DNA ANALYSIS OF HUMAN BLOOD RECOVERED FROM EXPLOSION DEBRIS

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A detailed study was initiated into natural gas migration in a residence and the effects of a natural gas explosion on both physical and biological trace evidence. Personnel from several Provincial and Federal agencies participated in this study [1]. The biological portion consisted of two experiments: 1. To determine if a simulated homicide scene could be recreated from blood spatter evidence recovered from the explosion debris. 2. To determine the effects of a brief, but high temperature, natural gas explosion on the ability to obtain reliable DNA typing results from exposed bloodstains.

This paper summarizes the results obtained from DNA analysis of bloodstains subjected to the destructive forces of a natural gas explosion in a residence. The experimental conditions were chosen to be as realistic as possible, with a fully furnished three bedroom house used as a test model. The following experimental parameters were examined.

1. To determine if bloodstain location relative to the ignition point and the nature of the surface material used, is important for both recovery and reliability of DNA typing results.
2. To compare and contrast DNA typing results obtained from dried bloodstains and stains made during the actual explosion from a pool of liquid blood.
3. To determine the quality and quantity of DNA isolated from exposed bloodstains.

MATERIALS AND METHODS

A fully furnished bungalow of approximately 80 sq m was used to study the effects of a natural gas explosion on bloodstain evidence. The surfaces tested and their composition are listed in TABLE 1.

TABLE 1: EXPERIMENTAL BLOODSTAINED SURFACES

1. Bathtub (enamel)	14. Bed Linen, BedRm #3 (cloth)
2. Bathroom sink (ceramic)	15. Mattress-7, BedRm #3 (cloth)
3. Bathroom tile (synthetic)	16. Pants, BedRm #3 (polyester)
4. Mattress-1, BedRm #1 (cloth)	17. Sweater, BedRm #3 (synthetic)
5. Crib frame, BedRm #1 (wood)	18. Kitchen Counter (synthetic)
6. Mattress-2, BedRm #1 (cloth)	19. Kitchen Fridge (enamel)
7. Mattress-3, BedRm #1 (cloth)	20. Kitchen Wall Paper (cellulose)
8. Hammer handle, BedRm #1 (wood)	21. Kitchen Sink (stainless steel)
9. Hammer head, BedRm #1 (metal)	22. Blouse (synthetic)
10. Mattress-4, BedRm #2 (cloth)	23. Sofa arm-1, LvgRm (cloth)
11. Mattress-5, BedRm #2 (cloth)	24. Sofa arm rest-2, LvgRm (cloth)
12. Window Frame, BedRm #2 (wood)	25. Stereo cabinet, LvgRm (wood)
13. Mattress-6, BedRm #3 (cloth)	26. Floor tile, LvgRm (synthetic)

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Bloodstains were prepared from fresh liquid blood collected in EDTA-containing blood collection tubes. A total of twenty five 200 ul stains were prepared one day prior to the explosion in all 6 rooms of the house. One stain (#17) was produced during the explosion, by dispersal of a fresh pool of blood placed on the chest surface of a mannequin. In general, all surfaces were in poor condition and no attempt was made to pre-clean any surface prior to application of blood. Surface controls were obtained either by placing an equivalent test volume of blood on a fragment of the surface, or by removing an equivalent test volume of blood from an adjacent test area with a cotton swab.

The house was filled with natural gas to a final 10% mixture with air and ignited by an electrical spark placed in the living room. Spot fires were extinguished by Fire Department personnel using water shortly after the explosion. Bloodstains were exposed to the elements for approximately 3 hours prior to their collection. Those found on cloth surfaces were removed by cutting out a fragment. Those present on solid surfaces were removed using a moistened cotton swab. A total of 7 hours elapsed between the explosion and storage at -20°C. The weather for the experiment was calm and sunny with an air temperature of approximately 7°C.

DNA was extracted from control and test bloodstain samples using standard proteinase K digestion, phenol-chloroform extraction, and ethanol precipitation methods [2]. DNA quality was evaluated by agarose gel electrophoresis and ethidium bromide fluorescence staining. The quantity of human DNA isolated was evaluated using a human specific alpha-satellite probe, D17Z1 [3]. Purified DNA was analyzed for restriction fragment length polymorphisms (RFLP) using *Hae* III restriction digestion and Southern blot hybridization techniques. Radioactively labelled, single locus, variable number of tandem repeat (VNTR) probes D17S79 and D4S139 were used to detect DNA alleles. Alpha satellite probes D7Z2 and DYZ1 were used as control probes to evaluate electrophoretic migration and *Hae* III digestion.

RESULTS AND DISCUSSION

A. Explosion and Bloodstain Recovery

The house was completely destroyed by the explosion, with the majority of the debris falling over a 50 m radius. Only 15 of the 26 stained surfaces were recovered from the explosion debris (stains 1, 4, 5, 7, 8, 9, 16, 17, 18, 19, 21, 23, 24, 25, and 26), representing all but one room of the original house. All recovered bloodstains exhibited surface charring, caused either by the heat of the explosion or by exposure to the resulting fireball. Several mattresses were destroyed by fire. Other bloodstains were either destroyed by fire, disintegrated, or remained buried in the basement rubble. Bloodstains on large durable surfaces survived the explosion and were readily located in the explosion debris. Several bloodstains were exposed to additional environmental stresses including water damage (stain 4) and contact with soil/mud (stains 4, 8, 9, and 21)

B. Blood Identification Tests

All recovered stains tested positive for blood using the Hemastix (Miles Laboratory, Rexdale, Ontario) presumptive test, and by the hemochromogen (Takayama) crystal test.

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C. DNA Quality and Quantity
High molecular weight (HMW) DNA was isolated from 12 of the 15 recovered bloodstains (stains 1,5, 7, 8, 9, 16, 17, 19, 23, 24, 25, and 26). DNA isolated from three stains (4, 18, 21) exhibited partial degradation. Two of these stains (4 and 21) were found in contact with the soil. For test bloodstains yielding HMW DNA, recoveries varied from 5 to 80% when compared to surface control samples.

D. DNA Typing Analysis
The feasibility of obtaining reliable DNA typing profiles from the 15 recovered bloodstains was determined using single locus RFLP analysis. DNA typing results using VNTR probes D4S139 and D17S79, and alpha satellite probes D7Z2 and DYZ1 are summarized below:

1. Eleven bloodstains (1,5,16,17,18,19,21,23,24,25 and 26) matched[4] the DNA profile of donor control samples. Two stains (18 and 21) had very weak DNA band intensities due to the degraded nature of these samples.
2. Two bloodstains (7 and 8) showed partial *Hae* III digestion patterns. One of these stains (8) had been found exposed to the soil.
3. One bloodstain (4) exhibited altered electrophoretic migration. This stain was on a mattress surface which was found soaked with water and exposed to the soil.
4. One bloodstain sample (9) produced no VNTR profile. This stain was prepared on a hammer which was found exposed to the soil.

All DNA typing profile matches were within 1.8 % of the band size of donor control samples. All surface control samples yielded DNA profiles consistent with blood donor control samples. One surface control sample (23) displayed a partial *Hae* III digestion pattern. This was not observed with the test bloodstain sample.

CONCLUSIONS

1. Due to the destructive nature of a natural gas explosion, only a portion of the original bloodstains were recovered (15 of 26).
2. All recovered bloodstains exhibited surface charring, but tested positive for blood using the Hemastix and hemochromogen assays.
3. The majority of bloodstains (11 of 15) yielded DNA typing profiles consistent with the donor control samples. Partial digestion, altered migration, and weak intensity bands, were encountered with the remaining four samples. Three of these stains were found exposed to the soil.
4. This study indicates that bloodstains exposed to a high temperature natural gas explosion, can yield reliable RFLP DNA typing results in the absence of additional environmental stresses.

REFERENCES

1. Participants: Edmonton, Calgary and County of Parkland Fire Departments; Northwestern Gas Utility Ltd; Edmonton Police Service; RCMP Forensic Laboratory, Edmonton; RCMP Canadian Bomb Data Centre; Transportation Safety Board of Canada;
2. S.J. Sambrook, E.F. Fritsch, and T. Maniatis. 1989. Molecular Cloning - A Laboratory Manual, 2nd edition, Cold Spring Harbor Laboratory Press.
3. J.S. Waye, L.A. Presley, B. Budowle, G.G. Shutler, and R.M. Fourney. 1989. Biotechniques 7:852-855.
4. For a match to occur between two DNA profiles, all corresponding DNA bands must visually match, and their estimated lengths must be within a match window of ± 2.6% of their mean size (RCMP DNA Analysis Protocol).

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