Estimating Original Bloodstain Volume: The Development of a New Technique Relating Volume and Surface Area

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Abstract

Reliable original bloodstain volume estimates can be a valuable piece of evidence in crime scene investigation. Present methods of volume estimation are limited in their application, hence, a more flexible method would be beneficial. The method explored in this study examined the relationship between a known volume and a generated bloodstain surface area. Surface specific standard graphs were created by dropping known volumes of blood on three surfaces. Digital images were taken of each stain and analyzed with Ultimage Pro (version 2.5), which calculated the surface area of each stain. Surface area vs. volume was then graphed and the best-fit line and equation calculated. Unknown volumes were then dropped onto each of the three surfaces and the surface area of each stain was calculated. The volume of each unknown was then estimated using its respective standard graph and equation. This method provided fairly accurate volume estimations and was applicable regardless of whether the stained surface was porous or non porous. The average errors for the surfaces were as follows: vinyl flooring 27%, carpet 18%, and drywall 13%. Further studies should be conducted to include larger volumes and vertical surfaces.

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CHAPTER 1
INTRODUCTION

1.1 General Background

Bloodstain pattern analysis has become very important tool in crime scene reconstruction (e.g., Bevel and Gardner, 2002, Lee, 2002, Pizzola *et a.l*, 1986a, Pizzola *et al.*, 1986b). The majority of the research, up until now, has focused mainly on spatter velocity and origin (Bevel and Gardner, 2002, MacDonell, n.d. and James and Ekert, 1999). Little research has been done to increase the accuracy of the existing methods for estimating the original bloodstain volume or to develop new methods (Bevel and Gardner, 2002 and James and Eckert, 1999). The volume of blood in a particular stain may become important in cases where there is a large volume of blood at one or more scenes, no body is found, and the probability of death is important to building a case. This study proposes a new method of determining original bloodstain volume by quantifying the relationship between the volume of blood in a stain and the surface area covered by that volume.

1.2 Background Material

Several techniques have been developed to aid the investigator in estimating the original bloodstain volume (e.g., Lee, 1986, Bevel and Garner, 2002, MacDonell and Lige, n.d.).

The following methods can be used to estimate bloodstain volume when the original stain is available to be analyzed. For blood left on non-absorbent surfaces Lee (1986) suggests scraping the blood from the surface, weighing the blood-crust, and then multiplying the weight by the dry weight constant of 4.167 to find the original blood

volume. The dry weight constant was developed by taking into consideration that 1 ml of liquid blood has a mass of 10.2 mg and the dry weight of 1 ml of blood has a mass of 2.4 mg (Lee, 1986). The weight lost during the drying process was then divided by the dry weight, resulting in a constant of 4.167 (Lee, 1986).

Lee devised an alternative method for estimating volume when a stain is found on an absorbent surface. In this case, the weight of one square unit of the matrix that does not contain a stain is subtracted from the weight of one square unit of the bloodstained matrix (Lee, 1986). The bloodstain volume is then found by multiplying the weight difference by the dry weight constant.

Bevel and Gardner (2002) developed a method of estimating volume by reconstructing, in the laboratory, the stains found at the scene. The first step in this method is to get an accurate description of the bloodstain by measuring its length and width as well as noting the topography (Bevel and Gardner, 2002). The next step is to obtain an unstained piece of the same surface and recreate the stain as best as possible (Bevel and Gardner, 2002). The volume needed to reproduce the stain is then taken to be the volume of the original stain (Bevel and Gardner, 2002).

MacDonell and Lige (n.d) developed a method that involved creating a stain the same size and on the same surface as the original stain and then immediately aspirating the blood with a micropipette. The volume of blood is then measured. MacDonell and Lige (n.d.) found that when blood is collected off of non-porous surfaces 90.1% of the blood deposited was lifted. The volume collected must therefore be multiplied by a factor of 1.1. When blood is lifted off of porous surfaces less blood was collected and the measured volume must be multiplied by a factor of 1.2 (MacDonell and Lige, 1971).

Indirect methods are also available in cases where there is no way of measuring the blood directly. The first method involves placing a grid overlay on the bloodstain and counting the number of units required to cover the stain (Lee, 1986). The weight of one unit of unstained surface is subtracted from the weight of one unit of stained surface. The difference between the two weights is then multiplied by the number of units of overlay required to cover the stain. This result is called the total weight of blood. To find the total volume of blood the total weight is multiplied by the dry weight constant (Lee, 1986).

A second method is available when the analysis can only be done from what is shown in photographs (Lee, 1986). The bloodstain is cut out of the photograph and weighed. A one-unit piece of a non-bloodstained photograph is then cut out as a control. The weight of the bloodstained area is divided by the weight of the one-unit photograph control. The total bloodstained area is found by multiplying the dividend by the unit area of the bloodstain. The next step is to find a piece of the surface on which the bloodstain was made. A one-unit piece, equal to the one unit of photograph used to find the total area, is cut out. A measured volume of blood is then poured onto the surface until it is covered. Finally, the total volume of blood can be calculated by multiplying the total area of the bloodstain by the volume of blood needed to cover one unit of surface (Lee, 1986).

While all of these methods can provide a broad volume estimate, evidence given at a criminal trial often requires a quantifiable degree of certainty and error. Only MacDonell and Lige (n.d.) provided results of their study and an error rate. None of the methods listed above performed any form of statistical analysis to support the claim that their methods are accurate. It is important that investigators know how accurate each of

the methods for estimating original bloodstain volume are so they can choose the most appropriate method for each crime scene.

While each method listed above provides the investigator with an important tool they fail to address some key issues of practicality. Lee's (1986) method of scraping the blood from a crime scene would prove an impossible task at many scenes. Also, no details of how to scrape, collect or store the scrapings were provided. It would be important that all of the scrapings were collected for this method to be accurate and if that was not possible that the percentage of powder residue left behind be quantified.

The method of comparing a stained piece of a surface with an unstained piece of a surface is only useful if the density of the surface is consistent. Lee (1986) mentioned that this method would be useful for blood volume estimations on soil. In cases where the density of the soil is not consistent it would not be possible to determine whether the difference in the weight between the stained and unstained portion was due to the presence of blood or to unknown substances in the surface.

Using the photograph unit method to estimate weight is very rough. The many calculations and steps increase the number of estimations made and therefore increase the rate of error. The photograph unit method fails to account for the angle and height from which the photograph was taken. These two factors would greatly influence the size in which the stain appeared in the photograph and therefore the estimation of what volume of blood made the stain.

The bloodstain reconstruction method proposed by Bevel and Gardner (2002) failed to account for dying time and absorption rate. The test stain should be in the same

state as the original stain when the volume estimation is made. Bevel and Gardner (2002) make no mention of how they compensate for these factors.

MacDonell and Lige (n.d.) also fail to address the issue of absorption. In their micropipette method the blood is dropped on an absorbent surface and immediately collected. It is unlikely that investigators would arrive at a crime scene in time to collect drops of blood immediately after they have fallen. The blood would most likely have soaked into to surface or dried. Once this occurred removing the stain with a micropipette would be impossible.

While the many unknown variables and disturbances that affect bloodstain formation at a crime scene make exact volume determination impossible it is desirable to have a more accurate, quantifiable method available.

1.3 Purpose of Study

The purpose of this study is to determine whether a quantifiable relationship exists between the volume of a bloodstain and the surface area covered by the stain in forensic contexts. To that end, a reproducible method of quantifying the area of a bloodstain and determining the volume of blood at the scene would be of great assistance to investigators.

CHAPTER 2
MATERIALS AND METHODS

2.1 Materials

Three different surface materials were used in this experiment: short fiber carpet, vinyl flooring and drywall. These materials were chosen due to their common use in homes. Forty-eight square feet of each surface was tested in order to complete four tests for each volume. Each surface was cut into 12inch by 16 inch pieces resulting in twenty-five pieces of each material. These measurements were arbitrarily chosen. It was important that the surfaces be a manageable size to transport and store during the drying phase and that they be easily photographed with available equipment.

The drywall was primed with Bullseye 1-2-3 interior/exterior white primer sealer and allowed to dry for a period of one hour. Two coats of Behr Premium Plus acrylic paint was then applied one hour apart and allowed to dry.

The carpet used in this experiment was a pale rose, Textured Saxony, nylon twist. The carpet was also coated with a scotch guard. No underlay was used during the experiment. It was thought that this would add another dimension to the analysis and it was felt that proof of a relationship between volume and surface area should supersede any confounding factors. The carpet was new and therefore the only preparation needed was to remove all loose carpet fibers.

The vinyl flooring used in this experiment was a white and gray plaza marble sheet flooring (felt# 48351) with a silicone wear layer. The only preparation of this surface was to dry wash, removing any debris.

Two and a half liters of sterile sheep's blood were purchased to complete this study. The blood was purchased from the Canadian Food Inspection Agency- Animal Disease Research Institute. The blood was treated with 1% sodium fluoride anticoagulant and preservative. The blood was refrigerated until needed to keep it in a steady liquid state until the completion of the experiment

The blood was gently agitated before use to insure that the cells had not settled and then measured using a graduated cylinder. A 5mL pipette and bulb were used to distribute the blood on the surfaces. For each surface, the blood was dropped at random heights, between 1 and 30 cm, at random velocities and in random location over the surface, thereby accounting for the type of variation that may occur at a crime scene. Care was taken to ensure that the blood did not flow over the sides of the surface. Each surface was given a number which was later used to identify the volume used to create the stain.

Volumes of 10mL, 20mL, 30mL, 40mL and 50mL were used as test volumes. Four trials of each volume, on each surface type were performed. Five unknown volumes for each surface were also dropped to test the validity of the theory. The unknown volumes were measured and poured into unmarked containers by a colleague. The actual volume of each of the unknowns was not known to the author until after the analysis was complete. All bloodstains were air-dried for a minimum of three days, in a secure room, to insure that the surfaces were not tilted or be disrupted in a way that would distort the bloodstain and interfere with later analysis.

A photograph center was set up in a location where the lighting could be controlled. The ambient fluorescent light and two 60-Watt light bulbs were the only light sources used. A Nikon Coolpix 990 digital camera was mounted on the photograph stand

and the distance set to 55 cm. This distance ensured that only the 12in by 16 inch piece would be included in the photograph. A level was placed on top of the camera to ensure that the images were all taken at a 90-degree angle. A scale was placed in all photographs. Digital images, in JPEG format, were taken individually of each bloodstain and saved to the memory card in JPEG format. Each photograph was taken at high image quality and full image size.

2.2 Computer Analysis

Each photograph was downloaded from the memory card onto a compact disk. The images were then loaded into Adobe Photoshop version 6.0. Each photograph was cropped to remove any unwanted background and reduced to 75% of the original size. The reduction of image size was necessary to allow the larger images to be analyzed by Ultimage Pro Graft DKE version 2.5 (1994 serial number 782510039).

Upon loading the images into Ultimage Pro a scaling factor was calculated using the scale in the images themselves. The scaling factor is the number of pixels equal to 1 cm. The scaling factor for this experiment was found to be 0.023. The next step in preparing the images for analysis was to convert the multi-coloured images into binary or two-coloured images. The program then distinguished, based on colour, what should be included in the surface area calculation and what should not. The distinction between colours was adjusted by colour thresholding. In cases like that of the marbled vinyl flooring, the program could not distinguish between the gray colour in the flooring and the pale bloodstains produced by the blood running across the surface. By adjusting the colour threshold the analyst can customize what is included in the analysis. Thresholding

is done based on hue, brightness and contrast. In this experiment, everything included in the surface area calculation was coloured red. Everything omitted was coloured black. The thresholding of colour was set for each stain. In adjusting the colour threshold we were able to ensure that only the blood was included in the analysis. The surface area was then calculated.

The standard bloodstains of known volume were used to create a standard graph for each surface type. Surface area was plotted against known volume. The graphing was done using Microsoft Excel. The best-fit line was then calculated and added by the program. These standard graphs were then used to estimate the volume of the unknown stains according to their calculated surfaces areas. The equation of the best-fit line found by performing a Pearson Correlation statistical analysis was also used to calculate the original bloodstain volume by substituting the surface area into the equation. The results of the two methods of determining stain volume were then compared.

2.3 Statistical Analysis

A Pearson Correlation was performed on the results of this study to determine whether they were statistically significant. The correlation coefficient (r) describes the direction of the relationship, either as a positive value indicating that one value is increasing as the other is increasing, or as a negative value indicating that one value is increasing as the other is decreasing. The absolute value of the (r) value also describes the strength of the relationship between two factors. The Pearson correlation also describes what percentage of variance that appears in the dependent variable (volume) can be

attributed to a change in the independent variable (surface area). This is the variance explained and is reported as the (r_2) value.

CHAPTER 3
RESULTS

3.1 General Results

The characteristics of each bloodstain were drastically different for each surface and volume. The random speed and height at which the blood was dropped produced stains of varying density and surface area (Figure 3.1, 3.2 and 3.3). The surface areas calculated by Ultimage Pro can be found in Appendix I. The surface areas calculated were graphed against their respective volumes to produce the standard graphs.

Two methods of volume estimation were used. The first method used the standard graphs developed in the first part of this experiment (Figure 3.4, 3.5, and 3.6). The second method calculated the original bloodstain volume using the equation of the best-fit line on each of the graphs.

A Pearson correlation determined the statistical significance of the results obtained in this study. The results of the Pearson correlation showed high correlation coefficients (r) and regression coefficients (r_2). The correlation coefficient describes the amount of dispersion seen in the values. A perfect relationship is given an (r)=1. The regression coefficient reflects the direction of the relationship as well as the strength of the linear relationship between the surface area and volume of blood in a stain. All values are significant at $p<0.005$.

3.2 Vinyl Flooring Results

The blood dropped on the vinyl flooring flowed freely over the surface, expanding until it reached its own level. The 10 ml and 20ml blood volumes produced small pools

Figure 3.1 Bloodstains on the Vinyl Flooring

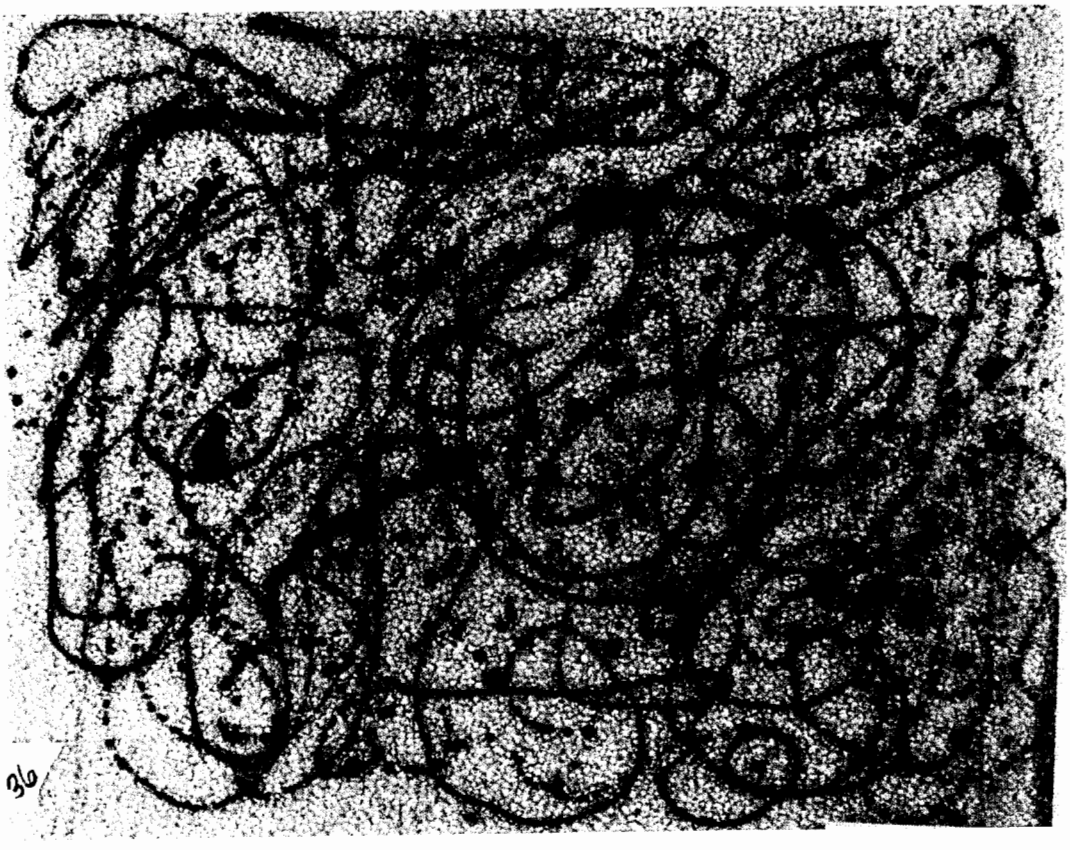

Figure 3.2 Bloodstains on the Carpet

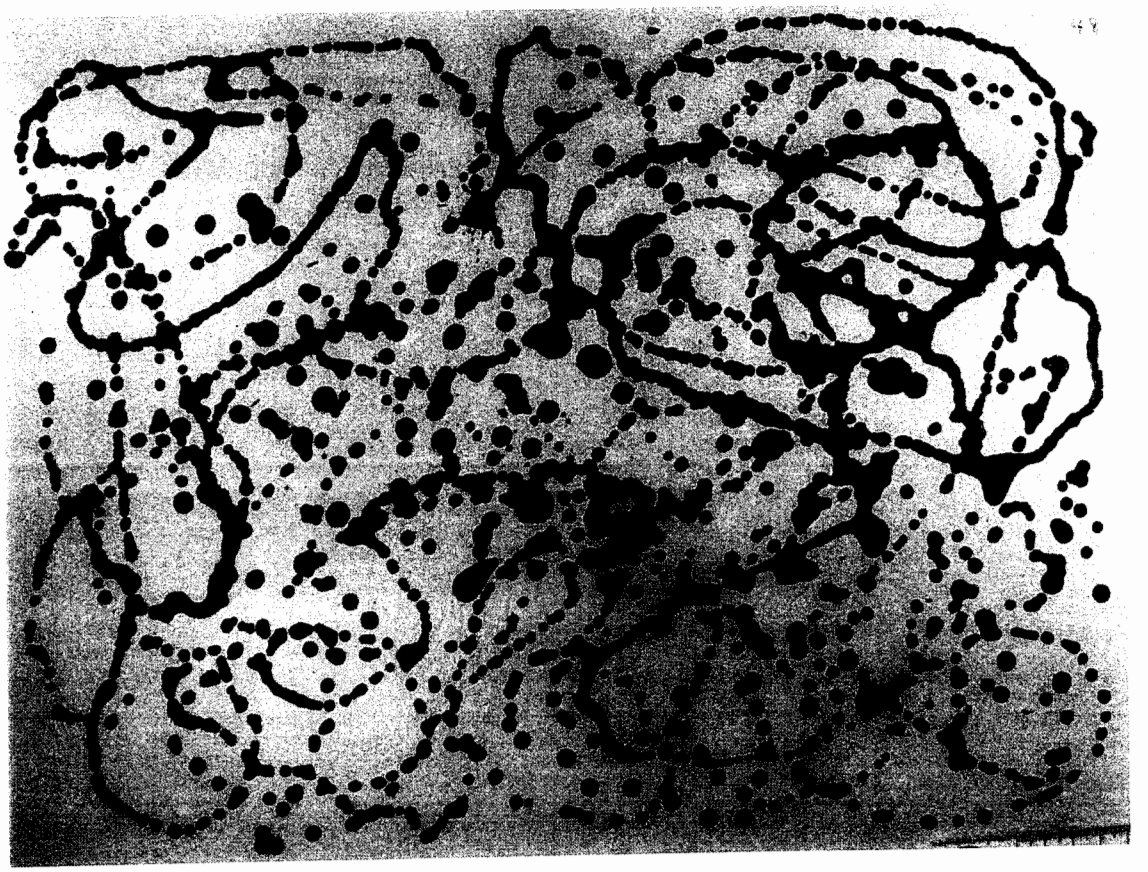

Figure 3.3 Bloodstains on the Drywall

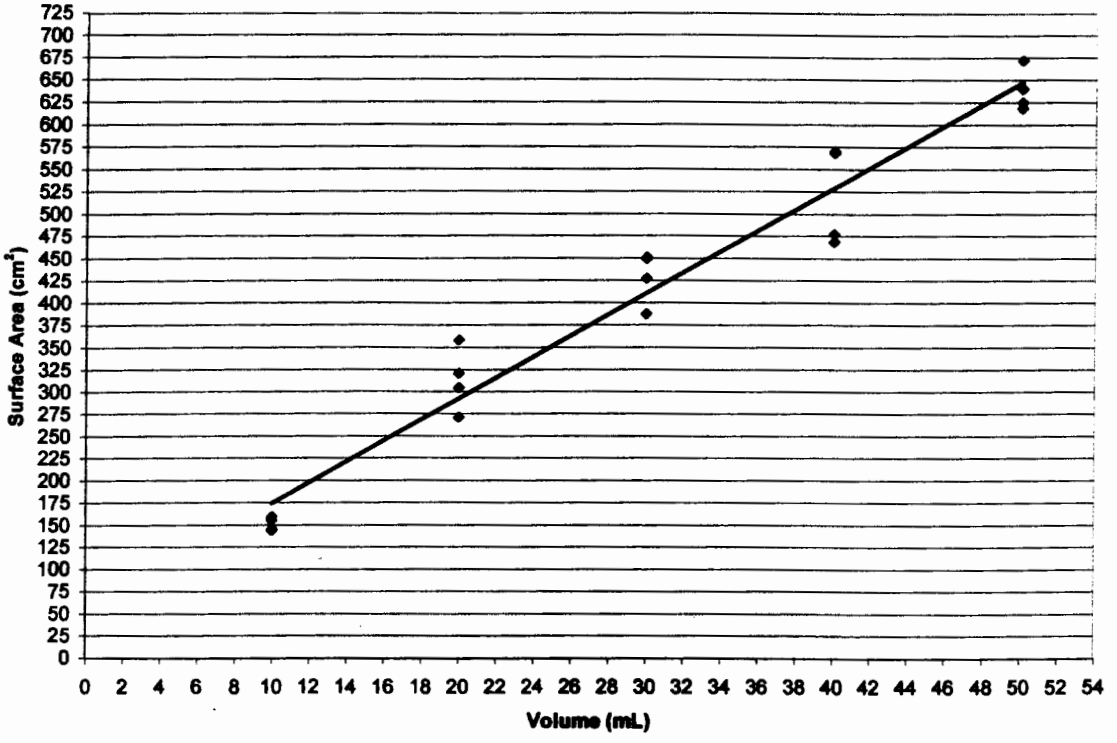

Figure 3.4 Standard graph for vinyl flooring representing the volume-surface area relationship

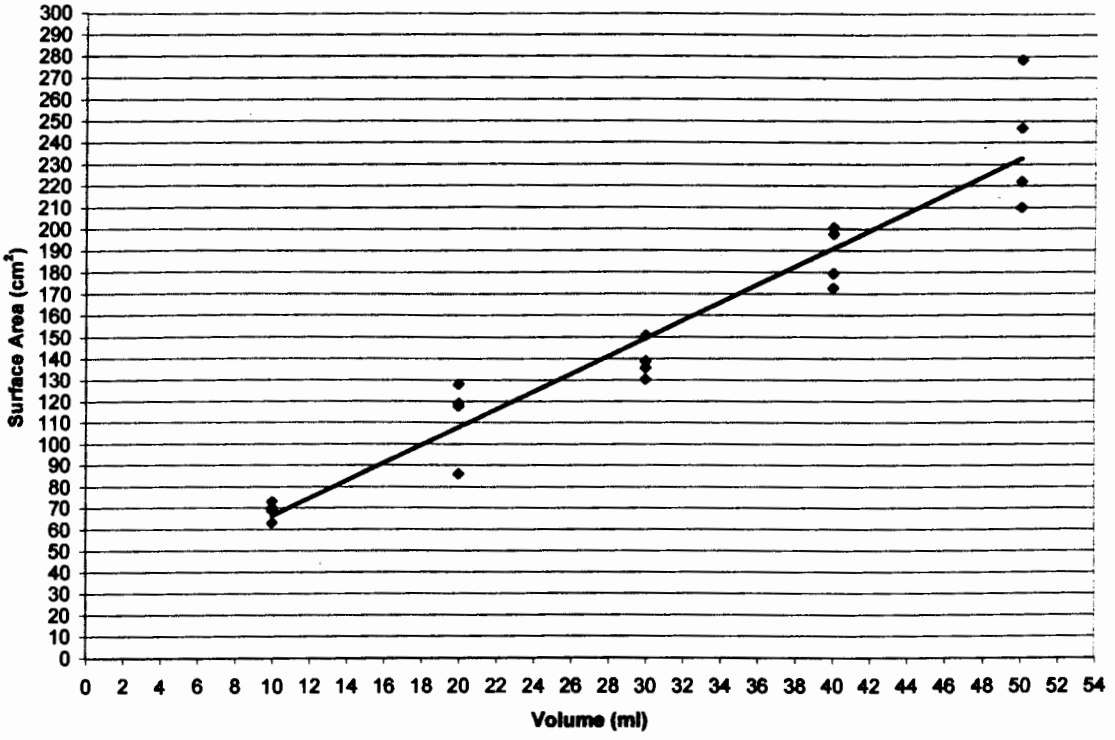

Figure 3.5 Standard graph for carpet representing the volume-surface area relationship

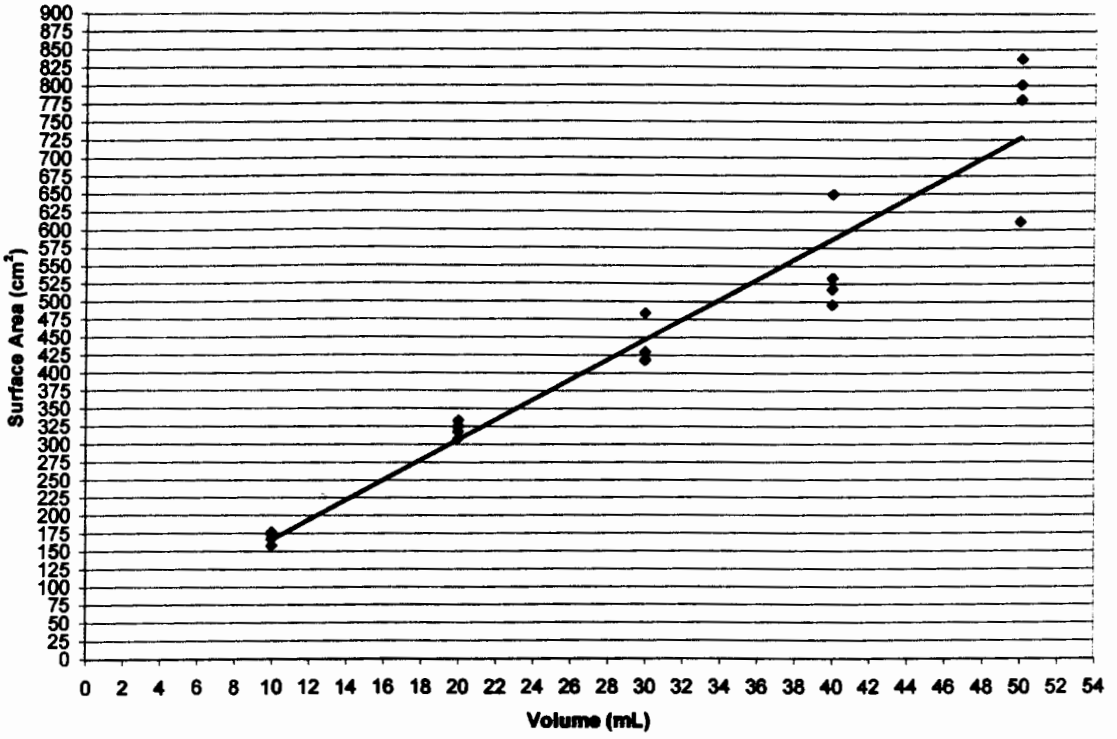

Figure 3.6 Standard graph for drywall representing the volume-surface area relationship

around the surface. However, the 30 ml, 40 ml and 50 ml volumes produced one large pool in the middle of the surface.

No overlap in the range surface areas covered by each volume was found on this surface. However, the ranges were only a minimum of 17.99 square centimeters apart.

The equation of the best-fit line for vinyl flooring was y=0.0663x + 0.085. The standard error of estimation was + 3.92 ml. The (r) value was 0.965 indicating there was no great amount of dispersion of the surface area-volume values from the best-fit line. The (r^2) value was 0.931 indicating that 93.1% of the variance in volume was explained by the change in surface area.

3.3 Carpet Results

The absorption of the blood into the carpet caused the blood to stay in the place where it was dropped. The volumes used in this study were small enough that the blood never soaked right through the carpet.

No overlap in range of surface areas covered by each volume was found on this surface. Each range was distinct from the ranges on either side with a minimum distance of 2.4 square centimeters. However, the range covered by each unit volume of 10 ml was very close to the ranges on either side.

The equation of the best-fit line for the carpet was y=0.220x –3.079. The standard error of the estimation was + 4.59 ml. The (r) value was 0.951 again showing that there was no great amount of dispersion of the surface area-volume values from the best-fit line. The (r^2) value was 0.905 indicating that 90.5% of the variance in volume was explained by the change in surface area.

3.3 Drywall Results

The drywall did absorb the blood to a certain extent. This was demonstrated in this study by dropping blood on the drywall only five hours after it had been painted. The blood ran to a much greater extent than it did on the drywall that had been painted a month prior to the experiment. However, the stains made on drywall expanded at a slower rate than the stains made on the vinyl flooring. For the 10 ml and 20 ml volumes, little expansion was observed. The 30 ml, 40 ml, and 50 ml volumes ran to the extent that they formed small pools.

The range of surface area covered by the 10 ml, 20 ml and 30 ml volumes were very distinct from one another. An overlap did occur between the 30 ml and 40 ml ranges and the 40 ml and 50 ml ranges.

The equation of the best-fit line for the drywall was $y=0.080x +3.322$. The standard error of the estimate was 2.99 ml. The (r) value was 0.980 indicating that there was no great amount of dispersion of the surface area-volume values. The (r^2) value was 0.960 indicating that 96.0% of the variance in the volume was explained by the change in surface area.

3.4 Summation

Tables 3.1, 3.2 and 3.3 compare the accuracy of the volume estimations for the unknowns using the standard graphs and the calculations using the equation of the best-fit line. Original bloodstain volumes were most accurately determined on the drywall using both the standard graph and equation of the best-fit line. One sample was estimated to the exact volume using the standard graph. The results using the standard graph method were

Table 3.1 Comparison of volume estimates and calculations to the actual bloodstain volume on vinyl flooring.

	Actual Volume(mL)	Estimated Volume(mL) Graph	Percent Error	Estimated Volume(mL) Equation	Percent Error
L1	10.0	11.5	11.5	12.62	26.2
L2	20.0	28.0	40.0	26.14	30.7
L3	30.0	47.5	58	43.11	43.7
L4	40.0	34.0	17.6	31.40	21.56
L5	50.0	53.0	6.0	45.52	8.96
		Average % Error	26.8	Average % Error	26.2

Table 3.2 Comparison of the volume estimates and calculations to the actual bloodstain volume on carpet.

	Actual Volume(mL)	Estimated Volume(mL) Graph	Percent Error	Estimated Volume(mL) Equation	Percent Error
C1	10.0	7.0	30.0	12.68	26.8
C2	20.0	18.5	7.5	19.58	2.1
C3	20.0	24.5	22.5	23.73	18.6
C4	30.0	31.0	3.0	30.70	2.3
C5	50.0	55.0	10.0	77.19	54.4
		Average % Error	14.6	Average % Error	20.84

Table 3.3 Comparison of the volume estimate and calculations to the actual bloodstain volume on drywall.

	Actual Volume(mL)	Estimated Volume(mL)	Percent Error	Estimated Volume(mL)	Percent Error
D1	12.0	14.5	20.8	14.90	24.17
D2	21.0	25.9	23.3	27.61	31.48
D3	27.0	27.0	0	29.82	10.44
D4	36.0	35.5	1.4	38.22	6.17
D5	46.0	44.0	4.34	48.44	5.50
		Average % Error	9.97	Average % Error	15.51

never more than 4.9 ml away from the actual volume. The results from the equation of the best-fit line were not quite as accurate but still produced estimates within 6.61 ml.

The second most accurate volume estimations, for both methods, were made on the carpet. The standard graph method produced estimates within 5ml. The equation of the line produced estimates within 3.73 ml except in all but one case.

The vinyl flooring produced the least accurate results for both the standard graph method and the equation of the best-fit line. Estimates from the standard graph were within 17.5 ml and the within 13.58 ml using the equation of the best-fit line.

The over all results of both methods show promise of narrowing the original bloodstain volume estimates now given at crime scenes.

Chapter 4
Discussion

4.1 Discussion

The accuracy of this method is dependent on how well the individual volumes can be discriminated one from another on the graph or by the equation. On the graph this would be affected by the difference in surface area covered by each sequential volume. Any overlap would be expected to affect the ability to discriminate between the two volumes. While it might be expected that the area of separation between two sequential volumes would decrease as the volume increased, because of the greater volume available to cover a variable amount of surface area, this was not shown to be true. The degree of separation was not found to follow any trend. Only two volumes overlapped, one on the carpet between the 20ml and 30 ml volumes and one on the drywall, between the 40 ml and 50 ml volumes. The overlap did not have an effect on the ability to discriminate. Estimates made at these intervals were more accurate than some estimates made where the interval difference was greater.

Another factor that may have an effect on accuracy is how far the points on the graph are scattered from the best-fit line or the heteroscedasticity. This is because the greater the scatter the less linear the relationship and the less accurately the equation of the best-fit line represents the actual data. In this study, heteroscedasticity did not appear to affect the results; however, further study is needed into how the surface areas of larger volumes vary from one another.

Field size and method of distribution may affect the amount of heteroscedasticity. In this study, smaller volumes of blood could be dropped over the whole area without

covering the same area twice. It is therefore more likely that the whole volume of blood contributed to the overall surface area. However, when the larger volumes of blood were dropped on the same size of field, almost the whole surface was covered with blood. It was more likely that blood was dropped in the same area more than once and therefore, that the whole volume of blood did not contribute to the overall surface area. While this appeared to be more of an issue with the less absorbent surfaces, because of the greater expansion of the blood the chances that it would overlap still increases, however, on the carpet. In an attempt to account for this, the blood was dropped from random heights.

The analysis of volume using photographs requires the conversion of a three-dimensional situation into a two-dimensional image. This prevents a direct assessment of the amount of absorption. With this in mind it was assumed that the volume estimates on the vinyl flooring would have been the most accurate of the three surfaces. This was due to the non-absorbent properties of vinyl which would eliminate the third dimension of depth, however, this was not found to be the case.

Volume estimates made on the vinyl flooring showed no trend in increasing or decreasing accuracy. The most accurate volume estimates using the standard graph were made at the 10ml and 50ml volumes. The points at these two volumes showed the least amount of variation in the surface area and displayed the least amount of heteroscedasticity. However, the most accurate estimates using the equation of the best-fit line were made at the 40ml and 50ml volumes. Comparing these results would indicate that heteroscedasticity and surface area variation may not affect the accuracy of this method.

Volume estimates made on the carpet were most accurate at the 20ml and 30ml volumes. The amount of surface area variation and the degree of heteroscedasticity appear to have little effect on the accuracy of either method on this surface. The surface variation and heteroscedasticity seen at the 20ml volume is greater than that at the 40ml volume and yet the 20ml volume was estimated more accurately. However, two unknown volumes dropped on the carpet had volumes of 20ml. The second volume of 20ml was predicted less accurately than was the first. This demonstrates the amount of possible variation that could occur in the volume estimates.

The trend in the volume estimates made on the drywall was that accuracy increased as volume increased. The surface area variation and heteroscedasticity were also seen to increase with volume therefore it may be assumed that these two factors have no affect on the accuracy of the estimates. It must, however, be taken into consideration when making this assumption that there was only five unknown samples which is not a good representation of the many possible surface areas each volume could cover.

The overall accuracy of the volume estimates on the vinyl flooring was found to be less accurate than those on the carpet and drywall. This can perhaps be explained by the curling of the vinyl at the corners, causing the blood to form a pool in the center. While the blood did leave behind a stain in the original location where it was dropped, the colour of the stain was pale in comparison to the pooled blood. This may have offset the surface area calculations. As previously mentioned in the materials and methods the colour threshold had to be adjusted to account for this discrepancy in colour. Perhaps controlling for the flatness of the vinyl would help improve the level of accuracy equal to

or greater than that of the drywall. Small pieces of vinyl would not be commonly found at a crime scene and therefore this would most likely not be an issue for investigators.

It was also expected that the volume estimations given by the equation of the best-fit line would have produced similar or more accurate results than that of the estimations derived from the standard graph. Since the equation was the same regardless of the surface area examined, the volume estimate would not be affected by human error. For the vinyl flooring the equation and the standard graph produced estimates with an equal rate of error. The average error for volume estimates on the carpet and drywall were lower when using the standard graph. How accurately the standard graph and the equation of the best-fit line-estimate volume depends greatly on the structure of the surface being examined.

The effects of how the physical properties of the surface affect the bloodstain characteristics can be seen when we examined the drywall. The time between when the drywall was painted and when the experiment was conducted was found to have an effect on the size of the stain. Half of the drywall used in this study was painted a month before the experiment began while the other half was painted only five hours prior to the first attempt. The blood dropped on the freshly painted drywall was not absorbed at all and ran off the surface. The characteristics of these stains were not consistent with the characteristics of the stains made on the drywall painted a month prior. Therefore the freshly painted drywall was allowed to dry for three days, a period that allowed the paint to dry completely, and then a second attempt was made. This produced consistent results.

Besides the chemical properties of the carpets, the presence of an underlay must also be considered when conducting volume estimates. The underlay may increase the

amount of blood absorbed. The reason for not including underlay in this study was because it was felt that it was important to first establish a strong relationship between surface area and volume before adding other factors.

Laber (n.d.) and Parker *et al.* (1982) found that the stain characteristics were dependent on the surface on which the stain was made, the distance the blood fell, the velocity at which the blood traveled, the object used to distribute the blood, and the temperature of the blood. In order to simulate crime scene conditions, the blood was dropped from random heights and at random velocities. The number of trials that could be performed in this study was limited due to financial constraints due to the cost of the blood. This limited the study to only one method of blood distribution. Also, the blood had to be kept at a temperature of approximately 8 °C in order to prevent it from coagulating, therefore; body temperatures of 37 °C could not be simulated.

Using sheep's blood as a substitute for human blood in this study was done for several reasons. Firstly, sheep's blood is more readily available. Accessing blood through the Canadian Blood Services was difficult due to uncertain and limited availability of expired blood products. Due to the time constraints it was necessary that all of the blood be available at the beginning of the experiment since the tests were conducted over a period of a few days. Secondly, in purchasing the sheep's blood through a government regulated agency, the blood was guaranteed to be sterile. While Canadian Blood Services routinely tests the blood they receive for a variety of diseases, it is impossible for them to guarantee sterility. While the usual biohazard precautions were taken, safety was always an issue.

Sheep's blood has been proven to be a suitable substitute for human blood in the laboratory setting (e.g., Didisheim, 1985, Goodman, 1998, Soloviev *et al.*, 1998, Yang *et al.*, 1996 and Christman, n.d.). Sheep's blood and organs are often used to test medical procedures prior to their use on humans (e.g., Goodman, 1998, Soloviev *et al.*, 1998, and Yang *et al.*, 1996). While the cellular composition of human and sheep blood is the same, the ratio in which each of the cellular components is present varies between the two. Sheep have approximately eleven million red blood cells per microliter while human have only five million cells per microliter (Didisheim, 1985). The human white blood cell count is between four and eleven thousand cells per microliter while sheep have between seven and ten thousand per microliter (Didisheim, 1985, and Marieb, 1998). The number of platelets was also found to be higher in sheep than in humans (Didisheim, 1985). Kotowski and Grieve (1986) did not find that these differences had any effect on the bloodstain characteristics. Sheep's blood and heart so resemble that of humans that they are the most widely used models for testing prosthetic hearts and other medical procedures (Goodman, 1998).

Christman (n.d.) performed a comparative hematological study using human, bovine, equine, swine and sheep's blood, as well as ink. He found that the animal blood and the ink produced identical stains when spattered in the same manner. Christman (n.d.) performed tests at different angles as well as at different velocities, and found that all of the stains produced looked extremely similar. Taking this information into consideration, sheep's blood was determined to be an acceptable test medium for this study.

It is important that the blood be in a steady state for the duration of the study in order to produce consistent results. It was therefore necessary that a small amount of anticoagulant be present in the blood. Sodium fluoride, which was used as both the anticoagulant and the preservative for the blood, was added by the supplier. While Christman (n.d.) used citrate dextrose and heprin, he found that the presence of an anticoagulant had no the effect on size of the stain. However, Parker *et al.* (1982) found a ten percent difference between the size of a blood drop with the anticoagulant and one without. It was not stated what the overall effect of this increase in drop size would have on the size of the bloodstain. It is, however, important to be aware of the possible changes that the addition of the anticoagulant might have on stain characteristics.

A small amount of error may have occurred when adjusting the colour threshold. It was difficult to ensure that the threshold was even for all surfaces. Consistency could not be guaranteed in situations where the threshold was set manually, as with vinyl flooring.

Error may have occurred when interpreting the standard graph. The unit interval was an average of 25 ml to accommodate for the large range of surface area covered by the five volumes. The amount of interpretation error is dependant on the abilities of the individual using the standard graphs.

While this study is still in the beginning stages, it shows promise of being a useful tool for estimating original bloodstain volume at crime scenes. This method is much more practical than those previously discussed since it can accommodate changes in surface properties. Standard graphs and equations can be developed according to whether the surface is absorbent or non-absorbent material and whether the blood is wet or dry.

Another benefit of this method is that the only step that must be performed at the crime scene is the photography of the bloodstains. This method would therefore decrease the amount of time needed to process the bloodstain evidence at the scene. Also, the use of photography allows the bloodstain evidence to be processed in a sequential and organized manner, allowing for easy documentation. With further study it is possible that this method will provide the necessary blood-volume information needed by the investigators.

CHAPTER 5
CONCLUSION

5.1 Summation

The use of standard graphs to estimate original bloodstain volume has eliminated many of the shortcomings of previous methods. The strong linear relationship allows the volume to be estimated accurately by comparing it to the calculated surface area. Having both the standard graphs and the equations of the best-fit lines provides the investigator with two methods by which to verify the original bloodstain volume estimates.

5.2 Conclusions and Recommendations

While it was attempted to make the parameters of this study as similar as possible to those found at a crime scene, several recommendations can be made in order to improve the accuracy of this method. Firstly, increasing the number of trials for each known volume used to compile the standard graph will allow for greater specificity of the volume estimates.

Secondly, the linear relationship must be examined at larger volumes. The blood volumes found at a crime scene have a larger diversity than what was explored in this study. Since pooling often occurs with bloodstains, larger volumes and field sizes must be examined. This will help determine whether the linear volume-surface area relationship holds true at larger volumes.

Thirdly, quantifying the heteroscedasticity between points at any given volume on the standard graphs would allow confidence levels to be established. Unknown stains could then be given a range in which their original volume fall into rather than an exact

value. This analysis was not performed in the study conducted since it was felt that more data points were needed to make the results statistically significant.

The research conducted in this study provides a foundation for the development for further research into the relationship between volume and surface area. These improvements are necessary to make this method practical for original volume estimation at crime scenes and admissible in court.

Surface Areas found by Ultimage Pro for the Vinyl Flooring

Volume (mL)	Average Surface Area (cm2)	Actual Surface Area (cm2)	Error
10	151.1	145.43	5.67
10		159.51	8.41
10		155.73	4.63
10		143.72	7.38
20	313.8	321.10	7.30
20		271.30	42.50
20		304.67	9.13
20		358.13	44.33
30	428.58	450.61	22.03
30		449.26	20.68
30		387.27	41.31
30		427.16	1.42
40	521.01	477.30	43.71
40		468.60	52.41
40		570.05	49.04
40		568.10	47.09
50	638.92	624.78	14.14
50		618.52	20.40
50		672.06	33.14
50		640.30	1.38

Surface Areas found by Ultimage Pro for the Carpet

Volume (mL)	Average Surface Area (cm2)	Actual Surface Area (cm2)	Error
10	68.75	68.87	0.12
10		69.98	1.23
10		73.04	4.29
10		63.10	5.65
20	112.63	118.96	6.33
20		117.67	5.04
20		85.92	26.71
20		127.98	15.35
30	147.39	135.67	11.72
30		150.77	3.38
30		172.73	25.34
30		130.38	17.01
40	182.07	150.77	31.30
40		179.73	2.34
40		197.77	15.70
40		200.00	17.93
50	239.28	246.67	7.39
50		278.50	39.22
50		210.00	29.28
50		221.96	17.32

Surface Areas found by Ultimage Pro for the Drywall

Volume (mL)	Average Surface Area (cm2)	Actual Surface Area (cm2)	Error
10	169.57	158.25	11.32
10		174.90	5.33
10		177.21	7.64
10		167.92	1.65
20	320.24	304.95	15.29
20		317.88	2.36
20		324.93	4.69
20		333.19	12.95
30	437.31	482.97	46.66
30		429.90	7.41
30		417.20	20.11
30		418.17	19.14
40	548.88	649.12	100.24
40		495.74	53.14
40		517.48	31.40
40		533.19	15.69
50	757.22	610.85	146.37
50		836.94	79.72
50		780.23	23.01
50		800.86	43.64

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