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TECHNICAL NOTE

CRIMINALISTICS

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Evaluation of Bluestar® Forensic Magnum and Other Traditional Blood Detection Methods on Bloodstained Wood Subjected to a Variety of Burn Conditions*,†

ABSTRACT: Accurate blood detection is a primary concern for forensic scientists, especially in highly compromised situations. In this study, blood was added to wood blocks and subjected to a variety of fire treatments: the absence or presence of accelerant, burn time (1, 3, or 5 min), and extinguishment method (smothering or dousing with water). Burned blocks were given a qualitative burn score, followed by removal of half of the char from each block and subsequent testing of each half for blood using luminol (13% positive; $n = 96$), Bluestar® Forensic Magnum (5.2% positive; $n = 96$), and combined phenolphthalein tetramethylbenzidine test (0% positive; $n = 192$). Luminol and Bluestar® Forensic Magnum performed similarly, both outperforming PTMB. Additionally, positive results were more likely from samples that were smothered, had a low burn score, and had more concentrated blood solutions (neat or 1:2). Overall, it is extremely unlikely that blood would be detected on combustible substrates exposed to direct fire.

KEYWORDS: forensic science, blood detection, fire, Bluestar Forensic Magnum, luminol, phenolphthalein tetramethylbenzidine

Blood evidence is commonly encountered in a variety of crime scenes, and the ability to accurately detect blood is a primary concern for forensic scientists. Specifically at fire scenes, it is difficult to recover testable evidence that has been directly subjected to fire's highly compromising conditions. Moreover, blood evidence may be more often encountered at arson scenes than other fire scenes because a primary motive for arson is to cover up a crime such as murder (1–3). This evidence may be further adulterated due to standard water suppression efforts taken to extinguish the fire. Although firefighters are trained to preserve evidence to the best of their ability and be cautious when suppressing the fire (4), these efforts can nonetheless reduce the integrity of the probative evidence found at the scene.

There are numerous blood detection reagents available to the forensic community (e.g., luminol, fluorescein, Bluestar® Forensic, phenolphthalein, and tetramethylbenzidine). Under ideal, uncompromised conditions, it is fairly easy to detect bloodstains using any of these methods, with some reporting sensitivity levels of 1:100 to 1:100,000,000 (5–14). Luminol is the among the oldest and most common blood enhancement test, but a variety of preparations have been evaluated through its nearly 70-year history, with the Grodsky preparation continuing to be the most commonly used formulation (15–18). It is not uncommon to encounter inconsistent results across studies with respect to how these blood tests perform relative to one another (13,16,19). It has also been noted that Bluestar® Forensic Latent Bloodstain Reagent (abbreviated as "Bluestar"; Bluestar®, Monte-Carlo, Monaco) and luminol can interfere with subsequent phenolphthalein or tetramethylbenzidine (TMB) testing for blood samples more dilute than 1:100 (20). A combined phenolphthalein tetramethylbenzidine (PTMB) has also been adopted by a small percentage of forensic laboratories and/or cited in the literature (21–24). This combined test is advantageous because it provides both basic (with phenolphthalein) and acidic (with TMB) testing environments, which eliminates false positives that may occur in either condition. However, it is only as sensitive as the least sensitive of the two reagents, which tends to be phenolphthalein (8).

Among the newest of the blood detection reagents is Bluestar® Forensic Magnum Bloodstain Reagent (abbreviated as "Bluestar Magnum"; Bluestar®, Monte-Carlo, Monaco), a product claiming to be three times more powerful than the original formulation Bluestar (25). Very few studies involving Bluestar Magnum exist in the literature, the most noteworthy of which assessed sensitivity of this and other luminol-based blood detection tests (12). This study concluded that Bluestar Magnum was the most sensitive of the blood tests evaluated for both porous and nonporous substrates, detecting as little as 50 μL of a 1:100,000 dilution.

New research questions continue to arise not only to evaluate newer products, but also to address challenging types of samples

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(e.g., burned bloodstains). Studies involving the effects of fire on biological evidence have demonstrated that extreme heat, close proximity to fire, and presence of soot negatively impact the sensitivity of presumptive blood tests and/or to obtain full DNA profiles, especially from combustible substrates (16,19–21). Researchers have attempted to combat soot from interfering with presumptive blood tests by wiping it away (23), but this does not seem to be an effective approach for combustible substrates such as wood. Interestingly, full DNA profiles have been obtained from samples failing to yield a positive presumptive blood test (23), extinguished via water by firefighters (23,26), and even from noncombustible and nonabsorbent samples exposed to temperatures of 1000°C (27). Full profiles have also been obtained from wood substrates, as long as the samples were not completely charred (26).

Although a plethora of studies has been conducted on blood detection reagents, none have been identified to evaluate the relatively new blood detection reagent, Bluestar Magnum, with bloodstained samples exposed to fire. For this type of evidence, one of the main drawbacks of the analysis process stems from relying on presumptive blood test results to guide, and often dictate, subsequent testing. This is problematic as many laboratories require a positive presumptive blood test on a suspected blood sample prior to DNA testing. Some laboratories take this a step further and require a positive microchemical/hemochromogen presumptive test (e.g., phenolphthalein and TMB) regardless of if a positive result was obtained from a blood enhancement test (e.g., luminol and fluorescein) (23)—likely because the latter are more subject to false positives. If this policy is strictly adhered to, many samples with potentially informative DNA profiles will go untested. This is supported by testimony from a forensic case in 2007 where a suspected bloodstain was recovered from a porous, combustible material exposed to high heat (though not burned via flame). It did not yield a positive result after repeated attempts using phenolphthalein. Despite these negative results, a nearly full DNA profile was recovered using the AmpF*l*STR® Identifiler® PCR Amplification Kit (Applied Biosystems, Foster City, CA).

In this study, wood blocks with different blood dilutions (neat, 1:2, 1:5, and 1:10) were exposed directly to fire with varying burns time (1, 3, or 5 min). Accelerant (unleaded gasoline) was added to half of these samples prior to burning. After removal from the fire, samples were either extinguished via smothering or dousing with water, the latter of which was intended to mimic standard suppression efforts employed by firefighters. In an attempt to combat the presence of soot and char, the charred upper layer was scraped away from half of each block with the hopes of exposing a char- and soot-free layer containing a detectable amount of blood. Lastly, samples underwent various blood testing methods—including Bluestar Magnum, luminol (Grodsky formula) and PTMB—in order to evaluate the suitability of Bluestar Magnum to detect burned bloodstains on a porous, combustible substrate.

Materials and Methods

Overview of Experimental Design

This study was designed to evaluate the ability of three presumptive blood tests to detect nonpreserved (i.e., no EDTA added) blood exposed to fire under several conditions. Treatments included: (i) blood dilution (neat, 1:2, 1:5, 1:10); (ii) burn time (1, 3, or 5 min); (iii) presence/absence of accelerant (unleaded gasoline); (iv) extinguishment method (smothered with an aluminum pan versus dousing with water); (v) testing the charred area directly versus scraping away the char and testing the area beneath the char; and (vi) blood detection test (luminol, Bluestar Magnum, and a combined phenolphthalein tetramethylbenzidine test).

Sample Preparation

A total of 116 untreated pine wood blocks (7.7 × 7.7 cm) were prepared for this study. For each of four dilutions of pig's blood (neat, 1:2, 1:5, and 1:10), 28 blocks were prepared by adding 500 μL of a dilution to one surface of the block; four of each dilution served as positive controls. Additionally, 500 μL of water was pipetted to each of four blocks to serve as negative controls. All samples were air dried for 2 days.

Sample Burning

A controlled fire was set inside a metal burn barrel that had holes in the bottom for airflow. Four samples of the same dilution were burned at a time, in the following order: neat, 1:2, 1:5, and 1:10 for 1 min, then 3 min, and 5 min. Immediately prior to burning, 500 μL of unleaded gasoline was pipetted on to the bloodstained surface of two of the four blocks. All four blocks were then secured in a wire cooking rack (to allow for easier addition to and recovery from the fire), placed into the fire, and removed after the designated burn time. Next, two of the four samples were smothered with an aluminum pan and two samples were doused with water. One sample with and without accelerant was extinguished using each of the two methods. None of the controls were burned, but two (half) of each control were doused with water. Samples cooled/air dried for 2 days prior to packaging in paper bags and transporting to the laboratory for blood testing.

It should be noted that the wire cooking rack broke and was not used after the 3 min burn of the 1:5 blood dilutions. Additionally, the fire began to die down during the end of the 1 min burns, and leaves were added as additional kindling to help revive the fire during the 5 min burn of the 1:5 blood dilutions.

Burn Score

In an effort to account for the inability to sustain the fire at the same intensity for the duration of the study (e.g., it began to die down), a burn score of 0–5 was assigned to each block to qualitatively assess the degree of burn, with "5" being the most burned. Descriptions and representative samples for each burn score are noted in Fig. 1.

Blood Detection Tests

In preparation for blood testing, half of the char was scraped off of each burned block with a metal knife (see Fig. 2). Half of the scraped side was swabbed with a sterile cotton swab and half of the charred side was swabbed with a second swab; positive and negative control blocks were swabbed in a similar fashion. These swabbings (192 treatment and 40 controls) were collected for PTMB testing. Half of the sample/control blocks (one for each treatment) were tested with luminol and the other half were tested with Bluestar Magnum. Descriptions of these three tests are below.

PTMB—For the PTMB test, reduced phenolphthalin (0.3 g of phenolphthalin, 7.5 g of potassium hydroxide pellets, 30 mL of

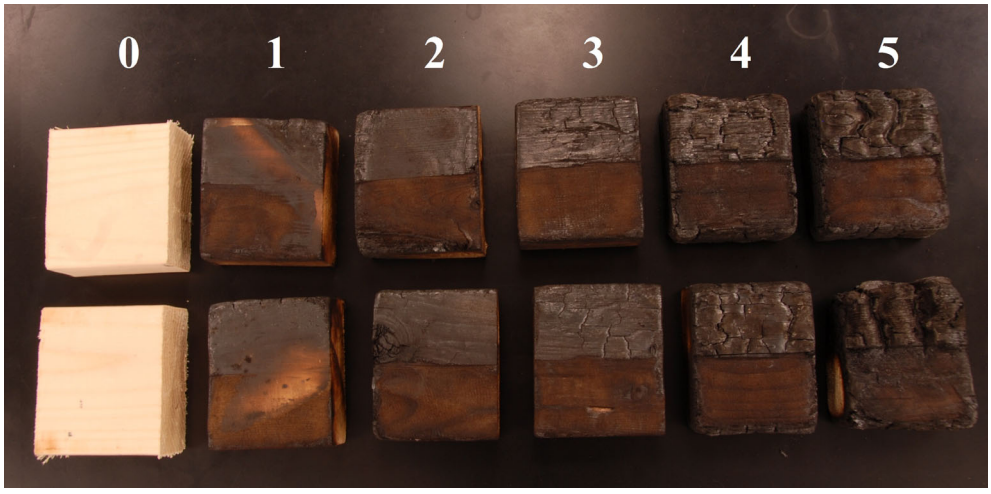

FIG. 1—*Burn score scale. Following burning, blocks were assigned a burn score from "0" to "5." "0" was reserved for samples that were not burned at all (i.e., the controls). "1" represented samples with a blackened surface that was not fully charred. The remainder exhibited a fully blackened surface but had varying levels of alligatoring (surface cracking), with "2" exhibiting none, "3" minimal, "4" moderate, and "5" deep. Representative examples of each score are shown above. [Color figure can be viewed at wileyonlinelibrary.com]*

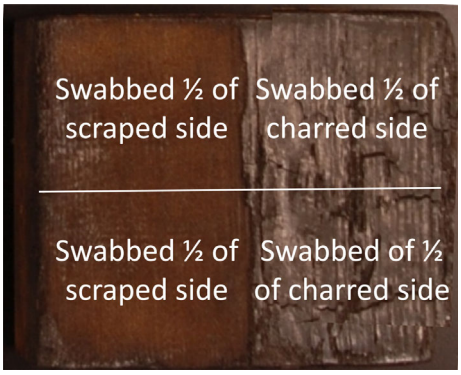

FIG. 2—*Preparing blocks for blood tests. To determine whether the presence of charred wood interfered with blood testing, the char was removed from half of each burned block via scraping. One cotton swab was then used to swab half of the charred side, and a second swab was used to swab half of the scraped side. These swabs were used for PTMB testing. Each block would also be sprayed with either luminol or Bluestar Magnum for additional testing. [Color figure can be viewed at wileyonlinelibrary.com]*

sterile water and ~3 g of zinc flakes until colorless) and TMB (0.01 g of TMB and 30 mL of glacial acetic acid) reagents were prepared the day before testing the swabs. Prior to use, the reagents were tested with a positive (1:6 pig's blood) and negative (sterile swab) control. For each sample/control swab, reagents were applied using a transfer pipette (Globe Scientific Inc. Item#: 137030; tip diameter 2.7 mm) in the following order: one drop of sterile water, 95% ethanol, phenolphthalin (colorless), and 3% hydrogen peroxide, followed by 2–3 drops of TMB. Observations were noted after the addition of each reagent. Regardless of the presence of blood, no color change should occur following the addition of the first three reagents (any color change at these points would be considered inconclusive). A positive result was indicated by a pink color after the addition of hydrogen peroxide (signifying the conversion to phenolphthalein), and a blue-green color after the addition of TMB. A negative result was indicated by no color change after the addition of hydrogen peroxide and TMB. An inconclusive result was indicated by any other reaction not categorized as positive or negative, including a "positive" reaction for one test and a "negative" reaction for the other. (24)

Luminol—The luminol reagent was prepared by combining 0.2 g of luminol, 10 g sodium carbonate, 200 mL of deionized water, and 1.4 g sodium perborate into a spray bottle; the reagent was used immediately (15). Prior to use, the prepared reagent was tested with a positive (1:6 pig's blood) and negative (unstained floor tile) control.

Given a large number of samples and controls to be tested, these were arranged into two groups of 29 blocks based on extinguishment method (see Figure S1). Samples and respective positive and negative controls were tested by applying two fine-mist spray applications to each block in complete darkness. In an effort to ensure accurate interpretation of results, two researchers were present to perform the test and observe the results. A positive result was indicated by an immediate blue chemiluminescence, while a negative result was indicated by the absence of chemiluminescence. Inconclusive results were indicated by any result that did not coincide with the expected result for a positive or negative reaction.

Bluestar Magnum—Bluestar Magnum was prepared as directed for the manufacturer by adding 125 mL of Bluestar® Forensic Magnum chemiluminescent solution and three supplied tablets to a spray bottle. The contents were swirled gently in a circular motion for two minutes, and the reagent was used immediately. Prior to use, the prepared reagent was tested with a positive (1:6 pig's blood) and negative (unstained floor tile) control.

Samples and the respective positive and negative controls arranged as described above for luminol testing and were tested by applying two fine-mist spray applications to each block in complete darkness. Results were interpreted the same as with the luminol test.

Statistical Analyses

Paired *t*-tests were performed in Excel with respect to treatments with two variables (extinguishment, accelerant, and testing

scraped versus charred side). Chi-square contingency tests with as-needed partitioning (28) were performed in R (R Core Team, Vienna, Australia; [29]) for test result based on blood test performed with respect to treatments with three or more variables (blood test, burn score, and blood dilution). A three-way ANOVA (analysis of variance) with post hoc Tukey HSD (honest significant difference) was performed in R for burn score to assess the influence of burn time, extinguishment, and accelerant. Lastly, a SIMPER ("similarity percentages"; [30]) analysis was performed in IBM SPSS Statistics software (IBM Inc., Armonk, NY) to determine which variables (i.e., treatments) were contributing the most to the dissimilarity in results between the three blood tests.

Results and Discussion

Burn Score

In this study, it was expected that an increase in burn time would increase the degree to which a block was burned, which would lead to a decrease in positive blood test results. However, the fire was not sustainable at the same intensity for the duration of the study. The fire first began to die down during the 3 min burns, with substantial loss in intensity at the 5 min 1:5 dilution burn set. To remedy this, a burn score variable was introduced to account for the degree of burn in an attempt to correlate burn score (rather than burn time) with blood test result. It was hypothesized that burn score could have been impacted not only by burn time, but also the presence of accelerant and/or extinguishment method. Furthermore, it was hypothesized that burn score would increase as burn time increased, as well as in the presence of accelerant, but would decrease for blocks extinguished via dousing with water, as compared to smothering with an aluminum pan.

Average burn scores were calculated for each of these variables, and a three-way ANOVA revealed that burn time did significantly impact burn score ($p = 0.003$) but the presence of accelerant ($p = 0.930$) and extinguishment method ($p = 0.068$) did not (see Fig. 3). When examining burn time and burn score further, it should be noted that the 3 min burn samples had the highest average burn score (3.9 ± 0.9), while the 5 min burn samples had the lowest burn score (2.9 ± 1.6). Furthermore, the only significant difference observed between burn times was between the 3 and 5 min burns (Tukey HSD, $p = 0.002$). This observation would have been unexpected if the fire intensity had remained the same throughout the study, but it is consistent with the fact that the fire did substantially die down during the 5 min burns.

Blood Test Results

Of the 384 blood tests performed on the 96 treatment samples, there were 17 positive test results from a total of ten blocks (see Fig. 4). Some blocks tested positive on both the scraped and charred side, while others only tested positive on one side. When examining the results based on blood test, it was noted that positive results were only obtained from luminol and Bluestar Magnum (see Fig. 5). In addition to no positive results being obtained from PTMB, this test was also the only one to yield inconclusive results (3% of the 192 swabs tested). Lastly, without taking into account any other variables, the differences in results were determined to be statistically significant between PTMB and both luminol and Bluestar Magnum (chi-square with partitioning, $p < 0.001$), but not between luminol and Bluestar Magnum (chi-square with partitioning, $p = 0.127$).

With respect to burn score, 71% of positive test results ($n = 17$) were from blocks with a burn score of 1, and 58% of blocks with a burn score of 1 ($n = 13$) tested positive with luminol or Bluestar Magnum (see Fig. 6). This was expected given that low burn scores signify low degrees of burn, which were hypothesized to be the most likely to yield positive blood test results. Without taking into account any other variables, the differences in results were determined to be statistically significant between samples with a burn score of 1 and all other burn scores, but not between burn scores of 2, 3, 4, or 5 (chi-square with partitioning, $p < 0.001$). It should also be noted that there were 60% more blocks with a burn score of 1 that were tested with luminol (eight blocks) than there were for Bluestar Magnum (five blocks). Unfortunately given the nature of this study, it was not possible to evenly distribute burn scores across the blood tests, and the increased number of blocks with burn scores of 1 for luminol likely explains the higher rate (although not statistically significant) of positive test results obtained from luminol, as compared to Bluestar Magnum.

There were only three blocks with a burn score other than 1 that had one or more positive test results: their burn scores ranged from 3 to 5; two tested positive with luminol and one tested positive with Bluestar Magnum; all were neat blood or 1:2 dilutions; all were burned for five minutes; all were smothered; two had accelerant; and two had positive reactions on both the scraped and charred sides (see Fig. 7). Interestingly, the block with a burn score of 5 that had a positive test result (with Bluestar Magnum) only tested positive on the scraped side. It is suspected that since this was from a neat blood sample, the blood-soaked down far enough into the wood that even after removing the charred surface, Bluestar Magnum was able to detect the blood.

When examining extinguishment method, 88% of positive test results ($n = 17$) were from blocks that had been smothered after burning, and 17% of smothered blocks ($n = 48$) tested positive with luminol or Bluestar Magnum (see Fig. 8). The differences observed between extinguishment method were statistically significant (paired t-test, $p < 0.001$). From these results, it seems likely that the blocks doused with water were further compromised and perhaps a significant portion of the detectable blood that remained on the block after burning was then washed away completely or to undetectable levels during the dousing step. However, it is noteworthy to revisit burn score with respect to extinguishment method at this point. The average burn score was lower for smothered compared to doused samples (3.1 ± 1.2 and 3.6 ± 1.2, respectively; see Fig. 3), but these differences were not significant ($p = 0.068$). Since the p-value is <0.10, extinguishment method may have some small influence on burn score, which has been shown to significantly influence the outcome of the blood test result. However, the mechanism in which smothering lowers burn score relative to dousing (or in which dousing increases burn score relative to smothering) remains unclear. Thus, the decreased rate of positive results obtained from doused samples may be due to a combination of water washing away residual blood and the extinguishment method's (possible) minor influence on burn score.

Upon review of the use of unleaded gasoline as an accelerant, it was observed that 59% of positive test results ($n = 17$) were from blocks that had accelerant added immediately prior to burning, and 13% of blocks with accelerant ($n = 48$) tested positive with luminol or Bluestar Magnum (see Fig. 9). The differences observed between the presence and absence of accelerant were

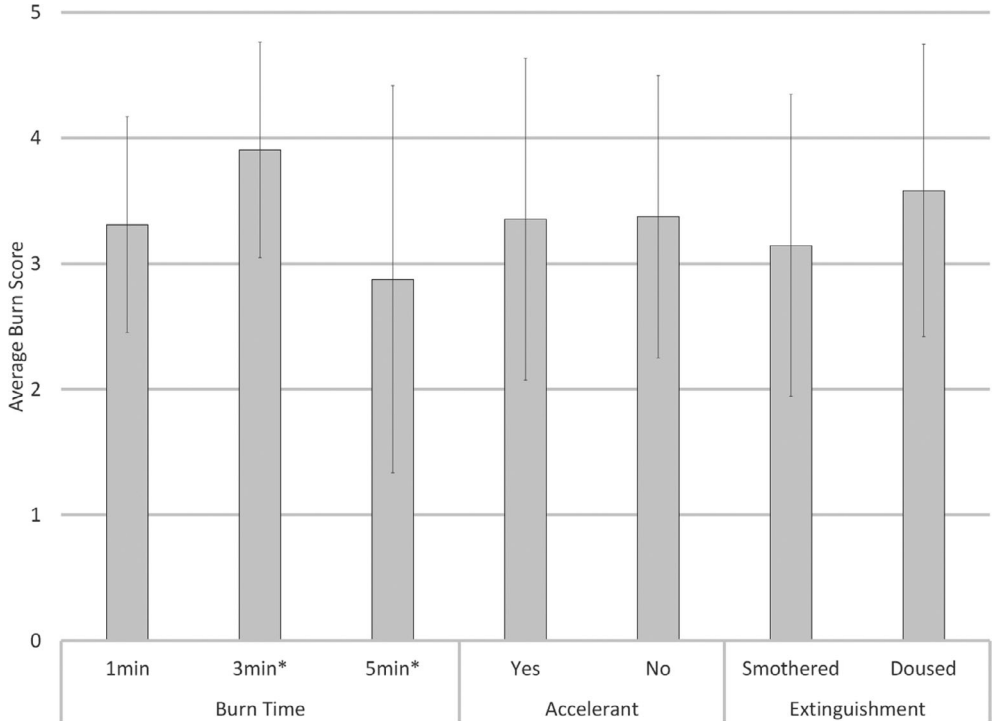

FIG. 3—*Variables potentially impacting burn score. Average burn scores were calculated for each of the three burn times, presence/absence of accelerant, and two extinguishment methods. A 3-way ANOVA determined that burn time significantly impacted burn score* (p = *0.003), but accelerant* (p = *0.930) and extinguishment* (p = *0.068) did not. **A Tukey HSD test on the burn times determined that the differences seen for the burn times were only significant between 3 and 5 min* (p = *0.002).*

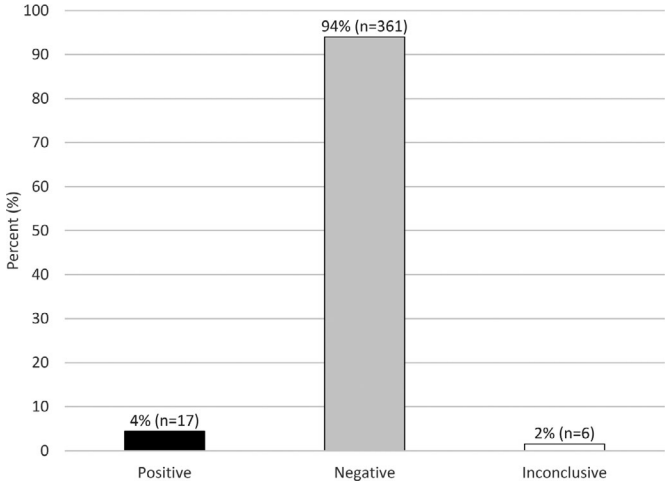

FIG. 4—*Summary of all test results. Of the 384 blood tests performed in this study, only 4%* (n = *17) were positive. The vast majority of all tests were negative for blood.*

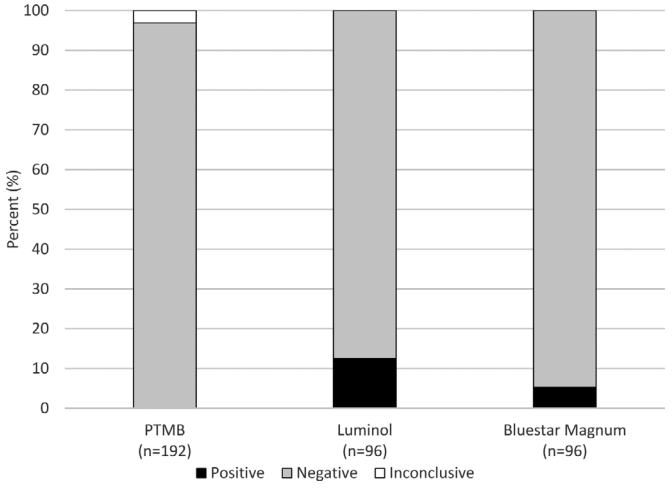

FIG. 5—*Summary of results based on blood test. When examining the results based on which blood test was performed, it was observed that positive results were only obtained using luminol and Bluestar Magnum; no positive results were obtained using PTMB. Overall, 13% of all blocks treated with luminol tested positive and 5% of all blocks treated with Bluestar Magnum tested positive. The differences between test results obtained between luminol and Bluestar Magnum were not significant (chi-square with partitioning,* p = *0.127), but those between PTMB and luminol/Bluestar were (chi-square with partitioning,* p < *0.001).*

not statistically significant (paired *t*-test, $p = 0.10$). These results were not expected, as it was hypothesized that use of accelerant would increase the degree of burn, thereby increasing burn score and reducing the ability to obtain a positive blood test result. Thus, positive results would have been more likely from blocks without accelerant than those with accelerant. Further review of the experimental design revealed potential flaws regarding the use of accelerants in this study. One flaw includes adding unleaded gasoline directly to the blocks prior to burning, with the hypothesis that the accelerant would increase the degree of burn. Accelerants are traditionally used to spread fires more quickly and/or to other areas, not to cause them to burn at a higher temperature. Furthermore, wood and gasoline actually burn at roughly the same temperature (4). Other studies have shown that not only are accelerants consumed very quickly (e.g., 500 mLis consumed in ≤60 sec), but that they may also protect the underlying surface, resulting in less damage than from

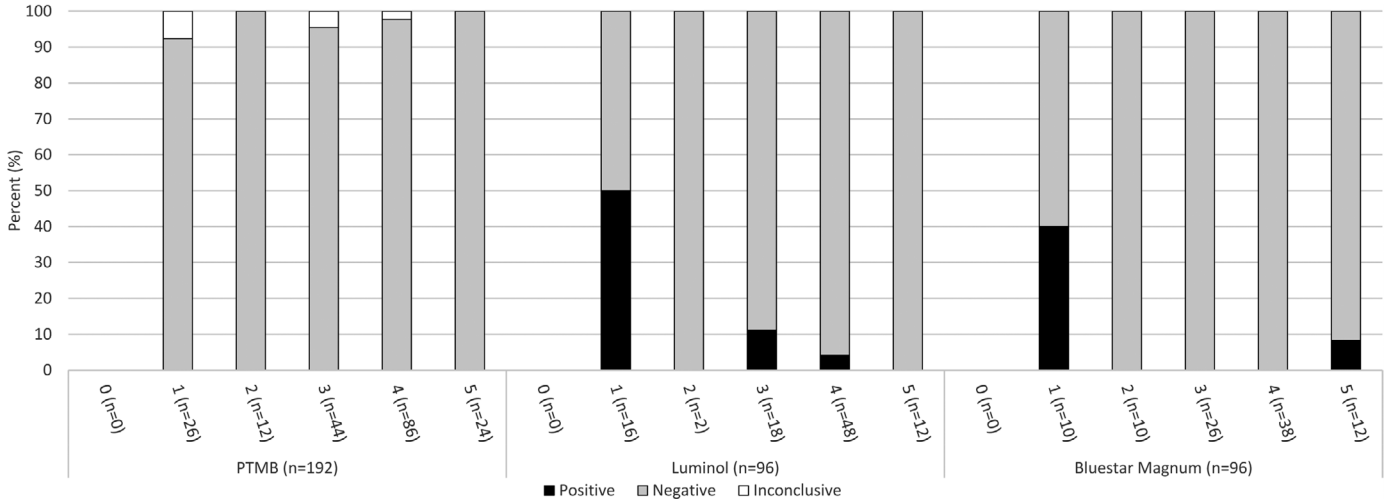

FIG. 6—*Blood test result versus burn score. Test results are displayed for each of the three blood detection tests with respect to burn score. Overall, 71% of all positive test results were from blocks with a burn score of 1, and 58% of all blocks with a burn score of 1 tested positive with luminol or Bluestar Magnum (none tested positive with PTMB). The differences in results were determined to be statistically significant between samples with a burn score of 1 and all other burn scores (chi-square with partitioning,* $p < 0.001$*). Additionally, 43% of the dissimilarity between the three blood tests was accounted for by blocks with a burn score of 1 (61% accounted for by blocks with a burn score ≤ 3; SIMPER analysis).*

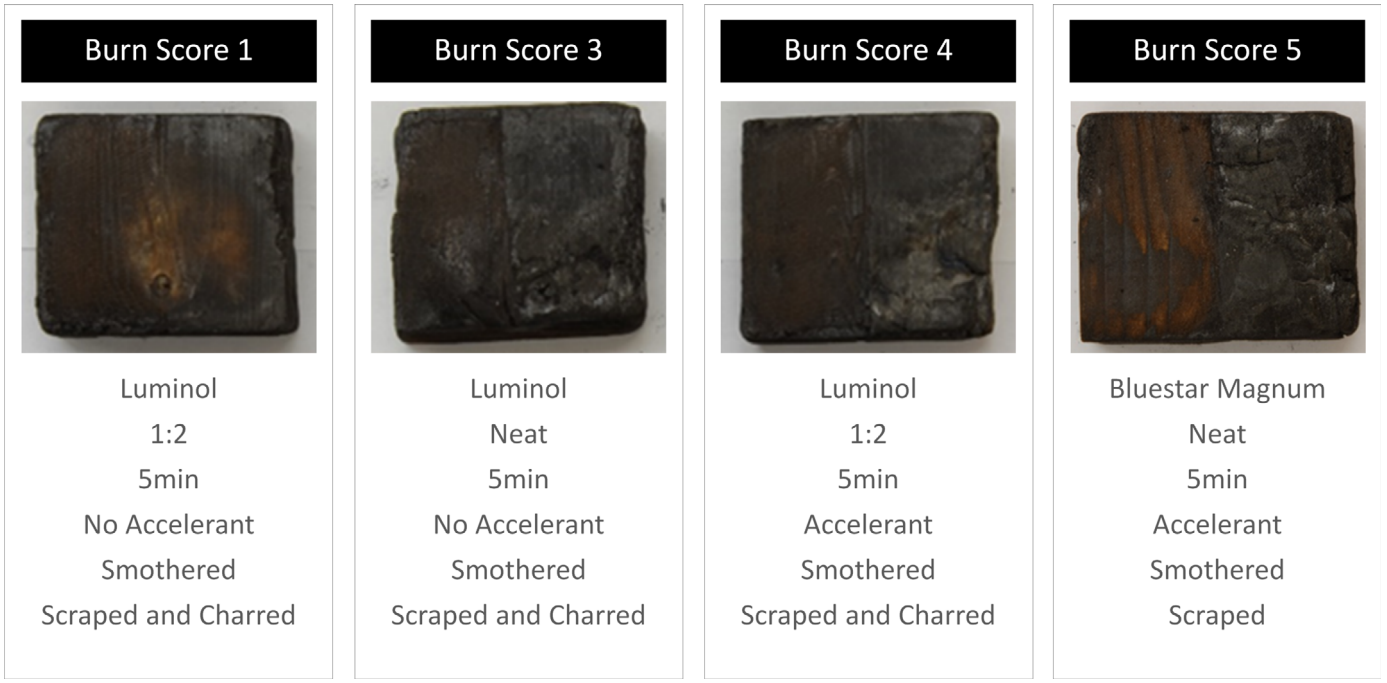

FIG. 7—*Examples of blocks testing positive for blood. Above are four of the ten blocks that tested positive for blood. The block with a burn score of 1 (far left) is a representative sample of the seven blocks with a burn score of 1 that tested positive for blood. The remaining three blocks are the only ones that tested positive for blood but had a burn score greater than 1. Treatments are summarized below each block. [Color figure can be viewed at wileyonlinelibrary.com]*

nonaccelerated fires (31,32). Thus, the application of 500 μL of unleaded gasoline (the same volume as blood) to the accelerant-treated samples may have been too small to significantly alter the fire because it would have been consumed very quickly, or it may have provided some protection to the block and decreased the degree of burn.

A second flaw may be attributed to the manner in which the samples were grouped together during burning. Four samples were burned together at a time—all had the same blood dilution; half with accelerant and half without; and half smothered and half doused. Since accelerant was added directly to individual blocks immediately prior to burning, it was assumed that the effects of the accelerant would be isolated to the block itself. However, given that blocks with and without accelerant were burning alongside one another, the presence of accelerant on half of those blocks may have altered the conditions of the fire as a whole (e.g., increased heat/intensity), thereby exposing blocks without accelerant to the same/similar conditions. However, it is difficult to discern what effects could have been transferred to nearby blocks given that wood and gasoline burn at about the

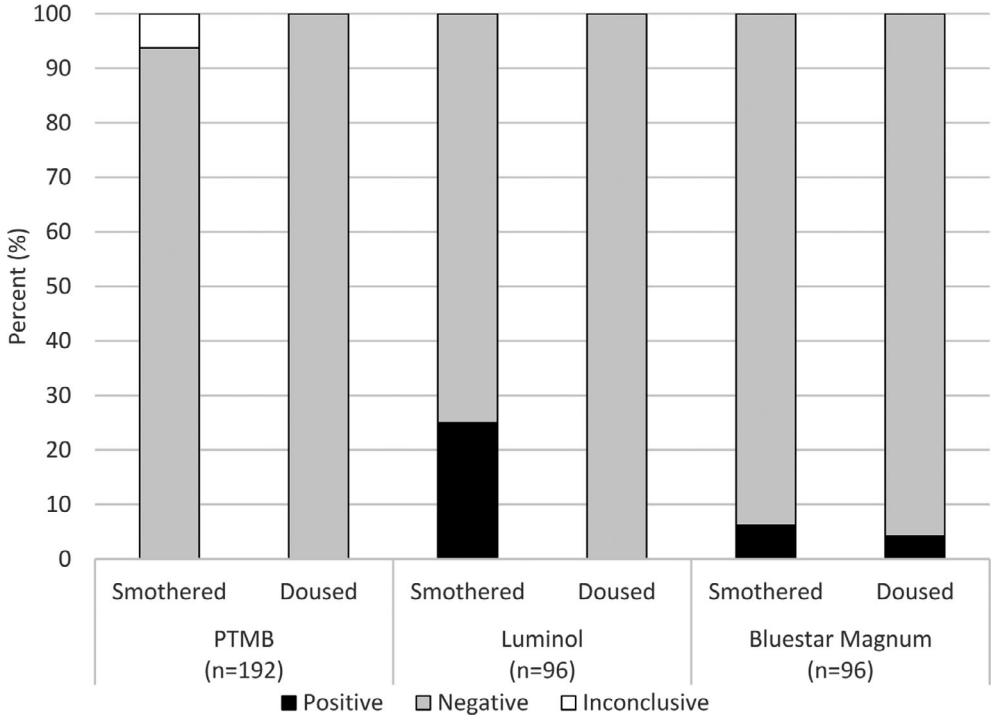

FIG. 8—*Blood test result versus extinguishment method. Test results are displayed for each of the three blood detection tests, with respect to extinguishment method. Overall, 88% of all positive test results were from blocks that had been smothered after burning, and 17% of all smothered blocks tested positive with luminol or Bluestar Magnum (none tested positive with PTMB). The differences in results were determined to be statistically significant between the two extinguishment methods (paired* t*-test,* $p < 0.001$*). Additionally, 80% of the dissimilarity between the three blood tests was accounted for by smothering (SIMPER analysis).*

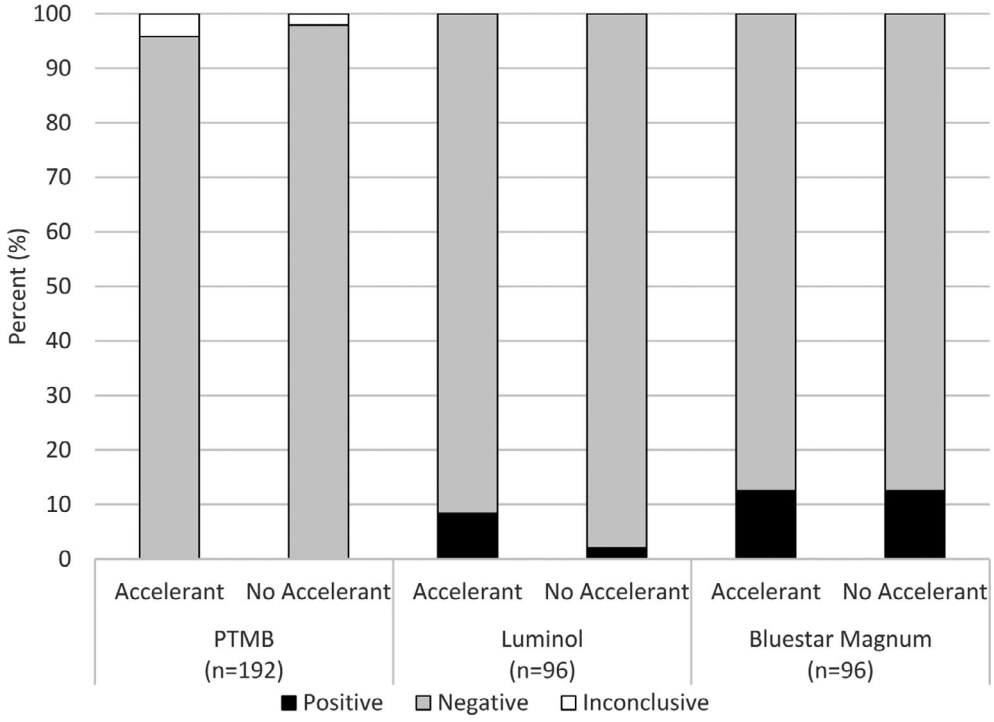

FIG. 9—*Blood test result versus accelerant. Test results are displayed for each of the three blood detection tests, with respect to the presence/absence of accelerant. Overall, 59% of all positive test results were from blocks that had accelerant added immediately prior to burning, and 13% of all blocks with accelerant tested positive with luminol or Bluestar Magnum (none tested positive with PTMB). These differences were not significant (paired* t*-test,* $p = 0.10$*).*

same temperature and that the accelerant was likely consumed quickly. Given these potential flaws, future testing is needed to better assess the use of accelerants.

Next, the impact of blood dilution on test result was examined. Overall, 71% of positive test results ($n = 17$) were from blocks that had either a neat blood or a 1:2 dilution, and 15% of

blocks with neat or 1:2 (n = 48) tested positive with luminol or Bluestar Magnum (see Fig. 10). It was expected that a higher percent of positive test results would arise from samples with less dilute blood, but it was uncertain at what point blood would no longer be detectable for more dilute samples. Without taking into account any other variables and looking at the four blood dilutions individually, the differences observed between blood dilutions were not statistically significant (chi-square, p = 0.184). When neat and 1:2 dilutions were grouped together and compared to 1:5 and 1:10 dilutions both separate and combined, the differences were not statistically significant (chi-square with partitioning, p = 0.115 and 0.127, respectively).

Lastly, testing the scraped versus charred (not scraped) side of a block was examined (see Fig. 11). Overall, 47% of positive test results (n = 17) were from the scraped side of the block, illustrating that scraping the char off of the blocks did not have a statistically significant impact on the result of the test (paired t-test, p = 0.639). For this particular variable, it was hypothesized that the presence of char would reduce the ability to obtain a positive blood test result because the blood initially present in the charred area would have been sufficiently destroyed during the act of burning and because the char itself may interfere with interpretation of the PTMB test. It was also hypothesized that scraping away the charred wood might expose detectable levels of blood that had soaked down into the wood, thus increasing the ability to obtain a positive test result. Conversely, it was also thought that scraping away the char may result in removing detectable levels of blood that may still be present in the charred layer. The results did not clearly support one single hypothesis. It is likely that a combination of these and other explanations may be the cause of the observed results.

It should be noted that regardless of whether the scraped or charred side was swabbed for PTMB testing, both swabs had black soot on them, but the swabs from the charred side did have more soot than the scraped side. As mentioned previously, the presence of this soot may have interfered with the interpretation of the PTMB test. However, the PTMB test has also already been shown to be less sensitive than luminol (10), so it is possible that even if the results had not been obscured by soot, all PTMB results may have been negative.

There was one additional noteworthy finding regarding the testing of the scraped versus charred side—all of the six blocks that tested positive with luminol did so on both the scraped and charred side. The same could not be said of Bluestar Magnum; of the four blocks that tested positive with Bluestar Magnum, one tested positive on both sides, one tested positive on the scraped side only and the remaining two tested positive on the charred side. In an effort to ensure accurate result interpretation, two researchers were present to perform these tests and observe their results. However, it is important to mention the possibility of subconscious bias during the observation time following the application of the enhancement reagent. After identifying a positive result on one half of the block, it is possible that the researchers subconsciously looked harder for a positive result on the other half.

In the results provided above, chi-square and paired t-tests were applied to evaluate which variables (blood test, burn score, extinguishment, accelerant, blood dilution, and scraped/charred) yielded statistically significant differences in the test results. These gave a rough idea of which variables influenced test result —blood test used, burn score, and extinguishment method. However, there are three main limitations on these tests: (i) the

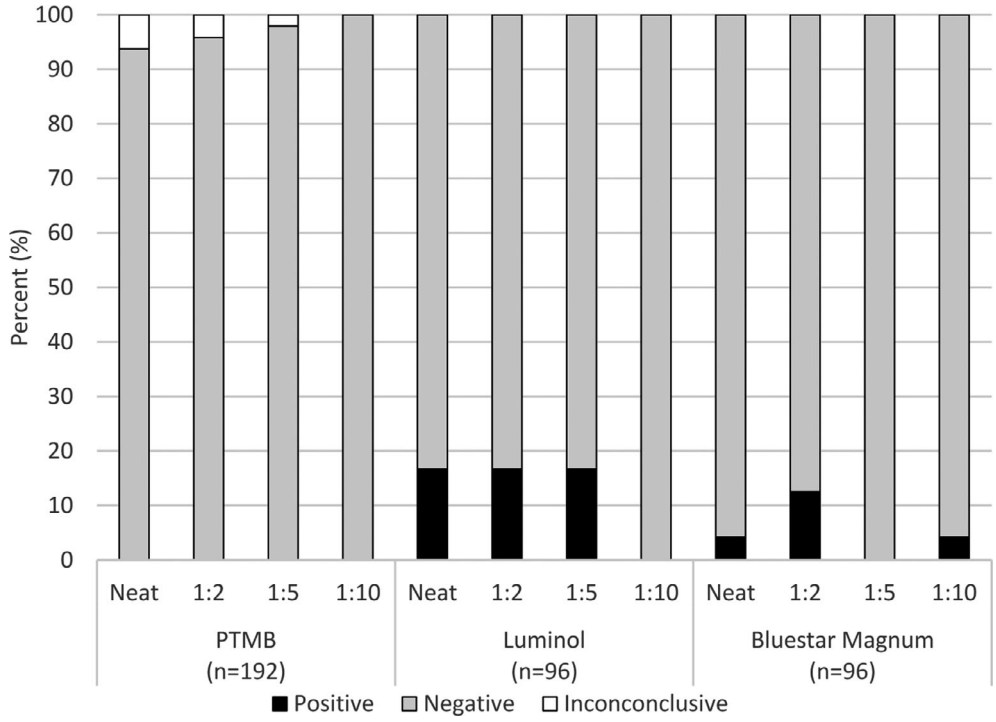

FIG. 10—*Blood test result versus blood dilution. Test results are displayed for each of the three blood detection tests, with respect to blood dilution. Overall, 71% of all positive test results were from blocks that had either a neat blood or a 1:2 dilution, and 15% of all blocks with neat or 1:2 tested positive with luminol or Bluestar Magnum (none tested positive with PTMB). Looking at each of the four blood dilutions independently as well as with partitioning, differences were not significant using chi-square (*p*-values ranged from 0.115 to 0.184); however, 75% of the dissimilarity between the three blood tests was accounted for by using neat blood or a 1:2 dilution (SIMPER analysis).*

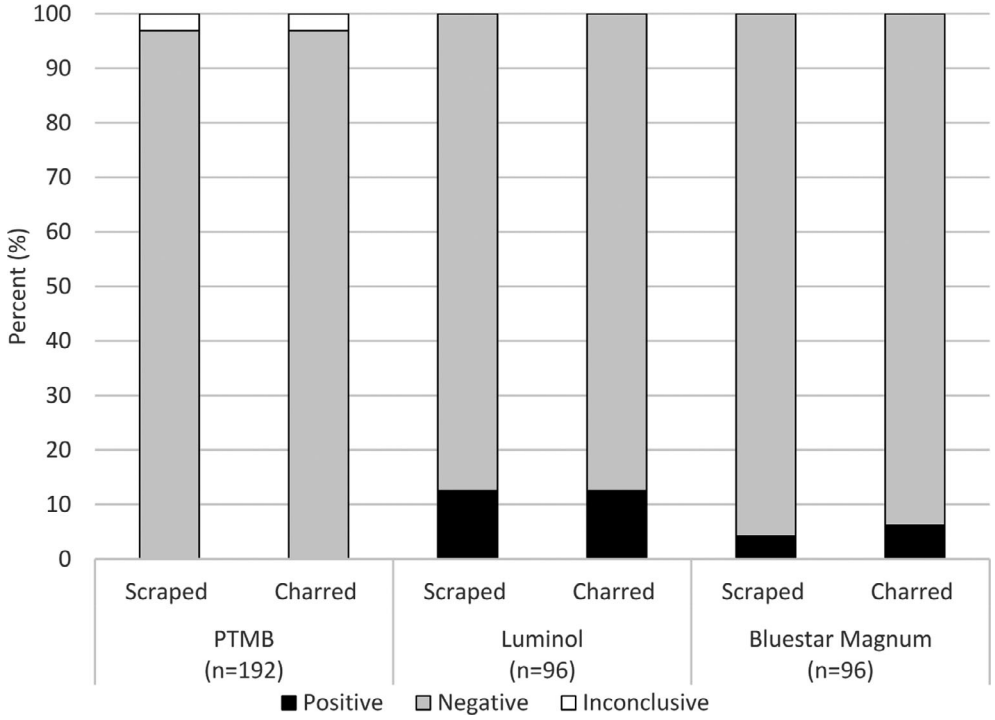

FIG. 11—*Blood test result versus char. Test results are displayed for each of the three blood detection tests, with respect to testing the scraped or charred (not scraped) side. Overall, 47% of all positive test results were from the scraped side of the block, which was not significantly different from the 53% that were from the charred side (paired* t*-test,* p = *0.639).*

chi-square tests only took into account the variable being tested (e.g., blood dilution); (ii) despite the fact that the paired *t*-tests compared replicates under the same treatment conditions (burn time, presence/absence of accelerant, extinguishment method, etc.), burn score was not the same for all test pairs given the nature of the experiment; and (iii) the degree to which each of these variables influenced test result was not assessed relative to each other.

Attempts to apply orthogonal statistical analyses proved to be challenging due to the limited number of positive test results (n = 17). In the end, a SIMPER test was performed to determine which treatments were contributing the most to the dissimilarity between the three blood detection tests. From this test, it was determined that for the dissimilarity observed between the blood detection tests:

- 80% was accounted for by smothering
- 75% was accounted for by using neat blood or a 1:2 dilution
- 43% was accounted for by blocks with a burn score of 1
- 61% was accounted for by blocks with a burn score $\leq$3

These results were fairly consistent with the findings observed using chi-square and paired *t*-tests. However, in addition to quantifying the magnitude in which these variables may impact test results, blood dilution was also noted as having a possible impact when neat blood and 1:2 dilution were grouped together (it had not been identified as significant using chi-square when keeping each dilution separate or using partitioning). Furthermore, SIMPER analysis suggests that extinguishment method may contribute to differences between test results across the three methods.

Lastly, given the experimental design and the manner in which samples were grouped in sets of four for burning, the treatments that were most controlled for in this study were the use of accelerant, extinguishment method, and testing the scraped versus charred side. The other variables (blood dilution, burn time, and blood detection test) were less controlled for because different treatments were not burned at the same time. Attempting this would have resulted in a large number of samples being burned at one time, which would have added another variable—positioning of samples in the fire itself.

Conclusion

In an evaluation of a newer blood detection reagent (Bluestar® Forensic Magnum Bloodstain Reagent) and two other blood tests (luminol and PTMB) for use with burned bloodstains on a porous, combustible substrate such as wood, it has been concluded that obtaining a positive result was especially difficult for all methods. However, positive blood test results are more likely to be obtained from luminol/Bluestar Magnum than PTMB. In fact, obtaining a positive PTMB result is extremely unlikely, even if the swab used for PTMB testing is collected prior to spraying the suspected bloodstain with luminol or Bluestar Magnum. Applying a qualitative burn score has been shown to be an effective means of assessing overall degree of burn and can be used as a predictor of test result. This is useful, as burn score takes into account burn time and temperature, as well as any other variable that may impact degree of burn. Given the potential flaws surrounding the accelerant-treated samples, no conclusions regarding the use of accelerant should be drawn from this particular study. Unfortunately, the standard suppression effort of extinguishing fires via water does negatively impact the ability to detect blood; it is unclear at this point if that also increases burn score, which in turn reduces the likelihood of a positive result. Given that arson is also used to cover up crimes such as murder, if the arsonist also attempted to clean up any bloodstains prior to the fire, the remaining residual blood would be even less

likely to be detected after the fire. This study has also shown that scraping away the charred upper layers of a porous, combustible material can expose a detectable amount of blood for Bluestar Magnum, but doing so may not provide an advantage over not removing the char.

Though obtaining DNA profiles was outside the scope of this study, many laboratories require a positive hemochromogen test result prior to attempting to obtain a DNA profile from a suspected blood sample. This study has demonstrated that a positive hemochromogen result will not be obtained from a charred, combustible material like wood that has had direct fire exposure for as little as 1 min. In reality, evidence is more often than not exposed to fire for longer periods of time, making it even less likely that positive results will be obtained. Thus, laboratories should be especially cautious of the practice of requiring a positive hemochromogen test, as samples may go untested because these tests are not sensitive enough to detect blood on burned items of evidence. Perhaps a new work flow should be established for burned samples in which a positive enhancement test —such as luminol or Bluestar Magnum—provides grounds for attempting a DNA profile.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Samples Tested with PTMB, Luminol and Bluestar Magnum.

Table S1. Blood test results.