipes and the entire surface of the end caps were wiped with ELIMINase (Decon Laboratories Inc., Bryn Mawr, PA) and rinsed with de-ionized water. The PVC end caps lacking a drilled hole were glued to each of the PVC pipes using PVC cement. The cardboard boxes and backpacks were autoclaved and UV irradiated on all surfaces for 15 minutes.

Assembly and handling of IEDs

Velcro was used to attach the cell phone or radio, battery, and circuit board to the plywood base, and screws were used to anchor the clamp that held the pipe bomb in place. The 18 containers, pipes, and mock triggers were placed separately into brown paper bags and then assigned a number from 13 – 30 (1 – 12 were used in a previous study). The cardboard boxes were assigned an even number and the backpacks were assigned an odd number. The steel pipe assignations ranged from 13 – 21 and the PVC, 22 – 30. Mock triggers that incorporated a cell phone versus a radio were assigned numbers so that there was an even distribution of each among the different combinations of cardboard box to backpack and steel to PVC (Table 1).

Eighteen volunteers randomly drew a number ranging from 13 – 30 that was used by them to identify which container, pipe, and mock trigger they were assigned. They also drew a letter that was recorded next to their number on a sheet of paper maintained by the principal investigator. Buccal swabs were obtained from each participant as DNA

reference samples and labeled with their respective letter designator. The cardboard boxes and pipes were handled for roughly 30 seconds to 1 minute. The backpacks were utilized in place of the participant's own for ten days. The mock triggers, which were the focus of this study, were removed from the brown paper bags, disassembled, handled briefly, and then re-assembled (resulting in the handling of each component for roughly 20 seconds).

Table 1. Type of components included in each IED

Device number	Explosive case material	Cell Phone/Radio	Container type
13	Steel	Cell Phone	Box
14	Steel	Cell Phone	Backpack
15	Steel	Radio	Box
16	Steel	Radio	Backpack
17	Steel	Cell Phone	Box
18	Steel	Cell Phone	Backpack
19	Steel	Radio	Box
20	Steel	Radio	Backpack
21	Steel	Cell Phone	Box
22	PVC	Cell Phone	Backpack
23	PVC	Cell Phone	Box
24	PVC	Radio	Backpack
25	PVC	Radio	Box
26	PVC	Cell Phone	Backpack
27	PVC	Cell Phone	Box
28	PVC	Radio	Backpack
29	PVC	Radio	Box
30	PVC	Cell Phone	Backpack

Each component of the IED was handled by the same individual. The mock trigger, explosive, and container were all analyzed separately, with the analysis of the mock trigger being the focus of this study.

Deflagration and recovery of IEDs

Deflagrations took place at the Lansing Fire Fighting Training Facility's (Lansing, MI) smoke room. At that location, 1.5 ounces of Green Dot Smokeless Shotshell Powder (Alliant Powder Co., Radford, VA) were added to the pipes, the second end cap was set into place, and a 45 second fuse was inserted through the hole drilled in the end cap. The pipe bomb was affixed to the mock trigger and then placed inside the container with the fuse showing. The IED was placed inside a metal crate designed to allow pressure from the blast to escape while containing the larger pieces of debris. After deflagration the remains of the container, pipe bomb, and mock trigger were collected separately and placed in brown paper bags for transport to the laboratory. The level of damage done to each was assessed based on the following 0 – 5 scale:

0. No components were badly damaged or fragmented
1. At least one or two components were moderately damaged, but mostly intact
2. At least two or three components were moderately damaged with some fragmentation
3. At least three or four components were moderately damaged with some fragmentation
4. At least three or four components were moderately damaged with high degrees of fragmentation
5. At least four or five components were highly damaged/fragmented

DNA isolation

The components of the mock-triggering mechanisms were swabbed separately and labeled with their respective device number and a lettered designator, a – j, specific to the location the swab was taken (Table 2). Three sets of swabs were used to cover all surfaces of the front, back, and sides of the cell phones or radios, two sets of swabs were used for the front and back of the circuit boards and plywood, and one set of swabs was

used for the entire surface of the batteries and wire (Figure 4). Since some of the areas that were being swabbed were large, extra digestion buffer was applied to the first swab to keep it moist throughout the process. The swabs were placed into a 2 ml tube with 600 µl of digestion buffer (400 µl for reference samples) and 6 µl of proteinase K (5 µl for reference samples) and incubated overnight at 55ºC. The swabs were then placed into spin baskets with collection tubes and centrifuged for 1 minute at 13,000 rpm and discarded. The extracted liquid was pipetted back into the original tubes. The organic extraction was carried out as above using an equal volume of phenol and chloroform as extracted liquid (650 µl for unknowns and 400 µl for reference samples).

Table 2. Letter designators assigned to each swab set

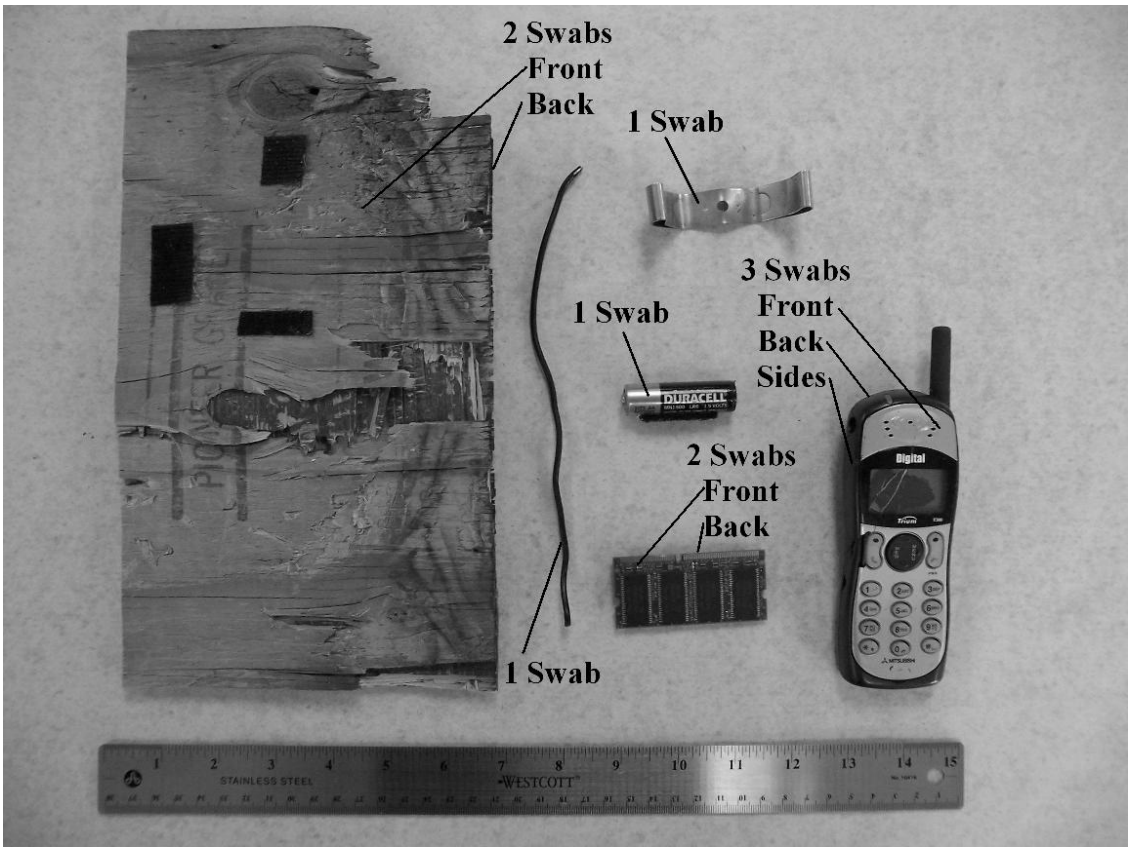
Letter designator	Trigger component
a	Phone/Radio front
b	Phone/Radio back
c	Phone/Radio sides
d	Circuit board front
e	Circuit board back
f	Battery
g	Clamp
h	Wire
i	Plywood front
j	Plywood back

Each swab set was labeled with the IED number followed by the respective letter designator.

Preliminary tests showed the use of Microcon YM-100 spin columns, instead of YM-30 spin columns, resulted in more complete PCR amplification of the unknown DNAs so they were used for the remainder of this study (except for reference samples).

Five hundred microliters of the aqueous layer from the organic extractions were added to the spin column and centrifuged for 25 minutes at 500 x g. The flowthrough was discarded and the remainder of the aqueous layer was added to the spin column followed by centrifugation for 20 minutes at 500 x g. Two hundred microliters of TE were added and centrifuged through the column at 500 x g until almost all of the liquid had passed through (around 12 minutes). Roughly 5 µl of TE remained against the wall of the spin column so 15 µl of TE was added to bring the total volume up to 20 µl. The column was left for 3 minutes then inverted into a clean tube and centrifuged for 1 minute at 1,000 x g. The reference DNA was cleaned as above and eluted at a final volume of 50 µl.

Figure 4. Areas of the mock trigger targeted for DNA collection



Ten swabs were taken; however, the plywood samples were not used in the analysis.

Quantification of DNA and determination of PCR inhibition

A Quantifiler Human DNA Quantification Kit was used to quantify DNA and determine if PCR inhibitors were present. Amplification was performed and detected on an iCycler thermal cycler with an iQ5 multi-color real-time PCR detection system (Bio-Rad, Hercules, CA). The reaction was carried out in 0.2 ml dome cap tubes (Dot Scientific, Burton, MI) and was set up according to the manufacturer's protocol at a final volume of 15 µl (7.5 µl reaction mix, 6.3 µl primer mix, and 1.2 µl DNA). The kit's 200 ng/µl standard DNA was serially diluted per the manufacturer's protocol providing eight standards ranging from 50 ng/µl to 0.023 ng/µl. Parameters for thermal cycling included a 95ºC hold for 10 minutes followed by 40 cycles of 95ºC for 15 seconds and 60ºC for one minute. Total DNA was calculated by multiplying the concentration of DNA (ng/µl, determined by quantification) by the sample volume (µl). This value was reported as the DNA quantity. Sample inhibition was determined by comparing the IPC cycle threshold (Ct) of an unknown sample to the standards. If the Ct of an unknown IPC was more than one cycle higher than the standards, then that sample was noted as possibly containing PCR inhibitors.

Amplification of STRs

An AmpFlSTR Minifiler PCR Amplification Kit was used to amplify DNA isolated from the mock triggers. The total volume in the reaction was reduced to 10 µl, including 4 µl of the AmpFlSTR Minifiler Master Mix, 2 µl of the AmpFlSTR Minifiler Primer Set, and 4 µl of sample DNA or 0.5 µl of the control DNA (0.1 ng/µl) plus 3.5 µl of sterile water. Parameters for thermal cycling included an initial 95ºC hold for 11

minutes followed by 33 cycles of 94ºC for 20 seconds, 59ºC for 2 minutes, and 72ºC for 1 minute, ending with a final 60ºC hold for 45 minutes. Select DNAs that yielded only a partial or no profile were re-amplified using 1 µl if the quantification indicated sufficient DNA or presence of inhibitors in those samples. Amplifications using 30 and 35 cycles were also tested during optimization. Results were improved using a higher cycle number; however, 35 resulted in an increase in artifacts. Re-amplifications of some inhibited samples were set-up using 250 or 500 ng/µl bovine serum albumin (BSA). There were no improvements over the first amplifications so the use of BSA was not continued.

PowerPlex 16 System (Promega) was used to amplify reference DNAs. The total volume of the reaction was 20 µl and included 0.3 µl AmpliTaq Gold DNA polymerase, 1 µl Gold ST★R 10X buffer, 1 µl PowerPlex 16 10X Primer Pair Mix, 7 µl sterile water, and 1 µl of a 1:10 dilution of reference DNA. Parameters for thermal cycling included one cycle of 95ºC for 11 minutes, 10 cycles of 94ºC for 30 seconds, 60ºC for 30 seconds, and 70ºC for 45 seconds, 22 cycles of 90ºC for 30 seconds, 60ºC for 30 seconds, and 70ºC for 45 seconds, and one cycle of 60ºC for 45 minutes.

Determination of genetic profiles and assignations

Two microliters of unknown amplified DNA (1.5 µl for the amplified control DNA and Minifiler allelic ladder) were combined in a 0.5 ml tube with 0.5 µl of GeneScan 500 LIZ Size Standard (Applied Biosystems) and 24.5 µl of deionized formamide. Following centrifugation, the samples were incubated at 95ºC for 3 minutes

and immediately put on ice for 3 minutes. The tube lids were cut off and one drop of mineral oil was added.

Amplified DNA was electrophoresed on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems) with GS STR POP4 (1 ml) G5 v2.md5 as the run module (GS STR POP4 (1 ml) A.md4 for the reference samples), performance optimized polymer 4 (Applied Biosystems), and 1X buffer with EDTA (Applied Biosystems). Electrophoresis parameters for DNAs amplified with Minifiler included a 15 kV injection for 5 seconds and a 15 kV run for 28 minutes at 60ºC. To increase peak heights of some samples that were well below threshold, amplified DNAs were cleaned using Millipore Montage Spin Columns and injected for 20 seconds and 60 seconds. The STR results were not improved so the standard injection conditions detailed above were used for all samples.

GeneMapper ID software v3.2.1 (Applied Biosystems) was used to analyze the data using Minifiler_GS500_HID_v1 as the analysis method, Minifiler_GS500_v1 as the panel, CE_G5_HID_GS500 as the size standard, and DS-33 Matrix 7-12-07 as the matrix. A threshold of 50 relative fluorescence units (RFU) was used for data interpretation. Parameters for reference DNAs amplified with PowerPlex included a 15 kV injection for 3 seconds and a 15 kV run for 30 minutes at 60ºC. The GeneMapper ID settings used for data analysis were: Analysis PP 16 SH as the analysis method, PowerPlex_16_ID3.1.0 as the panel, ILS600 Advanced as the size standard, and Matrix – UMSTest as the matrix.

DNA profiles from the unknown samples were compared to reference profiles and each locus was categorized on a scale from A – F (Appendix A):

A. Presence of both handler alleles without others
B. Presence of both handler alleles with others

C. One single handler allele present
D. One handler allele with others present
E. Amplification but of unexpected alleles
F. No callable alleles

A consensus genetic profile was produced by comparing allele calls at each locus of the eight DNA samples obtained from the mock triggers. All interpretations of the unknowns were done before seeing reference profiles. If two major alleles (or one allele for homozygotes) were present at least twice, then those were recorded as the consensus for that locus, otherwise all possible alleles were recorded. The consensus profile was then compared to the reference profile and results for each locus were placed into one of four categories:

1. Both alleles were consistent with the handler with no other calls included
2. Both handler alleles were present among other possible calls
3. Only one handler allele was present among other possible calls
4. Neither handler allele was called

Amplification using 1 µl DNA

Based on the Quantifiler internal PCR controls and the STR results, PCR inhibition and/or degradation was most likely the cause of incomplete or absence of STR amplification for many of the DNA samples. Select DNAs whose quantification results indicated the presence of human DNA but had little or no amplification were re-amplified using 1 µl of DNA in an attempt to dilute any inhibitors. This also helped determine whether these samples were affected more by inhibition or degradation. A second consensus profile was generated (as described above) using data from the re-amplifications and the initial amplifications combined.

An additional six IEDs (numbered 31 – 36) were prepared and deflagrated as above (with the exception of a slightly increased handling time). DNA quantities in the sample extracts were not determined. Amplifications were set up in duplicate with 1 µl and 4 µl of DNA. Data from these amplifications were interpreted as above.

Statistical calculations

Minitab 16 software (Minitab Inc., State College, PA) was used for statistical calculations. The normality of a set of data was determined first using a protocol in Minitab 16 called "Normality". Since data sets were not normally distributed their P values were determined using the Mann-Whitney test. An alpha of 0.05 was used.

RESULTS

Post deflagration and DNA isolation observations

There was a wide range of damage among triggers, wherein some were minimally affected by the blast and others were completely destroyed. The damage and fragmentation was much higher with the triggers that incorporated a steel pipe bomb than those with a PVC pipe bomb (Table 3). In some instances the steel pipe bombs caused the lid of the crate to fly open allowing components of the triggers to be strewn about the containment room. The crate remained secured during deflagration of PVC pipe bombs, where typically the only debris that escaped were pieces small enough to fit through the holes in the crate, including many fragments of the pipe itself. PVC bombs caused more charring, and increased unburned and partially burned smokeless powder. In most cases there was black residue on the plywood base, and of those, about half had partially burnt powder imbedded into some of the trigger components that left small yellow/brown marks. The amount of residue/unburned powder varied among the mock triggers and did not seem to relate to the amount of overall damage observed.

Table 3. Degree of fragmentation for each mock IED trigger

Steel		
Device #	Phone/Radio	Classification
13	Phone	1
14	Phone	3
15	Radio	3
16	Radio	4
17	Phone	3
18	Phone	5
19	Radio	4
20	Radio	4
21	Phone	5

PVC		
Device #	Phone/Radio	Classification
22	Phone	0
23	Phone	1
24	Radio	3
25	Radio	1
26	Phone	1
27	Phone	0
28	Radio	0
29	Radio	1
30	Phone	1

Each mock trigger was rated as to how much damage was incurred during deflagration. A mock trigger was rated a 0 when there was virtually no damage, and 5 if almost all components of the device were damaged. Devices 13 – 21 were deflagrated using a steel pipe bomb while PVC was used for devices 22 – 30.

There were also trends in the amount of damage done to the individual components. Generally the phone or radio suffered the greatest amount of visible damage, followed by the plywood and then the metal clamp. Most of the batteries and wires appeared relatively unaffected, except in some cases where the battery shell or wire coating was stripped off. The least damaged component was the circuit board; in only two cases was it obviously affected by deflagration.

Recovery of the individual trigger components for subsequent DNA isolation depended on their level of fragmentation. Generally all of the components were recovered in their entirety. When components were highly fragmented, some of the

pieces were usually not recovered, especially in cases where the lid to the containment crate was forced open; however, at least some fragments from every component were recovered for each trigger.

Swabbing the components collected visible residue on their outer surfaces. As a result, most swabs were black in color and some had solid pieces of adhering smokeless powder. The residue was mostly separated during phenol/chloroform extraction; however, a very fine black residue was visible on the Microcon filters of some samples. The swabs of the plywood base were black or yellow/brown in color. The black residue was removed as above, but a yellow discoloration remained in the aqueous layer after organic extraction. During Microcon filtration the yellow substance did not filter through, but remained with the DNA. Initial amplification of these DNAs (from the first few triggers processed) failed due to high levels of PCR inhibition, and analysis of plywood samples was halted at that point.

DNA quantification

The total amount of DNA isolated from swab pairs of the trigger components ranged from 0.0 – 5.22 ng (not including the negative control), with an average of 0.497 ± 0.80 ng (Table 4). There were 17 swab pairs that contained greater than 1 ng of DNA, 67 with greater than 0.1 ng but less than 1 ng, and 52 with less than 0.1 ng. The negative control swab pairs contained no DNA except for the front of the phone and the battery, which had 0.452 ng and 0.075 ng, respectively. All reagent negative controls contained no DNA. Total DNA isolated from each mock trigger ranged from 0.302 – 9.081 ng. Of the 67.62 ng of DNA recovered, 24.485 ng (36.2%) came from the cell phones and

radios, 17.146 ng (25.4%) from the circuit boards, 10.456 ng (15.5%) from the clamps, 8.159 ng (12.1%) from the wires, and 7.312 ng (10.8%) from the batteries.

The total amount of DNA isolated from devices that incorporated a steel pipe bomb was not significantly different than the amount from triggers with a PVC pipe bomb ($p = 0.736$) (Table 5). Thirty and three tenths nanograms of DNA were obtained from 7 triggers (average of 4.3 ± 0.84 ng, median of 3.78) that utilized a steel pipe bomb, and 37.3 ng DNA (average of 4.7 ± 0.77 ng, median of 3.04) were obtained from 8 triggers where a PVC bomb was used. There did not seem to be any correlation between the amount of trigger damage and DNA recovered, where the average quantity of DNA isolated from category 0 was 5.8 ng, 1 was 3.7 ng, 3 was 5.0 ng, 4 was 1.6 ng, and 5 was 3.5 ng (no mock triggers were in category 2).

Table 4. Quantity of DNA isolated from each trigger

	Amount of DNA (ng) recovered from each trigger component								
Device number	Phone/radio front	Phone/radio back	Phone/radio sides	Circuit board front	Circuit board back	Battery	Clamp	Wire	Total
13**	0.452	0.000	0.000	0.000	0.000	0.075	0.000	0.000	0.527
14	5.22	0.122	0.117	0.522	0.050*	0.000*	1.34	0.488	7.85
15	**0.714**	0.515	**1.48**	1.18	0.000	0.297	0.836	0.794	5.81
16	**0.103**	**0.575**	**0.414**	0.000*	0.000*	**0.197**	**0.353**	**1.05**	2.69
17	**0.000**	0.458	**0.417**	0.263*	**0.534**	1.58	**0.821**	0.798	4.87
18	**0.393**	**0.000***	**0.309**	3.63*	**0.000***	0.000*	**0.630**	0.000	4.96
19	**0.278***	**0.145***	**0.878***	0.024*	0.000	0.311	0.061	0.000	1.70
20	0.000*	**0.000**	0.077*	0.000*	**0.000**	0.095	**0.000**	**0.147**	0.319
21	**0.122**	0.000*	**0.000**	0.000*	0.000*	**0.516**	**0.006**	**1.44**	2.08
22	**0.052**	**0.798**	0.084	**0.222**	**0.144**	**0.744**	**0.149**	0.626	2.82
23	0.000	**0.384**	**0.558**	**0.570**	**0.050**	**0.737**	**0.066**	**0.674**	3.04
24	0.188	0.240	0.192	0.000	0.216	0.027	0.126	0.395	1.38
25	**0.338**	**0.446**	**0.825**	**0.941**	**1.08**	**0.804**	**0.000**	**0.573**	5.00
26	0.000*	0.025*	0.015	0.000*	0.549	0.336	0.365	0.887	2.18
27	**2.19**	**1.74***	**2.45**	**1.56**	**0.000***	**0.518**	**0.504**	**0.125**	9.08
28	0.000*	0.042	0.000*	0.162	0.618	0.107	4.46	0.000	5.38
29	**0.041**	**0.030**	0.079	0.079	0.000	0.000	0.000	**0.074**	0.302
30	0.173	0.521	0.752	1.68	**3.08**	**1.11**	0.750	0.093	8.16
Total	9.81	6.04	8.64	10.8	6.31	7.38	10.5	8.16	67.6

The amount of DNA isolated from each swab set ranged from 0.00 ng – 5.22 ng with an average of 0.497 ± 0.80 ng. Total DNA for each component (not including quantities from the negative control) and trigger are also shown. Numbers in bold indicate samples where both handler alleles were obtained from at least 2 loci when amplified with Minifiler. * Internal PCR Control indicated the presence of inhibition ** Device served as a negative control

Table 5. DNA obtained compared to trigger damage classification

Steel		
Device #	Total DNA Obtained (ng)	Damage Classification
13*	0.527	1
14	7.85	3
15	5.81	3
17	4.87	3
16	2.69	4
19	1.70	4
20	0.319	4
18	4.96	5
21	2.08	5

PVC		
Device #	Total DNA Obtained (ng)	Damage Classification
22	2.82	0
27	9.08	0
28	5.38	0
23	3.04	1
25	5.00	1
26	2.18	1
29	0.302	1
30	8.16	1
24	1.38	3

The amount of damage done to the mock triggers during deflagration was rated on a scale of 0 to 5, with 0 being the lowest and 5 being the highest. The average amount of DNA obtained from triggers in each damage category was 5.76 ± 3.15 ng for category 0, 3.73 ± 3.06 ng for 1, 4.98 ± 2.7 ng for 3, 1.57 ± 1.19 ng for 4, and 3.52 ± 2.04 ng for 5.
* Device served as a negative control

Detection of PCR inhibition

Twenty-six DNAs showed PCR inhibition (Table 6). Twenty of those were from triggers deflagrated with a steel pipe bomb and seven were from PVC IEDs. Twelve of the inhibited DNAs were from the phone/radios, 12 from circuit boards, 2 from batteries, and 0 from the metal clamps or wires. Ten of the inhibited samples quantified as having some DNA, ranging from 0.024 – 3.63 ng, of which only three produced STR profiles of five or more loci where both handler alleles were obtained. The remaining 16 inhibited DNAs quantified at 0, four of which had STR profiles where both handler alleles were obtained from more than five loci.

Table 6. STR analysis of inhibited samples

	14e*	14f*	16d*	16e*	17d	18b**	18d	18e**	18f**	19a	19b	19c	19d
D13	F	F	F	F	F	B	F	B	F	A	E	F	F
D7	F	F	F	F	F	A	F	F	F	F	F	F	F
Amel.	F	F	F	F	F	A	F	A	F	A	C	A	F
D21	F	F	F	F	F	A	F	B	F	A	C	F	F
D16	C	C	F	C	F	C	F	C	F	F	F	F	F
D18	F	F	F	F	F	A	F	A	F	A	B	A	F
CSF	F	F	F	C	A	B	F	A	F	A	A	A	F
FGA	F	F	F	F	F	F	F	A	F	C	F	F	F

	20a**	20c	20d**	21b**	21d**	21e**	26a**	26b**	26d**	27b	27e	28a**	28c**
D13	F	D	F	F	F	F	F	F	F	A	A	F	F
D7	F	E	F	F	F	F	F	F	F	A	A	F	F
Amel.	C	C	F	F	F	F	F	F	F	B	A	F	F
D21	C	F	F	F	F	F	F	F	F	A	F	F	F
D16	E	D	F	F	F	F	F	F	F	D	A	F	F
D18	A	D	F	F	F	F	F	F	F	B	A	F	F
CSF	E	B	F	F	C	F	F	F	F	A	A	F	F
FGA	F	F	F	F	F	F	F	F	F	A	C	F	F

Twenty-six DNAs had indication of PCR inhibition based on Quantifiler. A scale from A – F was used to represent the number of handler's alleles obtained from STR analysis, where A represents the presence of both handler alleles without others, B, both handler alleles present with others, C, one handler allele with no others, D, one handler allele with others, E, non-handler alleles only, or F, no callable alleles. *250 ng/µl BSA used **500 ng/µl BSA used

Analysis of STR electropherograms

Of the 1088 loci analyzed from initial amplifications (Appendix A) the number that fell into each category was: A – 229 (21%), B – 120 (11%), C – 77 (7%), D – 43 (4%), E – 29 (3%), and F – 590 (54%). Thirty-two had no indication of DNA, 9 of which produced a partial profile containing at least two loci with both handler's alleles. A full, clean profile of the handler was not obtained from any DNA sample; however, there were 14 where both handler alleles were present at all loci (with some loci having other alleles). Consensus profiles generally provided much more information than did any profile by itself. There were 68/136 (50%) consensus loci that fell into category 1, 20/136 (15%) in category 2, 3/136 (6%) in category 3, and 40/136 (29%) in category 4 (Table 7). Based on the consensus, 10/17 triggers had a profile where at least 3 or 4 loci had only the handler's alleles present, 4 of which had only the handler's alleles called at all loci.

Table 7. Evaluation of consensus profiles from initial amplifications

	D13	D7	Amel.	D21	D16	D18	CSF	FGA
14	4	4	1	4	4	4	4	4
15	2	2	1	2	2	2	1	1
16	1	1	1	1	1	1	1	1
17	1	1	1	1	2	1	1	1
18	1	1	1	2	2	1	1	2
19	1	4	1	1	4	1	1	3
20	2	2	1	2	2	2	2	3
21	1	1	1	1	2	1	1	3
22	2	1	1	1	2	1	2	4
23	1	1	1	1	1	1	1	1
24	3	4	4	4	4	4	1	4
25	1	1	1	1	1	1	1	1
26	4	4	4	4	4	4	4	4
27	1	1	1	1	1	1	1	1
28	4	4	4	4	4	4	4	4
29	4	3	2	3	3	1	3	4
30	4	4	1	4	4	4	1	4

A scale from 1 – 4 was used to represent the number of handler's alleles obtained using the consensus profile, where 1 represents the presence of both handler alleles only (including homozygotes), 2, both handler alleles with others present, 3, one handler allele by itself, and 4, no handler alleles were obtained. These are based on results from the original amplifications using 4 µl of sample DNA.

Re-amplification of DNAs after clean-up using Microcon YM-100 spin columns

The three sets of DNAs that were re-cleaned with Microcon YM-100 columns and re-amplified yielded more complete profiles than previous amplifications (Table 8). Twenty three of twenty four (96%) loci had both handler alleles present when DNAs were cleaned with YM-100 columns as compared to only 8 of 24 (33%) with YM-30 columns. No handler alleles were obtained from 13 of 24 (54%) loci analyzed after

initial amplification, while at least one handler allele was obtained from every locus as a result of re-cleaning and amplification.

Table 8. Comparison of STR results when DNAs were amplified following clean-up with Microcon YM-30 spin columns versus Microcon YM-100

	Microcon YM-30			Microcon YM-100		
	23g	**27b**	**27c**	**23g**	**27b**	**27c**
D13	B	F	F	B	A	A
D7	A	F	F	A	A	B
Amel.	A	F	A	A	B	B
D21	C	F	F	A	A	A
D16	D	F	F	A	D	B
D18	E	F	B	B	B	B
CSF	C	A	A	B	A	A
FGA	A	F	F	A	A	A

Original STR data from DNAs concentrated using Microcon YM-30 columns after extraction yielded little information. Select samples were re-purified with Microcon YM-100 columns and amplified again. A scale from A – F was used to represent the number of handler's alleles obtained from STR analysis, where A represents the presence of both handler alleles without others, B, both handler alleles present with others, C, one handler allele with no others, D, one handler allele with others, E, non-handler alleles only, or F, no callable alleles.

Level of trigger fragmentation and pipe bomb type compared to STR results

There was no indication that the amount of damage to the triggers correlated with STR analysis outcomes. Triggers at the highest damage classification yielded handler alleles while some with low damage had few or no handler alleles (Table 9). All triggers from IEDs deflagrated with steel pipe bombs had a consensus profile where at least one locus had only the handler's alleles, as opposed to seven of nine triggers from IEDs deflagrated with PVC pipe bombs. Full consensus profiles were obtained from three triggers associated with PVC and one with steel.

Table 9. STR results compared to damage classification and pipe bomb type

Steel		
Device #	Total number of loci	Damage Classification
14	1	3
15	3	3
17	7	3
16	8	4
19	5	4
20	1	4
18	5	5
21	6	5

PVC		
Device #	Total number of loci	Damage Classification
22	4	0
27	8	0
28	0	0
23	8	1
25	8	1
26	0	1
29	1	1
30	2	1
24	1	3

The amount of damage done to the mock triggers during deflagration was rated on a scale from 0 to 5, 0 being the lowest and 5 the highest. These damage classifications were compared to the total number of loci in the consensus profile for each trigger where only the handler's alleles were called.

Comparison of STR results among trigger components

Based on the scale from A – F that was used to rate each locus for the presence of the handler's alleles, the cell phones/radios had an average of 56 loci (of 136) (41%) in categories A and B, the clamp 47 (35%), the battery 36 (26%), the circuit board (average of front and back) 35 (26%), and the wire 29 (21%) (Table 10). No handler alleles were obtained from 66 (49%) of the cell phone or radio DNAs (average of the three swabs taken), 74 (54%) of the clamps, 81 (60%) of the batteries, 86 (63%) of the circuit boards (average of the two samples), and 94 (69%) of the wires.

Table 10. STR analysis compared to individual mock trigger components

	Phone or Radio Front	Phone or Radio Back	Phone or Radio Sides	Circuit Board Front	Circuit Board Back	Battery	Clamp	Wire
Total A	28	32	38	20	29	25	35	22
Total B	25	21	23	10	11	11	12	7
Total C	12	5	7	7	19	10	9	8
Total D	6	7	6	1	3	9	6	5
Total E	6	4	4	1	6	4	1	3
Total F	59	67	58	97	68	77	73	91

A scale of A – F was used to represent the number of handler's alleles obtained, where A represents the presence of both handler alleles without others, B, both handler alleles present with others, C, one handler allele with no others, D, one handler allele with others, E, non-handler alleles only, or F, no callable alleles.

STR amplification using 1µl DNA and the second set of triggers

Seven of ten triggers (devices 14, 15, 19, 24, 26, 28, and 30) showed an improvement in STR results when re-amplified using 1 µl of DNA, with an average increase of 33% (13% – 46%) in the number of loci that fell into categories A and B. Two triggers, 26 and 28, had no STR amplification using 4 µl of DNA, while re-amplification resulted in 15% and 46%, respectively, of loci falling into categories A and B. DNAs from the remaining 3 mock trigger DNAs amplified using 1 ul (triggers 20, 22, and 29) gave an average decrease of 17% of loci in categories A and B.

Combining data from the amplifications of 1 and 4 µl DNA to build a consensus profile gave better overall results than using only the initial amplifications (Table 11). Ninety-one of one hundred thirty six (67%) loci fell into category 1, 33/136 (24%) in category 2, 11/136 (8%) in category 3, and 1/136 (1%) in category 4. Fourteen of seventeen triggers had a profile in which at least 3 or 4 loci had only the handler's alleles

present, 11/17 had a profile with at least 5 or 6 loci, 8/17 had at least 7, and 5/17 had only the handler's alleles called at all loci.

The amount of damage to triggers 31 – 33, which used a steel pipe bomb, was similar to previous trials; however, much less damage was done to 34 – 36, which used PVC, due to the end cap of the pipe coming off during deflagration. STR analysis (based on consensus profiles) resulted in 38/48 (79%) of loci falling into category 1, 8/48 (17%) in category 2, 1/48 (2%) in category 3, and 2/48 (2%) in category 4. Five of six triggers had a profile where at least 6 loci had the alleles present, 4 of which had at least 7, with 2 of those having only the handler's alleles called at all loci.

Table 11. Evaluation of consensus profiles from initial amplifications and re-amplifications of 1 µl DNA combined

	D13	D7	Amel.	D21	D16	D18	CSF	FGA
14	2	1	1	3	2	3	1	2
15	1	1	1	1	1	2	1	1
16	1	1	1	1	1	1	1	1
17	1	1	1	1	2	1	1	1
18	1	1	1	2	2	1	1	1
19	1	1	1	1	1	1	1	1
20	2	2	1	2	2	2	2	2
21	1	1	1	1	2	1	1	3
22	1	2	1	1	2	2	1	1
23	1	1	1	1	1	1	1	1
24	1	3	3	2	1	2	2	1
25	1	1	1	1	1	1	1	1
26	3	3	1	2	3	3	2	4
27	1	1	1	1	1	1	1	1
28	1	2	1	2	2	2	2	1
29	3	2	3	2	2	1	2	1
30	1	1	1	1	2	1	1	1
31	1	2	1	1	1	1	1	1
32	1	3	1	1	1	1	1	1
33	1	1	1	1	1	1	1	1
34	1	1	1	1	1	1	1	1
35	1	1	1	1	4	2	1	1
36	1	2	1	2	2	2	2	2

A scale from 1 – 4 was used to represent the number of handler's alleles obtained using the consensus profile, where 1 represents the presence of both handler alleles only, 2, both handler alleles with others present, 3, one handler allele only, and 4, no handler alleles. The data include results from the original amplifications using 4 µl of DNA combined with selected samples that were re-amplified using 1 µl. Also shown are the second set of mock triggers (31 – 36) that were amplified using both DNA volumes.

DISCUSSION

Previous analyses of IEDs have focused mainly on class characterizations of the components used, analyzing trace evidence, chemical testing of the explosive charge, and examination for possible fingerprints (Shachtman 2007). With the increase in both international and domestic IED threat, it is important that the investigative techniques used for analyzing attacks continue to develop. In recent studies, DNA obtained from the explosive charge has shown some success in providing a genetic profile of individuals that may have come into contact with the device (Esslinger et al. 2004; Kremer 2008; Foran et al. 2009); however, the amount and quality of DNA is generally insufficient to reliably obtain the handler's profile on a case by case basis. The focus of this study was to determine if the chance of identifying the handler of an IED after deflagration could be increased by analyzing other components of the IED, such as electronic triggering mechanisms. There are several reasons why the trigger may produce more reliable and consistent results as compared to the explosive charge itself. One is the increased distance of the trigger components to the explosive charge where the heat and pressure from deflagration would have less of an impact on DNA. In this study a sort of "worst case scenario" was tested with the trigger components being positioned within 6 inches of the explosive device. As a result of their close proximity, many of the mock triggers were extensively damaged. It is likely that DNA on the components was also affected, which may be one of the reasons that only a partial or no profile was obtained from many of the trigger component DNAs, as opposed to the analysis of DNA from IED containers where a full profile of the handler was almost always obtained (Hoffmann et al. 2011);

however, there was still an increase in success over previous studies that focused on the explosive charge alone (Esslinger et al. 2004; Kremer 2008; Foran et al. 2009). Petrovick and Harper (2011) carried out a similar study where approximately 10 ng of extracted buccal cell DNA was deposited on IED triggers that were then placed 1 – 5 meters from an explosive device (using C4, Composition B, and ammonium nitrate) to determine if the effects of deflagration on DNA varied at different distances. They found that the amount of DNA lost during deflagration decreased as the distance of the trigger to the explosive increased and that at 1 meter approximately 49% of the handler's alleles were obtained while at 5 meters it increased to 95%. Given the drop-off in success from 5 to 1 meter, it is not surprising that even fewer alleles were detected in this study when the trigger was placed very close to the explosive, particularly considering that shed epithelial cells were the source of DNA in this study, rather than high molecular weight DNA. If the mock triggers were placed further away from the pipe bombs it is likely that there would have been an increase in success of determining the handler's profile.

Another potential advantage of analyzing electronic triggers results from their construction. Since the mechanisms are made of multiple individual components, there may be less fragmentation of each as they separate during deflagration, resulting in easier recovery and swabbing. In most cases the components in the current study remained intact but separated from the plywood base, however many times they were completely destroyed. The tendency for the phones and radios to fragment more than the other components may have been due to their size and complexity in that they were the largest elements and consisted of many smaller parts that separated during deflagration. This was especially true for the radios, which were made with a brittle plastic. The other

components were smaller and generally comprised of a single solid piece and constructed of metal (clamps, batteries, and wire) or tougher plastic (circuit boards). Since deflagration was done in a metal crate rather than out in the open some of the fragmentation potentially occurred when the components collided with the container. It is difficult to determine the ease of 'real world' trigger component recovery compared to the explosive device since both remained inside of the containment crate in this study. Generally large pieces of the steel pipes were recovered while both large and small pieces of the triggers remained. Collecting pieces of the PVC pipe was difficult considering that it fragmented into numerous small pieces during deflagration.

A third advantage of analyzing the IED trigger over the explosive charge was that trigger assembly might require longer handling time resulting in more accumulated DNA. In a study by Gomez (2009), multiple pipe bombs (PVC and steel) were swabbed after deflagration and an average of 0.023 ± 0.022 ng (median of 0.007 ng) DNA was obtained from each pipe. A significantly higher amount of DNA was obtained per swab set from the triggers used in this study, with an average of 0.497 ± 0.800 ng (median of 0.206 ng; $p = < 0.01$); however, it was originally thought that even more DNA would be obtained considering increased handling of the trigger components. Kisilevsky et al. (1999) showed that DNA transferred through sloughed epithelial cells persists on rougher surfaces longer than smooth surfaces. All of the components incorporated in the mock triggers had smooth surfaces (except for the unanalyzed plywood bases), which may have affected the retention of DNA after handling. The only rough areas on any of the triggers were the mounted electrical components on some circuit boards. Swabbing these areas was extremely difficult since the cotton from the swab often became snagged. Petrovick

and Harper (2011) recommend the use of foam swabs for rough surfaces, and while not used in this study, they may provide advantages over cotton swabs for the circuit boards or other rough/sharp areas. Lowe et al. (2002) proposed that some people leave behind more DNA than others and different conditions such as hand washing, length of handling, and time after contact affect DNA deposition as well. It is possible that a combination of these factors had an impact on the number of skin cells that remained on the triggers. In this study participants were asked to handle each of the components for roughly 20 seconds. Had they been handled for a longer period of time, which would closer resemble actual assembly of an IED that incorporated a triggering mechanism, more cells/DNA would presumably have been deposited. This would have likely increased overall success in determining the handler's profile. For the second set of mock IEDs that were used to address this, participants handled the components for an extended period of time. As a result of longer handling and multiple amplifications (discussed below) there was an increase in success of obtaining the handler's profile.

One of the goals of this project was to determine which IED trigger components were the best for analysis. Petrovick and Harper (2011) recovered very little DNA from wire and asserted that the probability of obtaining a profile from it is low. In this study, similar quantities of DNA were isolated from the wire as the battery and clamp, and partial profiles were obtained from each. Therefore, it seems that all components of the trigger can retain enough DNA for analysis and should not be overlooked, although the greatest amount of DNA and highest number of handler's alleles originated from the phones and radios, perhaps making them the "best" component for analysis.

It was originally thought that by using multiple swabs to cover larger surfaces, more DNA would be recovered, consequently three swab sets were used for the phones/radios (front, back, and sides) and two for the circuit boards (front and back); however, it is possible that more handler alleles could have been obtained if the DNA was collected onto one swab rather than being separated onto multiple swabs. While a comparison of using one swab vs. multiple swabs to collect the handler's DNA was not included in this study, work by Richert (2011) showed that using one swab to cover all surfaces of a firearm was more advantageous than using multiple swabs. Had DNA from the phones/radios and circuit boards been combined, it is possible that more complete profiles could have been obtained from those two components. On average each sample from the front, back, and sides of the phones/radios yielded 0.40 ng human DNA while the average from the front and backs of the circuit boards was 0.52 ng (excluding the negative control and device #14 which had an unusually high amount of DNA on the phone's front, presumably from contamination), so generally less than 0.5 ng was being amplified since the entire extract was never consumed for one amplification. If the DNA had been collected together, the average amount of total human DNA would have likely been around 1.2 ng for the phones/radios and 1.04 ng for the circuit boards, which would have resulted in more DNA available for amplification. A potential disadvantage of using one swab is that multiple handlers would result in a mixture. Using multiple swabs in these circumstances may be beneficial in differentiating individuals. The effects of this would not have been seen in this study because the components were cleaned in advance and only one volunteer handled them.

Since there was no statistical difference in the amount of DNA obtained from triggers when a steel or PVC pipe bomb was used, it is possible that the pipe material does not play a role in DNA retention or recovery from IED triggers; however, more damage was done to the mock triggers of IEDs that incorporated a steel pipe bomb, even though the same type of propellant was used. Due to the increased strength of steel over PVC there was much more pressure built-up during deflagration and subsequently greater concussion on objects around the explosive, with more physical damage done. Further, there was more partially burned and unburned powder on the components when PVC was used, which stemmed from the weaker casing separating before all of the powder was completely consumed. Other evidence of this comes from video of one of the mock IED deflagrations that used a PVC pipe: substantial flames came out of the metal container during deflagration indicating that there was a great deal of burning powder being expelled from the casing.

The quality of DNA isolated from the IED triggers was likely affected by deflagration. Initial STR analysis of DNA from trigger 23 components resulted in almost no usable data, even though quantification indicated there was enough DNA to obtain a genetic profile and PCR inhibition was not detected. This discrepancy may have resulted from DNA degradation. The Quantifiler amplicon size is 62 bases (Quantifiler User Guide) whereas the Minifiler size range is roughly 75 – 285 bases (Minifiler User Guide). The smaller amplicon will be less influenced by degradation, so there was possibly a reduced effect on quantification compared to STR amplification. To test this, selected samples were re-cleaned using Microcon YM-100 columns, which have a nucleotide cutoff of 125 bases (as opposed to the YM-30 cutoff of 50 bases). Though these samples

were not re-quantified for a direct comparison to the STR amplification, by filtering out the smaller DNA fragments more complete profiles were obtained.

The black residue on the swabs from the trigger components was removed during extraction; however, it is possible that other contaminants remained. The IPC included in Quantifiler indicated inhibition in 26 samples, 19 of which originated from mock triggers incorporating a steel pipe bomb. Based on these findings it seems possible that inhibition from the steel pipe bombs was greater, even though there were larger amounts of black residue on the trigger components of IEDs using PVC.

Minifiler provides improved amplification of DNA containing inhibitors as compared to standard STR kits (Mulero et al. 2008) still, many of the trigger DNAs had little or no STR amplification, even when quantification results indicated the presence of DNA. This could have resulted from the amount of DNA/inhibitor being added to each reaction. One and two tenths microliters of template DNA was added to Quantifiler reactions (15 µl total volume) while 4 µl was added to Minifiler reactions (10 µl total volume). If inhibitors were present, they would have a higher impact on the Minifiler reactions where the DNA sample makes up 40% of the total reaction volume compared to 8% of the Quantifiler reaction. Additional evidence of PCR inhibition comes from the success of re-amplifications using less template DNA, where decreasing the input from 4 to 1 µl in the STR PCR resulted in more complete profiles from 7 of 10 triggers tested. For the re-amplifications in which profile success declined, the already low amount of DNA was likely decreased too much. Combining data from 1 and 4 µl amplifications resulted in an increase of overall success in determining the handler's profile. Subsequent findings supported this, where some amplifications from the second set of

IEDs gave better results using 1 µl DNA, while 4 µl was more suitable for others. Conversely, 32 samples quantified as having 0 ng of DNA, 9 of which produced a partial profile of two or more loci with the handler's alleles. This discrepancy may be attributed to a number of things, including the higher amount of DNA added to the Minifiler reaction. If stochastic sampling effects influenced both Minifiler and Quantifiler assays, Minifiler, with nine loci targeted, would have a higher chance of some loci amplifying than would Quantifiler, which targets only one locus. In addition, PCR inhibitors could have affected the Quantifiler reaction more than the Minifiler reaction. The buffer used with Minifiler was improved for amplification of low quality DNAs with inhibitors (Minifiler User Guide), including a "carrier protein" that is not found in the Quantifiler buffer. It is also possible that there was a mutation at the binding site of a Quantifiler primer or the TaqMan probe in some samples, which would decrease or even prevent amplification/detection of DNA. Either way, the data show that a zero quantification value from Quantifiler does not always indicate the absence of human DNA, which should be considered when determining whether to amplify a particular sample or not.

Rarely did analysis of trigger DNA result in a pristine profile. Often, degraded DNA profiles appear on an electropherogram as a "ski slope", where the peaks of the smaller amplicons are higher, and decrease in height as the size of the amplicons increase (Bessetti 2007). Sometimes this can lead to drop-out of the larger loci, resulting in a partial profile. Though this was occasionally observed, especially at FGA and D21S11, all loci seemed to be susceptible to drop-out. This indicates greater effects due to PCR inhibition than DNA degradation.

More than two peaks were present at several loci, making it difficult to determine which were the handler's alleles. Though contamination at any step could not be ruled out, it seems unlikely since there was very little DNA obtained from the negative control (device #13) indicating that cleaning prior to handling was effective and that methods used for analysis did not introduce extraneous DNA. Furthermore, there was no evidence of alleles matching lab personnel or any of the bomb squad members who may have come into contact with the device. The swabs taken from the front of the negative control phone recovered 0.45 ng DNA, which could have been carryover from its original owner. This might explain extra peaks in some samples from donated phones, though elimination standards were not obtained to confirm this. Benschop et al. (2011) found that increased forward (+1 repeat from the parent allele) and back (-1 repeat from the parent allele) stutter contributed to many of the drop-in peaks when multiple LCN DNAs were analyzed. They also noted that when the number of PCR cycles was increased, so did the ratio of stutter to the parent allele. The recommended PCR cycle number for Minifiler is 30 (Minifiler User Guide), while in this study 33 were used. In many of the samples analyzed in this study, increased stutter may have accounted for some of the extra alleles. This made interpretation of these profiles difficult, where in some cases a homozygote with high stutter was interpreted as a heterozygote. A similar problem was encountered with high heterozygote peak imbalances, which may also be in part attributed to the increased number of PCR cycles (Bright et al. 2010). However, in many of the interpretable profiles the peak heights were low. If the cycle number was reduced those peaks would likely have dropped out. This occurred in initial amplifications of

samples from device 23 using 30 cycles; however, an extensive study comparing cycle numbers was not undertaken.

The use of consensus profiles increased the reliability of typing the handler's DNA and was effective for excluding many drop-in alleles, including stutter. In addition, complete (or nearly complete) handler profiles were determined for some of the triggering mechanisms. In contrast, interpretation of individual profiles from each of the component DNAs resulted in elevated drop-in alleles and few or no full handler profiles. In many instances drop-out of handler alleles occurred at different loci for separate component's DNA amplifications. If those profiles were interpreted by themselves the result would be a partial profile. A more conservative approach was taken to build the consensus profiles in this study by including alleles that amplified from 2 or more trigger component DNAs, although this did result in some extra alleles. If an allele had to amplify three times to be included in the consensus, more of the extraneous peaks could be eliminated; however, fewer handler alleles would also have been called. This can be demonstrated by re-interpreting the data from the initial amplifications of 4 µl of DNA. Increasing the number of replicate alleles to three would have resulted in no change for 98/136 (72%) loci, drop-out of one handler allele for 16/136 (12%) of loci, drop-out of all alleles for 18/136 (13%) of loci, and inclusion of only the handler's alleles (where other extraneous alleles were previously included) for 4/136 (3%) of loci.

A potential drawback to the consensus approach used here is that by comparing DNA profiles isolated from different components of the trigger, there is an assumption that the same individual's DNA resides on each. That assumption is accurate for this study because all trigger components were cleaned in advance and only handled by one

individual. In a "real world" scenario the same may not be the case, and certainly they are unlikely to have been cleaned in advance. A better approach may be to amplify DNAs of each component in replicates (possibly using different volumes of template DNA) and determine a consensus profile using only those amplifications.

CONCLUSION

With an ever-increasing threat of IEDs, both domestically and internationally, it has become essential that investigative techniques into attacks be improved. Using the sensitivity of today's DNA analysis technologies, it is possible to use biological evidence from exploded IEDs to help investigators identify the perpetrator. However, there are many challenges associated with such analyses, considering the effects deflagration has on DNA.

Analysis of IED trigger components yielded more DNA and more complete genetic profiles than the explosive device; however, heat and pressure from deflagration still have a large impact on the quality of DNA isolated. Considering that IEDs are generally fabricated by hand, any of the components may retain enough DNA for analysis and should not be overlooked. In this study the handler's DNA was obtained from all trigger components in some instances, including a cell phone or radio, circuit board, battery, pipe bomb clamp, and wire. Since the amount of DNA recovered from IED trigger components is generally very small, LCN techniques such as the use of miniSTRs, increased PCR cycling, and amplifying in replicates with different volumes of DNA may be necessary. Finally, developing a consensus profile from multiple DNAs taken from the same triggering device results in a more complete profile of the handler.

Though there are still challenges that must be overcome when dealing with DNA samples taken from deflagrated IEDs, the analysis of triggering devices may be an important tool for investigating IED attacks, and depending on the circumstances of the attack could give investigators the best chance of determining the assembler. Due to the

increased complexity of IEDs today it is probable that bombs will include such a device, making them a worthwhile focus for DNA collection in future IED investigations.

APPENDIX A. By locus categorization of allele calls for each mock trigger

In order to evaluate the success in determining the expected allele calls from the interpretation of the unknown DNA samples, after comparison to the reference samples taken from the IED handlers, each locus was categorized on a scale from A – F:

A - Presence of both handler alleles without others
B - Presence of both handler alleles with others
C - One single handler allele present
D - One handler allele with others present
E - Amplification but of unexpected alleles
F - No callable alleles

Below are the classifications for every locus analyzed from the initial amplification of DNA taken from each mock IED trigger, except for 13 which served as a negative control.

	14a	**14b**	**14c**	**14d**	**14e**	**14f**	**14g**	**14h**
D13	F	F	F	F	F	F	F	F
D7	F	F	F	F	F	F	F	F
Amel.	F	F	F	F	F	F	F	F
D21	F	F	F	F	F	F	F	F
D16	F	F	F	F	C	C	F	F
D18	F	F	F	F	F	F	F	F
CSF	F	F	F	B	F	F	F	F
FGA	F	F	F	F	F	F	F	F

	15a	**15b**	**15c**	**15d**	**15e**	**15f**	**15g**	**15h**
D13	B	F	B	C	F	F	F	F
D7	F	F	A	F	F	F	F	F
Amel.	A	F	A	F	C	F	F	F
D21	A	F	A	F	F	F	F	F
D16	F	F	B	F	F	F	F	F
D18	B	B	F	F	F	F	F	F
CSF	B	F	A	C	C	F	A	F
FGA	A	F	A	F	F	F	F	F

	16a	16b	16c	16d	16e	16f	16g	16h
D13	B	A	B	F	F	C	B	F
D7	B	A	B	F	F	D	B	F
Amel.	A	B	A	F	F	A	A	F
D21	F	C	A	F	F	F	A	F
D16	B	A	B	F	C	F	B	F
D18	A	A	B	F	F	B	A	A
CSF	B	A	B	F	C	B	D	A
FGA	D	B	B	F	F	A	A	F

	17a	17b	17c	17d	17e	17f	17g	17h
D13	A	F	B	F	C	F	B	F
D7	F	F	A	F	F	F	A	F
Amel.	A	F	A	F	A	F	A	F
D21	F	F	D	F	A	F	A	F
D16	F	F	A	F	C	F	A	F
D18	F	F	B	F	A	F	A	F
CSF	A	A	A	A	A	A	A	A
FGA	C	F	B	F	C	F	A	F

	18a	18b	18c	18d	18e	18f	18g	18h
D13	D	B	A	F	B	F	A	F
D7	F	A	A	F	F	F	B	F
Amel.	A	A	A	F	A	F	A	F
D21	A	A	A	F	B	F	B	F
D16	C	C	C	F	C	F	A	F
D18	A	A	C	F	A	F	A	F
CSF	B	B	B	F	A	F	A	F
FGA	A	F	A	F	A	F	B	F

	19a	19b	19c	19d	19e	19f	19g	19h
D13	A	E	F	F	F	F	F	F
D7	F	F	F	F	F	F	F	F
Amel.	A	C	A	F	F	F	F	F
D21	A	C	F	F	F	F	F	F
D16	F	F	F	F	F	F	F	F
D18	A	B	A	F	F	F	F	F
CSF	A	A	A	F	F	A	F	F
FGA	C	F	F	F	F	F	F	F

	20a	20b	20c	20d	20e	20f	20g	20h
D13	F	D	D	F	A	F	B	D
D7	F	E	E	F	F	F	B	F
Amel.	C	A	C	F	C	C	A	F
D21	C	D	F	F	A	F	C	F
D16	E	B	D	F	E	F	E	A
D18	A	E	D	F	D	E	C	D
CSF	E	B	B	F	A	F	A	A
FGA	F	C	F	F	F	F	D	F

	21a	21b	21c	21d	21e	21f	21g	21h
D13	A	F	A	F	F	A	F	A
D7	A	F	F	F	F	C	F	C
Amel.	A	F	A	F	F	A	F	A
D21	D	F	C	F	F	A	F	F
D16	A	F	B	F	F	A	A	B
D18	B	F	A	F	F	D	A	A
CSF	A	F	C	C	F	A	C	A
FGA	E	F	E	F	F	D	F	E

	22a	22b	22c	22d	22e	22f	22g	22h
D13	A	B	F	A	B	B	B	F
D7	D	B	F	F	A	A	F	F
Amel.	C	A	F	C	A	A	A	F
D21	C	B	F	A	C	D	C	F
D16	E	F	F	B	B	D	F	F
D18	D	B	F	A	C	D	A	D
CSF	B	A	A	B	A	B	A	A
FGA	C	A	F	F	C	D	A	F

	23a	23b	23c	23d	23e	23f	23g	23h
D13	F	B	A	F	F	A	B	A
D7	E	A	A	A	F	C	A	C
Amel.	F	A	A	D	A	A	A	A
D21	F	A	C	B	F	A	A	C
D16	F	A	A	A	D	A	A	A
D18	C	A	B	B	C	A	A	C
CSF	F	A	A	A	A	A	B	B
FGA	F	A	A	C	F	B	F	B

	24a	24b	24c	24d	24e	24f	24g	24h
D13	F	F	F	F	D	F	F	F
D7	F	F	F	F	F	F	F	F
Amel.	F	F	F	F	F	F	F	F
D21	F	F	F	F	F	F	F	F
D16	F	F	F	F	F	F	F	F
D18	F	F	F	F	F	F	F	F
CSF	B	F	F	F	B	F	F	F
FGA	F	F	F	F	F	F	F	F

	25a	25b	25c	25d	25e	25f	25g	25h
D13	B	B	B	B	B	B	A	A
D7	B	A	B	B	B	B	D	B
Amel.	A	A	A	A	A	A	A	A
D21	B	B	B	B	A	B	F	A
D16	B	B	B	A	B	B	D	A
D18	B	B	A	A	A	A	D	A
CSF	B	B	A	A	A	A	C	B
FGA	B	A	B	B	B	B	D	A

	26a	26b	26c	26d	26e	26f	26g	26h
D13	F	F	F	F	F	F	F	F
D7	F	F	F	F	F	F	F	F
Amel.	F	F	F	F	F	F	F	F
D21	F	F	F	F	F	F	F	F
D16	F	F	F	F	F	F	F	F
D18	F	F	F	F	F	F	F	F
CSF	F	F	F	F	F	F	F	F
FGA	F	F	F	F	F	F	F	F

	27a	27b	27c	27d	27e	27f	27g	27h
D13	B	A	A	B	A	A	C	F
D7	B	A	B	A	A	C	C	C
Amel.	A	B	B	A	A	A	A	A
D21	A	A	A	A	F	F	F	C
D16	B	D	B	A	A	C	A	A
D18	B	B	A	A	A	A	F	C
CSF	B	A	A	A	A	C	C	A
FGA	B	A	A	A	C	C	F	F

	28a	28b	28c	28d	28e	28f	28g	28h
D13	F	F	F	F	F	F	F	F
D7	F	F	F	F	F	F	F	F
Amel.	F	F	F	F	F	F	F	F
D21	F	F	F	F	F	F	F	F
D16	F	F	F	F	F	F	F	F
D18	F	F	F	F	F	F	F	F
CSF	F	F	F	F	F	F	F	F
FGA	F	F	F	F	F	F	F	F

	29a	29b	29c	29d	29e	29f	29g	29h
D13	D	E	E	F	F	F	F	D
D7	F	D	D	F	C	D	C	B
Amel.	B	B	A	A	A	F	F	B
D21	E	D	C	F	E	F	F	F
D16	C	D	E	C	E	E	F	E
D18	A	A	F	F	C	D	F	E
CSF	C	D	F	E	C	C	F	D
FGA	C	A	D	C	F	F	F	C

	30a	30b	30c	30d	30e	30f	30g	30h
D13	F	F	F	F	E	E	F	F
D7	F	F	F	F	E	F	F	F
Amel.	F	F	F	F	A	A	F	F
D21	F	F	F	F	C	F	F	F
D16	F	F	F	F	B	B	F	F
D18	F	F	F	F	B	F	F	F
CSF	F	F	F	A	A	A	A	F
FGA	F	F	F	F	E	E	F	F

APPENDIX B. STR amplification using 1 µl of DNA compared to 4 µl

Below is a side-by-side comparison of the STR results obtained from separate amplifications of 4 µl and 1 µl DNA with the total number and percentage of each classification, A – F, used to determine the success in the interpretation. A represents the presence of both of the handler's alleles without others, B, both handler alleles present with others, C, one handler allele with no others, D, one handler allele with others, E, amplification but of unexpected alleles, or F, no callable alleles.

Amplification of 4 µl DNA

	14a	14c	14d	14g	14h
D13	F	F	F	F	F
D7	F	F	F	F	F
Amel.	F	F	F	F	F
D21	F	F	F	F	F
D16	F	F	F	F	F
D18	F	F	F	F	F
CSF	F	F	B	F	F
FGA	F	F	F	F	F

Tot. A	Tot. B	Tot. C	Tot. D	Tot. E	Tot. F
0	1	0	0	0	39
0%	3%	0%	0%	0%	98%

Amplification of 1 µl DNA

	14a	14c	14d	14g	14h
D13	B	F	B	A	A
D7	D	F	B	C	A
Amel.	A	A	A	A	B
D21	E	C	D	F	F
D16	E	E	D	A	E
D18	E	E	D	C	F
CSF	B	A	B	A	A
FGA	E	E	C	D	F

Tot. A	Tot. B	Tot. C	Tot. D	Tot. E	Tot. F
11	6	4	5	8	6
28%	15%	10%	13%	20%	15%

Amplification of 4 µl DNA

	15a	15b	15c	15f	15g	15h
D13	B	F	B	F	F	F
D7	F	F	A	F	F	F
Amel.	A	F	A	F	F	F
D21	A	F	A	F	F	F
D16	F	F	B	F	F	F
D18	B	B	F	F	F	F
CSF	B	F	A	F	A	F
FGA	A	F	A	F	F	F

Tot. A	Tot. B	Tot. C	Tot. D	Tot. E	Tot. F
9	6	0	0	0	33
19%	13%	0%	0%	0%	69%

Amplification of 1 µl DNA

	15a	15b	15c	15f	15g	15h
D13	C	D	B	D	B	A
D7	A	F	B	C	D	B
Amel.	A	A	A	C	A	A
D21	A	A	B	B	A	B
D16	A	B	B	B	A	A
D18	D	D	A	D	C	E
CSF	B	A	B	A	C	A
FGA	A	D	B	D	C	A

Tot. A	Tot. B	Tot. C	Tot. D	Tot. E	Tot. F
19	13	6	8	1	1
40%	27%	13%	17%	2%	2%

Amplification of 4 µl DNA

	19a	19b	19c	19f
D13	A	E	F	F
D7	F	F	F	F
Amel.	A	C	A	F
D21	A	C	F	F
D16	F	F	F	F
D18	A	B	A	F
CSF	A	A	A	A
FGA	C	F	F	F

Tot. A	Tot. B	Tot. C	Tot. D	Tot. E	Tot. F
10	1	3	0	1	17
31%	3%	9%	0%	3%	53%

Amplification of 1 µl DNA

	19a	19b	19c	19f
D13	C	D	B	A
D7	B	C	A	A
Amel.	A	A	A	A
D21	D	F	A	C
D16	A	C	B	A
D18	A	C	B	A
CSF	C	A	A	A
FGA	A	F	A	B

Tot. A	Tot. B	Tot. C	Tot. D	Tot. E	Tot. F
17	5	6	2	0	2
53%	16%	19%	6%	0%	6%

Amplification of 4 µl DNA

	20a	20b	20c	20g	20h
D13	F	D	D	B	D
D7	F	E	E	B	F
Amel.	C	A	C	A	F
D21	C	D	F	C	F
D16	E	B	D	E	A
D18	A	E	D	C	D
CSF	E	B	B	A	A
FGA	F	C	F	D	F

Tot. A	Tot. B	Tot. C	Tot. D	Tot. E	Tot. F
6	5	6	8	6	9
15%	13%	15%	20%	15%	23%

Amplification of 1 µl DNA

	20a	20b	20c	20g	20h
D13	F	C	F	F	F
D7	F	F	F	F	F
Amel.	F	C	A	F	F
D21	F	D	E	F	F
D16	F	F	F	B	A
D18	F	F	F	F	E
CSF	F	B	F	F	A
FGA	F	E	C	F	F

Tot. A	Tot. B	Tot. C	Tot. D	Tot. E	Tot. F
3	2	3	1	3	28
8%	5%	8%	3%	8%	70%

Amplification of 4 µl DNA

	22a	22b	22d	22e	22f	22h
D13	A	B	A	B	B	F
D7	D	B	F	A	A	F
Amel.	C	A	C	A	A	F
D21	C	B	A	C	D	F
D16	E	F	B	B	D	F
D18	D	B	A	C	D	D
CSF	B	A	B	A	B	A
FGA	C	A	F	C	D	F

Tot. A	Tot. B	Tot. C	Tot. D	Tot. E	Tot. F
13	11	7	7	1	9
27%	23%	15%	15%	2%	19%

Amplification of 1 µl DNA

	22a	22b	22d	22e	22f	22h
D13	F	B	A	F	B	F
D7	F	D	F	F	C	F
Amel.	F	A	C	C	C	F
D21	C	A	F	F	F	F
D16	E	B	B	B	B	F
D18	D	B	F	F	E	E
CSF	F	B	A	A	E	B
FGA	F	B	F	F	D	F

Tot. A	Tot. B	Tot. C	Tot. D	Tot. E	Tot. F
5	10	5	3	4	21
10%	21%	10%	6%	8%	44%

Amplification of 4 µl DNA

	24a	24b	24c	24e	24f	24g	24h
D13	F	F	F	D	F	F	F
D7	F	F	F	F	F	F	F
Amel.	F	F	F	F	F	F	F
D21	F	F	F	F	F	F	F
D16	F	F	F	F	F	F	F
D18	F	F	F	F	F	F	F
CSF	B	F	F	B	F	F	F
FGA	F	F	F	F	F	F	F

Tot. A	Tot. B	Tot. C	Tot. D	Tot. E	Tot. F
0	2	0	1	0	53
0%	4%	0%	2%	0%	95%

Amplification of 1 µl DNA

	24a	24b	24c	24e	24f	24g	24h
D13	C	A	D	E	F	F	A
D7	F	F	F	C	F	F	F
Amel.	C	F	C	C	F	C	C
D21	C	F	C	E	F	F	F
D16	C	C	C	C	C	F	E
D18	F	C	C	C	F	F	F
CSF	A	A	B	A	E	F	E
FGA	C	C	A	D	F	F	F

Tot. A	Tot. B	Tot. C	Tot. D	Tot. E	Tot. F
6	1	19	2	5	23
11%	2%	34%	4%	9%	41%

Amplification of 4 µl DNA

	26a	26e	26f	26g	26h
D13	F	F	F	F	F
D7	F	F	F	F	F
Amel.	F	F	F	F	F
D21	F	F	F	F	F
D16	F	F	F	F	F
D18	F	F	F	F	F
CSF	F	F	F	F	F
FGA	F	F	F	F	F

Tot. A	Tot. B	Tot. C	Tot. D	Tot. E	Tot. F
0	0	0	0	0	40
0%	0%	0%	0%	0%	100%

Amplification of 1 µl DNA

	26a	26e	26f	26g	26h
D13	F	E	F	C	F
D7	F	F	E	C	F
Amel.	F	C	C	C	C
D21	F	E	B	E	A
D16	F	C	E	A	F
D18	F	D	E	C	F
CSF	F	E	B	A	A
FGA	F	F	F	F	F

Tot. A	Tot. B	Tot. C	Tot. D	Tot. E	Tot. F
4	2	8	1	7	18
10%	5%	20%	3%	18%	45%

Amplification of 4 µl DNA

	28a	28c	28d	28e	28g
D13	F	F	F	F	F
D7	F	F	F	F	F
Amel.	F	F	F	F	F
D21	F	F	F	F	F
D16	F	F	F	F	F
D18	F	F	F	F	F
CSF	F	F	F	F	F
FGA	F	F	F	F	F

Tot. A	Tot. B	Tot. C	Tot. D	Tot. E	Tot. F
0	0	0	0	0	40
0%	0%	0%	0%	0%	100%

Amplification of 1 µl DNA

	28a	28c	28d	28e	28g
D13	E	A	A	E	A
D7	F	F	C	F	B
Amel.	F	A	A	F	A
D21	F	A	F	A	B
D16	E	F	F	A	B
D18	B	F	E	E	B
CSF	F	B	E	A	B
FGA	E	C	C	E	A

Tot. A	Tot. B	Tot. C	Tot. D	Tot. E	Tot. F
11	7	3	0	8	11
28%	18%	8%	0%	20%	28%

Amplification of 4 µl DNA

	29a	29c	29h
D13	D	E	D
D7	F	D	B
Amel.	B	A	B
D21	E	C	F
D16	C	E	E
D18	A	F	E
CSF	C	F	D
FGA	C	D	C

Tot. A	Tot. B	Tot. C	Tot. D	Tot. E	Tot. F
2	3	5	5	5	4
8%	13%	21%	21%	21%	17%

Amplification of 1 µl DNA

	29a	29c	29h
D13	F	E	F
D7	F	E	F
Amel.	A	F	F
D21	F	F	F
D16	F	C	D
D18	F	F	E
CSF	F	F	F
FGA	F	F	F

Tot. A	Tot. B	Tot. C	Tot. D	Tot. E	Tot. F
1	0	1	1	3	18
4%	0%	4%	4%	13%	75%

Amplification of 4 µl DNA

	30a	30b	30c	30d	30e	30f	30g
D13	F	F	F	F	E	E	F
D7	F	F	F	F	E	F	F
Amel.	F	F	F	F	A	A	F
D21	F	F	F	F	C	F	F
D16	F	F	F	F	B	B	F
D18	F	F	F	F	B	F	F
CSF	F	F	F	A	A	A	A
FGA	F	F	F	F	E	E	F

Tot. A	Tot. B	Tot. C	Tot. D	Tot. E	Tot. F
6	3	1	0	5	41
11%	5%	2%	0%	9%	73%

Amplification of 1 µl DNA

	30a	30b	30c	30d	30e	30f	30g
D13	A	A	A	A	A	A	B
D7	F	C	A	D	A	C	A
Amel.	A	A	F	A	B	A	A
D21	F	A	A	A	A	C	C
D16	A	A	B	B	E	A	F
D18	A	A	D	A	A	A	C
CSF	A	A	B	A	A	A	C
FGA	F	F	F	A	A	A	C

Tot. A	Tot. B	Tot. C	Tot. D	Tot. E	Tot. F
34	5	7	2	1	7
61%	9%	13%	4%	2%	13%

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