Effect of Temperature and pH on Bloodstain Evidence

Kannika Sutthapodjanarux[1], Nathinee Panvisavas[1,2], and Nopadol Chaikum[1,3]

[1] Forensic Science Graduate Programme, [2] Department of Plant Science, [3] Department of Chemistry , Faculty of Science, Mahidol University, Rama VI Road, Bangkok, Thailand.

Abstract

Bloodstains can be successfully recovered through search techniques, so that forensic examinations could then be performed to link the perpetrator, the victim, and the crime scene. In this study, the effect of temperature and pH on alternate light source searching (ALS), presumptive chemical tests, and DNA analysis of bloodstain were investigated. The results showed that ALS-search for bloodstain on two different surfaces was not affected by temperature and pH of the stain. Presumptive testing for blood, both Kastle Myer (KM) and leuco-malachite green (LMG) tests, gave negative results when the bloodstain was treated at temperature higher than 250°C. Concentrated acidic and alkali solutions did not affect the chemical presumptive tests for blood, but affected DNA analysis. However, DNA analysis results could be obtained from bloodstains treated with 0.1M NaOH and temperature lower than 250°C.

Key word : *Bloodstain, Forensic examination, Temperature, pH*

Introduction

Bloodstains found in the crime scenes often became crucial evidences to solve cases. If bloodstains are successfully detected and analyzed, then the link between the contributor of the bloodstain. Bloodstains in forensic science is used to identify suspects, victims and persons who at the crime scene. And the crime scene could then be established. The science of bloodstain identification is still in high ranking in forensic science base on the fact.

Uncontrolled conditions of crime scenes affect the quality of biological evidence, including bloodstains. Degradation of biological trace can be caused by heat, moisture, microorganisms, etc. Moreover, biological evidence can also be deteriorated by chemical insult. And bloodstains could still be recovered and identified which had never been demonstrated before to show the effecting factor of pH for forensic examination. The result will be usefulness for forensic scientist when identify suspect stain in crime scene. Here, we reported the effect of temperature and pH on bloodstain evidence using alternate light source (ALS), presumptive chemical tests, and DNA analysis.

Material and Methods

1. Preparation of stains

To study the effect of temperature, 6-9 replicates of 20 μL of human blood were spotted on two different surfaces, i.e., cotton fabric, and tile. The blood was left overnight to dry, and treated in different temperatures: 1) Low temperatures at 0°C, 25°C, 40°C and uncontrolled for 1 day, 1, 2, 3, and 5 weeks. 2) High temperatures at 100, 150, 200, 250, 300, and 350°C by leaving the bloodstains in the oven for 10 minutes.

To study the effect of pH, 0.5 ml. human blood was mixed with HCl or NaOH at 0.1, 0.5, and 1 M. Then the blood of different pH were spotted on cotton fabric and tile, 6-9 replicates per surface per set were examined.

2. Search and Examination of bloodstains

2.1 Bloodstain was investigated using the alternate light source (mini crime scope MC-400) at 450 nm.

2.2 Chemical presumptive tests were performed using the Kastler-Myer (KM) and Leucomalachite Green (LMG) reactions. Untreated bloodstain and negative control were also included.

2.3 DNA analysis

2.3.1 DNA extraction & quantification. DNA was extracted from the treated bloodstains using QIAamp® DNA Mini Kit (QIAGEN, CA, USA), following the manufacturer protocol. The DNA extract was quantified by spectrophotometry (NanoDrop™ 1000 Software Version 3.6.0). The absorbance was measured at the wavelengths of 260 and 280 nm.

2.3.2 DNAamplification

2.3.2.1 STR analysis, human genotype was generated by using the AmpliSTR® Identifiler kit (Applied Biosystems, USA). A number of 15 STR loci and the anelogenin sex- determination marker were analysed. PCR was carried out in a total volume of 6.25 µL containing 2.6 µL of AmpliSTR® Identifiler PCR Reaction Mix, 1.4 µL of each primer set, 0.1 µL of AmpliTaq Gold, 1.1 µL of H_2O, and 1 µL DNA Polymerase. The following thermocycle condition was applied; initial denaturation at incubation step at 95°C for 11 min; 28 cycles of denaturing at 94°C for 1 min, annealing at 59°C for 1 min and extension at 72°C for 1 min, final extension at 60°C for 60 min.

2.3.2.2 Mitochondrial DNA analysis. Human *cytochrome* b, 160-bp, on the mitochondrial DNA was PCR amplified. Primer sequence were Forwarded 5'-ATCTGAGGAGGCTACTCAGTAGACA-3', Reverse 5'-ATCGGAATGGGAGGTGATTCCTAGG-3 PCR was carried out in a total volume of 25 µL containing 2.5 µL of 10X PCR buffer, 1.5 µL of 1.5 mM $MgCl_2$, 0.5 µL of 10 mM dNTPs, 1 µL of each primer, 0.2 µL of AmpliTaq DNA Polymerase, and 2 ng of DNA template. The following thermocycle condition was applied; initial denaturation at 95°C for 3 min; 30 cycles of denaturing at 95°C for 30 seconds, annealing at 60°C for 30 seconds and extension at 72°C for 1 min, final extension at 72°C for 10 min.

2.3.3 Analysis of amplification products

2.3.3.1 Amplification products of nuclear STRs were analysed using the ABI 310 Genetic analyzer. PCR fragment sizes and genotype (allele types) were determined using the Gene mapper ID version 3.1 software.

2.3.3.2 Amplification products of the human *cytochrome* b gene fragment were analysed by ethidium bromide stained-Agarose gel electrophoresis.

Results and discussion

1. The effect of temperature

In this study, 2 ranges of temperatures were investigated: low (0, 25, and 40°C, and uncontrolled), and high temperatures. Bloodstains left at low temperatures could be easily distinguished from the surface deposited by the naked eyes. In contrast, when bloodstains on cotton fabric were treated at high temperatures, both bloodstains and surface became charred, thus it was difficult to distinguish the stain from the surface by the naked eyes. Then alternate light source at 415 nm was used to expose the treated bloodstains. The results showed that the position of the stain could be located in all treatments, including the samples treated at high temperature.

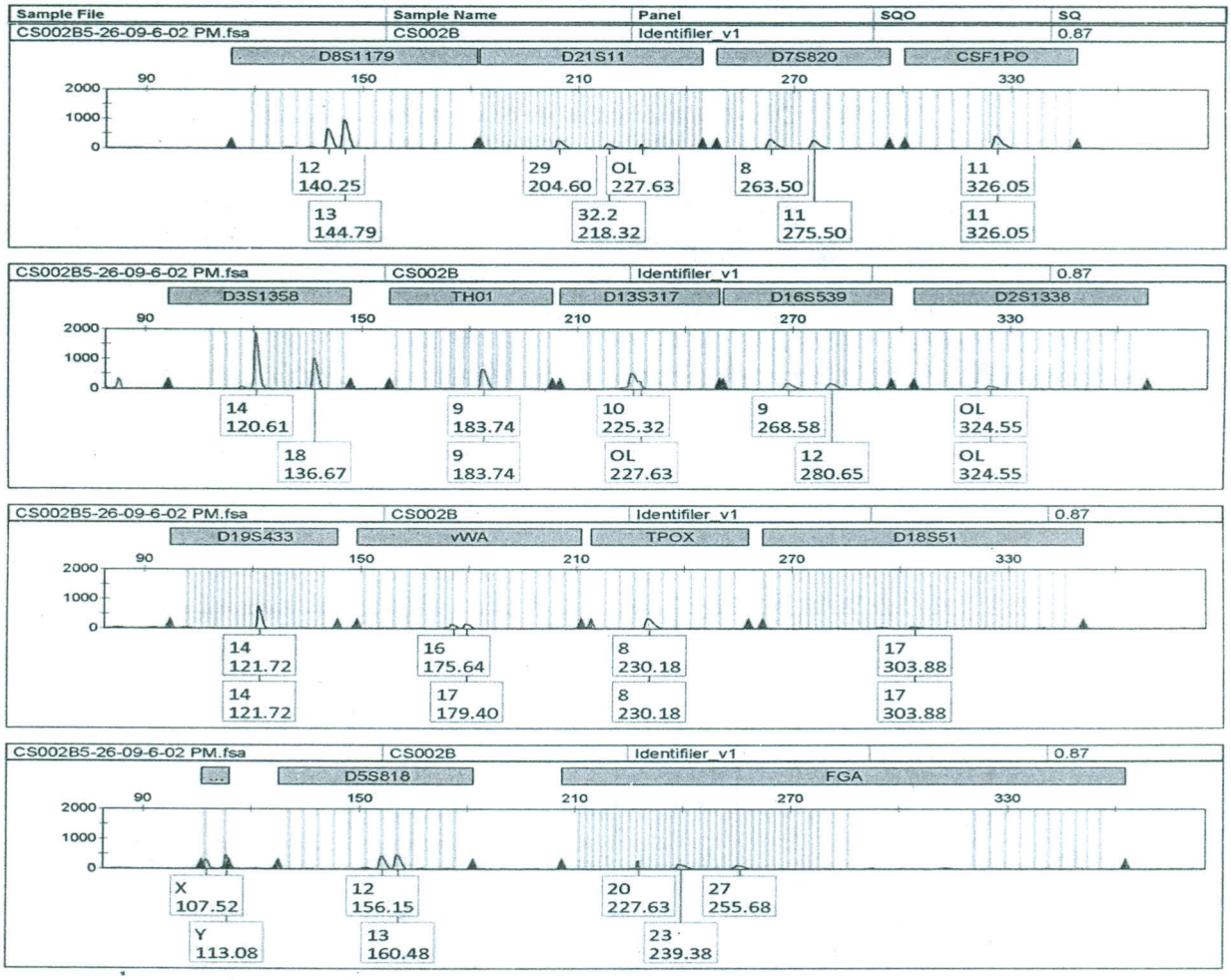
Treated bloodstains were then presumptively tested with KM and LMG reagents. All bloodstains left at low temperature showed positive results, giving a pink and blue green color change to the KM and LMG reagents, respectively, as the positive bloodstain control. However, no color change was observed when the samples were exposed to temperatures higher than 250°C.

In the present of Fe^{2+} and phenolphthalein, LMG would be oxidized, resulting in the color change of the chemical tests. High temperature caused oxidation of Fe^{2+} to Fe^{3+}. Therefore, no color change would be observed in the absence of Fe^{2+}.

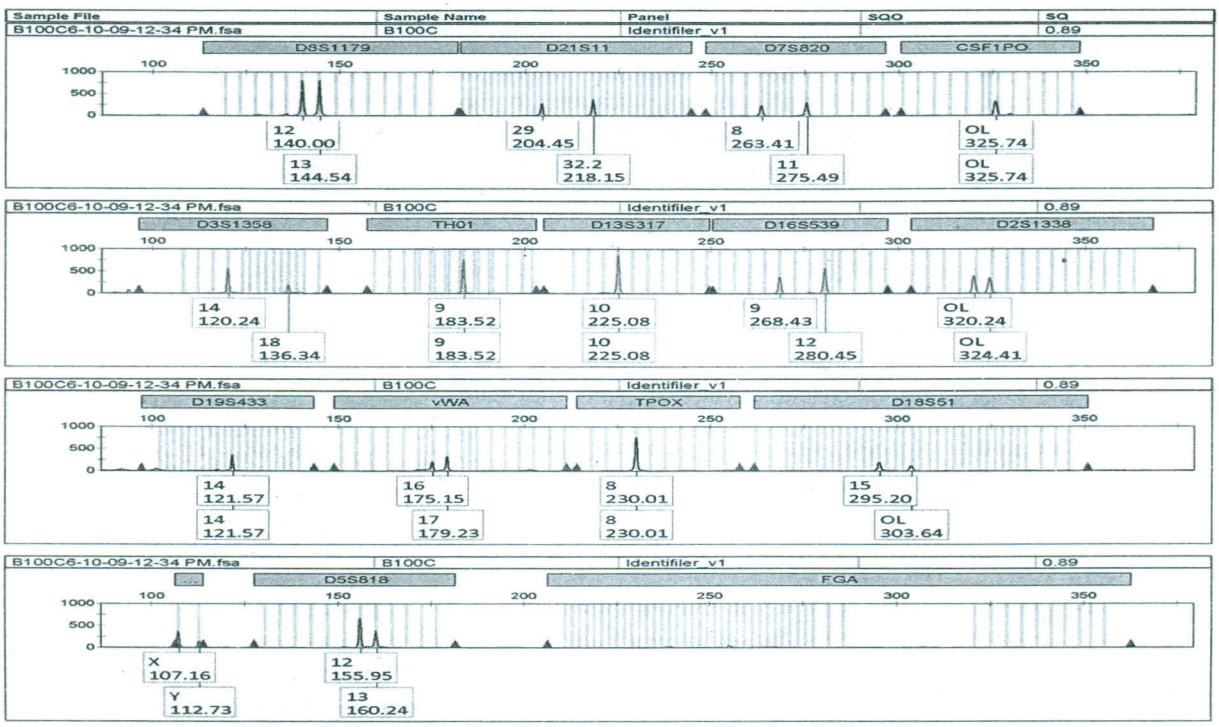
DNA was then extracted from the treated bloodstains for PCR-based DNA analysis. Full STR profiles were obtained from all samples treated in low temperature for up to 5 weeks. PCR Amplification of the human *cytochrome* b gene fragment was performed to check the bloodstain samples treated in high temperature prior to STR typing. The results showed that the 160-bp DNA fragment of human *cytochrome* b gene was amplified from samples treated in 100, 150, and 200°C; but not 250, 300, and 350°C. Then 2 samples, one from the sample group which PCR-amplified the 160-bp *cytochrome* b fragment and the other from the group which gave no PCR- product, were selected for STR typing.

As depicted in Figure 1, partial or incomplete DNA profiles were obtained from both samples with different level of allele dropout, only one genetic locus was dropped out from the partial DNA profile obtained from the bloodstain treated at 100°C, i.e. FGA. In contrast, only one allele was obtained from the DNA profile generated from the blood treated at 250°C.

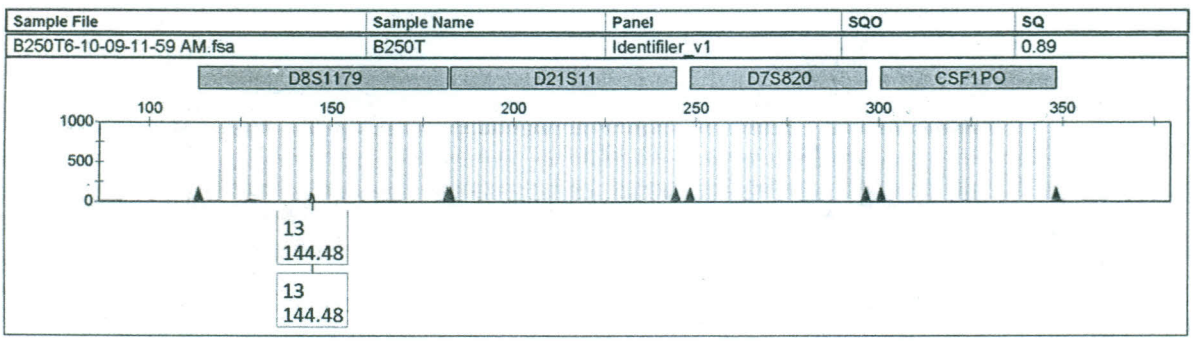
The results suggested that the physical appearance of the bloodstains could be altered by high temperatures (>100°C), and made the observation by naked eyes difficult. Alternate light source could aid the detection of stain in this case. Moreover, the temperature higher than 250°C could alter the blood composition (heme) and destroy the genetic material, then affecting both chemical presumptive tests for blood and DNA analysis.



Sample A: Reference DNA profile obtained for bloodstain



Sample B: DNA profile obtained for bloodstain treated at 100 °C



Sample C: DNA profile obtained for bloodstain treated at 250 °C

Sample A: Reference	Amel	D5	FGA	D8	D21	D7	CSF1	D3	THO1	D13	D16	D2	D19	Vwa	TPOX	D18
DNA profile	X	12	23	12	29	8	11	14	9	10	9	18	14	16	8	17
	Y	13	27	13	32	11	11	18	9	10	12	19	14	17	8	17
Sample B: DNA	X	12		11	29	8	11	14	8.3	10	9	18	13	15.2	8	14.2
profile 100 °C	Y	13		13	32	11	11	17	8.3	10	12	19	13	17	8	17
Sample C: DNA	X															
profile 250 °C	Y			13												

Figure.1 Partial or incomplete DNA profiles

2. The effect of pH

Blood was clotted and separated from the liquid serum when mixed with HCl and NaOH solutions. Different concentration of acidic and alkali pH bloodstains reacted similar to the untreated bloodstain when exposed to the alternate light source at 415 nm. (Fig. 2)

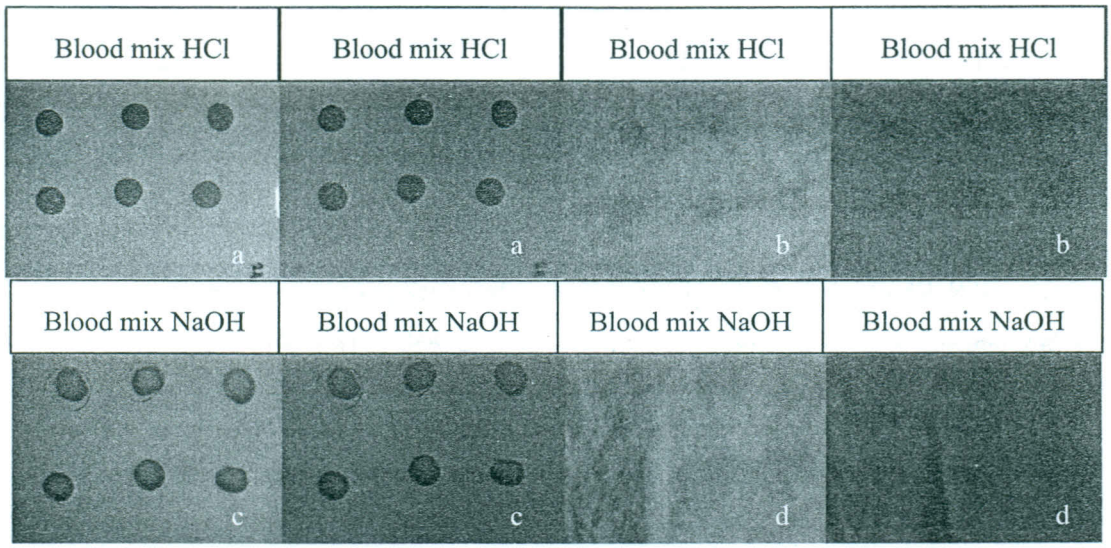


Figure 2. The result of bloodstain samples treated with HCl (upper) or NaOH (lower) when exposed to the alternate light source at 415 nm.

However, the intensity of the stains was slightly in the control. Also positive color change when the stains were chemically presumptively tested with KM and LMG reagents. The acidic and alkali pH bloodstains were then subjected to PCR-based DNA analysis. The 160-bp human *cytochrome* b gene fragment was PCR amplified from only one, sample, i.e., bloodstain mixed with 0.1 M NaOH. Three samples were then selected for human STR analysis, one was the bloodstained mixed with 0.1 M HCl and the other two was bloodstains mixed with 0.1 M, and 0.5 M NaOH. A partial DNA profiles were obtained from the alkali pH bloodstain. Acidic conditions can cause hydrolysis of nucleic acids to their smaller molecular units (bases, sugar, and phosphate) (6).Therefore the DNA strands would then be destroyed, resulting in no DNA profile. Alkali pH denatures DNA by disrupting the hydrogen bonding. The effect of alkali in the pH range of 7-8, which is near the physiological range, would be more subtle on the DNA structure (6) resulting in the presence of partial DNA profiles.

The results suggested that elevated pH of the bloodstains did not affect the search and chemical presumptive testing. However, because acidic and alkali pH destroyed the DNA structure, thus affecting DNA analysis of the bloodstains.

Conclusion

Both temperature and pH did not affect searching of bloodstains using alternate light source at 415 nm, but affected the downstream examinations, both presumptive testing and DNA analysis. Bloodstains treated in temperature higher than 250°C could not be presumptively tested with KM and LMG reagents. Partial STR profiles were obtained from bloodstains treated in temperature higher than 100°C and weak alkali pH (0.1 M NaOH). In conditions which temperature is over 250°C, acidic and alkali pH, no DNA profile would be generated from the bloodstain sample.

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