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Chemical Enhancement Techniques of Bloodstain Patterns and DNA Recovery After Fire Exposure*

ABSTRACT: It is common in forensic casework to encounter situations where the suspect has set a fire to cover up or destroy possible evidence. While bloodstain pattern interpretation, chemical enhancement of blood, and recovery of deoxyribonucleic acid (DNA) from bloodstains is well documented in the literature, very little information is known about the effects of heat or fire on these types of examinations. In this study, a variety of known types of bloodstain patterns were created in a four-room structure containing typical household objects and furnishings. The structure was allowed to burn to flashover and then it was extinguished by firefighters using water. Once the structure cooled over night, the interior was examined using a bright light. The bloodstains were evaluated to see if the heat or fire had caused any changes to the patterns that would inhibit interpretation. Bloodstain patterns remained visible and intact inside the structure and on furnishings unless the surface that held the blood was totally burned away. Additionally, a variety of chemical techniques were utilized to better visualize the patterns and determine the possible presence of blood after the fire. The soot from the fire formed a physical barrier that initially interfered with chemical enhancement of blood. However, when the soot was removed using water or alcohol, the chemicals used, fluorescein, luminol, Bluestar®, and Hemastix®, performed adequately in most of the tests. Prior to DNA testing, the combined phenolphthalein/tetramethyl benzidine presumptive test for the presence of blood was conducted in the laboratory on samples recovered from the structure in an effort to assess the effectiveness of using this type of testing as a screening tool. Test results demonstrated that reliance on obtaining a positive presumptive result for blood before proceeding with DNA testing could result in the failure to obtain useful typing results. Finally, two DNA recovery methods (swabbing the stain plus cutting or scraping the stain) were attempted to evaluate their performance in recovering samples in an arson investigation. Recovery of DNA was more successful in some instances with the swabbing method, and in other instances with the cutting/scraping method. Therefore, it is recommended that both methods be used. For the most part, the recovered DNA seemed to be unaffected by the heat, until the temperature was 800°C or greater. At this temperature, no DNA profiles were obtained.

KEYWORDS: forensic science, bloodstain patterns, chemical enhancement, DNA, arson, fire

Fires are frequently used by perpetrators to cover up homicides or other violent crimes (1,2). The purpose of this project was to determine how fire and firefighting efforts cause changes to occur in dried bloodstain patterns burned in a typical structural fire. In addition, samples were recovered from these dried bloodstain patterns in an effort to assess the effect of fire and firefighting efforts on the ability to obtain quality DNA profiles under measurable temperature conditions.

Bloodstain patterns can be challenging to interpret due to soot build-up and firefighting efforts (3). In research conducted on bloodstains exposed to an environmental temperature maximum of 115°F (46.1°C), no change was observed in the overall characteristics of bloodstain patterns (4). This environmental temperature maximum, however, is low relative to temperatures reached during a fire.

Luminol and fluorescein are well documented as not having any ill effects on either traditional serological analysis or DNA (5–7). Therefore these two methods, along with Bluestar® (8,9), were selected to better visualize any residual bloodstain patterns. Fluorescein was selected for its ability to provide fluorescence, whereas luminol and Blue Star® were chosen for their ability to provide chemiluminescence. Other commonly used chemical enhancements would not exhibit the necessary color contrast with the soot and char anticipated by the fire event. This research primarily addressed whether or not bloodstain patterns can remain unaltered and therefore interpretable upon exposure to a fire and the subsequent efforts to extinguish the fire, and whether currently used chemical enhancement techniques assist in the visualization of the patterns. In addition to the use of the listed chemical enhancement reagents, and because many crime scene investigators require a positive blood screening result before they will recover a suspected bloodstain, Hemastix® strips were used during the processing of the mock fire scene prior to collection of samples. Given their portable nature, ease of use and interpretation, Hemastix® strips are commonly used in the field. In this project, samples were recovered even if a Hemastix® strip yielded a negative result.

In the laboratory setting, many biologists are required to obtain a positive blood screening test result prior to proceeding with DNA analysis, regardless of the blood screening results obtained by the crime scene investigators. Accordingly, the combined phenolphthalein (P)/tetramethyl benzidine (TMB) presumptive test for blood (10) was conducted in the laboratory immediately prior to the placement of samples in tubes for DNA processing. DNA testing was conducted on all samples, even those with negative

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or inconclusive presumptive blood screening results. This allowed exploration of the reliance on positive blood screening results as a mandatory requirement in the laboratory prior to proceeding with DNA analysis, specifically, if reliance on a positive blood screening result prior to DNA analysis would result in failure to process samples that ultimately yielded a quality DNA testing result.

It is a widely held belief that obtaining DNA testing results from blood samples recovered from fire scenes can be problematic due to extreme heat and firefighting efforts. Because the ability to interpret the bloodstain patterns, coupled with the ability to identify the blood source, lends the most value to crime scene investigators, we explored which temperature extremes interfered with the ability to obtain a full short tandem repeat (STR) DNA profile using the PowerPlex® 16 System. DNA testing was also conducted on samples exposed to chemical enhancement procedures. Whenever possible, samples intended for DNA analysis were both swabbed and cut (or scraped), enabling a comparison of the efficacy of each recovery method, as well.

Methods and Materials

Construction

A four-room structure was built at the Bureau of Alcohol, Tobacco, Firearms, and Explosives (ATF) National Laboratory Center, Fire Research Laboratory (FRL) in Ammendale, Maryland, to simulate a small apartment (Fig. 1). The entire structure measured 30 ft by 15 ft and was 8 ft high. The exterior walls comprised 5/8-inch thick gypsum wallboard mounted to a 2-inch by 4-inch wood frame. The ceiling was comprised of 5/8-inch gypsum wallboard. The floor was a single layer of 5/8-inch plywood protected by a layer of 0.5-inch gypsum. The interior walls consisted of 0.5-inch gypsum wallboard. The structure floor plan consisted of a living area that had one door and one window. A dining area was adjacent to the living area. The dining area had one window and two open doorways, one that led to a kitchen and the other that led to a bedroom. The bedroom also had a window. The interior of the structure held normal household artifacts such as furniture, curtains, articles of clothing, a stove, cabinets, a computer monitor, a bed, bedding, glassware, plastic,

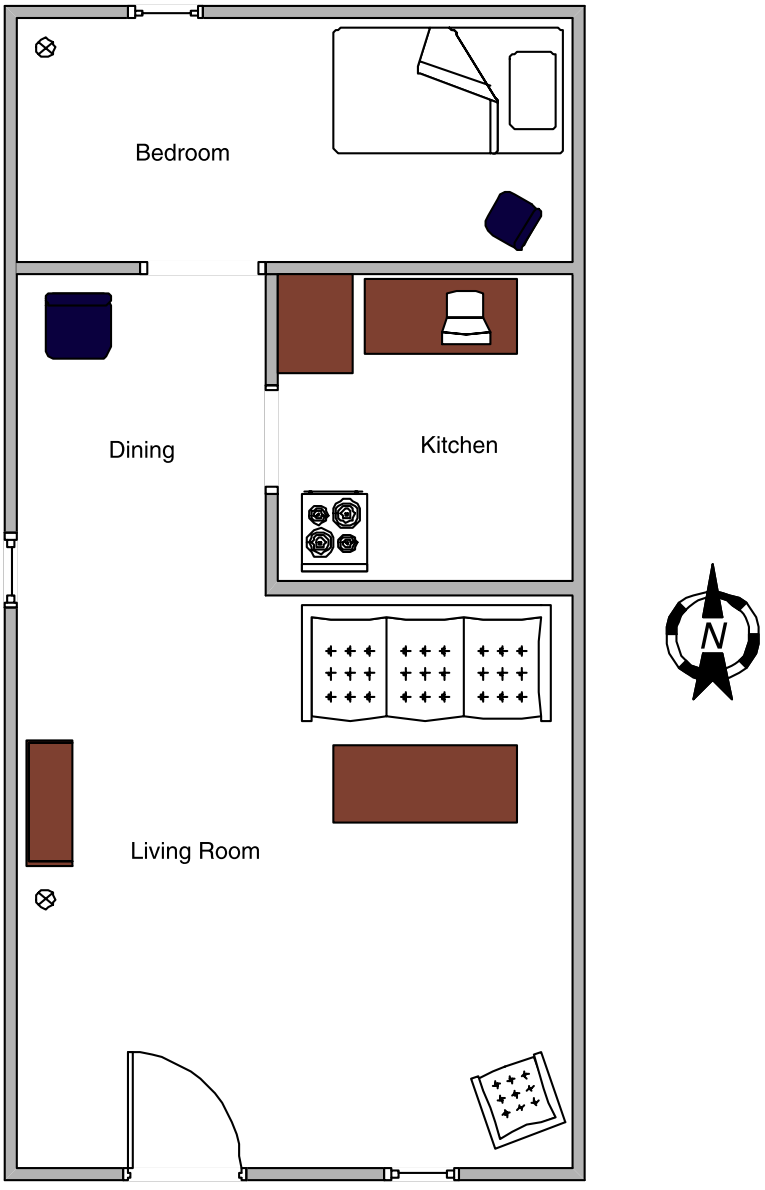

FIG. 1—*Floor plan of structure.*

bottles of alcohol, and a weapon. Most of the household artifacts were purchased from a second-hand store. A variety of flooring material was utilized in the structure: 100% nylon carpet, vinyl floor covering, and hardwood floor substrates (Fig. 2).

Bloodstain Patterns

A pint of human blood was provided by the Michigan Community Blood Centers (Grand Rapids, MI). The blood was placed on surfaces and substrates within the structure (Figs. 3*A* and 3*F*) in typical bloodstain patterns normally observed at a crime scene (11). The patterns were documented and allowed to dry overnight. Prior to the burning of the structure, all of the stains appeared to be dry with the exception of a pooled bloodstain pattern in the bedroom. The created patterns included: impact stains, castoff, transfer, dripping patterns, projected blood, and blood pools.

Bloodstain Pattern Terminology

Blood pools. A large volume of blood in a single area.
Cast-off pattern. Blood drops that are flung or cast from some object as a result of motion.
Dripping pattern. Result from blood dripping into blood.
Impact pattern. Bloodstain pattern created when blood receives a blow or force resulting in the random distribution of smaller drops of blood.
Passive drop (bleeding). Bloodstain drop(s) created or formed by the force of gravity acting alone.
Projected blood patterns. Produced by blood released under pressure.
Transfer pattern. An image created when a wet, bloody surface comes into contact with a secondary surface. A recognizable image or part of the object may be observed in the pattern.

Fire Source

The structure was exposed to a simulated "accidental" sofa fire. The sofa was positioned in the living room directly across from the shut window and door of the living room. The initiating fire source in this scenario was a natural gas sand burner that was positioned adjacent to the armrest of the sofa. Direct flames from the burner were applied for 300 sec to ignite the sofa. After the initial 300 sec, the gas was shut off and the fire was allowed to develop naturally, for *c*. 45 min, until flashover conditions were reached

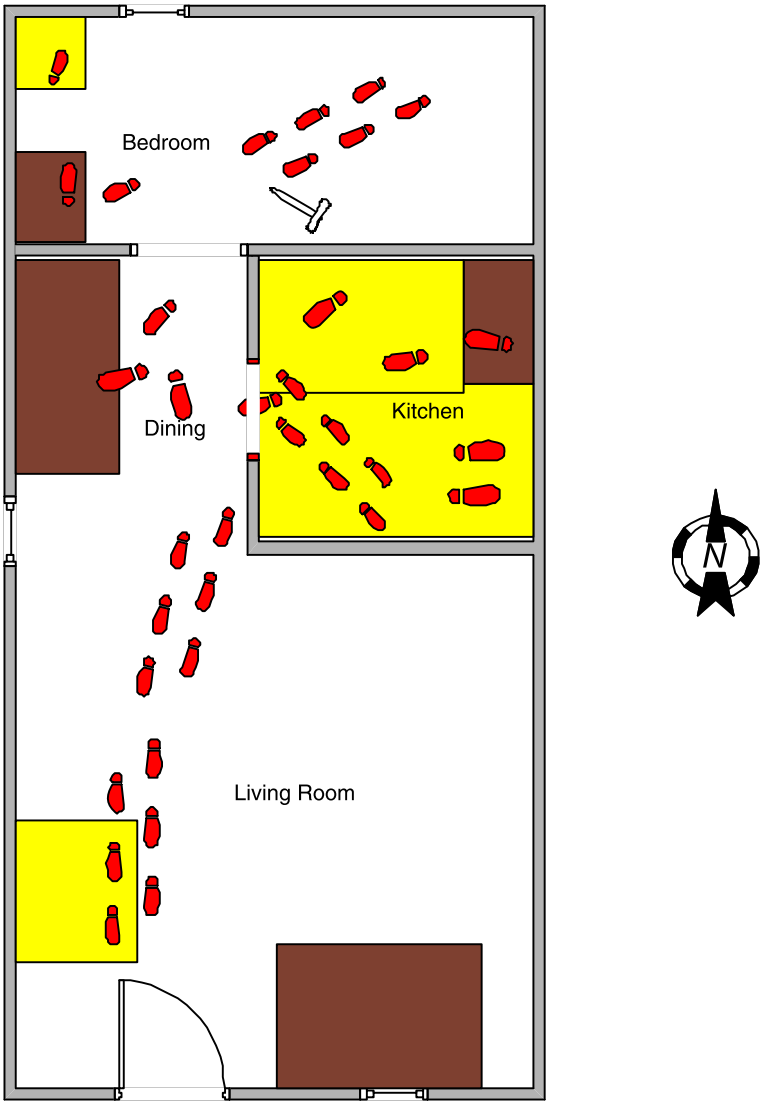

FIG. 2—*Flooring and footwear impressions: light gray–vinyl floor covering; dark gray–hardwood floor substrate; white–carpet.*

FIG. 3—*(A) Creation of transfer stain pattern in living room. (B) Creation of projected blood pattern in living room.*

(Fig. 4). The fire was then put out by firefighters using water. The maximum and minimum temperatures of each room at the ceiling and floor levels were recorded (Table 1). After the structure cooled, the structure was documented for changes in the previously applied bloodstain patterns, chemical enhancement techniques were utilized to better visualize any patterns, and samples were collected for DNA analysis.

Chemical Enhancement

Three different reagents, luminol, fluorescein, and Bluestar® (Roc Import Group, Monaco), were utilized to better visualize the

FIG. 4—*The structure approaching flashover.*

TABLE 1—*Maximum and minimum temperatures recorded by thermal couplers.*

	Temperature	
Location	Lowest	Highest
Living room	282°C	923°C
Dining room	179°C	646°C
Kitchen	152°C	375°C
Bedroom	146°C	307°C

bloodstain patterns after the burn. In most instances, one chemical enhancement technique was randomly selected for use on the various types of bloodstain patterns in each of the rooms. Luminol was selected as a subsequent spray to several negative fluorescein reactions in the living room, as luminol can yield a positive reaction after a negative fluorescein reaction has been noted. Although fluorescein can cause a false-positive reaction if used after a luminol application, the reverse is not true.

Luminol was made using a two-part solution. Part 1 comprised 0.1 g luminol, 5.0 g sodium carbonate and 90 mL water. Part 2 was 10 mL of 3% hydrogen peroxide, which was added to part 1 prior to spraying. The fluorescein product (K.P.S. Technologies, L.L.C., Georgia State University) was a two-part liquid that required no mixing. The remaining visualization method was Bluestar® (Roc Import Group) and was prepared fresh according to manufacturer's recommendations. Using aerosol sprayers, each test reagent was applied to various bloodstains such that the entire surface of the stain was covered. The bloodstains that were sprayed with fluorescein were observed in the dark using an alternative light source (450 nm) and orange safety goggles, while the bloodstains that were sprayed with luminol and Bluestar® were observed in the dark with the unaided eye. With fluorescein, the reaction was deemed positive when fluorescence was noted. With luminol and Bluestar®, the reaction was deemed positive when chemiluminescence was noted. For all three chemical enhancement reagents, the reaction was determined to be negative when no fluorescence or chemiluminescence was noted. Hemastix® strips (Henry Schein, Pittsburgh, PA), which utilize TMB chemistry (12), were used to conduct presumptive blood screening tests at the time of sample recovery on some of the patterns.

In certain instances, bloodstain patterns were wiped with a Kaydry® (Fisher Scientific, Atlanta, GA) that had been saturated with

distilled water prior to testing and/or sample collection in an effort to remove some of the soot. Other bloodstain patterns were wiped with FisherBrand premoistened 70% isopropyl alcohol wipes (Fisher Scientific) prior to testing and/or sample collection, also in an effort to remove soot.

Documentation

The structure, contents, and bloodstain patterns were sketched and photographed using both a 35-mm camera and a digital camera prior to burning. The bloodstain patterns were also documented with measurements. After the burn, the patterns were re-photographed. Subsequently, a portion of the bloodstains were chemically enhanced and the reactions photographed. When luminol (Fig. 5) and Bluestar® were used, the photographs were taken in a relatively dark environment to visualize the chemiluminescence produced by a positive reaction. The recommended film speed of 400 ASA and exposure times of 20 to 30 sec for the chemiluminescent reactions were utilized. The camera was set on a tripod to keep the camera motionless during the exposure (13). The fluorescein reactions were photographed in the same manner; however, an alternative light source was utilized to illuminate any positive reactions and an orange filter (450 nm) was placed over the camera lens. In addition, the burn was documented via digital photography and video by the ATF FRL Staff. As is standard practice, the ATF Data System collected the fire measurement data, including temperature measurements from floor to ceiling in each room within the structure.

DNA Sampling and P/TMB Testing

Whenever possible, the samples for DNA analysis were collected using the following two methods: (i) the stains were swabbed using sterile polyester tip swabs moistened with type I water and (ii) a portion of the stained substrate was cut or blood scraped from its surface. Type I water is ultra purified water having a minimum electrical resistivity of 18.0 Megohm-cm at 25°C and a maximum total organic content of 100 μg/L.

Prior to preparing the samples for DNA analysis in the laboratory, they were photographed using a digital camera and subsequently subjected to presumptive blood screening using the combined P/TMB method. The P/TMB presumptive screening method comprised four sequential steps: the addition of ethanol to a portion of a sample followed by the addition of 3% hydrogen peroxide, a working solution of phenolphthalin, and saturated solution of TMB. A sample is deemed to yield a positive reaction when no color changes are noted following the addition of the ethanol and the hydrogen peroxide, a pink color is noted after the addition of the phenolphthalin, and a green–blue color is noted following the addition of the TMB. A sample is determined to have a negative reaction when no color changes are noted after the addition of any of the chemicals. An inconclusive finding results when any color changes, or lack thereof, are noted that differ from those expected for a positive reaction.

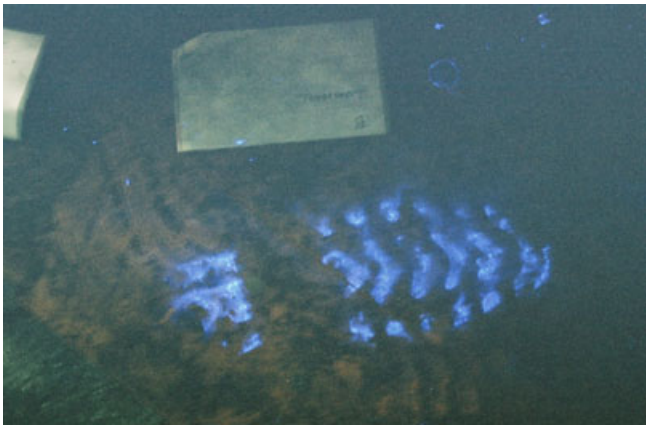

FIG. 5—*A positive luminol reaction of a footwear impression.*

DNA Testing

DNA was extracted organically followed by DNA purification and concentration through Microcon YM-100 filters (Amicon, Beverly, MA). DNA recovery was determined using both a 1% agarose yield gel and the Quantiblot kit (Applied Biosystems, Foster City, CA). Given the sensitivity limitation of the Quantiblot kit, all samples were amplified and typed, even those with no Quantiblot quantification result. Polymerase chain reaction (PCR) amplifications utilized the PowerPlex® 16 System (Promega, Madison, WI) and AmpliTaq Gold® DNA polymerase (Applied Biosystems) with each amplification tube containing 2.5 μL Gold ST*R 10× buffer, 2.5 μL PowerPlex® 16 primer pair mix, 0.8 μL AmpliTaq Gold, 9.2 μL type I water, and 10 μL template DNA (target of 0.75 ng). Amplification reactions were performed using a Perkin–Elmer GeneAmp® 9600 Thermal Cycler (Foster City, CA) following the parameters recommended by the Promega Corporation for this instrument (14), for 10/20 cycles. After amplification, a portion of each amplicon was evaluated on a 3% test gel. The analysis of the amplification products was conducted on an ABI PRISM® 310 Genetic Analyzer (Applied Biosystems) with 1 μL amplicon/ladder added to a loading solution comprised of 24.0 μL Hi-Di Formamide (Applied Biosystems) plus 1 μL Internal Lane Standard (ILS) 600 (Promega). Injection times were either 3, 5, or 10 sec, depending on the amount of amplified product observed on the 3% test gel.

After a preliminary review of the DNA results obtained, additional experiments were designed to assess the effect of soot with regard to the recovery of DNA as well as the effect of the water used to extinguish the fire with regard to the recovery and amplification of DNA. Accordingly, *c*. 5 months after the aforementioned structural burn, a sample of water used by the ATF FRL to extinguish fires was obtained. The ATF FRL chemically "scrubs" and recycles the water used in their facility to extinguish fires. The approximate pH, alkalinity, and free chlorine levels of the ATF water were obtained using InstaTEST Pool & Spa Test Strips (LaMotte, Chestertown, MD).

Fiber Analyses

The fiber content of the carpeting, curtains, and items of clothing contained within the burned structure was confirmed via light and polarized light microscopy and/or FTIR (15).

Results

Bloodstain Pattern and Chemical Enhancement Results

The living room sustained the worst amount of fire damage (Figs. 6*A* and 6*B*). The highest temperature of 923°C reached during the course of the fire was recorded in the area of the ceiling in the living room (Table 1). The walls that abutted the ignition source (couch) were burned extensively. Bright light was used on

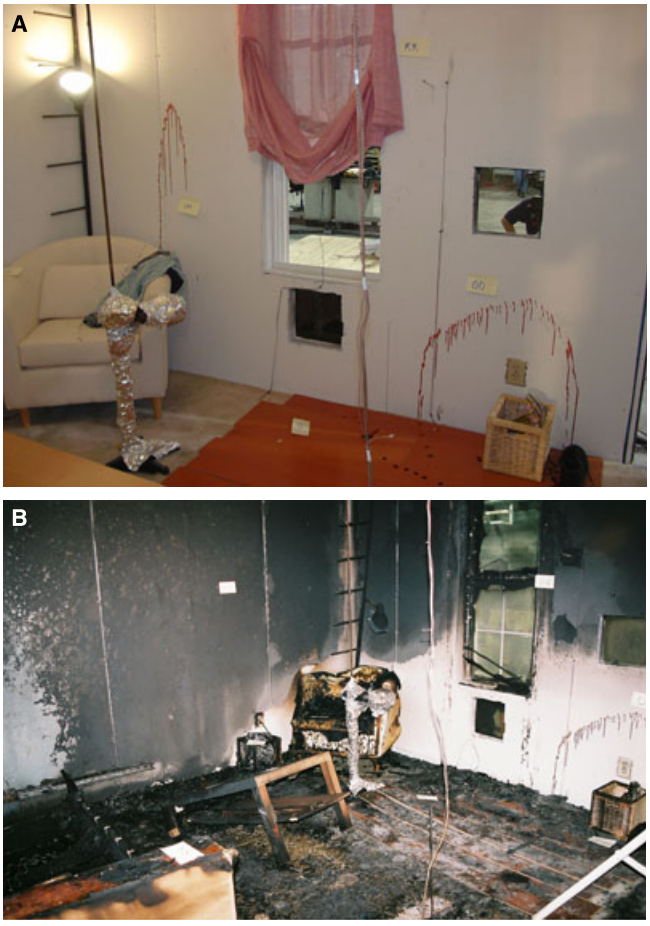

FIG. 6—*(A) Living room across from fire source before burn. (B) Living room across from fire source after burn.*

these walls; however, no visible bloodstain patterns were observed. The couch and the coffee table that was positioned in front of the couch were also burned extensively. No bloodstain patterns were visible on these two items. Fluorescein was sprayed on areas of the couch and coffee table, with one area on the top surface of the coffee table reacting positive. Fluorescein, luminol, and Bluestar® processing were attempted on the two badly burned living room walls and couch, with each application providing a negative result. No positive reactions were noted with applications of fluorescein followed by luminol in these areas of the living room, as well.

Bloodstain patterns could be observed in areas that were less burned in the living room utilizing a bright light. These stains were not altered by fire fighting efforts, only blackened with soot. When a visible stain pattern was observed, the chemical enhancement testing results were positive, with the exception of the visible stain patterns on the ceiling in the southwest corner and on the phone book and ceramic plate on the bookshelf against the west wall. The bloodstain patterns that were placed on the floor on the living room carpet either burned or were destroyed by the firefighting efforts.

The remainder of the structure (dining area, kitchen, and bedroom) had similar effects to the less burned areas of the living room, having received a lesser degree of heat and smoke damage (Figs. 7*A* and 7*B*). All of the bloodstain patterns, including the footwear impressions created in the bedroom and kitchen on the wood flooring product and the vinyl, could be visualized utilizing a bright light. All of the chemical enhancement products utilized gave positive results. The lowest temperature recorded in the structure was in the area of the bedroom floor, reaching 146°C (Table 1).

Hemastix® Results

The results of the Hemastix® tests in the living room were immediately positive on the bloodstain patterns that were still visible, with the following exceptions. One cast-off stain pattern on the ceiling in the southwest corner of the room was initially negative when tested with a Hemastix®. This cast-off stain pattern area was then treated with fluorescein, which also yielded a negative result. The Hemastix® testing was retried, again yielding a negative result. After wiping this fluorescein treated area with an alcohol wipe in an effort to remove soot, a positive Hemastix® result was obtained. Similarly, a portion of the bloodstain pattern on the wall behind the bookshelf against the west wall of the living room failed to yield a positive Hemastix® result but did yield a positive result with Bluestar® subsequent to wiping the area with a distilled water saturated Kaydry® prior to spraying.

All of the Hemastix® test results were positive on samples tested on substrates in the dining room, kitchen, and bedroom.

Sample Recovery and P/TMB Results

Two methods of recovery (swab plus cutting or swab plus scrapings) were attempted from 36 bloodstain patterns in an effort to elucidate if a particular sampling technique would be the best for DNA testing. Of the 36 sets of paired samples, a difference in the number of callable loci was noted in seven of the sets (19.4%). In two of the swab and cutting sets, the DNA results from the swab yielded a greater number of loci. In three of the swab and cutting sets, the DNA results from the cutting yielded a greater number of loci. In two of the swab and scraping sets, the DNA results from the scraping yielded a greater number of loci.

Screening of samples with the P/TMB method was conducted at the laboratory prior to DNA testing on a total of 94 samples (Table 2). Of the 64 of these samples that yielded full DNA profiles, 55 samples (86%) tested positive with P/TMB, eight samples (12.5%) tested negative, and one sample (1.5%) yielded an inconclusive testing result. Of the seven of these samples that yielded a partial DNA profile, five samples (71.4%) tested positive with P/TMB and two samples (28.6%) tested negative. Of the 23 of these samples from which no DNA profile was obtained, seven samples (30.5%) tested positive with P/TMB, 13 samples (56.5%) tested negative, and three samples (13%) yielded an inconclusive testing result. The samples with inconclusive results tested negative for the phenolphthalein portion of the test and positive for the TMB portion, and were all collected from the living room.

The P/TMB results of 16 samples collected as paired sets (swab plus cutting or swab plus scrapings) were mixed, meaning that if the swab tested positive for P/TMB, the cutting or scrapings results were negative or vice versa. In the living room, six additional samples collected as paired sets also had mixed results. For these, however, one of the samples in the pair yielded an inconclusive P/TMB testing result and the other sample yielded either a positive or negative P/TMB result.

DNA Results

A summary of the DNA testing results is depicted in Table 3. Of the 98 samples processed, 69% yielded a full DNA profile, while the remainder of the samples yielded either a partial DNA profile (7%) or no DNA profile (24%). The samples that yielded

FIG. 7—*(A) Dining room before burn. (B) Dining room after burn.*

partial DNA profiles fell into three categories: (i) sample flooded with water when fire extinguished (28.5%); (ii) sample on glass substrate (28.5%); and (iii) probable inhibitor present or reason not apparent (43%). The samples that yielded no DNA profiles fell into five categories: (i) sample subjected to extremely high temperature (26.1%); (ii) sample in close proximity to fuel source/burned away (17.4%); (iii) sample flooded with water when fire extinguished (26.1%); (iv) dilution of sample by chemical enhancement reagent (17.4%); and (v) probable inhibitor present (13%). An inhibitor is a substance that interferes with or prohibits the PCR affecting the ability to obtain a complete DNA profile. Samples that were obtained in an attempt to determine if low levels of DNA, such as those from the wearer of the garment, were recoverable yielded full DNA profiles. In some instances, these wearer DNA profiles were mixtures.

The results of the additional experiments designed to assess the effect of soot with regard to the recovery of DNA demonstrated no measurable effect on the recovery of DNA upon the addition of 1 ng of soot and 4 ng of soot to samples. The water sample obtained from ATF did not have a measurable level of free chlorine and the pH was *c*. 8.4. The addition of the ATF water to blood samples did not have any measurable effect on the isolation of DNA, nor did the addition of the ATF water directly into the amplification mix have any adverse effect on sample amplification.

Discussion

It is encouraging that the majority of bloodstain patterns were readily observable using bright light following the fire. Accordingly, bloodstain patterns are interpretable after a fire depending on the extent of damage caused by the fire and firefighting efforts. Bright light should be used to initially attempt to locate bloodstain patterns that have not been burned away by the fire. All chemical enhancement techniques utilized worked; however, due to the sooty conditions, it was easiest to photograph the positive fluorescein results and avoid overdiluting the stains. The luminol and Bluestar® required additional amounts of reagent than are normally required to achieve a positive result. When attempts were made to remove soot from the bloodstains using water and 70% isopropanol wipes, the chemical enhancements gave positive results quicker than when having to penetrate the soot. Unless excessive amounts of soot were present, no difficulties were encountered when using either a chemical enhancement technique or the Hemastix® test strips. None of the chemical enhancement reagents interfered with the ability to obtain a DNA profile as has been previously published.

TABLE 2—*Phenolphthalein/tetramethyl benzidine testing results and DNA results.*

P/TMB Result	Full DNA Profile Obtained	Partial DNA Profile Obtained	No DNA Profile Obtained
Positive	55	5	7
Negative	8	2	13
Inconclusive	1	0	3

The coffee table adjacent to the fuel source in the living room sustained extensive damage, and yet a positive fluorescein result was obtained from its surface. Subsequent testing could not prove that the observed reaction was due to the dripping bloodstain that was placed on the table. The surface of the table where the blood had been was burned away during the fire. In addition, the fluorescent pattern noted upon treatment of the remaining table surface with fluorescein was different than that of the dripping bloodstain. Regardless, the extent of the damage to the surface of the coffee table from the fire made the ability to obtain a DNA profile from this bloodstain highly unlikely.

Since many agency protocols call for their crime scene technicians to obtain a positive presumptive blood test result before recovering a sample for DNA testing, these findings assist in making recommendations for fire scene processing. When possible, it is

TABLE 3—*Summary of samples processed for DNA from highest temperature to lowest.*

Location/Substrate	Temperature	No. Loci in DNA Profile	Notes
Living room East wall	904°C	0/16	Stained area burned away Cutting and swab—fluorescein
Living room Ceiling, southwest corner	861°C	0/16	Cuttings—no treatment, fluorescein, fluorescein + alcohol wipe Swab—fluorescein + alcohol wipe
Living room South wall	848°C	0/16	Majority of stained area burned away Cutting and swab—no treatment
Dining room North wall	391°C	0/16	Flooded with water Cutting and swab—no treatment
Living room Coffee table	328°C	0/16	Stained area burned away Cutting and swab—fluorescein
Bedroom Ceiling	297°C	16/16	Cutting—no treatment
Dining room Sweatpants	291°C	0/16	Flooded with water Cutting—upper front, no treatment Swab—lower leg, no treatment Swab—lower leg, luminol
Dining room Sweatpants	291°C	10*/16	Flooded with water Cutting—lower leg, no treatment
Dining room Sweatpants	291°C	16/16	Classic degradation pattern noted, flooded with water Cutting—lower leg, luminol
Living room Shoe	279°C	16/16	Cutting and swab—no treatment Swab for wearer—no treatment
Kitchen North wall	277°C	16/16	Cutting and swab—luminol
Kitchen East wall	273°C	16/16	Cutting and swab—no treatment Cutting and swab—Bluestar® + luminol
Kitchen South wall	259°C	16/16	Cutting and swab—Bluestar®
Kitchen Glass monitor	253°C	0/16	Dilution by chemical enhancement reagent Scrapings and swab—luminol
Kitchen Glass monitor	253°C	4/16	Glass surface Swab—no treatment
Kitchen Glass monitor	253°C	16/16	Glass surface Scrapings—no treatment
Kitchen Countertop surface Wooden handle of fork on countertop	240°C	16/16	Cutting and swab, countertop—no treatment Swab, wooden handle of fork—no treatment
Kitchen Stovetop surface Ceramic pot on stovetop	232°C	16/16	Scrapings and swab, stovetop surface—no treatment Swab, stovetop surface—luminol Swab, ceramic pot—luminol
Bedroom Curtain on north wall	204°C	0/16	Flooded with water Cutting—no treatment, more soot
Bedroom Curtain on north wall	204°C	6/16	Flooded with water Cutting—no treatment, less soot
Bedroom Telephone on back of chair	198°C	16/16	Scrapings and swab—no treatment
Living room West wall	196°C	6/16	Cutting—no treatment
Living room West wall	196°C	12/16	Swab—Bluestar® + water wipe
Living room West wall	196°C	16/16	Classic degradation pattern noted Swab—no treatment Cutting—Bluestar® + water wipe Cutting and swab—Bluestar® + alcohol wipe
Dining room Piece of glass on floor	179°C	16/16	Classic degradation pattern noted Glass initially in frame on wall—would have been at a temperature closer to 412°C for some portion of the fire Swab—no treatment
Dining room Laminate floor Sponge on floor Carpet Underpants on floor Boot on floor	179°C	16/16	Scrapings and swab, laminate floor—no treatment Cutting, sponge—no treatment Cutting and swab, carpet—fluorescein Cuttings (2), underpants—no treatment Swab, boot—no treatment
Bedroom Chair arm	161°C	7*/16	Low temperature, not flooded with water or diluted by chemical enhancement reagent Possible inhibitor Cutting—no treatment
Bedroom Chair arm Plate on chair Silverware on chair	161°C	16/16	Swab, chair arm—no treatment Scrapings and swab, plate—no treatment Scrapings and swab, silverware—no treatment

TABLE 3—*Continued*

Location/Substrate	Temperature	No. Loci in DNA Profile	Notes
Dining room	160°C	16/16	Cutting and swab—fluorescein + water wipe
West wall			
Bedroom	157°C	16/16	Cutting and swab—no treatment
East wall			
Living room	153°C	12/16	Swab—fluorescein
Plate on bookshelf against west wall			
Living room	153°C	16/16	Classic degradation pattern noted
Plate on bookshelf against west wall			Scrapings—fluorescein
Kitchen	152°C	16/16	Cutting from bloodstain—no treatment
Pants on floor			Swabs for wearer from pockets and waistband—no treatment
Bedroom	146°C	0/16	Dilution by chemical enhancement reagent
Hardwood flooring			Swab, hardwood flooring—luminol
Vinyl floor			Swab, vinyl floor—luminol
Bedroom	146°C	16/16	Scrapings and swab, hardwood flooring—no treatment
Hardwood flooring			Cutting and swab, vinyl floor—no treatment
Vinyl floor			Swab, vinyl floor—Bluestar®
Flat sheet from bed			Cutting and swab, flat sheet—fluorescein
Fitted sheet from bed			Cutting and swab, fitted sheet—no treatment
Jeans on floor			Cutting and swab, jeans—no treatment, Bluestar®
Wool jacket from floor			Cutting and swab (for wearer), wool jacket—no treatment
Hatchet on floor			Swabs, hatchet handle and head—no treatment
Carpet			Cutting and swab, carpet—no treatment
Living room	111°C	0/16	Low temperature, not flooded with water or diluted by chemical enhancement reagent
Phonebook on bookshelf			Possible inhibitor
			Cutting and swab—no treatment
			Cutting—fluorescein

*Additional alleles noted.

best to perform a presumptive screening test on a suspected bloodstain pattern using either traditional chemical reagents or a tool such as a Hemastix® test strip. When able to obtain a positive result, this avoids the addition of a chemical enhancement reagent that may dilute the sample, which can be problematic for subsequent DNA testing. It is strongly recommended to use a procedure designed to facilitate the removal of soot, such as the wiping techniques described herein or the liquid latex procedure recently described by Larkin (16), on patterns that initially yield a negative presumptive screening test result before proceeding to the use of any chemical enhancement reagents. Should a negative presumptive test result still be obtained, it is recommended for fire scenes that the crime scene team attempts the use of the fluorescein chemical enhancement technique first. This recommendation follows from findings that less fluorescein was needed to obtain a positive result.

Based on the P/TMB findings, it is necessary to provide a caution for laboratories requiring a positive chemical screening test result for the presence of blood prior to initiating DNA testing. As shown by the shaded area of Table 2, this approach is likely to result in the failure to process samples that can clearly yield DNA profiles suitable for comparison purposes. While it is noted that for the most part, samples with full DNA profiles also had positive P/TMB results, *c*. 11% of samples that did not yield a positive P/TMB testing result did yield DNA profiles suitable for comparison purposes. Accordingly, for fire scenes, the use of a chemical screening test for the presence of blood as a tool to eliminate samples to be subjected to DNA testing is not recommended. For those scenes where the most probative samples are likely to be closest to the fuel source, it is advised that DNA testing be conducted on these samples in an attempt to obtain a DNA profile even if the chemical screening test for blood is negative or inconclusive. It is also suggested for fire scenes where limited samples are recovered that DNA testing be conducted on all samples regardless of blood screening test results. Should the DNA typing result only have probative value if the biological sample is blood, a portion of the sample can be subjected to an immunochromatographic or messenger RNA-based testing procedure available for body fluid identification (17–20).

In order to make recommendations regarding the processing of fire scenes for bloodstain patterns and the recovery of DNA samples, it is important to have a clear understanding of when useful DNA testing results were obtained, as identification of the blood source is often a goal. As noted above, the DNA testing results were primarily successful. Table 4, however, depicts the lack of DNA typing results for samples affected by high temperature and/or close proximity to the ignition source. In particular, six samples from two of the hottest points in the fire, the ceiling of the living room and the south wall in the living room, did not yield DNA profiles. No DNA profiles were obtained from the other hottest point in the fire, the east wall in the living room; however, this bloodstain pattern burned away during the fire. Given that the temperature measured in areas of the living room ceiling and the

TABLE 4—*Samples affected by high temperatures and/or close proximity to ignition source.*

Location/ Substrate	Temperature	No. Loci in DNA Profile	Notes
Living room	904°C	0/16	High temperature
East wall			Stained area burned away
Living room	861°C	0/16	High temperature
Ceiling, southwest corner			
Living room	848°C	0/16	High temperature
South wall			Majority of stained area burned away
Living room	328°C	0/16	Stained area burned away
Coffee table			

south wall were in excess of 845°C, it is not surprising that no DNA typing results were obtained using current DNA typing technology. It is noted that the greatest amounts of soot will collect closest to the ceiling during a fire event. In addition, greater amounts of soot will collect on the surfaces closest to the fuel source. Therefore, while these areas would also have had greater amounts of soot adhering, the additional testing conducted did not show that even extraordinarily large amounts of soot would interfere with the ability to successfully extract the DNA. Thus, it still seems most probable that the excessive temperature is responsible for the failure to obtain DNA profiles from the samples recovered from the living room ceiling and south wall.

A series of samples directly in the "line of fire" from the water hose used to extinguish the blaze yielded somewhat unexpected results. Primarily, these bloodstains were recovered from the north walls in the dining room and bedroom and objects near the walls. Of the nine samples recovered from this area, six did not yield a DNA profile, two yielded a partial DNA profile, and one yielded a full DNA profile, albeit with a classic degradation pattern (Table 5). Figure 8 depicts the areas in the structure on which the water was concentrated during the effort to put the fire out. The "halo" where less soot is noted defines this area. Because many other samples yielded full DNA profiles when the temperatures to which they were exposed were even higher than these areas, it is unlikely that temperature alone is responsible. In addition, these samples tended to have less soot deposits given the force of the water directed on them. It is not likely that the soot was an interfering factor as many other samples from which full DNA profiles were obtained had heavier soot deposits. Based on these findings, further testing was performed to determine if there was something unusual about the water used by the ATF FRL that may have interfered with the ability to obtain DNA testing results. Our subsequent testing on a sample of water obtained from the Laboratory did not show any affect of this water on the ability to isolate or amplify DNA. It is important to note, however, that this sample of water was obtained months after our structure fire was extinguished and may have had different properties than the water that was actually used. It is surmised by the process of elimination, therefore, that either the force of the water as it was expelled from the fire hose may be in some way responsible for this observed phenomenon or something in the composition of the water.

Throughout the testing, there are numerous examples of full DNA profiles being obtained when positive luminol, fluorescein, or Bluestar® reactions were obtained on bloodstain patterns sprayed with these enhancement reagents prior to sample recovery. The only exceptions to this were two samples in the kitchen and two samples in the bedroom (Table 5) where the patterns were "oversprayed" or super-saturated with reagent, resulting in the pattern being observably washed away by the enhancement reagent. Only luminol and Bluestar® will have this ill effect on bloodstains because of the short life span of the reaction, which may require multiple applications of the reagents if the photographer is not prepared to capture the reaction quickly. Under these conditions, it is not surprising that no DNA profile could be obtained.

Table 6 highlights results from samples affected by their proximity to glass or other factors. The DNA profiles obtained from samples recovered from glass or ceramic items tended to yield a classic degradation pattern. A classic degradation pattern is observed when lower molecular weight STRs amplify successfully and those of higher molecular weight either amplify partially or not at all due to the degradation. Glass, because it contains no moisture like wallboard and other items, heats to temperature more quickly and is slower to return to room temperature. This phenomenon explains why, then, the classic DNA degradation pattern is observed as blood on the surface of glass items is exposed to the elevated temperatures for a longer period of time. It is recommended, therefore, that the crime scene team recover bloodstain patterns from glass items only when absolutely necessary—and that they recover alternate samples from other substrates when at all possible.

Also of note is the cutting sample from the arm of the chair from the bedroom that yielded a partial DNA profile (Table 6). As the swabbing of the blood pattern from this same area yielded a full DNA profile and the temperature in this area of the structure

TABLE 5—*Samples affected by flooding with water and/or dilution by chemical enhancement reagents.*

Location/ Substrate	Temperature	No. Loci in DNA Profile	Notes
Dining room North wall	391°C	0/16	Flooded with water Cutting and swab—no treatment
Dining room Sweatpants	291°C	0/16	Flooded with water Cutting—upper front, no treatment Swab—lower leg, no treatment Swab—lower leg, luminol
Dining room Sweatpants	291°C	10*/16	Flooded with water Cutting—lower leg, no treatment
Dining room Sweatpants	291°C	16/16	Flooded with water Classic degradation pattern noted Cutting—lower leg, luminol
Kitchen Glass monitor	253°C	0/16	Dilution by chemical enhancement reagent Scrapings and swab—luminol
Bedroom Curtain on north wall	204°C	0/16	Flooded with water Cutting—no treatment, more soot
Bedroom Curtain on north wall	204°C	6/16	Flooded with water Cutting—no treatment, less soot
Bedroom Hardwood flooring	146°C	0/16	Dilution by chemical enhancement reagent Swab—luminol
Bedroom Vinyl floor	146°C	0/16	Dilution by chemical enhancement reagent Swab—luminol

*Additional alleles noted.

FIG. 8—*View depicting locations saturated with water when the fire was extinguished, with "halo" effect evident against the back wall.*

TABLE 6—*Samples affected by other factors.*

Location/ Substrate	Temperature	No. Loci in DNA Profile	Notes
Kitchen	253°C	4/16	Glass surface
Glass monitor			Swab—no treatment
Living room	196°C	6/16	Cutting—no treatment
West wall			
Living room	196°C	12/16	Swab—Bluestar® + water wipe
West wall			
Living room	196°C	16/16	Classic degradation pattern noted
West wall			Swab—no treatment
			Cutting—Bluestar® + water wipe
Dining room	179°C	16/16	Glass initially in frame on wall
Piece of glass on floor			—would have been at a temperature closer to 412°C for some portion of the fire
			Classic degradation pattern noted
			Swab—no treatment
Bedroom	161°C	7*/16	Possible inhibitor: low temperature, not flooded with water or diluted by chemical enhancement reagent
Chair arm			Cutting—no treatment (full profile with swab)
Living room	153°C	12/16	Ceramic/glass
Plate on bookshelf against west wall			Swab—fluorescein
Living room	153°C	16/16	Ceramic/glass
Plate on bookshelf against west wall			Classic degradation pattern noted
			Scrapings—fluorescein
Living room	111°C	0/16	Possible inhibitor: low temperature, not flooded with water or diluted by chemical enhancement reagent
Phonebook on bookshelf			Cutting and swab—no treatment
			Cutting—fluorescein

*Additional alleles noted.

TABLE 7—*Summary of DNA results based on temperature and location.*

Room	Maximum Temperature Reached in Room	% Full DNA Profile	% Partial DNA Profile	% No DNA Profile
Living room	923°C	33.3	12.5	54.2
Dining room	646°C	66.7	5.5	27.8
Kitchen	375°C	86.4	4.6	9.0
Bedroom	307°C	85.3	5.9	8.8

remained low, the finding of a partial DNA profile when the fabric cutting was included in the extraction tube is most indicative of an inhibitor being present in the fabric. It is known that inhibitors can co-extract with DNA and thus affect the ability to obtain a full DNA profile. In addition, an inhibitor may also have affected the samples from the telephone book, which yielded no DNA profile. It was noted upon sample collection that the blood deposits had an unusual ''bubbled'' appearance, indicating the possibility of an interaction between the brightly colored printed surface of the telephone book and the blood upon exposure to the relatively high and prolonged temperature in the room. Also of interest are the four samples from the west wall of the living room, two of which yielded partial DNA profiles and two which yielded full DNA profiles with observable degradation patterns. Given that the temperature in this area was relatively low (196°C), the area did not appear to have been flooded with water when the fire was extinguished, nor was it flooded with chemical enhancement reagent, no clear reason for these results is evident. It was noted that these samples were recovered from areas on the wall that were in close proximity to the telephone book.

Table 7 clearly demonstrates the adverse effect that elevated temperatures had on the ability to obtain DNA typing results in the structure. This data show that rooms in the structure with higher maximum temperatures had a greater percentage of partial or no DNA profiles obtained. This trend provides valuable information for individuals who process fire scenes. In general, samples from structure fires recovered for DNA analysis will have a greater likelihood of yielding a full DNA profile the farther they are from the fuel source and, essentially, the closer they are to the floor.

Based on the findings, a multiple sample recovery method should be used for sample collection. In some instances, the portion of the sample recovered onto a swab yielded a higher DNA recovery and a better DNA profile while in others the same could be said for the portion of the sample recovered as a cutting or scraping. This multicollection protocol at fire scenes will help to ensure that the best DNA testing results achievable will be obtained.

While the research conducted answered numerous questions regarding bloodstain pattern interpretation, use of chemical enhancement products, and DNA recovery after a fire, it also presented new questions for possible future study. These areas of interest include: (i) subjecting samples exposed to high temperatures to a mini-STR study (21) to determine whether any typing results could be obtained; (ii) further testing of samples in the ''line of fire'' of a fire hose (force/pressure vs. ATF recycled water); and (iii) additional work on brightly printed materials to determine why the DNA results were compromised on the telephone book and the surrounding area.

References

1. Gerberth VJ. Practical homicide investigation tactics, procedures and forensic techniques, 3rd edn. Boca Raton, FL: CRC Press, 1996.
2. Fisher BAJ. Techniques of crime scene investigation, 6th edn. Boca Raton, FL: CRC Press, 2000.
3. James SH, Eckert WG. Interpretation of bloodstain evidence at crime scenes, 2nd edn. Boca Raton, FL: CRC Press, 1998.
4. Brady T, Tigmo J, Graham G Sr. Extreme temperature effects on bloodstain pattern analysis. IABPA News 2002;18(2):3–20.
5. Grispino RR. The effect of luminol on the serological analysis of dried human bloodstains. Crime Lab Digest 1990;17(1):3–23.
6. Budowle B, Leggitt JL, Defenbaugh DA, Keys KM, Malkiewicz SF. The presumptive reagent fluorescein for detection of dilute bloodstains and subsequent STR typing of recovered DNA. J Forensic Sci 2000;5(5):1090–2.
7. Gross AM, Harris KA, Kaldon GL. The effect of luminol on presumptive tests and DNA analysis using the polymerase chain reaction. J Forensic Sci 1999;4(4):837–40.
8. Blum LJ, Esperanca P, Rocquefelte S. A new high-performance reagent and procedure for latent bloodstain detection based on luminol chemiluminescence. Can Soc Forensic 2006;39(3):81–100.
9. Jakovich C. STR Analysis following latent blood detection by luminol, fluorescein and Bluestar®. J Forensic Ident 2007;57(2):193.
10. Virginia Department of Forensic Science. The forensic biology procedure manual, section II—presumptive and confirmatory tests for biological substances. 2006. Available at: http://www.dfs.virginia.gov/services/forensicBiology/manuals/procedures/02%20-%20II-Presumptive%20and%20Confirmatory%20Tests/05%20-%20Chapter%201%20-%20DETECTION%20OF%20BLOOD.pdf, Accessed May 2008.
11. Bevel T, Gardner RM. Bloodstain pattern analysis, 2nd edn. Boca Raton, FL: CRC Press, 2002.
12. Saferstein R, editor. Forensic science handbook volume I, 2nd edn. Upper Saddle River, NJ: Prentice Hall, 2002; 272–6.
13. Zweidinger RA, Lytle LT, Pitt CG. Photography of bloodstains visualized by luminol. J Forensic Sci 1973;18(4):296–302.

14. Promega Corporation. PowerPlex® 16 System Technical Manual for use of products DC6530 and DC6531 [part # TMD012] 2001. Madison, WI: Promega Corporation, 2001.
15. Robertson J, Grieve M, editors. Forensic examination of fibers. London: Taylor and Francis, 1999; 109–12.
16. Larkin T. A case study of a dismemberment homicide scene subjected to an arson attack—soot removal techniques for BPA and fingerprint examinations. Proceedings of the 2006 International Association of Blood Pattern Analysts Annual Meeting, October 18–20, Corning, NY, 2006. Available at: http://www.iabpa.org, Accessed September 2008.
17. Johnston S, Newman J, Frappier R. Validation study of the Abacus Diagnostics ABAcard® HemaTrace® membrane test for the forensic identification of human blood. Can Soc Forensic 2003;36(3):173–83.
18. ABAcard® HemaTrace® technical information sheet 2003. West Hills, CA: Abacus Diagnostics, Inc., 2003.
19. Nussbaumer C, Gharehbaghi-Schnell G, Korschineck I. Messenger RNA profiling: a novel method for body fluid identification by real-time PCR. Forensic Sci Int 2006;3:181–6.
20. Juusola J, Ballantyne J. mRNA profiling for body fluid identification by multiplex quantitative RT-PCR. J Forensic Sci 2007;52(6):1252–62.
21. Butler J. Forensic DNA typing: biological, technical, and genetics of STR markers, 2nd edn. Burlington, MA: Elsevier Press, 2005.

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