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PAPER

CRIMINALISTICS

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Investigative Studies into the Recovery of DNA from Improvised Explosive Device Containers*,‡

ABSTRACT: Apprehending those who utilize improvised explosive devices (IEDs) is a national priority owing to their use both domestically and abroad. IEDs are often concealed in bags, boxes, or backpacks to prevent their detection. Given this, the goal of the research presented was to identify IED handlers through postblast DNA recovery from IED containers. Study participants were asked to use backpacks for 11 days, after which they served as containers for pipe bombs. Eleven postdeflagration backpack regions likely to be handled were swabbed and analyzed via mini-short tandem repeats (miniSTRs) and alleles were called blind. An experimental consensus method was examined in which profiles from all regions were considered, to help identify spurious drop-in/out. Results were correct for all loci, except one that remained ambiguous. The results show that recovering DNA from IED containers is a viable approach for aiding in the identification of those who may have been involved in an IED event.

KEYWORDS: forensic science, improvised explosive device, backpack, miniSTR, consensus DNA profile, bomb container, MiniFiler, QuantiFiler, DNA typing

Improvised explosive devices (IEDs) have gained substantial publicity owing to their extensive use in ongoing conflicts in the Middle East, and have become the weapon of choice for insurgents and terrorists. Their effectiveness overseas has lead to concerns about an increased domestic threat, causing local, state, and federal officials to allocate substantial resources to their detection and defeat. This concern is well founded, given that the United States has shown vulnerability to IED attacks in the past, as exemplified by the Centennial Olympic Park and Oklahoma City bombings.

Ideally, an IED event is disrupted before the device is ever activated. Owing to this, the majority of U.S. research and funding toward IED defeat has been targeted at developing preventive measures against attacks (1). Unfortunately, the continuous evolution of IEDs, combined with the unlimited number of targets, probably makes complete IED defeat impossible. In the event that an IED is activated, the focus must then turn toward mitigating the effects of the device and apprehending those responsible. Traditionally, forensic analyses of postblast IED components have been restricted to fingerprint and trace examinations, with varying degrees of effectiveness.

Advances in the field of forensic biology have the potential to generate definitive identification of the manufacturers of IEDs. The discovery that brief contact between a person and an object is sometimes all that is needed to recover the handler's DNA (2) has resulted in genetic profiles being obtained from an array of handled objects (reviewed in [3]). However, working with limited quantities of DNA can be challenging as analyses are often marked by allele drop-in or drop-out, heterozygote peak imbalance, and increased stutter (4,5). Furthermore, DNA from shed skin cells is expected to be degraded, again hindering the ability to obtain DNA profiles. Finally, it is not uncommon for handled evidence to harbor substances that co-extract with DNA and inhibit its amplification (reviewed in [6]).

These difficulties are likely to be exacerbated in postblast IEDs in that the cells/DNA left on a device will have experienced tremendous heat and pressure, increasing DNA degradation. Large amounts of smoke, soot, and other potential PCR inhibitors can also be anticipated. Still, it is possible that DNA could be recovered from postblast IEDs, leading to identification of its assembler. Toward this end, the Forensic Science Program at Michigan State University, in conjunction with the Michigan State Police, has been investigating genetic identification of those who utilize IEDs. Initial attempts to generate DNA profiles from exploded pipe bombs utilized standard short tandem repeat (STR) methodology (7). That research was not conducted blind (i.e., profiles were known in advance), and only one full profile and a few partial profiles were obtained. Following this, blind mitochondrial DNA (mtDNA) profiling using a closed population of DNA contributors was undertaken, which resulted in greatly increased identification success (8). There, 18 of 38 bombs were correctly assigned to a single donor, seven were correctly assigned to a subset of donors with shared haplotypes, 12 bombs were not assigned, and only one was incorrectly assigned. These improved results likely stemmed from advantageous characteristics of mtDNA, including its high copy number (9) and overall resistance to degradation (10). However, the nature of mtDNA is that it is not individualizing, a substantial drawback in determining who assembled an IED, particularly when

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specific suspects have not been identified. The early research into obtaining DNA directly from IEDs was followed by a small number of more targeted studies, including preliminary investigations into how postblast evidence is collected, processed, and analyzed (11–13), which again met with varying success.

In the research presented here, a new approach was sought for identifying assemblers of explosive devices. IEDs are often concealed in a secondary container, such as a backpack, bag, or box, and it is plausible that a handler's DNA profile might be more efficiently obtained from the container than the IED itself, for several reasons. First, during the manufacturing and transportation process a perpetrator is likely to have extended contact with the IED container, allowing ample opportunity to deposit epithelial cells. Second, porous surfaces of IED containers may retain shed cells better than the smoother surfaces of pipe bombs. And finally, DNA on the container might undergo less destruction than that on the IED itself, leading to improved amplification and typing success. In the current study, participants used backpacks in everyday activities, which subsequently served as containers for pipe bombs that were deflagrated in a controlled environment by members of the Michigan State Police Bomb Squad. Backpack fragments were returned to the Michigan State University Forensic Biology Laboratory, where different sections were swabbed. The DNA was amplified using MiniFiler (Applied Biosystems, Foster City, CA), which is optimized for degraded and inhibited DNA (14). STR results were called blind, and were only later compared with known (buccal swab) results. The utility of considering consensus STR profiles was also explored, in which handlers' genotypes were predicted blindly based on all DNA typing results from a backpack, with a goal of identifying/omitting sporadic drop-in/out and assessing the effectiveness of this methodology.

Materials and Methods

IED Preparation and Deflagration

Ten pipe bombs (five galvanized steel and five PVC) were assembled. Bombs were 1 foot in length, 1 inch in diameter, with a pair of end caps, one of which had a 1/4 inch hole drilled in the center for fuse placement. Pipes and end caps were soaked for 1 h in 10% bleach, rinsed with distilled water, then placed in a Spectrolinker XL-1500 UV Crosslinker (Spectronics Corporation, Westbury, NY) for 10 min (*c.* 5 J/cm^2), turning halfway through. ELIMINase (Decon Laboratories, Bryn Mawr, PA) was applied to all surfaces according to the manufacturer's instructions, which were then rinsed twice using sterile water. Bomb components were dried in a laminar flow hood. End caps without the hole were affixed to PVC pipes using PVC cement; steel end caps were not fastened. Components for each bomb were placed in new paper bags. Steel bombs were assigned numbers 3–7, whereas PVC bombs were assigned numbers 8–12 (bombs 1 and 2, PVC and steel respectively, were used in preliminary studies).

600D polyester backpacks (LEED'S, Pittsburgh, PA) were autoclaved for 45 min at 135°C, followed by 45 min of drying at 100°C. The backpacks were irradiated in the UV crosslinker for 15 min (*c.* 7.5 J/cm^2) per side. This procedure eliminated all DNA on two backpacks used in preliminary studies (backpacks 1 and 2; no amplifiable DNA recovered), thus the remaining 10 backpacks were used in IED experiments. Backpacks 3–10 were randomly distributed to eight volunteers who used them for *c.* 11 days. Backpack 11 acted as a positive control in which, predeflagration, a participant handled the 11 targeted areas (five zippers, the top handle, the left and right strap, the neck region, the front middle region, and the front tab; Fig. 1) three times a day for 3 days, alternating the order in which the regions were handled. Backpack 12 served as a negative control. Backpacks were randomly assigned a number that was paired (anonymously) with a numbered buccal swab from each volunteer, which were not revealed until all DNA analyses were completed. Volunteer use followed guidelines established by the University Committee on Research Involving Human Subjects.

FIG. 1—*The regions of a backpack swabbed postdeflagration. Eleven areas of each backpack were targeted for postblast DNA recovery including the five zippers (Z1–Z5), top handle (TH), front tab (FT), front middle region (FM), left strap (LS), right strap (RS), and the neck region (NR). Also shown is an intact zipper with all four components including the base (B), pull (P), string (S), and tab (T). In many instances fewer than 11 targeted regions were recovered.*

Backpacks and pipe bombs were transported to a Lansing (MI) Fire Department firefighting training facility, and deflagrations were conducted in the facility's smoke room. Immediately preceding deflagration, a member of the Michigan State Police Bomb Squad filled the pipes with 1.5 ounces of Green Dot Smokeless Shotshell Powder (Alliant Powder Co., Radford, VA) and affixed the drilled end cap to the device. A fuse was inserted and the pipe bomb was placed inside the main pocket of the corresponding backpack, with only the fuse exposed. The backpack bomb was set inside a steel crate (Fig. 2

FIG. 2—*Exemplary backpack destruction inside the crate following deflagration of a PVC pipe bomb. Backpacks housing steel pipe bombs suffered considerably more fragmentation. The crate (inset), approximately 3 feet wide, was designed to limit the dispersal of IED and backpack fragments. Walls and lid were constructed of steel with holes cut in them to relieve pressure from the blast; the floor was made of wood.*

inset) and the fuse was lit through the circular hole in the front of the crate. Following deflagration, bomb and backpack fragments were collected and placed in a new paper bag, which were returned to the Michigan State University Forensic Biology Laboratory. The steel crate and smoke room were swept between deflagrations to remove any uncollected debris. All investigators involved in the deflagrations wore disposable sleeves, facemasks, and gloves.

DNA Recovery and Purification

Backpacks were individually processed in a laminar flow hood that had been wiped with 10% bleach and UV irradiated for 10 min. Each backpack was swabbed with sterile cotton swabs (Puritan Medical Products, Guilford, ME) in the 11 targeted areas (Fig. 1). Occasionally some of these areas were destroyed by the blast or were unrecoverable; the remainder were sampled.

All solutions, reagents, and consumables were filter sterilized (0.2 μm filter) and/or subjected to UV irradiation as appropriate prior to use. The double swab technique (15) was utilized in which a swab moistened with 150 μL of digestion buffer (20 mM Tris, 50 mM EDTA, 0.1% SDS, pH 7.5) was thoroughly passed over the targeted region, followed by a dry swab that was immediately applied to the same section. Both swabs (wet and dry) were placed in the same 1.5 mL tube and stored at −20°C.

DNA extractions were performed by adding 350 μL of digestion buffer (total volume of *c.* 500 μL when including the 150 μL previously added to the swabs) and 6 μL of proteinase K (20 mg/mL) to tubes containing the swabs, which were vortexed and incubated overnight at 55°C. Reagent blanks were initiated for each backpack. Following incubation, swabs were placed in a spin basket, centrifuged for 1 min at 17,950 ×***g*** (13,000 revolutions per minute) and discarded. The flow through was pipetted back into the original tube. An equal volume of phenol (500 μL) was added to the tube, which was vortexed and centrifuged at 17,950 ×***g*** for 6 min. The aqueous layer was transferred to a new tube and an equal volume of chloroform was added. The tube was vortexed and centrifuged for 6 min at 17,950 ×***g***. The aqueous layer was transferred to a Microcon YM-30 spin column (Millipore Corporation, Billerica, MA), and 100 μL of TE (10 mM Tris, 1 mM EDTA, pH 7.5) was added. The column was centrifuged for 12 min at 14,000 × *g*, followed by one wash with 200 μL TE and centrifuged for 8 min. The retentate was brought to 20 μL with TE, and stored at −20°C. Reference buccal swabs were extracted using a ChargeSwitch® Forensic DNA Purification Kit (Invitrogen, Carlsbad, CA) as per the manufacturer's protocol, and DNAs stored at −20°C.

DNA Amplification and Analysis

DNAs extracted from the backpack swabs were amplified using an AmpF*l*STR MiniFiler PCR Amplification Kit. Reactions were carried out in 10 μL volumes, including 2 μL of the MiniFiler primer set, 4 μL of the MiniFiler Master Mix, and 4 μL of DNA template. The template volume represented the maximum available for 10 μL reactions, which, based on our previous work on deflagrated IEDs [7,12,13] and our unpublished observations) rarely meets the desired DNA amounts for STR analysis. Reference sample reactions contained 1 μL of DNA diluted 1:100 in TE and 3 μL TE, whereas positive controls contained 3 μL of 007 control DNA (0.1 ng/μL; Applied Biosystems) and 1 μL of TE. Amplification conditions included an 11 min incubation at 95°C, followed by 30 cycles consisting of a 20 sec denaturation at 94°C, 2 min of primer annealing at 59°C, and a 1 min extension at 72°C, followed by a 45 min final extension at 60°C.

DNAs were prepared for electrophoresis by adding 2 μL of amplified product (1.5 μL for reference samples and allelic ladders) to 24.5 μL of deionized formamide and 0.5 μL of GeneScan™ 500 LIZ® Size Standard (Applied Biosystems). Electrophoresis was performed on an ABI PRISM® 310 Genetic Analyzer (Applied Biosystems) using the GS STR POP4 (1 mL) G5 v2.md5 run module (5 sec injection at 15 kV). Data were analyzed using GeneMapper® ID software v3.2.1 (Applied Biosystems).

Electropherograms were manually reviewed, and callable alleles (50 relative fluorescence units and above) were recorded. Mixtures that had clearly dominant peaks (i.e., a major contributor) were also noted. Next, data from each backpack were assessed concurrently to see if a single "consensus" profile could be developed based upon concordance of allele calls among the backpack samples, investigating if common alleles were attributable to the handler. No steadfast criteria were used for establishing consensus profiles due to the preliminary nature of this approach, although we continue to develop and assess such criteria based on this and other data sets. After handler profiles were called, a different investigator compared them to the known (buccal) profiles to evaluate the utility of this strategy.

The ability to successfully obtain results from the different backpack regions was examined for each locus, placing them into one of six categories based on the known profiles:

- The locus contained only the handler's alleles.
- The handler's alleles constituted the major profile (one or two dominant peaks), but other allele(s) existed.
- The locus had multiple alleles that included the handler's, but the handler's could not be distinguished as the major profile.
- The locus had one of the handler's alleles, either solely or with extraneous peaks.
- None of the alleles matched the handler's.
- No alleles were called.

The number of MiniFiler loci that met the first three criteria was categorized as: all 9, 8 or 7, 6 or 5, or <5. Each backpack region was analyzed across all handlers to assess which was most likely to produce an accurate DNA profile.

DNA Quantification Using Real-Time PCR

DNA volumes for amplifications were maximized in the above experiments as very low postblast DNA yields have been encountered in our previous IED experiments. Retrospectively, DNA yields from each backpack region were estimated using a Quantifiler Human DNA Quantification Kit (Applied Biosystems) to determine if DNA quantities correlated with STR results. Detection was performed on an iCycler thermal cycler and an iQ5 multicolor real-time PCR detection system (Bio-Rad, Hercules, CA). PCR reactions were carried out in 15 μL volumes consisting of 6.3 μL primer, 7.5 μL reaction mix, and 1.2 μL DNA. Standards were run in duplicate and unknowns in triplicate. Thermal cycling parameters included a 10 min incubation at 95°C followed by 40 cycles of 15 sec at 95°C and 1 min at 60°C. Average DNA concentrations and standard deviations for replicates were calculated.

Results

Deflagrations and DNA Recovery

Overall, the majority of backpack fragments were retained within the crate (Fig. 2), zippers being the exception. Areas of the backpacks that were stitched or made of stronger material, such as the

top handle, withstood the blast better than others. The backpacks containing steel pipe bombs suffered more damage than those housing PVC bombs. Backpack 8 briefly caught fire after the deflagration, with flames shaken out by a member of the Bomb Squad.

Seventy-five regions from the eight backpacks were recovered, ranging from seven from backpack 7 to 11 from backpack 10, with most of the variability caused by the failure to retrieve zippers; 28 of 40 zippers were located, four of which retained all components (Fig. 1). The origin of 21 of the 28 zippers could not be determined. The front middle section of backpack 3 was the only nonzipper region not recovered. DNA from one other region (front middle region of backpack 8) could not be analyzed due to a spin column malfunction during processing. Swabs were often soiled with residue from the explosives resulting in discoloration of the digestion buffer, most of which disappeared during the DNA extraction process.

MiniSTR Analysis

The postdeflagration positive control backpack produced handler's alleles from all regions tested, with two zippers experiencing substantial allelic drop-out. The negative control backpack had one zipper that had allele calls at all nine loci, which were not consistent with anyone involved in the study (researchers or participants). The remaining eight regions from that backpack produced a total of 10 callable peaks, all of low intensity. The reagent blanks processed with both control backpacks did not produce any callable alleles. The eight reagent blanks processed with backpacks 3–10 produced four low intensity alleles: the reagent blank for backpack 10 produced peaks for X and Y at amelogenin and allele 10 at CSF, whereas backpack 4 produced an 11 allele at D7. Positive and negative amplification controls processed in parallel with each set of backpack swabs gave expected results. One investigator (SGH) could not be excluded as a potential contributor to mixed samples in three instances, whereas remaining personnel did not contribute to any of the other mixtures observed.

Results from the different regions of experimental backpacks varied greatly; in some instances full profiles were obtained, whereas others showed signs of allelic drop-in/out, peak imbalance, and/or mixtures. Placing the >650 loci analyzed in this study into one of six quality categories (see Materials and Methods) produced 591 (88.7%) that contained the handler's alleles (categories 1–3 above; Fig. 3), half of which had alleles solely from the handler. Forty-one additional loci had one of two heterozygote handler alleles, 27 of which showed no other alleles. Thirty-one loci did not have any callable alleles, 16 occurring at the D7 locus. Only three loci solely contained callable alleles that did not originate from the handler.

The ability to recover DNA from the various backpack regions differed substantially (Table 1). Forty-six of the 74 regions analyzed had the handlers' alleles at all nine loci (i.e., all loci fell into categories 1–3), 30 of which had either the handlers' alleles only or the handler was the major contributor. The top handle was the most effective area for recovering DNA, producing the volunteers' full profiles in all instances. Other regions ranged from an average of 8.9 loci (neck region) down to 7 loci (front tab). Swabs of the zippers averaged the handlers' alleles at 7.4 loci, with intact zippers producing 8.8 loci and zippers missing both the base and tab averaging 5.3.

There was also variability among backpacks/handlers (Fig. 4). For instance, all swabs from backpack 4 produced full handler's profiles, and backpack 8 had full handler's profiles in nine of 10 swabs. Backpack 6 had the lowest number of full profiles (three),

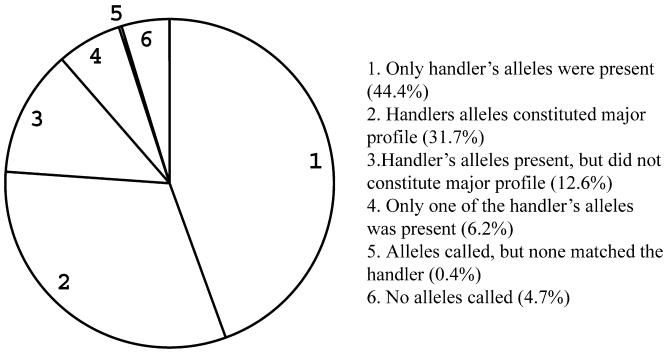

FIG. 3—*Percentage of loci from deflagrated backpack samples placed in each of six categories. The first three categories denote loci in which the handlers' alleles were present in their entirety (88.7%). The second and third categories denote loci that had callable alleles besides the handlers' (44.3%). The last three categories denote missing handlers' alleles (11.3%). The consensus method allowed data from all categories to be considered when developing a profile.*

TABLE 1—*Number of loci with handler alleles broken down by backpack region.*

	Number of Loci with Handler Alleles				
Swab Location	9	7 or 8	5 or 6	<5	Average
Zippers					
Intact	3	1	–	–	8.75
No tab	3	2	2	–	7.43
Only tab	2	2	–	–	8
Only string	1	2	–	–	7.67
No base	3	–	1	–	8
No base or tab	2	1	1	2	5.3
Top handle	8	–	–	–	9
Left strap	6	2	–	–	8.75
Right strap	4	2	1	1	7.75
Neck region	7	1	–	–	8.88
Front middle	5	1	–	–	8.83
Front tab	2	3	2	1	7
Total	46	17	7	4	7.97

The top handle produced all handlers' alleles in all cases (based on MiniFiler), followed by the neck region, front middle, left strap and intact zippers, right strap, and front tab. Fragmented zippers had more variable results.

but four swabs had the handler's alleles at seven or eight loci. Eleven swabs produced the handler's alleles at six or fewer loci, seven of which came from backpacks 9 and 10.

DNA Quantitation

DNA yields varied over several orders of magnitude across backpacks and regions swabbed (Table 2). The highest yield was 1.25 ng/μL from the neck region of backpack 4 whereas the lowest was 0.0014 ng/μL for the front tab of backpack 10. Fifty-three of 74 samples (71.6%) had DNA quantities below 0.2 ng/μL, whereas 11 had quantities above 0.4 ng/μL, nine of which originated from backpacks 4, 7, and 8. The majority of samples from these three backpacks had DNA quantities above 0.1 ng/μL, with only three samples below that value (a zipper and front tabs). In comparison, no samples from backpacks 6, 9, and 10 had a DNA quantity in excess of 0.1 ng/μL.

The top handle, straps, and neck region returned the highest average DNA quantities (*c.* 0.3 ng/μL), whereas the front tab

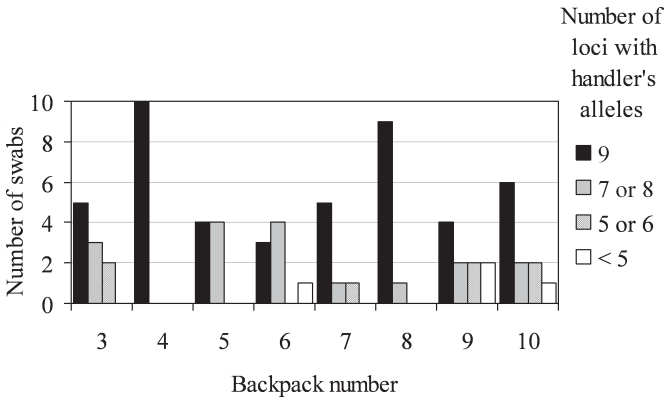

FIG. 4—*Number of loci containing handlers' alleles from backpacks based on the MiniFiler kit (nine loci). The majority of swabs (85%) had the handler's alleles at seven or more loci (black and gray bars). The highest percentage of swabs with the correct handler's alleles at all loci came from backpacks 4 (100%) and 8 (90%). Eleven swabs had the handler's alleles at six or fewer loci (striped and white bars) with seven coming from backpacks 9 and 10.*

averaged the lowest of the nonzipper regions (0.067 ng/μL). DNA yields from zippers averaged 0.091 ng/μL, with the four zippers recovered intact averaging 0.615 ng/μL. The next largest quantity of DNA was obtained from zippers missing just a base (0.162 ng/μL) and having only a tab (0.15 ng/μL), whereas swabbing only the string produced 0.041 ng/μL. Quantification further revealed that just two samples (3RSP and 7RSP) showed signs of inhibition based upon poor amplification of the QuantiFiler internal positive control; in both cases genomic DNA was still detected and alleles were produced.

The Feasibility of Developing Consensus Profiles

When all tested regions from a backpack were considered concurrently, extraneous alleles and potential drop-in/out became apparent (Table 3, and exemplified in Fig. 5). In total, seven of eight blindly predicted consensus profiles were identical to the participants' reference sample (Table 4). The lone exception was backpack 9, in which D21 produced a 30 homozygote from three swabs and a 30,31 heterozygote from six swabs, the latter being the correct call; the eight other loci were correctly identified.

Discussion

The research presented here demonstrates that it is feasible to recover DNA from postblast IED containers and correctly identify the handler of the container using miniSTRs. The high level of success in recovering and typing DNA from IED containers could stem from multiple factors, including the overall degree of contact with a handler, the ability of backpacks to harbor and retain shed cells, and/or the STR kit employed. Clearly, placing an IED in a container and transporting it is likely to generate ample opportunity to deposit shed cells. In this study, participants utilized the backpacks for *c.* 11 days and in all instances their DNA was recovered. The backpack regions selected for swabbing were based on those presumed to be used/handled by volunteers, however, not all regions were equally productive in producing alleles. A possible reason for this is that certain parts of the backpack, such as the front tab, were simply not utilized by the handler, resulting in limited DNA. The overall level of contact with any given backpack region remains unknown of course, and probably varied among individuals, but normal backpack use involves the straps being handled to wear it, including grasping and then putting the wearer's arms through them. Likewise, top handles are commonly used for backpack transport. The neck region, which often produced quality DNA profiles, would generally rub against clothes, skin, and hair during backpack use, again depositing cells. Unlike the potentially unused front tab, however, the poorer DNA results from zippers is probably not attributable to frequency of use, given they are an integral part of backpacks. There was noticeable variation in zipper results depending on the components that were swabbed (Fig. 1 and Table 1), with complete zippers producing the largest quantities of DNA, and zippers that retained tabs generally producing better results than those that did not. Once again utilization could influence the effectiveness of DNA typing, as the design of the zippers was such that the string and plastic tab would most likely be clasped to access the pockets.

The various backpack regions themselves may have also contributed to typing success. Total surface area was probably key to DNA recovery given that the zippers and front tab, the smallest regions sampled, routinely produced the poorest results. This

TABLE 2—*Quantities of DNA recovered from backpack swabs.*

	Backpack								
Swab	3	4	5	6	7	8	9	10	Average
Z1U	0.0991	0.165	0.00933	0.00857	0.104	0.165	0.0180	0.0256	*
Z2U	0.0686	0.393	0.00955	0.00230	–	0.242	0.0107	0.0125	*
Z3U	0.0476	0.149	–	–	–	0.149	0.00700	–	*
Z4U	–	0.251	–	–	–	0.0916	–	–	*
Z3	–	–	–	–	–	–	–	0.0286	0.0286
Z4	0.109	–	–	–	–	–	0.0268	0.0303	0.0553
Z5	0.0246	–	–	–	–	0.296	–	0.0132	0.111
TH	0.215	0.732	0.324	0.0570	0.307	0.566	0.0302	0.0804	0.288
LS	0.224	0.892	0.182	0.0401	0.189	0.805	0.0534	0.0658	0.306
RS	0.418	1.233	0.0488	0.0529	0.213	0.271	0.0356	0.0573	0.291
NR	0.292	1.250	0.0169	0.0641	0.192	0.534	0.0579	0.0586	0.308
FM	–	0.848	0.0435	0.0325	0.507	–	0.0359	0.0159	0.247
FT	0.0279	0.385	0.0158	0.00810	0.0637	0.0158	0.0172	0.00141	0.0669

Quantities represent the average of three replicates and are reported as ng/μL. Multiplying the quantities by four gives an estimate of how much DNA was used in PCR. Swab abbreviations are detailed in Fig. 1. A 'U' after a zipper indicates its origin was unknown. (–)—that region of the backpack was not recovered. (*)—averages for zippers from unidentified locations were not calculated.

TABLE 3—*Exemplary allele calls from backpack 8 used to form a consensus profile of the handler.*

	Locus								
Sample	D13	D7	AMEL	D2	D21	D16	D18	CSF	FGA
8Z1U	8,12;10	**8,11**	X,Y	20,21;17	28,30;31	9,11;12	15,16;13	**11,13**	18,24;19
8Z2U	**8,12**	**8,11**	**X**	20,21;16,18,24	**28,30**	**9,11**	15,16;13,17	11,13;9	18,24;20
8Z3U	8,12;9,11	**8,11**	**X**	20,21;18,25,26	28,30;31	9,11;12,13	15,16;12,13,17	11,12,13*	18,24;20,21,22
8Z4U	8,9,11,12*	8,9,11*	X,Y*	18,20,21,23,25*	**28,30**	9,11,12,13*	12,14,15,16,18*	10,11,12,13*	18,20,21,23,24*
8Z5	8,12;13	**8,11**	**X**	20,21;22,23,26	**28,30**	**9,11**	15,16;17	**11,13**	18,24;20
8TH	8,12;13	**8,11**	X,Y	20,21;16,17,18,22	28,30;31.2,32.2	**9,11**	15,16;17	11,13;10	18,24;19,20
8LS	8,12;13	**8,11**	X,Y	20,21;16,17,18,19	28,30;31,31.2	**9,11**	15,16;13,17	11,13;10,12	18,24;21,22
8RS	8,12;13	**8,11**	**X**	20,21;17,25	**28,30**	**9,11**	15,16;12	11,13;12	18,24;19,20,21,22
8NR	**8,12**	**8,11**	X,Y	20,21;16,17,18	28,30;31	**9,11**	**15,16**	**11,13**	**18,24**
8FT	NC	**8,11**	**X**	14,21 +	**28,30**	**9,11**	**15,16**	10,11,13*	18,19,21,24*
Blank	NC	NC	NC	NC	NC	NC	NC	NC	NC
Handler call	8,12	8,11	X	20,21	28,30	9,11	15,16	11,13	18,24
Subject F	8,12	8,11	X	20,21	28,30	9,11	15,16	11,13	18,24

Allele designations from recovered backpack regions (here backpack 8) were tabulated in order to blindly call a consensus profile for the handler of the backpack. Resultant consensus profiles were checked for concordance with reference profiles from study participants (''Subject''). Samples were identified by backpack number and region swabbed (see Fig. 1 for abbreviations). A 'U' after a zipper indicates its origin was unknown. Alleles are placed in categories denoted as: (Bold)—the locus contained only the handler's alleles; (no denotations)—the handler's alleles (left of the semicolon) constituted the major profile, but other allele(s) existed; (*)—the locus had multiple alleles that included the handler's, but those alleles could not be designated as the major profile; (+)—the locus had at least one of the handler's alleles; (NC)—no alleles were called. Blank is the reagent blank. Note that many of swabs produced potentially ambiguous profiles, while consensus alleles were all consistent with the handler.

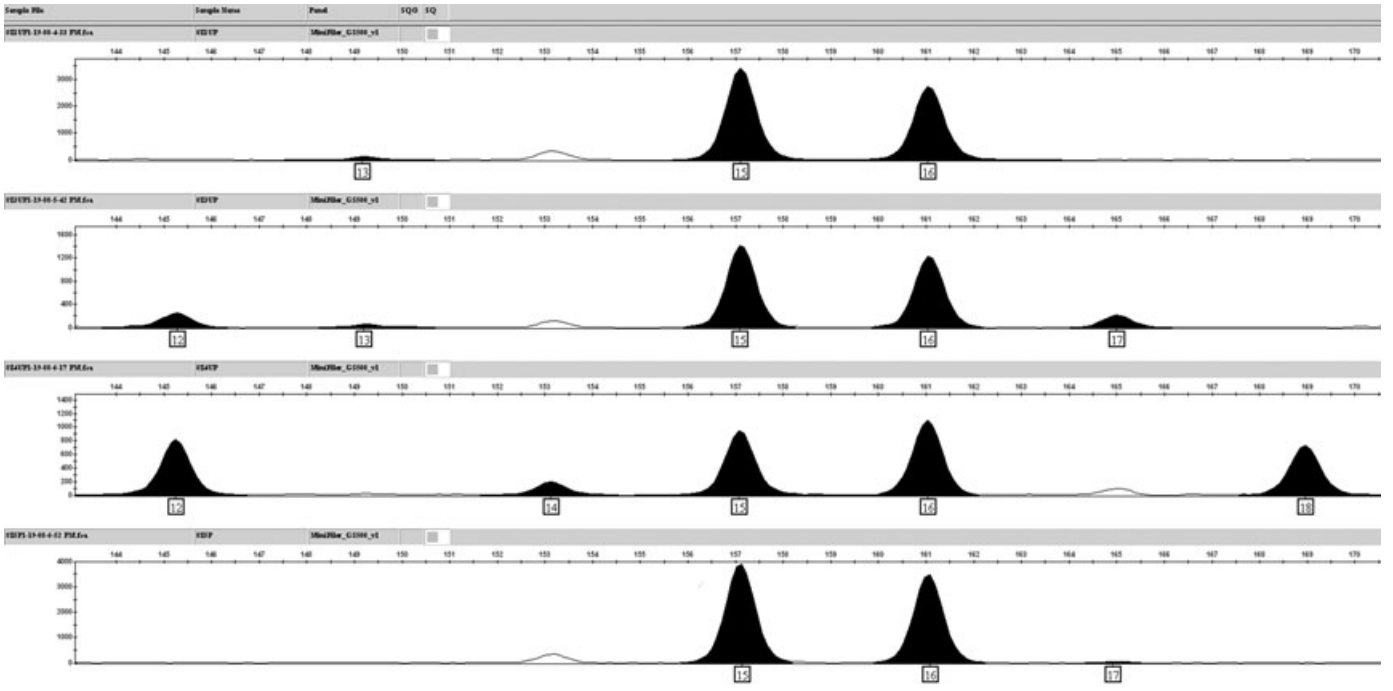

FIG. 5—*Exemplary consensus profile development based on D18 electropherograms from four backpack 8 zipper samples. All electropherograms show extra callable peaks that may lead to ambiguous profiles if considered individually or processed as a single swabbing. With the consensus procedures followed it becomes clear that the handler's alleles are likely 15,16.*

reinforces a common sense notion that swabbing larger surface areas will be more fruitful in general. Likewise, most surfaces were constructed of a polyester weave that seemed more prone to trapping cells than smooth surfaces, such as portions of the zippers. The most obvious disparity in surface texture was on the zippers, part of which were hard plastic, part metal, and part fabric. The former two were easy to swab, but their smooth surfaces may not have retained cells well, particularly after the violence of a deflagration. Furthermore, the zipper strings were often singed or melted, meaning cells might have been trapped within. Processing singed strings was difficult and frayed the cotton swab, which like other surface types may have affected cell/DNA recovery. The general poor recovery of DNA from the zippers was confirmed in the control handled backpack (#11), in which all zippers were handled three times daily, yet two of them produced very few alleles.

Use of the MiniFiler kit likely resulted in further advantages when typing DNA from postdeflagration backpacks. It is our experience that postdeflagration DNA is highly degraded, and thus smaller STR sizes undoubtedly led to more successful allele calls. Also, MiniFiler contains a proprietary buffer formulated to help

TABLE 4—*Consensus handler profiles from postblast backpacks and the study participant to which they corresponded.*

	Backpack							
Locus	3	4	5	6	7	8	9	10
D13	8,12	8,11	11,12	12	11,12	8,12	8,14	11,12
D7	10,11	9,10	11	10,11	8,12	8,11	8,10	9,12
Amel.	X	X	X	X	X	X	X	X
D2	23,24	20,25	23,24	20,24	20,21	20,21	17,20	19,25
D21	27,30	27,30	29,32	31,31.2	31,OL	28,30	*	29,30
D16	11,13	12,13	10	9,11	9,11	9,11	12,13	11
D18	15,17	13,16	16	13,14	17	15,16	13,16	13,16
CSF	11	12,13	11,12	11,12	10,11	11,13	12	12,13
FGA	21,24	21,25	21	20,25	22,23.2	18,24	20,22	22,23
Participant	B	E	G	I	J	F	A	H

Loci are from MiniFiler. All consensus allele calls were produced blind based on aggregate results from total swabs from a backpack (e.g., Fig. 5), which were then compared to known (buccal swab) results. In all instances the correct handler was identified. *Denotes there was no consensus between a 30 homozygote and a 30,31 heterozygote. OL was a consistent off ladder allele of 33.1.

alleviate PCR inhibition (14). It was not apparent from the QuantiFiler data that inhibition was a substantial problem, with only two samples showing reduced amplification of the internal control, both of which produced STR alleles. However, we have often encountered PCR inhibition in postblast samples, and the enhancers in the MiniFiler buffer may have helped improve results.

Finally, our previous research involving IEDs led us to investigate the utility of calling handler profiles using a consensus methodology. It should be noted that this method (examining allele agreement among swabs) is different than combining alleles/partial profiles from multiple swabs to *build* a profile, which could easily lead to errors. However, this approach *is* still experimental in nature and thus is not supported by the Scientific Working Group on DNA Analysis Methods (16), nor would a consensus profile be eligible for entry into the Combined DNA Index System under current guidelines. Instead, our research goal was to examine if alleles that were repeatedly seen from different regions of a backpack were attributable to the handler, whereas those that occurred once or very infrequently were spurious. This could be very useful if a person(s) other than the primary handler came into contact with some portion of the backpack. In this regard, the consensus profile experiment led to blindly generated, unambiguous, correct allele calls for all but one locus on one backpack, which was itself not incorrect, but simply could not be differentiated. Based on these preliminary results, ongoing research is being undertaken to further investigate the feasibility of the consensus methodology, with a focus on developing more definitive criteria for utilizing and implementing this approach.

Conclusions

The utilization of IEDs both domestically and abroad is a continuing concern, not only in light of the huge impact they have had in the Middle East, but also given their prevalence in domestic terror. Although disrupting all such devices before they are activated is the most desirable scenario, the constant evolution of IEDs probably makes that an unattainable goal. Given this, modern DNA profiling has the potential to be an important tool for identifying those who produce IEDs, as research in this area has shown a continuous improvement in our ability to identify assemblers/handlers of such devices. The results of this study demonstrate that targeting remnants of postblast IED containers for DNA collection and subsequent analysis can be advantageous for identifying a handler of an IED. With the interpretational methods employed, handlers' DNA profiles were fully discerned blind for seven backpacks, with the remaining backpack having only a single ambiguous locus. Furthermore, all 74 samples processed from the backpacks resulted in at least a partial profile, and oftentimes a full profile, of the handler. Naturally, the variability that IEDs display regarding their construction, concealment, and delivery will potentially influence DNA profiling results; however, targeting IED packaging proved to be a very legitimate tactic in this study. The information garnered from this research accentuates the importance of performing proper and exhaustive collections of postblast scenes, as information from any IED component may lead to valuable investigative information, including the DNA profile of a perpetrator.

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References

1. Wilson C. Improvised explosive devices in Iraq and Afghanistan: effects and countermeasures. CRS Report for Congress, November 21, 2007, http://assets.opencrs.com/rpts/RS22330_20071121.pdf (accessed November 19, 2010).
2. Van Oorschot RAH, Jones MK. DNA fingerprints from fingerprints. Nature 1997;387:767.
3. Wickenheiser RA. Trace DNA: a review, discussion of theory, and application of the transfer of trace quantities of DNA through skin contact. J Forensic Sci 2002;47:442–50.
4. Gill P. Application of low copy number DNA profiling. Croat Med J 2001;42:229–32.
5. Budowle B, Eisenberg AJ, van Daal A. Validity of low copy number typing and applications to forensic science. Croat Med J 2009;50:207–17.
6. Bessetti J. An introduction to PCR inhibitors. Profiles DNA 2007;10:9–10.
7. Esslinger KJ, Siegel JA, Spillane H, Stallworth S. Using STR analysis to detect human DNA from exploded pipe bomb devices. J Forensic Sci 2004;49:481–4.
8. Foran DR, Gehring ME, Stallworth S. The recovery and analysis of mitochondrial DNA from exploded pipe bombs. J Forensic Sci 2009;54:90–4.
9. Robin E, Wong R. Mitochondrial DNA molecules and virtual number of mitochondria per cell in mammalian cells. J Cell Physiol 1988;136:507–13.
10. Foran DR. Relative degradation of nuclear and mitochondrial DNA: an experimental approach. J Forensic Sci 2006;51:766–70.
11. Bille TW, Cromartie C, Farr M. Effects of cyanoacrylate fuming, time after recovery, and location of biological material on the recovery and analysis of DNA from post-blast pipe bomb fragments. J Forensic Sci 2009;54:1059–67.

12. Kremer SL. The use of miniSTRs and mitochondrial DNA to identify handlers of pipe bombs [thesis]. East Lansing (MI): Michigan State University, 2008.
13. Gomez KM. Recovery and quantification of nuclear and mitochondrial DNA from improvised explosive devices using comparative soaking and swabbing techniques [thesis]. East Lansing (MI): Michigan State University, 2009.
14. Applied Biosystems. AmpFlSTR® MiniFiler™ PCR amplification kit user guide. Foster City, CA: Applied Biosystems, 2006, http://www.appliedbiosystems.com.br/site/material/12tmnlnd.pdf (accessed November 19, 2010).
15. Sweet D, Lorente M, Lorente JA, Valenzuela A, Villanueva E. An improved method to recover saliva from human skin: the double swab technique. J Forensic Sci 1997;42:320–2.
16. Scientific Working Group on DNA Analysis Methods. SWGDAM interpretation guidelines for autosomal STR typing by forensic DNA testing laboratories, 2010, http://www.fbi.gov/about-us/lab/codis/swgdam.pdf (accessed February 15, 2011).

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