A Comparison Study On The New Formula of Bluestar® Latent Bloodstain Reagent and its Effects on DNA Typing/Amplification.

Justin Lautz*,[1] B.S.; Samantha Webb,[2] B.S.

[1] Saint Louis University Clinical Laboratory Sciences Department
[2]Saint Louis Metropolitan Police Department Laboratory Division

ABSTRACT

OBJECTIVE: This study was designed to test two kinds of latent blood reagents, luminol and Bluestar® Magnum, and to determine their ability to detect latent blood and to preserve DNA for genetic profile determinations.

DESIGN: Three dilutions of blood were applied to six substrates: vinyl tile, ceramic tile, treated and untreated wood, carpet and cinderblock. Each blood dilution, 1:1, 1:10 and 1:100, was cleaned with four cleaning agents: water, 10% bleach, hydrogen peroxide and Woolite® Oxyclean. The substrates were incubated for, two days, seven days, 42 days and 63 days before treatment with luminol or Bluestar Magnum® and subsequently sampled for DNA analysis.

SETTING: Saint Louis Metropolitan Police Department Laboratory Division Forensic Biology/DNA Lab.

SAMPLES: Blood was acquired from the Primary Investigator in EDTA tubes and from an expired blood unit donated by Saint Louis University Hospital.

INTERVENTIONS: Blood was sampled onto six varying substrates at three different dilutions. Each stain was cleaned by one of four cleaning agents and allowed to stand for four pre-determined time durations.

MAIN OUTCOME MEASURES: For each variable, chemiluminescence intensity was graded on a three point scale and mean values were compared for each latent bloodstain reagent. DNA results were also compared to a "blood control DNA profile" to determine if either reagent compromised DNA integrity.

RESULTS: Bluestar Magnum® gave better chemiluminescence than luminol when detecting latent bloodstains. Neither reagent showed any degradation to DNA.

CONCLUSION: Bluestar Magnum® has superior sensitivity and reactivity compared to luminol. Both reagents have comparable DNA results. Further studies with Bluestar Magnum® and cinderblock should be done.

ABBREVIATIONS USED: DNA = Deoxyribonucleic Acid, EDTA = ethylenediaminetetraacetic acid, PCR = Polymerase Chain Reaction, PI = Primary Investigator

INDEX TERMS: Latent Bloodstain Reagent, Luminol, Bluestar Magnum®, Chemiluminescence, Polymerase Chain Reaction, Capillary Electrophoresis

Introduction

Many pieces of evidence at a working crime scene are not initially apparent to the investigating officer. Sometimes key evidence is not observed during the initial investigation due to the scene being cleaned by the perpetrator of the crime, or the evidence is so small that the officer cannot easily see it with the unaided eye. One type of evidence in particular, blood evidence, is of great interest to the investigator. Blood evidence can be used to place a victim or suspect at the scene of a crime (through DNA) or give insights into the "acts" of the crime (blood spatter).

One tool an investigator has when latent blood evidence is suspected is the chemiluminescence reaction. Chemiluminescence has been used at the crime scene for many years to identify latent blood evidence. The first facet of this technology was luminol and it was introduced over a decade ago. Luminol is used to visualize blood that has been cleaned off a substrate, hence it is considered a latent blood reagent. Luminol reveals traces of blood with a light-producing chemical reaction between several chemicals in the luminol reagent and hemoglobin, an oxygen-carrying protein in the blood. The oxidation reaction of luminol and the heme component of hemoglobin (iron + protoporphyrin ring) causes a peroxidase-like reaction that emits a chemical luminescence which is visible in the dark.[1] In this reaction, the reactants have more energy than the products, causing the molecules to emit the extra energy in the form of visible light photons. Past studies on other chemiluminescent latent blood reagents such as Leucomalachite Green and Leucocrystal Violet have shown that these chemicals interfere with subsequent DNA typing. Consequently, since DNA technology is becoming the hallmark of forensic identification, luminol was intensely researched to determine if it compromises DNA typing.[2] These studies found that treating a latent bloodstain with luminol can have an effect on the ABO typing of blood stains using conventional serological blood typing, but it does not have an adverse effect on the subsequent analysis of these bloodstains using the DNA technique Restriction Fragment Length Polymorphisms (RFLP)[1]. RFLP determines variation in the length of a defined DNA fragment produced by restriction enzymes. Currently, fluorescent based Short Tandem Repeat (STR) analysis is used to type DNA and luminol has also shown not to adversely effect STR results[3]. Unlike RFLP, STR analysis does not require a high quality or defined sample amount. STR looks for short sequences of DNA that are repeated numerous times in a head-tail manner. Coupled with Polymerase Chain Reaction (PCR), a DNA amplification technique, STR is currently the most widely used technique for DNA analysis.

A few years ago, the ROC IMPORT Company released a luminol based chemical called Bluestar®. Its manufacturer claims that Bluestar® is much more sensitive, longer lasting and does not require complete darkness to visualize the presence of latent blood stains. Since Bluestar® has not been around as long as luminol, little research on its effects on DNA typing have been conducted. Four years ago, one study investigated the effect of Bluestar® on various blood dilutions and subsequent DNA typing but did not adequately investigate the effects of cleaning agents, time, and substrates on the ability to get a viable DNA profile after treatment with Bluestar®.[2] Furthermore, DNA extraction and quantification techniques have improved and there have been revisions to the Bluestar® formula since this four-year-old study. Therefore, an updated study is in order.

In this study, various substrates will be sampled with different blood dilutions. Each blood dilution will be cleaned by varying cleaning agents. The substrates will then sit for an allocated amount of time before treatment with the Grodsky formula of luminol and the newly introduced Bluestar Magnum®. Each treated substrate will be photographed and semi-quantified using a Likert scale in order to compare the quality of the two different latent blood reagents. Furthermore, DNA samples will be taken post-treatment to determine if the latent blood reagent, substrate, and/or the time exposure had any adverse effects on standard forensic DNA quantification, amplification and analyzation results.

Materials and Methods

Design

Blood for this study was acquired through standard venipuncture from the P.I. utilizing an EDTA anticoagulant and from an expired blood unit donated by Saint Louis University Hospital. Blood dilutions of 1:10 and 1:100 were made in addition to the 1:1 (undiluted blood) samples. Six substrates were selected in this experiment: untreated wood, treated wood flooring, vinyl flooring, ceramic tile, carpet and cinderblock. Each substrate was marked off into a 3X4 grid creating 12 squares. The vertical columns (3) represented the blood dilution used; 1:1, 1:10 and 1:100 respectively. The horizontal columns (4) represented the type of cleaning agent used to clean the blood stain. Of the six types of substrates, two of each type sat out for 62 days (nine weeks), two for 43 days (six weeks), two for seven days (one week) and two for two days after the blood stain was cleaned. Of the matching pair, one was assigned for treatment by Bluestar Magnum® and one was assigned for treatment by the Grodsky formula of luminol purchased from Doje's Forensic Supplies Inc. The blood stain was cleaned until no longer visible or until the stain was cleaned as much as the cleaning agent allowed. The cleaning agents selected for this study were: warm tap water, 10% bleach solution, hydrogen peroxide and Woolite™ Oxyclean carpet cleaner. The substrates were labeled for each "time duration" studied and for the type of latent blood reagent used creating six pairs for a total of 12 substrates for each time duration in the study.

Sample Application and Cleaning

Each substrate was sampled with one milliliter of pure or diluted blood per marked off square for a total of 12 milliliters of blood per substrate. After the sampling process was completed, the stains were left to sit for 10-15 minutes. After the 10-15 minutes, the samples were wiped with a paper towel to remove the majority of the stain. Once all substrates were wiped, each bloodstain was cleaned with their respective cleaning agent until the stain was completely removed or until the stain was cleaned as much as the cleaning agent would allow. Swabs from the latent bloodstains were taken from the 1:1/water square from all six different substrates before latent blood reagent treatment to act as our substrate control. The substrates then sat until their "treatment" date.

Treatment of the Bloodstains with Latent Blood Reagent and Sample Collection

Substrates were treated with either Bluestar Magnum® or luminol on their assigned date. Each latent blood reagent was made according to manufacturer's specifications and was used within the suggested time frame given for optimal reactivity/chemiluminescence. Before spraying the substrates, a digital camera optimized for dim light/slow exposure photography was set up on a tripod facing down. Each substrate was placed under the tripod and sprayed with the corresponding latent blood reagent and photographed. DNA samples were taken immediately after treatment from both the luminol and Bluestar Magnum® from the 1:1, water cleaned bloodstains from the seven day, 42 day and 63 day substrates only. Swabs were taken while the samples

were still damp from the spraying of the latent blood reagent. Samples were collected by swabbing with a cotton swab. The cotton swabs were allowed to dry, placed in a paper envelope, then in a desiccator, and stored in a -80°C freezer to await analysis.

Extraction and Quantification of DNA

Extraction of DNA from the swabs was achieved by organic means utilizing phenol/chloroform/isoamyl alcohol. Human DNA quantitation was completed utilizing the ABI 7000 and the Quantifiler System as outlined in the Saint Louis Metropolitan Police Department Lab Divisions *DNA Extraction/Quantification/Amplification and Analyzation Protocol*[4].

STR Amplification and Typing

PCR amplification was done by AmpF*l*STR Identifiler Loci PCR Amplification kit which DNA typed the STRs at the D8S1179, D21S11, D7S820, CFS1PO, D3S1358, THO1, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818 and FGA loci and Amelogenin. Post amplification genetic analysis was done on the ABI 310 Genetic Analyzer via capillary electrophoresis. The standard lab protocol for both the amplification and analysis was followed according to the manufacture of the kits and equipment.

Results

The intensity of chemiluminescence between Bluestar Magnum® and the Grodsky formula of luminol was photographed and recorded using a 0-3+ Likert scale according to the following criteria:

Reaction strength grading:
0 = chemiluminescence not seen
1+ = weak chemiluminescence
2+ = moderate chemiluminescence
3+ = strong chemiluminescence

These results were recorded on a data table as follows:

TWO DAY STUDY

Bluestar Magnum®

Vinyl Tile		1:1	1:10	1:100
	Water	3+	1+	1+
	10% Bleach	2+	0	0
	Hydrogen Peroxide	0	0	0
	Woolite® Oxyclean	1+	0	0

Luminol

Vinyl Tile		1:1	1:10	1:100
	Water	1+	0	0
	10% Bleach	0	0	0
	Hydrogen Peroxide	0	0	0
	Woolite® Oxyclean	0	0	0

* Vinyl tile example only*

Comparative visualization study data

The effect of time on luminol and Bluestar Magnum® chemiluminescence (Table 1) was analyzed by averaging all the reaction strengths at the 1:1 dilution, cleaned by water and considering all substrates for each time duration.

TABLE 1 – Effect of time duration on chemiluminescence

	BLUESTAR MAGNUM®	LUMINOL
2 DAY	2.83	1.00
7 DAY	2.67	0.83
42 DAY	2.50	0.50
63 DAY	2.50	1.33
OVERALL MEAN	2.63	0.92

Excluding Bluestar Magnum®, luminol exhibited the most chemiluminescence during the two day and 63 day study whereas Bluestar Magnum® gave strong chemiluminescence for all time durations with the two day and seven day being the strongest.

Likewise, the effect of the substrate on the ability of the latent blood reagent to chemiluminesce the bloodstain (Table 2) was calculated by averaging all the reaction strengths at the 1:1 dilution and considering all cleaning agents at every time duration for each substrate.

TABLE 2 – Effect of substrate type on chemiluminescence

	BLUESTAR MAGNUM®	LUMINOL
VINYL TILE	1.44	0.06
CERAMIC TILE	2.06	0.25
TREATED WOOD	1.44	0.13
CINDERBLOCK	3.00	2.25
CARPET	3.00	0.81
WOOD	2.90	0.44
OVERALL MEAN	2.31	0.66

The last three substrates, cinderblock, carpet and wood, showed a more intense reaction opposed to vinyl tile, ceramic tile and treated wood for both latent blood reagents. Overall, Bluestar Magnum® exhibited stronger chemiluminescence for all substrates.

The effect of the cleaning agent on chemiluminescence for each latent blood reagent (Table 3) was evaluated by averaging the reaction strengths at the 1:1 dilution, at every substrate and duration for each cleaning agent.

TABLE 3 – Effect of cleaning agent on chemiluminescence

	BLUESTAR MAGNUM®	LUMINOL
WATER	2.63	0.92
10% BLEACH SOLUTION	2.58	0.83
HYDROGEN PEROXIDE	2.04	0.46

WOOLITE® OXYCLEAN	1.96	0.42
OVERALL MEAN	2.30	0.66

Woolite® Oxyclean and hydrogen peroxide yielded the two lowest reaction strengths of the four cleaning agents for both luminol and Bluestar Magnum®. Again, Bluestar Magnum® illuminated stronger than luminol for all cleaning agents.

The effect of the blood dilution on the reaction strength (Table 4) was calculated by averaging the reaction strengths of every substrate and cleaning agent at all time durations for each dilution.

TABLE 4 – Effect of dilution on chemiluminescence

	BLUESTAR MAGNUM®	**LUMINOL**
1:1	2.30	.66
1:10	1.43	.39
1:100	0.73	.01
OVERALL MEAN	1.49	0.35

Chemiluminescence was shown to decrease as the dilution increased for both reagents. By and large, Bluestar Magnum® again demonstrated a greater reactivity than luminol.

The Bluestar Magnum®, luminol comparison (Table 5) was evaluated simply by looking only at the two day study and averaging those reaction strengths that fell under the 1:1 dilution, cleaned by water, for every substrate.

TABLE 5 – Bluestar Magnum® & luminol comparison

	BLUESTAR MAGNUM®	**LUMINOL**
TOTAL AVERAGE (2 DAY ONLY)	2.83	1.00

Generally, Bluestar Magnum® graded higher than luminol.

Comparative DNA study data

The results given in tables 6-8 were computed as a percentage of "usable" alleles for forensic identification. Alleles are deemed usable by standards set by the Saint Louis Metropolitan Police Department (SLMPD) Laboratory Division[4]. If a particular allele does not meet the criteria, it is omitted for use in forensic identification statistics. The PCR kit used by the SLMPD identifies a total of 15 loci. For this experiment, a pure blood control was typed and each locus analyzed. Blood used in the DNA part of this experiment came from one source. The pure blood control yielded two alleles at each locus for a total of 30 identifiable, usable alleles in the unaltered pure blood control. This profile was the standard to which each genetic profile obtained from the experiment was

compared. To obtain the percentages, the number of useable alleles obtained from one of the experimental runs (X) was divided by the total possible alleles that could be detected (30). It is important to note in this half of the study that the two day DNA samples were omitted due to the donor unit of blood obtained from St Louis University Hospital being leukocyte reduced, hence, greatly reducing the amount of DNA in the blood. Reduced leukocyte numbers were verified by a complete blood cell count (CBC) on the unit of blood obtained from the hospital.

In table 6, the substrates were compared by averaging the number of usable alleles of the 15 loci at the seven day, 42 day and 63 day studies for a specific substrate and dividing by the total number of possible alleles (30) for a percentage. This was done for both Bluestar Magnum® and luminol treated DNA samples to compare the effect of the substrate and Bluestar Magnum® or luminol treatment on a genetic profile.

TABLE 6 – Percentage of usable alleles for forensic ID comparing substrates

	BLUESTAR MAGNUM®	**LUMINOL**
VINYL TILE	0%	0%
CERAMIC TILE	6.6%	26.7%
TREATED WOOD	0%	0%
CINDERBLOCK	66.7%	100%
CARPET	98.9%	93.3%
WOOD	91.1%	84.4%

Both Bluestar Magnum® and luminol had the best percentages with cinderblock, carpet and wood. Vinyl tile and treated wood did not give any useable alleles for forensic identification for both latent blood reagents.

Table 7 compared the effect of time and Bluestar Magnum® or luminol treatment. This was accomplished by averaging the total number of usable alleles of the 15 loci for every substrate at each time duration divided by 30 for a percentage. This was done for both Bluestar Magnum® and luminol treated DNA samples.

TABLE 7 - Percentage of usable alleles for forensic ID comparing time durations

	BLUESTAR MAGNUM®	**LUMINOL**
2 DAY	N/A	N/A
7 DAY	48.9%	62.2%
42 DAY	52.2%	40.0%
63 DAY	30.6%	50.0%

Luminol gave the greatest amount of usable alleles relative to itself in the seven day study and the least in the 42 day study. Bluestar Magnum® presented the largest amount of usable alleles relative to itself in the 42 day study and the least in the 63 day study.

Table 8 was calculated by averaging the total number of alleles for all substrates and time durations for each latent blood reagent.

TABLE 8 - Percentage of usable alleles for forensic ID comparing latent blood reagents

	BLUESTAR MAGNUM®	LUMINOL
TOTAL AVERAGE % ACROSS ALL SUBSTRATES AND TME DURATIONS	43.9%	50.7%

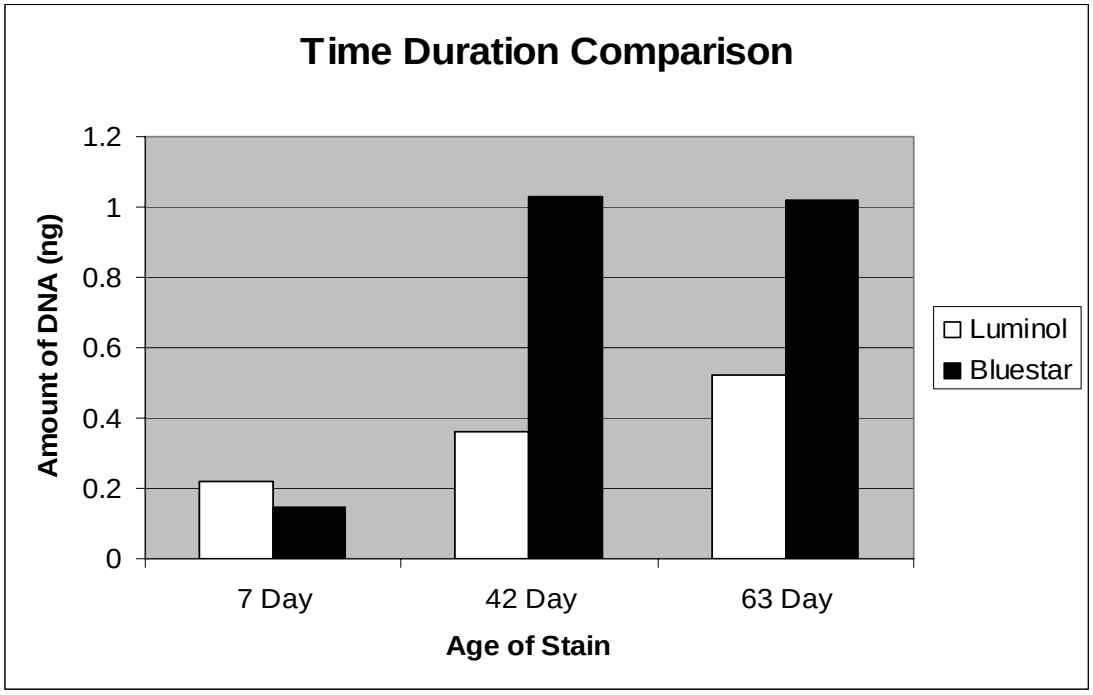
Generally, luminol yielded only a slightly higher percentage of usable alleles compared to Bluestar Magnum®.

Comparative quantification study data

Graphs 1-2 show the quantification data obtained from the ABI 7000 and the Quantifiler System in nanograms. Luminol and Bluestar Magnum® are compared on the basis of time duration (Graph 1) and substrate (Graph 2).

The effect of time duration on quantification of DNA (Graph 1) was evaluated by averaging the quantification amounts from four substrates for each duration for both luminol and Bluestar Magnum®. The substrates cinderblock and wood were eliminated from this data set due to these results being statistically significant outliers.

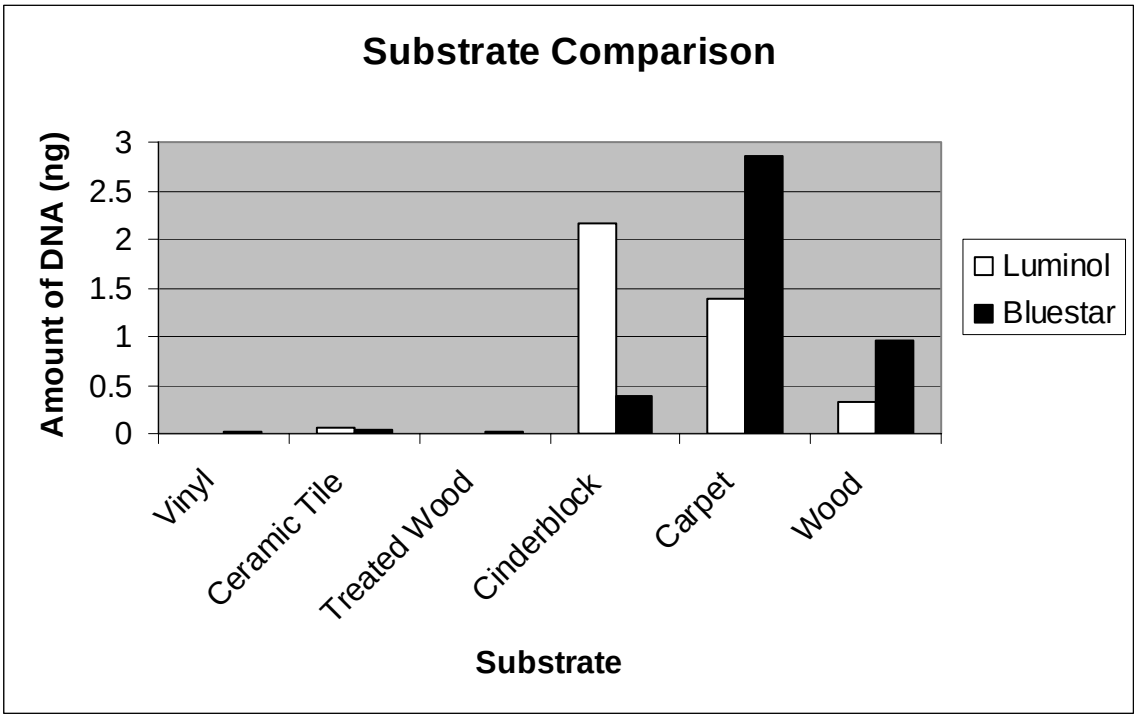
GRAPH 1 – Quantification of DNA comparing time durations



Both Luminol and Bluestar Magnum® exhibited greater quantities of DNA as the bloodstain aged. Also, Bluestar Magnum® yielded greater amounts of DNA compared to luminol on all time durations except the seven day.

The effect of the substrate on DNA quantities (Graph 2) was calculated by averaging the quantification amounts of the three time durations for each substrate for both luminol and Bluestar Magnum®. The quantifications from the 42 day cinderblock study and the 63 day wood study were omitted from this data set due to these results being statistically significant outliers.

GRAPH 2 – Quantification on DNA comparing substrates



Graph 2 shows that cinderblock, carpet and wood yielded the highest quantities of DNA of the six substrates for both Bluestar Magnum® and luminol.

Furthermore, since the presence or absence of a particular allele at a specific locus is each weighed statistically differently for forensic identification, probability statistics were calculated employing the Popstats™ statistics software (Table 9). Popstats is a statistics program that is commonly used by many police agencies around the country to calculate the likelihood of discovering the same genetic profile in the general population. The SLMPD Laboratory Division does not accept probabilities less than 1 in 2.8E+11 for identity statements claiming scientific certainty[4].

TABLE 9 - Popstat™ statistics (Frequency)

LUMINOL	Vinyl Tile	Ceramic Tile	Treated Wood	Cinderblock	Carpet	Wood
7 day	0	1 in 1.555E+8	0	1 in 1.843E+14	1 in 1.843E+14	1 in 1.843E+14
42 day	0	0	0	1 in 1.843E+14	1 in 7.429E+11	1 in 2.943E+8
63 day	0	1 in 2,974	0	1 in 1.843E+14	1 in 5.089E+13	1 in 1.843E+14

BLUESTAR MAGNUM®	Vinyl Tile	Ceramic Tile	Treated Wood	Cinderblock	Carpet	Wood
7 day	0	0	0	1 in 1.843E+14	1 in 1.843E+14	1 in 1.843E+14
42 day	0	1 in 776	0	1 in 1.843E+14	1 in 1.843E+14	1 in 4.023E+12
63 day	0	0	0	0	1 in 1.045E+13	1 in 9.425E+10

*Frequency = Probability of discovering the same profile in the general population (Caucasian population)

0 = too little DNA is present to make a conclusive statistical determination as to the source of the DNA

Overall, the Popstats™ suggested that a conclusive statistical determination as to the source of the DNA could not be made for the bloodstains sampled off the vinyl tile, ceramic tile and the treated wood for both luminol and Bluestar Magnum®.

Discussion

Comparative visualization study

First, when analyzing the effect of time on luminol and Bluestar Magnum®, luminol showed the strongest illumination in the two day and 63 day intervals whereas Bluestar Magnum® showed the strongest chemiluminescence for the first two time durations. However, in general, the time variable did not affect either latent blood reagent in their ability to visualize the bloodstain. Bluestar Magnum® showed stronger chemiluminescence for all time durations as compared to luminol. Second, analyzing the effect of the type of substrate on the visualization of the bloodstain, both latent blood reagents showed strongest chemiluminescence with the more porous or absorbent substrates; cinderblock, wood and carpet being the top three. It is important to note that these were also the only three substrate types where the bloodstains could not be completely removed and were still visible by the unaided eye due to their ability to "absorb" the bloodstain. The "smoother" substrates like vinyl tile, ceramic tile and treated wood, yielded the lowest reaction strength numbers. This was most likely due to the increased ease of cleanup on the non-porous surfaces. It is also important to note that with the smoother substrates, chemiluminescence often occurred around the perimeter of the marked off area, likely due to the cleaning technique "pushing" the blood to the borders. Reactivity was still graded taking the borders into consideration since all the smooth substrates were cleaned until no visible blood was seen. Third, looking at the effects of the cleaning agent on chemiluminescence, Bluestar Magnum® exhibited a greater ability to visualize the latent bloodstain as compared to luminol. Generally for both reagents, hydrogen peroxide and Woolite® Oxyclean did the most efficient job in bloodstain clean-up and minimization of chemiluminescence.

As analyzed in previous studies[5], we did not notice significant hypochlorite-induced chemiluminescence while using the 10% bleach solution with the luminol. Only in the circumstances where just the bleach cleaned bloodstains were visualized or bright flashes of chemiluminescence were seen, was hypochlorite-induced chemiluminescence suspected. On the other hand, this phenomenon could not be gauged effectively with the Bluestar Magnum® since it reacted intensely with almost every sample.

Fourth, gauging the effects of diluted blood on chemiluminescence, Bluestar Magnum® again proved to be more sensitive than luminol. Intuitively, for both latent bloodstain reagents, the blood dilution was inversely proportional to the reaction strength. For the most part, chemiluminescence with the 1:100 dilutions were not visible with the luminol on all substrates, whereas Bluestar Magnum® showed a 1+ to 2+ reaction for most substrates at the 1:100 dilution. Overall, Bluestar Magnum® demonstrated a much greater sensitivity and reactivity/intensity than the Grodsky formulation of luminol under all test conditions. Furthermore, the intensity of the chemiluminescence exhibited by Bluestar Magnum® faded much quicker than luminol but was immediately sensitive to subsequent sprayings to the latent bloodstain, whereas luminol was not. Because of this, it could be foreseen that DNA results could be harder to obtain due to a "dilution" of the blood sample in the effort to constantly re-illuminate the latent bloodstain for slow

exposure photography purposes. As a crime scene technician, one should be conscientious of this when treating the bloodstain with Bluestar Magnum®.

Comparative DNA typing study

The secondary purpose of this study was to determine if the application of either Bluestar Magnum® or luminol had any effect on the ability to extract, quantitate, amplify or analyze DNA obtained from latent bloodstains. Table 6 compares the percentage of usable alleles from the six substrates treated with either Bluestar Magnum® or luminol. Similar to the visualization study results, the worst results were obtained from the smooth, less porous substrates: vinyl, ceramic tile and treated wood, for both latent bloodstain reagents. This also held true for the quantification results (Graph 2). Ceramic tile did yield some usable alleles for forensic identification for both luminol and Bluestar Magnum®, but according to Popstats™ (Table 9), they were not statistically significant enough to make a conclusive decision as to the source of the DNA. Whereas no statistical probability could be made with the vinyl tile and treated wood due to the complete lack of any identifiable alleles. On the other hand, cinderblock, carpet and wood gave a large percentage of usable alleles, all statistically good enough for forensic identification except for the 42 day luminol wood study and the 63 day Bluestar Magnum® cinderblock and wood study. The results from the 63 day Bluestar Magnum® cinderblock study were of particular interest. Unexpectedly, this substrate yielded no alleles for identification. This raised concern since cinderblock consistently gave a full allele profile in the preceding time duration studies. Investigating further, for all time durations; cinderblock treated with Bluestar Magnum® gave constantly low quantitation volumes relative to luminol. Further studies should be conducted to determine if this result is just an artifact due to the lack of a completely controlled experiment (i.e.: inconsistent bloodstain swabbing technique, environmental insult, etc.), or if Bluestar Magnum® has an undesirable reaction with cinderblock that compromises DNA evidence.

Looking at the effect of the time duration on DNA analysis, the quantitation results (Graph 1) suggest that quantities of DNA obtained from the sample increase as the bloodstain ages. Conversely, table 7 shows no observable trend in the age of the stain and the number of usable alleles between the two latent bloodstain reagents. Table 8 suggests that Bluestar Magnum® and luminol are comparable in their capacity to preserve DNA evidence. Furthermore, if the possibly skewed data from the 63 day cinderblock study is excluded, this statement is even more evident with percents of 39.7 and 41.0 respectively.

Conclusion

Overall, Bluestar Magnum® showed superior sensitivity and reactivity compared to luminol in the visualization half of this study. From the data acquired, it can be concluded that the more porous/absorbent the substrate, the greater the likelihood of being able to visualize the bloodstain. This is likely due to the fact that these types of substrates are more difficult to completely clean compared to smooth substrates. Likewise, the more porous/absorbent substrates yielded a greater likelihood to extract a DNA profile suitable for forensic identification. Again, the ability of the substrate to "absorb" the blood greatly affected the amount of DNA extracted out of the substrate as illustrated in the quantitation graphs. The Grodsky formula of luminol demonstrated the ability to preserve DNA evidence agreeing with previous studies[3]. Similarly, Bluestar Magnum® also exhibited the ability to preserve DNA for genetic profile determinations. Considering there were a large amount of variables which created difficulty in maintaining variable control in this study, further studies are recommended. In particular, to verify these results, and to determine if Bluestar Magnum® applied on cinderblock adversely affects DNA extraction, quantitation, amplification or analysis. Also, a study which analyzes whether the quantity of latent bloodstain reagent used had any bearing on the amount of DNA recovered from a sample would be beneficial.

References

1. Centre Hospitalier Universitaire De Nates. Bluestar® Forensic Validation Study. *Operations Unit for Genetic Impinting,* 2003.

2. French Defense Department, Gendarmerie Nationale Criminal Research Institute Biology Department. The Effect of Bluestar® Blood Reagent on DNA Typing. *Aspirant Breniaux,* 2001.

3. Gross, Ann Marie, Kathy A. Harris, and Gary L.Kaldun. The Effect of Luminol on Presumptive Tests and DNA Analysis Using the Polymerase Chain Reaction. *The Journal of Forensic Science* 44.4 (1999)

4. Saint Louis Metropolitan Police Department Laboratory Division. ABI Prism 310 Genetic Analyzer Protocol for the Identifiler PCR Amplification Kit. (2005).

5. Kent, Erina J. M., Douglas A. Elliot, and Gordon M. Miskelly. Inhibition of Bleach-Induced Luminol Chemiluminescence. *The Journal of Forensic Science.* 48.1 (2003).

Acknowledgements

- First and foremost, the Saint Louis Metropolitan Police Department Laboratory Division for giving me the research opportunity.
- SLU professor Tim Randolph for his exceptionable guidance during my research and all throughout my academic career.
- Fellow SLMPD Lab interns: Amanda Finley, Jeff Donnellon and Meghan Hatfield.
- Home Depot and the Borkowski family for their generous donation of substrates for this study.