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TECHNICAL NOTE

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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*

ABSTRACT: This study investigated the effects of time, cyanoacrylate fuming, and location of the biological material on DNA analysis of post-blast pipe bomb fragments. Multiple aliquots of a cell suspension (prepared by soaking buccal swabs in water) were deposited on components of the devices prior to assembly. The pipe bombs were then deflagrated and the fragments recovered. Fragments from half of the devices were cyanoacrylate fumed. The cell spots on the fragments were swabbed and polymerase chain reaction/short tandem repeat analysis was performed 1 week and 3 months after deflagration. A significant decrease in the amount of DNA recovered was observed between samples collected and analyzed within 1 week compared with the samples collected and analyzed 3 months after deflagration. Cyanoacrylate fuming did not have a measurable effect on the success of the DNA analysis at either time point. Greater quantities of DNA were recovered from the pipe nipples than the end caps. Undeflagrated controls showed that the majority (>95%) of the DNA deposited on the devices was not recovered at a week or 3 months.

KEYWORDS: forensic science, DNA typing, polymerase chain reaction, Identifiler®, pipe bomb, cyanoacrylate fuming

A pipe bomb is a fairly simple form of an improvised explosive device. The basic components of the device (pipe nipple, end caps, black powder, and fuse) are readily available at common hardware stores and hobby shops. Approximately 3000 pipe bomb investigations were reported to the Bureau of Alcohol, Tobacco, Firearms and Explosives (ATF) over the last 10 years (ATF database). If the device is detected prior to deflagration, the device can be rendered safe through several means which leave any physical evidence relatively unharmed. In some instances, the only way to render the device safe is to cause the deflagration of it in a controlled manner. After a device is deflagrated, in a controlled or uncontrolled manner, any physical evidence that was on the device has now been subjected to extreme insults including exposure to high temperatures and the products of combustion, in addition to any other environmental insults.

In the past, the investigation of a pipe bomb incident involved several types of examinations within the laboratory, including the latent fingerprint, tool marks, and explosive residue examinations, but typically not DNA analysis. As knowledge and technology have improved in the collection, extraction, amplification, and typing of biological material, the range of evidence potentially suitable for DNA analysis has expanded to include touch evidence (1–3). It was hoped that new DNA technologies would enable the DNA section to aid in the investigation of these cases by potentially identifying the maker of the device through the analysis of the biological material transferred to the components during its assembly. The feasibility of this was demonstrated previously by Esslinger et al. (4). In that study, the pipe bomb components were handled by individuals prior to the deflagration. After deflagration, the fragments were recovered and short tandem repeat (STR) DNA analysis was performed. A full profile and multiple partial profiles were obtained.

Once it was determined that DNA of sufficient quantity and quality survived on post-blast pipe bomb fragments, it was important to investigate several practical aspects of the analysis. In this study, the following factors were investigated: time between the deflagration of the device and DNA analysis, cyanoacrylate fuming of the fragments soon after deflagration, and the location of the biological material on the device.

Most disciplines within the crime laboratory have significant backlogs and the DNA analysis section is typically no different. These backlogs can cause delays in the analysis of evidence for months. For dried blood or other biological evidence, this delay will have little to no effect on the success of the DNA analysis. It is unknown, however, what effect, if any, time has on the DNA analysis of post-blast bomb fragments that have been subjected to a different set of environmental insults. In addition to extreme heat, the DNA is also potentially exposed to the products of combustion which have unknown effects. If it is determined that the DNA is significantly degraded over time, then it may be necessary to prioritize pipe bomb cases to reduce the amount of time between the device deflagration and the DNA analysis of the collected fragments.

Latent print examination and DNA analysis are frequently requested on the same items of evidence. The effects of most of the common latent print chemicals have been investigated and have been found to have little to no detrimental effect on the DNA analysis (5,6). Recently though, cyanoacrylate fuming has been found to decrease the amount of DNA recovered from latent fingerprints (7). However, the differences in these results might be because of

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the different cyanoacrylate fuming methods used in each of the studies. To prevent damage or obliteration of latent fingerprints on items of evidence that may occur during transport or subsequent handling, the ATF Laboratory encourages its agents to cyanoacrylate fume evidence in the field, when practical. For the purposes of this investigation, it was hypothesized that cyanoacrylate fuming may protect the biological material deposited on the post-blast fragments by preventing the biological material from being scraped off and creating a barrier, thus preventing the products of combustion or other substances in the environment from degrading the DNA.

During the manufacture of the device, both of the main components of the device, the pipe nipple and the end caps, potentially may be handled by the individual. At the time of deflagration, the propellant contained within the sealed device burns rapidly and builds up extreme pressure to the point that exceeds the structural limits of one or more components of the device. The pressure built up in the device can be relieved in several ways (see Fig. 1). The end caps may be fragmented, the pipe nipple may rupture, or a combination of both can occur. The heat generated by the burning of the black powder inside the device will be transferred to the components. The "maximum temperature of explosion" for black powder is *c*. 2380°C (8). Similar temperatures would be expected for the powders used in this study. How much of the thermal energy reaches the outer surfaces of the device components is unknown, however. Because the biological material spotted on the sides of the end caps has two layers of metal between the surface and the interior of the device (threaded portion of the pipe nipple and the side wall of the end cap), it was thought that biological material on the outer surface of the pipe nipple would be subjected to greater temperatures because of the conductivity of the metal than material on the sides of the end caps. On the other hand, the end caps may demonstrate greater fragmentation and physical abrasion as a result of the deflagration. The proximity of the biological material to the point of rupture and thus release of the heated gases may affect the DNA more than the heat conducted through the pipe. This study compares the success of DNA typing on biological material deposited on the sides of the end caps and the pipe nipples to determine which components are more likely to be useful for DNA analysis.

Methods

To perform this study, six pipe bombs were assembled. The components (pipe nipple and end caps) for devices were purchased at a local hardware store. A ¼″ hole was drilled in the top of one end cap of each device to insert the fuse. The pipe nipples, 1″ × 8″ galvanized steel, and associated end caps were cleaned with 10% bleach (0.615% sodium hypochlorite) and then rinsed with 70% ethanol.

To deposit cells and thus DNA on the devices, Esslinger et al. (4) attempted to create a real world pipe bomb event by having individuals handle the components. Because a wide variation in the amount of cellular material deposited on an item by a single person or between persons during handling has been observed (9), in this study, a cell suspension was used to ensure a consistent amount of biological material on each of the areas analyzed. This protocol allows for a more direct examination of the effects of the deflagration without the variability in the initial quantity of DNA present. Two buccal swabs were collected from a female individual not involved in the study to create a cell suspension. The swab heads were placed in a 1.5 mL centrifuge tube with 1 mL of nuclease-free water and vortexed for 10 sec. The swab heads were removed and the tube was centrifuged at 12,500 × *g* for 3 min to pellet the cells. The supernatant was removed and the cells were resuspended in 1 mL of nuclease-free water. This process was repeated a second time to wash the cells. Finally, the cells were suspended in 1 mL of nuclease-free water. An approximate cell count was performed by microscopically counting the cells in three 2 μL spots and using the average. The cell concentration was estimated at 1000 cells per 2 μL. Six 10 μL aliquots of the cell suspension (*c*. 30 ng of DNA) were deposited on each of the end caps and pipe nipples in spots circled by an indelible marker to allow for easy post-blast collection of the cell spots. The cell spots were allowed to dry at room temperature overnight. Two control pipe nipples and end caps, not to be deflagrated, were prepared in a similar manner and transported with the rest of the devices. One set of components was to be cyanoacrylate fumed, the other was to be left untreated. Unfortunately, the control components were lost at the explosive range.

The actual assembly of the devices was performed immediately before deflagration by ATF explosive enforcement officers at the National Center for Explosives Training and Research at Fort AP Hill in Virginia. The officers wore latex gloves during the assembly process. Three different black powder substitutes were used in the devices: GOEX Pinnacle Powder™ (GOEX Powder, Dayline, LA) was used in devices 1 and 2; Jim Shockey's Gold Powder™ (American Pioneer Powder, Inc., Boca Raton, FL) was used in devices 4 and 5; and Triple 7 Powder™ (Hodgdon, Shawnee Mission, KS) was used in devices 7 and 8. Devices 3 and 6 were not used for this study. The devices were deflagrated in a manner to maximize the recovery of the pipe bomb fragments while maintaining at least some of the cell spots. In previous attempts, the pipe bombs were buried in sand and then deflagrated, which is the typical method employed to recover fragments for studies involving explosive residue testing. No DNA was recovered from any of the fragments recovered. It was thought that the physical abrasion on the surface of the fragments removed most, if not all, of the

FIG. 1—*Fragments collected from three of the devices post-blast. Different levels of fragmentation were observed depending on the brand of powder used in the device. The circles marking the areas where cell spots were deposited can be seen on some fragments. (A) Device #2, Go Ex Pinnacle, (B) Device #4, Jim Shockey's Gold, and (C) Device #7, Triple 7.*

FIG. 2—*Demonstration of how the devices were prepared to prevent cross-contamination of fragments and maximize the number of fragments collected. (A and B) The rolls of wire fencing were crimped on one end and (C) the rolls of wire fencing were placed in 2–3 foot deep trenches.*

biological material. In this study, a roll of wire fencing was crimped on one end (see Fig. 2) and the rolls were placed in individual trenches measuring *c.* 2–3 feet in depth. Each assembled device was then placed in the center of a roll of wire fencing. Pyrotechnic fuses and double primed electric matches were used to initiate the deflagration.

The fragments from each device were then collected by individuals wearing latex gloves and placed in individual metal paint cans. Fragments from the devices were found lying in the trench, embedded in the sides of the trench, and caught in various layers of the roll of wire fencing (see Fig. 3). The fragmentation of the devices varied depending on the type of powder used. For example, devices 1 and 2 resulted in extensive fragmentation of the pipe nipples as well as the end caps. In contrast, essentially only the end caps fragmented for the other devices. While the comparison of the fragmentation of the devices depending on the powder used was not an original intention of the study, the resulting fragmentation may have an effect on the DNA typing success.

The fragments were then transported to the laboratory for examination. The following day, the fragments from devices 2, 5, and 8 were cyanoacrylate fumed following the standard protocol used at the ATF Laboratory. The fragments were placed in a Foster and Freeman (Sterling, VA) MVC 3000 chamber set at 75% relative humidity. The process is comprised of the following steps: a 12-min humidifying cycle, a 10-min step in which the glue is heated at 120°C, and a final purge of 20 min.

The fragments were stored over the weekend at room temperature. The biological material from two cell spots from the fragments of the end caps and the pipe nipples of each device was collected on the tips of cotton swabs using the double swab technique (10) for four pairs of swabs per device for the initial analysis. Cell spots with obvious physical abrasions were avoided as were cell spots within scorched areas. The tips of each pair of swabs were cut into a single 2 mL centrifuge tube. By collecting the biological material on the tips of the swabs and only cutting off the relevant portion of the swabs, the volume of lysis buffer was sufficient to completely cover the swab material. Additionally, this protocol minimized the amount of swab material which may trap the cells, and therefore the DNA, during extraction process. The DNA was extracted and purified utilizing a slightly modified Qiagen QIAamp® DNA Micro Forensic Sample protocol (11). The swabs were incubated in 400 μL of Qiagen Buffer ATL and 20 μL of Proteinase K (20 mg/mL; Invitrogen, Carlsbad, CA) overnight at 56°C in a thermal mixer (Eppendorf, Westbury, NY) rotating at 900 rpm. The following day, 400 μL of Qiagen Buffer AL and 1 μg of carrier RNA (12) were added to the tubes. The tubes were vortexed for 15 sec and then incubated at 70°C in the thermal mixer rotating at 900 rpm for 10 min. The swab tips were then transferred to a SpinEze™ basket (Fitzco, Spring Park, MN) and the basket replaced in the tube. The tubes were centrifuged for 3 min at 12,500 × *g* to collect any lysate remaining in the swab tips. After centrifugation, the basket and swab tips were discarded and the lysate was transferred to the top of a Qiagen QIAamp® DNA Micro column. The lysate was passed through the column's membrane by centrifugation at 6000 × *g* for 1 min. The DNA bound to the membrane was washed with 500 μL of Qiagen Buffer AW1 and then 500 μL of Qiagen Buffer AW2. The membrane was then dried by centrifuging the columns at maximum speed for 3 min. The DNA was eluted with two 50 μL volumes of TE^{-4} (10 mM Tris–HCl, pH 8, and 0.1 mM EDTA) collected by incubation at room temperature for 5 min and then centrifugation at 13,500 × *g* for 1 min. The final elution volume of 100 μL was then concentrated down to *c.* 30 μL using a Microcon 100 filtration unit (Millipore, Billerica, MA). This was accomplished by

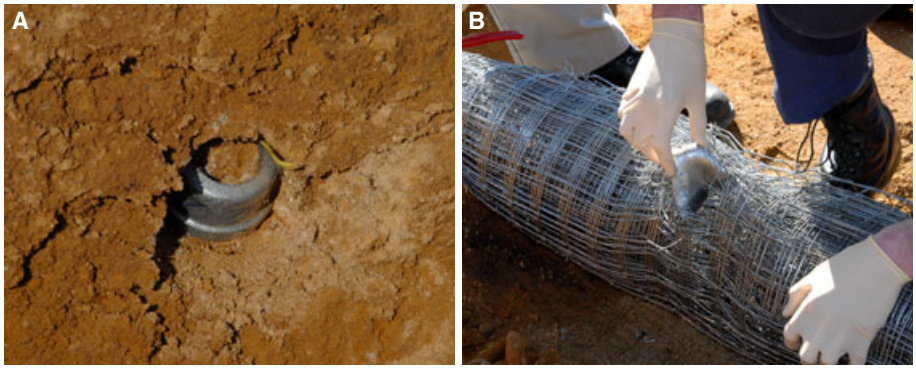

FIG. 3—*Collection of the post-blast fragments. (A) Portion of an end cap embedded in the wall of the trench and (B) portion of the pipe nipple protruding from the roll of fencing.*

transferring the 100 μL elution volume to the top of the Microcon filtration unit and centrifuging the device for *c*. 12 min at 500 × *g*. Prior to inverting the filtration unit into a centrifuge tube, 20 μL of TE^{-4} was added to the top reservoir. The final DNA extract was collected by centrifuging the device at 1000 × *g* for 3 min. The volumes of each sample were measured and then brought up to *c*. 30 μL by adding the necessary volume of TE^{-4}.

The concentration of DNA was determined by using the Applied Biosystems (AB) Quantifiler™ Human DNA Quantification Kit and the AB 7500 Real-Time Polymerase Chain Reaction (PCR) System (Foster City, CA). STR DNA analysis was performed using the AB AmpF*l*STR® Identifiler® Amplification Kit (AB) and the AB GeneAmp® PCR System 9700 (AB) following manufacturer's recommended cycling parameters: an initial incubation step of 95°C for 11 min; then, 94°C for 1 min, 59°C for 1 min, and 72°C for 1 min (28 cycles); and a final extension incubation at 60°C for 60 min. The following loci are amplified using the Identifiler® Amplification Kit: D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, FGA, and the gender determining locus Amelogenin. Fragment analysis was performed on the AB 3130 Genetic Analyzer with GeneMapper™ ID software (AB). The following parameters were used: 1 μL of amplified product mixed with 8.7 μL of Hi-Di™ formamide and 0.3 μL of GeneScan™–500 LIZ™, 3 min denaturation at 95°C, 3 min snap-cooling, and a 3 kV 5 sec injection. The analytical threshold for allele peak height detection is set at 50 RFU.

After the initial analysis, the process was repeated *c*. 3 months later to compare the DNA yield and DNA typing success. The pipe bomb fragments were stored in the paint cans in which they were collected at room temperature during the time interval. A total of 24 DNA samples were analyzed at each time point. Half of these samples were collected from fragments that had been cyanoacrylate fumed.

Results and Discussion

The first objective of this study was to investigate the effect of time on the success of DNA analysis on post-blast pipe bomb fragments. The results of this study indicate that the amount of DNA recovered from post-blast bomb fragments is a fraction of the initial amount of DNA deposited. In addition, the quantity of DNA recovered decreases greatly as time passes (see Tables 1 and 2). Assuming the rough approximation of the cell suspension concentration is close to 5000 cells and therefore 30 ng of DNA, on average only about 10–15% of the DNA from the end caps and 30–35% of the DNA from the pipe nipples was recovered after 1 week. The average concentration of DNA recovered from the end caps and the pipe nipples 3 months after deflagration was roughly an order of magnitude less than the concentration of DNA recovered within a week of the deflagration. In most cases, forensic laboratories do not have a 1 week turnaround time on routine cases. These results demonstrate that cases involving the analysis of post-blast pipe bomb fragments should be prioritized to minimize the loss of DNA.

Unfortunately, the control pipes for this study were lost. However, the control samples from a subsequent similar study provide valuable additional information (Table 3). Cells were spotted on both PVC and galvanized steel pipe nipples. The control samples were prepared following the same protocol except a smaller amount of cells was spotted. After 1 week, the DNA recovered from the cell spots on the PVC pipe was *c*. 5% of the amount of DNA recovered from the same volume of the cell suspension. The quantity of DNA recovered from the steel pipe cell spots was even lower at 1.7%. At the 3-month time point, the quantity of DNA recovered from the PVC cell spots was approximately the same as the quantity recovered at the 1-week time point. The DNA recovery from the steel pipe nipples at the 3-month time point was *c*. 50% compared with the 1-week time point. The cell spots dried on a different substrate from this study (the adhesive surface of electrical tape) demonstrated little to no loss after 1 week (data not shown). After 3 months, the decrease in recovery ranged from 10% to 50%. This data would suggest that a main factor causing the initial dramatic decrease observed in the amount of DNA recovered from the post-blast fragments is the ability to remove the cells from the surface of the pipe nipple or end cap. Another contributing factor to the loss of DNA could be the efficiency of the recovery of DNA from the swabs during the extraction process. The 90% loss of DNA from the 1-week to 3-month time point in the post-blast samples might be partially because of an increased difficulty in removing the cells from the surface of the components, but there appear to be other factors affecting the amount of DNA recovered also. From the control samples, there is no evidence that normal

TABLE 1—*DNA recovery from pipe bomb fragments 1 week after deflagration.*

	DNA Recovery 1 Week Post-Deflagration					
	Not Cyanoacrylate Fumed			Cyanoacrylate Fumed		
	Device	Sample Name	Quantity* (ng/μL)	Device	Sample Name	Quantity* (ng/μL)
End Cap	1	1E1	0.000	2	2E7	0.290
	1	1E2	0.335	2	2E8	0.309
	4	4E3	0.018	5	5E9	0.101
	4	4E4	0.185	5	5E10	0.000
	7	7E5	0.002	8	8E11	0.000
	7	7E6	0.244	8	8E12	0.230
Average			0.131			0.155
SD			0.144			0.140
Pipe Nipple	1	1P1	0.088	2	2P8	0.279
	1	1P2	0.107	2	2P9	0.285
	4	4P3	0.586	5	5P10	0.433
	4	4P4	0.463	5	5P11	0.394
	7	7P6	0.408	8	8P12	0.261
	7	7P7	0.373	8	8P13	0.229
Average			0.338			0.314
SD			0.200			0.081

*The final volume for all DNA extracts is 30 μL.

TABLE 2—*DNA recovery from pipe bomb fragments 3 months after deflagration.*

	DNA Recovery 3 Months Post-Deflagration					
	Not Cyanoacrylate Fumed			Cyanoacrylate Fumed		
	Device	Sample Name	Quantity* (ng/μL)	Device	Sample Name	Quantity* (ng/μL)
End Cap	1	1E13	0.013	2	2E15	0.000
	1	1E14	0.006	2	2E16	0.000
	4	4E17	0.003	5	5E19	0.034
	4	4E18	0.009	5	5E20	0.052
	7	7E21	0.045	8	8E23	0.000
	7	7E22	0.040	8	8E24	0.000
Average			0.019			0.014
SD			0.018			0.023
Pipe Nipple	1	1P14	0.027	2	2P16	0.043
	1	1P15	0.006	2	2P17	0.042
	4	4P18	0.050	5	5P20	0.041
	4	4P19	0.079	5	5P21	0.044
	7	7P22	0.021	8	8P24	0.031
	7	7P23	0.043	8	8P25	0.077
Average			0.038			0.046
SD			0.026			0.016

*The final volume for all DNA extracts is 30 μL.

TABLE 3—*DNA recovery from undeflagrated control pipes (PVC and steel) 1 week and 3 months after cell deposition.*

Sample Name	1 Week Total DNA (ng)	3 Months Total DNA (ng)
Control pipe-PVC 1	0.174	0.135
Control pipe-PVC 2	0.163	0.280
Control pipe-PVC 3	0.177	0.158
Average-PVC	0.171	0.191
Control pipe-steel 1	0.067	0.023
Control pipe-steel 2	0.018	0.036
Control pipe-steel 3	0.078	0.024
Average-steel	0.055	0.028
Cell suspension-1	3.360	3.680
Cell suspension-2	3.700	3.690
Cell suspension-3	2.350	2.770
Average-cell suspension	3.137	3.380

A different cell suspension with a reduced cell concentration was used in the making of the control samples compared with the test samples. Quantitation results for the DNA extraction from the same volume of the cell suspension are provided, as well.

degradation of the DNA over time accounts for the additional loss of DNA. The electropherograms do not demonstrate the typical downward slope attributed to DNA degradation on either the postblast fragments or the control samples.

In most cases in which DNA analysis is requested, other forensic examinations are requested as well. In these instances, it is necessary to determine an order of analysis which allows each discipline to conduct its testing without altering the evidence to the point that precludes testing by the remaining disciplines. Because the collection of biological material usually involves the swabbing of the substrate which would obliterate any latent prints, cyanoacrylate fuming is routinely performed prior to DNA analysis. In this study, it was hypothesized that the cyanoacrylate fuming may actually have a beneficial effect on the DNA analysis by protecting the biological material in two ways. First, the cyanoacrylate may prevent the biological material from being scraped off during transport or normal handling of the containers in which the fragments are stored. Second, the cyanoacrylate layer would prevent the products of combustion present on the fragments from reacting with moisture in the air over the course of time during storage. In this case, the fragments were already transported to the laboratory before the cyanoacrylate fuming was performed. There was, however, routine handling of the containers after the fuming occurred. The DNA concentrations recovered from the cyanoacrylate fumed fragments and the untreated fragments of the end caps are similar. This holds true for the fragments of the pipe nipples, as well. It should be noted that the sensitivity limit of the quantitation method used is *c*. 9 pg/μL and the precision is decreased at this concentration of DNA (e.g., 1 SD is approximately half of the target value). Any possible "protective" effect conveyed by the cyanoacrylate fuming would be expected to be most noticeable at the 3-month time point. The average DNA quantitation results again demonstrate similar DNA recoveries for each set of samples (see Tables 1 and 2). Four of the six fumed end cap samples did not yield any detectable DNA while several of the nonfumed samples demonstrated low concentrations of DNA (<10 pg of DNA/μL). Therefore, the cyanoacrylate fuming of the evidence did not demonstrate any protective qualities. A paper published after this study was performed may explain, in part, why no protective qualities were observed. Wargacki et al. (13) demonstrated that the cyanoacrylate layer deposited on the surface can actually be porous. Therefore, water or other molecules in the environment would still have access to the products of combustion and the biological material. There is also no conclusive evidence that cyanoacrylate fuming had a detrimental effect on the success of DNA analysis as has been reported previously (7). Although, as noted earlier, any difference in results when compared with other studies might be because of differences in the cyanoacrylate fuming methods used, which may result in varying amounts of cyanoacrylate deposition. As demonstrated by Pitilertpanya et al. (7), heavier deposition of cyanoacrylate decreased the subsequent DNA typing results. For example, better typing results were obtained from samples fumed for 20–30 min compared with those fumed for 40 min. Other factors, such as the glue heating temperature and the size of the fuming chamber, can significantly affect the amount of cyanoacrylate deposition. Some laboratories (7,14) have investigated replacing water or saline with acetone to moisten the swab used to collect biological material from cyanoacrylate fumed items, because of its

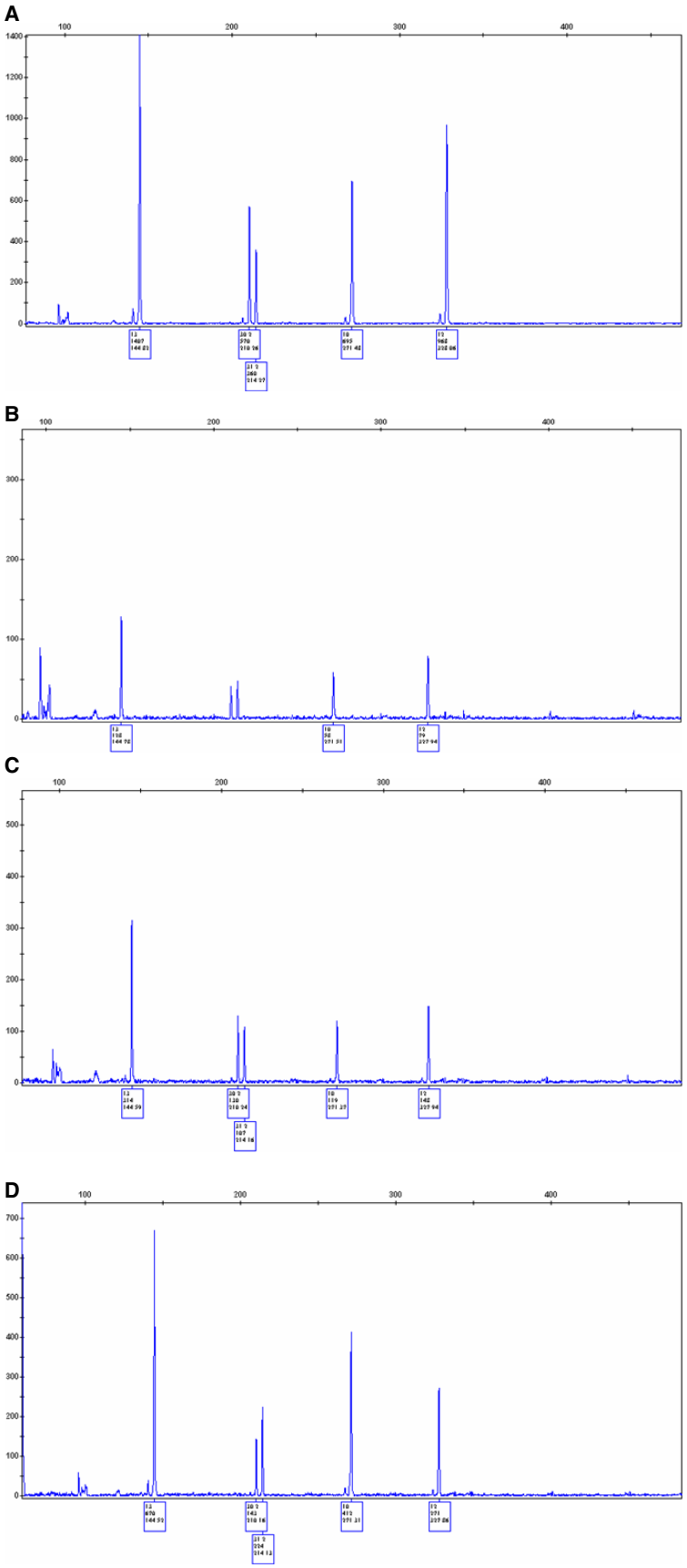

FIG. 4—*Examples of electropherograms from samples analyzed 1 week (A and B) and 3 months (C and D) after deflagration of the devices. The samples were amplified using the AmpFlSTR® Identifiler® Amplification Kit and analyzed on the AB 3130 Genetic Analyzer. Samples (A and D) are from pipe nipples. Samples (B and C) are from end caps.*

ability to dissolve the cyanoacrylate polymer. Initial studies at the ATF Laboratory indicate the use of acetone may increase the amount of biological material collected depending on the surface being swabbed.

As discussed in the Introduction, the location of the biological material on the device may affect its subsequent exposure during deflagration. How it would be affected was unknown. A combined total of 24 cell spots were analyzed from pipe nipple fragments (both time points, fumed and not fumed) which were compared with the 24 cell spots analyzed from end cap fragments (both time points, fumed and not fumed). On average, the quantity of DNA recovered from the pipe nipples demonstrated an approximate twofold increase over the quantity of DNA recovered from the end caps. It was estimated originally that the total quantity of DNA deposited on each spot was 30 ng (5000 cells). While this was a rough estimate calculated by counting the cells in several aliquots of the cell suspension under a microscope, it is a useful number to compare with the actual quantity of DNA recovered from the cell spots. The DNA extraction of the cell spots located on the pipe nipples from the analysis within 1 week post-deflagration yielded *c*. 10 ng of DNA on average, or one-third of the estimated original quantity of DNA. The total DNA recovered from the end caps for the same time point was only about 4 ng. The DNA concentrations recovered at the 3-month time point were *c*. 10% of the concentrations recovered at 1 week. The difference in the DNA recovery observed from the pipe nipples and the end caps may seem insignificant when compared with the total initial DNA. For example, the combined results of recovery of DNA were *c*. 1.7% of the initial DNA spotted on the end caps and 4.2% of the DNA spotted on the pipe nipples. However, five of the 12 DNA extracts from the end caps had concentrations below 50 pg/μL (three had concentrations of 0 pg/μL) while none of the 12 DNA extracts from the pipe nipples had a concentration below 50 pg/μL.

As mentioned in the Methods, it cannot be determined if the variability in the Esslinger et al. (4) results may have been because of the realistic method in which the cells were deposited on the components or the subsequent conditions the samples were exposed to. In this study, a consistent amount of cellular material was spotted on the components, yet there remains a wide range of results from a single component, from a single device, and between devices. This is an indication that exterior surfaces of the fragments are each exposed to unique insults which may affect the biological material present severely or relatively mildly.

Because of the manner in which the pipe bomb fragments were captured, the majority of the fragments had little to no soil contamination. This benefit allows for a more direct comparison of the effects of the deflagration without the extraneous effects of potential external factors such as PCR amplification inhibition because of the presence of soil (15,16). The electropherograms of the amplified product were consistent with expected results for the quantities amplified. Where sufficient DNA was amplified (>300 pg), complete profiles were observed with no indications of degradation (see Fig. 4). At low levels of template DNA, partial profiles were observed with the expected peak height imbalance, allele drop-out and/or locus drop-out. The same was true for the cell spots analyzed at the 3-month time point. A comparison of the DNA concentrations in Tables 1 and 2 to the corresponding number of alleles detected and indicated in Tables 4 and 5 may not always seem consistent. For example, sample 4E3 has a concentration of 18 pg/μL and 14 of the 27 possible alleles were detected. Sample 1E13 has a concentration of 13 pg/μL yet all 27 alleles were detected. This could be the result of two factors. First, as noted previously, the quantitation method used has a decreased precision at the lower end of the standard curve, i.e., for samples with low concentrations of DNA as in this study. In addition, the difference between an allele being "detected" and not "detected" could be a matter of a few RFU. In this instance, most of the alleles observed in sample 4E3 have RFU values between 50 and 100, with many allelic peaks visible below the 50 RFU threshold across the profile. The majority of the allelic peaks for sample 1E13 have values between 50 and 150 RFU. No indication of inhibition was detected by the internal PCR control in the quantitation nor was it indicated in the electropherograms.

One obvious, but still important, point did arise during the course of this study. When collecting items of evidence that potentially contain low levels of biological material, it is critical to take steps to prevent contamination from the individual collecting the items. In this case, the individuals collecting the fragments all wore latex gloves; however, one of the samples analyzed demonstrated the presence of a mixture consistent with the expected profile and the profile of the individual collecting the fragment. Even though gloves are worn, contaminating DNA may still be introduced by

TABLE 4—*DNA typing success of samples from pipe bomb fragments 1 week after the deflagration.*

	Alleles Detected 1 Week Post-Deflagration* (27 alleles possible)					
	Not Cyanoacrylate Fumed			Cyanoacrylate Fumed		
	Device	Sample Name	Alleles Detected	Device	Sample Name	Alleles Detected
End Cap	1	1E1	0	2	2E7	27
	1	1E2	27	2	2E8	27
	4	4E3	14	5	5E9	27
	4	4E4	27	5	5E10	0
	7	7E5	0	8	8E11	0
	7	7E6	27	8	8E12	27
Average			16			18
Pipe Nipple	1	1P1	NA†	2	2P8	27
	1	1P2	27	2	2P9	27
	4	4P3	27	5	5P10	27
	4	4P4	27	5	5P11	27
	7	7P6	27	8	8P12	27
	7	7P7	27	8	8P13	27
Average			27			27

*The target amount of template DNA used for amplification was *c*. 0.5–1 ng if available. All alleles with peak heights greater than 50 RFU were counted.
†This sample demonstrated the presence of a mixture and therefore was not used in this analysis.

TABLE 5—*DNA typing success of samples from pipe bomb fragments 3 months after the deflagration.*

	Alleles Detected After 3 Months* (27 alleles possible)					
	Not Cyanoacrylate Fumed			Cyanoacrylate Fumed		
	Device	Sample Name	Alleles Detected	Device	Sample Name	Alleles Detected
End Cap	1	1E13	27	2	2E15	0
	1	1E14	26	2	2E16	0
	4	4E17	4	5	5E19	27
	4	4E18	5	5	5E20	27
	7	7E21	26	8	8E23	0
	7	7E22	27	8	8E24	0
Average			19			9
Pipe Nipple	1	1P14	27	2	2P16	27
	1	1P15	26	2	2P17	27
	4	4P18	27	5	5P20	27
	4	4P19	27	5	5P21	27
	7	7P22	27	8	8P24	27
	7	7P23	27	8	8P25	27
Average			27			27

*The target amount of template DNA used for amplification was *c*. 0.5–1 ng if available. All alleles with peak heights greater than 50 RFU were counted.

any of a number of routes including sneezing, coughing, and sweating. This situation is similar to the analysis of ancient DNA, severely degraded skeletal remains, or other instances in which a minute amount of pristine DNA introduced by an exogenous source during the collection of the evidence can overwhelm the endogenous DNA. This is a further indication that personnel at a crime scene participating in the collection of handled objects or other items of evidence containing low levels of DNA should take elevated precautions to prevent contamination. These precautions can include changing gloves more frequently, wearing a mask/head covering, or wearing a full Tyvek® suit (Dupont, Wilmington, DE). It also demonstrates the importance of an internal staff DNA index containing the profiles of individuals who may come in contact with the evidence from the crime scene to the laboratory so that the possibility of contamination can be detected and investigated.

In summary, this study again demonstrates that it is possible to successfully perform nuclear DNA testing on biological material recovered from post-blast pipe bomb fragments. However, this study also indicates several factors that may affect the success of the DNA typing. One major factor is the ability to recover DNA from cellular material once it has dried on the surface of the pipe nipple or end cap. One possibility is that the cells become difficult to remove using the typical swabbing method, although there may be other causes. The time between the device deflagration and the collection and analysis of the biological material also had a dramatic effect on the recovery of DNA. On average, a 90% reduction in DNA recovery was observed in a 3-month time period. This data suggests that cases that involve post-blast bomb fragments should be prioritized to increase the chances of successful DNA analysis. The original location of the biological material also affected the amount of DNA recovered subsequently. Roughly double the quantity of DNA was recovered from the pipe nipples compared with the end caps. Finally, cyanoacrylate fuming did not demonstrate a measurable effect on the recovery of DNA or DNA typing success. Further studies should be conducted to determine the cause of the loss of DNA over time from post-blast pipe bomb fragments. If the cause can be determined, then preventative measures can be implemented to reduce this loss and increase the DNA typing success from this type of evidence. Additionally, other methods for collecting biological material from the surface of the pipe bomb components should be researched that will recover a greater quantity of the cells originally deposited through the handling of the objects.

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