# Evaluating the Accuracy of Bloodstain Pattern Analysis Using Hemodynamic Factors

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## Abstract

This comparative survey explores the relationship between the discipline of bloodstain pattern analysis (BPA) and hemodynamic blood properties, such as viscosity and hematocrit. In BPA, forensic scientists study the phase change of blood when in contact with air, but little forensic literature connects blood biomechanics, such as hematocrit levels, to BPA. Red blood cell count, or hematocrit, in females (37-48% of blood volume) is slightly lower than males (45-52% of blood volume) from menstrual red blood cell loss, etc. Strong evidence suggests that erythrocytes influence blood viscosity because of their high concentration (4-6 x $10^6$ RBC/$mm^3$ or 40-45% of blood volume in healthy individuals). When whole blood is altered by a disorder/disease or alcohol intake, hematocrit levels can be affected as well. With this knowledge, there is reason to believe that blood viscosity changes with individualistic hematocrit levels. Therefore, it is hypothesized that traditional BPA can produce inaccurate results. Intravenous blood samples were drawn from nine volunteers (all women, including eight with blood alterations and one healthy control) into collection tubes containing ethylenediaminetetraacetic acid. Each sample was tested for viscosity using a Cannon-Fenske viscometer and hematocrit levels using a ZipCombo centrifuge. Each sample was used to make several bloodstains at varying degrees of impact (10°, 30°, 60°, and 90°). ANOVA ($\alpha = 0.05$) and Tukey HSD statistics were used to compare angle of impact variables against each other within the nine participants. This survey connects hemodynamic properties to angle of impact tests in BPA by significantly showing how bloodstains can be misinterpreted. By examining blood viscosity among several individuals, this research assesses the accuracy of BPA by comparing experimental and expected bloodstain angles and creates a predictive framework for analyzing bloodstains created by physiologically altered blood. In conclusion, hemodynamic factors among individuals were found to influence traditional BPA methodology and future research is recommended to better understand hemodynamic properties and fulfill the recommendations made by the 2009 National Academy of Sciences (NAS) Report.

**Key words**: angle, blood, forensic science, hematocrit, hemodynamics, pattern analysis, viscosity

## Introduction

The purpose of this paper is to present a comparative survey among a variety of blood disorder patients capturing hemodynamic factors of blood outside of an individual's body. There is significant research on blood as an internal biological fluid, however there is little research on the influence of hemodynamic factors as blood exits the human body (Nordqvist 2017). This phenomenon is only seen in forensic science literature where bloodstain pattern analysis (BPA) is used to reconstruct crime scenes using blood outside of the human body. BPA is the interpretation of bloodstains at a crime scene in order to reconstruct and/or recreate the actions that caused the bloodshed and pattern. Forensic technologists examine the size, shape, distribution, and location of the bloodstains to form an opinion about what happened at the crime scene.

Additionally, analysts can determine an area of convergence (two-dimensional analysis) or area of origin (three-dimensional analysis) to better understand where the bloodstains originated from at a scene. BPA is a multidisciplinary approach that uses biology, physics, and mathematics to assist the analyst in crime scene reconstruction, corroborating witness statements, and

including or excluding potential perpetrators from the scene investigation. Analysts aim to categorize the bloodstains at a crime scene by gathering information from the spatter patterns, transfers, voids, and other marks that occurred after bloodshed. BPA differs greatly from the testing of blood which is left for serologists and DNA analysts (Koen and Goetz 2017).

Whole blood is composed of cellular elements that include erythrocytes or red blood cells (RBCs), leukocytes or white blood cells (WBCs) and thrombocytes (platelets). These cellular elements are suspended in an aqueous polymer solution called the plasma, which acts as a delivery system for the cellular components in blood. Plasma is composed of electrolytes, hormones, antibodies, enzymes, and other proteins in small concentrations (Bodnar et al. 2011). There is evidence that RBCs influence the mechanical properties of blood because of their high concentration (4-6 x $10^6$ RBCs/$mm^3$ or 40-45% of blood volume in healthy individuals) compared to the other cellular elements (Bodnar et al. 2011). The red blood cell count, or hematocrit, in females (37-48% of blood volume) is slightly lower than males (45-52% of blood volume) because of menstrual red cell loss (Shiel 2017). Low hematocrit levels are seen in individuals with abnormal hemoglobin, bone marrow complications, and anemia, while high hematocrit levels are seen in individuals living at high altitudes and in chronic smokers (Shiel 2017). Dehydration can also cause red blood cell levels to appear extremely high, although the individual may have physiologically normal blood.

When asked in a legal setting, a bloodstain pattern analyst generally describes blood as a Non-Newtonian viscoelastic fluid. Non-Newtonian fluids, such as paint, ketchup, toothpaste and blood have large particles that have limited time mobility when a force is applied quickly, resulting in the formation of a solid. A common example of this phenomenon is corn starch and water, in which a force can quickly change the liquid into a solid. Shear rates are used to measure the rate of change of velocity of two adjacent layers of fluid. They ultimately measure how the fluid is "worked" in the environment in which it is placed. Because blood encounters several types of vessels within the human body, it experiences shear stress, and therefore shear rates can be determined based on the viscosity of the blood. Viscosity, in centistokes (cSt), is the measure of a liquid's ability to resist deformation by force or tension. A fluid is shear-thinning if the viscosity decreases as the shear rate increases. Some shear-thinning fluids are known as pseudo-plastics which are used in industrial and biological processes. Shear-thinning, or a decrease in viscosity, is directly related to blood dynamics and the interactions of RBCs (Lanotte 2016). Conversely, shear thickening occurs when the viscosity of the fluid and the shear rate mutually increase. Plasma exhibits Newtonian fluid properties, in which the viscous stress from flow is dependent on the local strain rate, but whole blood follows non-Newtonian characteristics, especially at low shear rates.

In blood, shear rates occur when the velocity of one layer of fluid is different from the velocity of an adjacent layer. Blood acts as a non-Newtonian fluid because of three phenomena; RBCs tend to form three-dimensional microstructures while having the ability to breakup and/or align with the field of flow (Bodnar et al. 2011). Each of these phenomena can be influenced by changes in shear rates of blood throughout an individual's daily life. At low shear rates, whole blood tends to have very little strain from vessel walls making it viscous or thick. Conversely, high shear rates coerce blood into becoming less viscous allowing it to flow easily through narrowed blood vessels. Recently, Bodnar et al. (2011) proposed a model that can predict the viscoelastic response of blood while inside the human body. They explained that RBCs behave like viscoelastic fluids in venous blood flow because they are elastic membranes filled with fluid, but in stenosed vessels shear rates can follow non-Newtonian properties (Bodnar et al. 2011).

Research suggests that when whole blood is altered by a disorder/disease or alcohol intake, RBCs can be affected in several ways. Hypo- and hyperthyroidism can cause anemia and erythrocytosis leukopenia; two alterations to RBC count (Dorgalesh et al. 2013). Levothyroxine is a common oral medication for treating hypothyroidism and thyroid cancers, but it does not directly influence RBC count in individuals. Evidence shows that Thalassemia I and II increase reactive oxygen species in an individual causing RBC oxidative damage (Ko . 1997). This suggests RBC count and blood viscosity is altered in Thalassemic patients. Studies also show that diabetic patients experience a decrease in RBC deformability, causing RBC rupture from an overall loss of RBC fluidity (Ernst and Matrai 1987). High alcohol consumption reduces thiamine and folate absorption in the intestines. Both of these nutrients are used in RBC production and alcohol dependent individuals (74 drinks/week) can experience bone marrow abnormalities leading to a decrease in RBC production (National Institute on Alcohol Abuse and Alcoholism). In alcoholic anemia, an individual can even experience a decrease in platelet production leading to the inability to form clots, which may also lead to a stroke. It should be noted that the quantity and type of alcoholic beverage consumed, increase the variability in coagulant properties of platelets. Von Willebrand's disease (VWD) is a rare blood clotting disorder that alters platelet activity. It can be classified into three types with decreasing blood clotting activity with each type (Sadler et al. 2006). Prothrombin 20210 mutation or Factor II mutation is a genetic disorder that causes dangerous blood clots to form more frequently than individuals without the mutation (National Blood Clot Alliance). Both VWD and Prothrombin 20210 mutations do not alter red blood cell count, rather the genetic composition of an individual's platelets. Finally, research suggests that blood thinners, such as heparin, bind to thrombin and induce a conformational change in the enzyme to prevent blood clotting (Machovich 1975, Mellanby 1934). Xarelto® is a more expensive alternative to heparin blood thinners, but both have not shown to directly influence the RBC count in individuals.

The conditions stated above lie on a spectrum of direct and indirect influence on blood dynamics. Some disorders directly influence RBC production or degradation, such as hypothyroidism and Thalassemia, while others only affect platelet genotype. Previous research suggests that blood viscosity is dependent on hematocrit levels in individuals, and therefore blood viscosity could be moderately individualistic. As mentioned above, platelet count, activity, diet, and clotting factors can influence blood viscosity. This phenomenon happens because the slightest alteration to blood components in an individual can lead to applied shear stress and a change in blood viscosity. For example, if an individual is dehydrated and exercises for a long period of time their blood would appear more viscous; the dehydration and continuous exercise would increase the level of red blood cells in the blood (to increase oxygen delivery to muscles, tissues, and organs) and the blood vessels would begin to apply shear stress (decrease in blood viscosity) to allow the blood to travel more easily through the body. This example shows how easily blood viscosity can change in an individual throughout their daily life, but it is difficult to monitor this physiological change inside of the body for research.

Blood behaves to specific scientific principles, both inside and outside the human body, and therefore BPA experts can examine stains to draw conclusions as to how blood was shed at a crime scene. Analysts classify bloodstain patterns as either spatter patterns, transfers, voids, other marks, or a combination of these. Bloodstains assist investigators in reconstructing the sequence of events that occurred during or after bloodshed. Analysts are forced to recognize and interpret bloodstain patterns to determine how they were created (National Forensic Science Technology Center). Bloodstain pattern

analysis has proven to be one of the most legitimate pieces of evidence for crime scene reconstruction, but other forms of evidence, such as hair and impression evidence, have received the legal "chopping block". Interestingly, BPA is a traditional forensic identification science that uses subjective assumptions and guesswork. The field of forensic science is amid a paradigm shift to sound scientific foundations and justifiable protocols, especially after the National Academy of Sciences (NAS) Report of 2009 (Saks and Koehler 2005). Forensic scientists must consider revising their methods with more defensible and empirical foundations to remain legitimate in a legal setting. With further research, reliability and accuracy of BPA can increase the ways that bloodstains can be applied judicially.

Currently, there is a gap in scientific literature because blood possesses enigmatic properties once it has left the human body. The specific influence of viscosity and hematocrit on BPA has been researched extensively (Aplin et al. 2019, Kim et al. 2016), but hemodynamic factors are unrecognized in populations with physiologically altered blood. Kim S. et al. (2016) attempted to use standard values of hematocrit in bloodstain trajectory reconstruction but found two systematic errors: one on the blood viscosity, which depends on the blood hematocrit, and the second on the estimated impact diameter (Kim et al. 2016). These uncertainties found in BPA hemodynamics support the recommendations made by the 2009 NAS Report, which mentioned that "the uncertainties associated with BPA are enormous" (Committee on Identifying the Needs of the Forensic Sciences Community, National Research Council 2009). Kim S. et al. states that employing generalized correlations or hematocrit levels to BPA can lead to errors in determining the area of origin of the bloodshed. This research will address this finding and attempt to apply generalized correlations and hematocrit/viscosity levels to BPA in a population of physiologically altered blood patients.

Attinger et al. (2013) provide a comparative review of fluid dynamics of blood, current BPA research, and new BPA methodologies. Their comprehensive analysis indicates that BPA would benefit from a more scientific foundation and joint research between BPA and fluid dynamics is needed to increase BPA reliability (Attinger et al. 2013). More recently, Aplin et al. (2019) analyzed BPA at six varying hematocrit levels and found that differences in the hematocrit and viscosity values did not affect the accuracy of the forensic analysis ($p > 0.05$) (Aplin et al. 2019). This is consistent with previous studies that state hematocrit values of a blood source do not influence its origin. The problem with the proposed methodologies is that the researchers do not attempt to make generalized correlations between hematocrit values and physiological blood alterations, which could be individualistic of a person at the crime scene. This project will assess this concept and analyze impact patterns when the contributing blood source is known to be of a physiologically abnormal sample.

The purpose of this paper is to present a comparative survey among a variety of blood disorder patients capturing hemodynamic factors of blood outside of an individual's body. The work presented here significantly extends the preliminary results obtained by the primary investigator on 12 October 2018 where blood viscosity was compared among dehydrated and hydrated individuals. At first glance, the dehydrated blood was more viscous and appeared to alter BPA accuracy. The preliminary research continued to analyze the blood samples by creating traditional bloodstains using an angle of impact test and string method. Upon analysis of detailed angles of impact, the dehydrated blood samples deviated up to 10 degrees from the position at which the sample was dropped. Although this phenomenon was only examined in one participant, it left reason to believe that blood viscosity could influence the accuracy of BPA. The NAS Report of 2009 recommended that sound scientific research be performed on BPA to decrease its level of inaccuracy. By attempting to examine

blood viscosity and hematocrit levels among several individuals, this present research assesses the accuracy of BPA by comparing experimental and expected angles of impact of bloodstains.

# Methods

## Sample Collection

Intravenous blood samples were drawn from nine volunteers (all women, including eight with blood disorders/ alterations and one healthy control) at the University of Providence lab into tubes containing ethylenediaminetetraacetic acid as an anticoagulant. Female participants were selected because hematocrit levels vary greatly between sexes and this variable needed to be controlled to analyze how hematocrit and BPA are related. The participants were in their 60s, 40s, 30s, 20s and teens to display a varying age demographic among the sample. Participants were hand selected by the primary investigator to ensure each subject had a blood alteration. Among them were patients with Thalassemia and blood clotting disorders, such as VWD. Other participants were found based off research that suggested physiological conditions that alter blood viscosity (indirectly caused from RBCs), such as diabetic and hypothyroid patients and those on regular blood thinners. Additionally, a participant was asked to consume two alcoholic drinks (28 g) before having their blood drawn. In a separate draw, the same participant consumed four alcoholic drinks (56 g) and had their blood drawn once more. In both cases, the blood was analyzed to see whether low or high alcohol consumption influences BPA. Additionally, the blood alcohol content (BAC) was recorded to ensure the participant had levels of legal and illegal alcohol consumption, in regards to operating a motor vehicle.

## Hematocrit Testing of Blood Samples

A ZipCombo centrifuge, heparinized centrifuge tubes, and EZ Reader were purchased from LW Scientific©. Blood samples were taken up into heparinized microcapillary tubes and capped at both ends using a thick clay. For each participant, the microcapillary tube was centrifuged at 12,000 rpm (7500g) for 3 min. After the allotted time, the capillary tubes were placed into an EZ Reader and red blood cell count was determined by the percent of erythrocytes separated from cellular solution.

## Viscosity Testing of Blood Samples

A Cannon Fenske viscometer with model number 2700 was obtained from the University of Providence lab. It was cleaned with distilled water and left to dry before testing the viscosity of three standards. The standards chosen for viscometer calibration were toluene (0.6800 cSt), distilled water (1.0038 cSt), and absolute ethanol (1.5200 cSt). The three standards were used to measure the kinematics of the specific glassware used. Kinematic viscosity is measured using centiStokes (cSt) and can be used to quantify unknown viscosities, such as blood. To ensure that temperature did not alter viscosity, each standard was chilled to 20 $^0$C using an ice bath. Additionally, the glass viscometer was placed in a 20$^0$C-water bath using a ring stand. With this system, both the sample and the viscometer were at a constant 20$^0$C before running the liquid through the glassware to determine viscosity. The viscometer was leveled before each test to ensure that each sample flowed properly though the viscometer. Each standard was loaded into the viscometer in separate trials and the time for each to pass from line “C” to line “E” was recorded (Fig. 1).

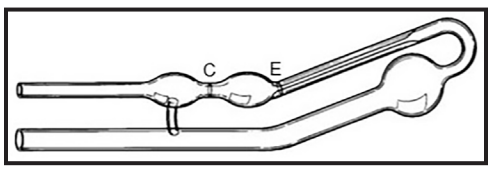



Figure 1. Cannon Fenske viscometer obtained from the University of Providence lab. Viscosity (in centistokes) was measured by timing a fluid between lines C and E. Image credit: Sigma Aldrich.

After chilling to 20°C, blood samples were loaded into the viscometer and timed in triplicate similarly to the standards. The same viscometer was used for standards and blood samples. Between each sample, the viscometer was washed thoroughly with acetone and left to dry to ensure no water influenced the viscosity testing. The time, in seconds, was plotted (Fig. 2) against the "known" viscosity of the standard to create a polynomial curve ($R^2 = 1$) that would be used to determine the unknown viscosities of the blood samples (Engineering Tool Box).

## Bloodstain Pattern Analysis

Traditional BPA was tested for accuracy by comparing experimental angles of impact to expected or dropped angles of impact (the angle at which the clipboard was fixed). Expected angles were formed using a clipboard and protractor set-up, in which a volunteer held the clipboard at the following angles: 10°, 30°, 60°, and 90°. A plastic pipette was used to drop the blood samples onto cardstock paper. Five drops were made for each of the four tested angles (Fig 3). Stains were made for each participant for a total of 180 bloodstains that would be used for comparison of each other. The cardstock paper was attached to the clipboard and set to the angle of impact before blood patterns were made. Drops were made approximately 0.3048 m (1 ft) above the paper and a new paper was used for each angle. Patterns were left to dry overnight before analysis and upon drying, the width and length of each stain was measured. Experimental angles of impact were obtained using the following equation:

$$\Theta_{\text{angle of impact}} = \sin^{-1}\left(\frac{w}{l}\right)$$

where $w$ and $l$ are the width and length of the blood droplets, respectively. The length of the pattern runs parallel with the tail of the blood drop, while the width runs perpendicular with the tail. In BPA, the length of the stain is measured by omitting the length of the bloodstain tail. The measured angles were averaged between the five stains on each paper. Experimental angles of impact were compared both physically and statistically to expected angles. Spines, satellite drops, and individualistic formations were also documented for each blood pattern.

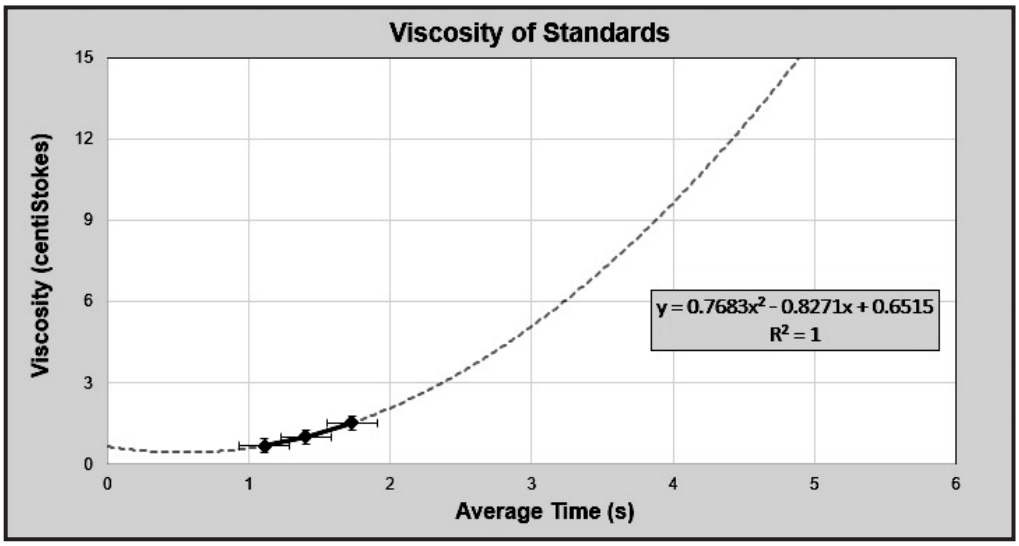



Figure 2. Polynomial curve of standards run through the Cannon Fenske viscometer. Known viscosities of the three standards are as follows: toluene (0.6800 cSt), distilled water (1.0038 cSt), and absolute ethanol (1.5200 cSt). Standard error was calculated to be ±0.25 s. for time calculations.

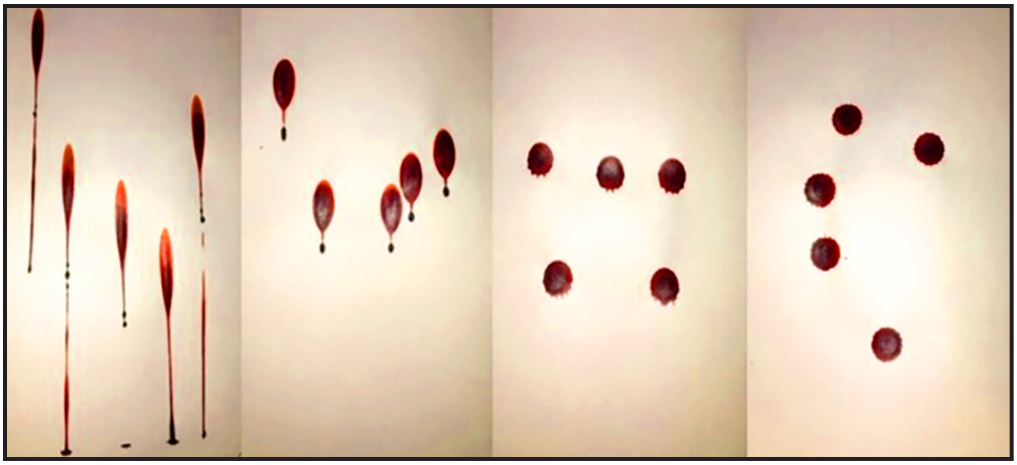


Figure 3. Traditional BPA at 10°, 30°, 60°, and 90° for the control blood sample. Satellite stains were documented if present. Five stains were made at each angle.

### Statistical Analysis of Blood Alterations and Angle of Impact

Each blood sample was compared using the following variables: hematocrit, viscosity (in cSt), and angle of impact. Viscosity and hematocrit levels ranged between participants, but statistically the blood samples were considered similar, and therefore were expected to produce bloodstains with similar measurements. Consequently, the average angles of impact between participants were statistically analyzed using analysis of variance (ANOVA). A confidence interval of 95 percent ($\alpha$=0.05) was used. The *F*-crit and *p*-values were used to find whether average angles of impact between blood alterations were significantly different and a Tukey Post Hoc test compared each participant between each other for individualism. Finally, a *t*-test was used to statistically compare expected angles to experimental angles for each participant. Both statistical analyses were used to evaluate the accuracy of BPA regarding hemodynamic influences.

## Results

### Viscosity and Hematocrit Levels Among Blood-Altered Participants

After averaging the time each blood sample took to cross between lines C and E on the Cannon Fenske viscometer, blood viscosity for each participant was found by using the polynomial curve created by the viscometer standards (Fig. 4). Blood viscosity between the participants varied between 10.29 and 21.22 cSt. The diabetic blood sample appeared to be the most viscous, while the participant with hypothyroidism showed the least viscous blood. The participant with low and high BAC levels showed similar viscosities between the two samples (12.55 and 12.86 cSt, respectively). Because the alcohol intake participant was the same individual for both tests, it is expected that the blood viscosity should not differentiate greatly. The participant had a BAC of 0.02 mg/L on the first blood draw and on the second draw, BAC increased to 0.11 mg/L. The reason for this, was to see how legal and illegal BAC levels (while operating a motor vehicle) could influence blood viscosity. In Figure 4, the viscosities of the alcohol intake participant (with low and high BACs) were similar, signifying that alcohol intake does not greatly alter blood viscosity in one individual. A polynomial trendline has been displayed on Figure 4 to show an increasing viscosity with each blood alteration. Upon testing blood samples for hematocrit levels (% RBC by volume), a figure was made to find the correlation between average blood viscosity and hematocrit (Fig. 5). Patients with hypothyroidism and those on

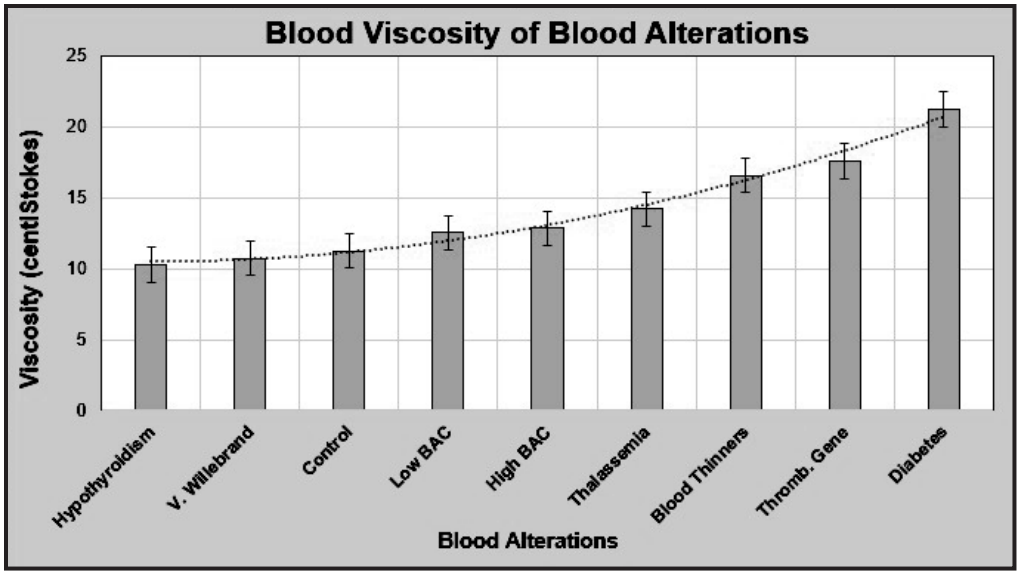



Figure 4. Varying blood viscosities among patients with altered blood. Viscosity ranges from 10.29 to 21.22 cSt. The estimated polynomial trendline shows increasing viscosity levels among the population of physiologically altered blood samples. The hypothyroid patient presents low blood viscosity, while the diabetic patient demonstrates high blood viscosity. Standard error was calculated to be ±1.25 cSt.

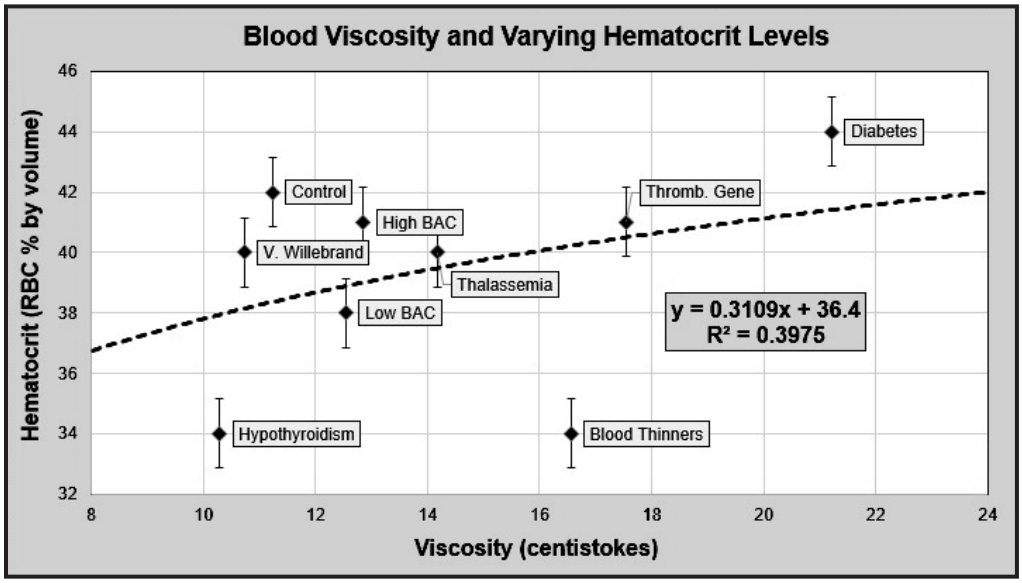



Figure 5. Logarithmic trendline ($R^2 = 0.3975$) shows that blood viscosity and hematocrit levels increase directly proportional to each other. The trendline includes all participants even though the hypothyroid and blood thinner participant appeared far from the trend. Standard error was found to be ± 1% of RBC by volume in this population. Most participants appear above the trendline, but the low alcohol intake patient appears slightly below.

regular blood thinners possessed the lowest hematocrit levels (34% RBC of blood volume). Alternatively, high hematocrit levels were found in the control and diabetic patients (42% and 44% of blood volume). Several of the hematocrit levels lie between 38 percent and 41 percent, and in most, a decreased RBC count was present. In Figure

5, a logarithmic trendline was created, showing that blood viscosity generally increases with increasing hematocrit levels ($R^2 = 0.3975$). In the participants with alterations that typically decrease red blood cell count, such as Thalassemia and Thrombin mutations, data appeared below the trendline. Interestingly, the low BAC participant also appeared below the trendline; the blood viscosity for this participant did not alter between alcohol consumptions, but hematocrit levels increased with alcohol intake. It is expected that alcohol consumption can reduce red blood cell count in heavy drinkers, but the participant was not known to consume alcohol regularly, and therefore red blood cell production may increase with alcohol consumption symptoms (increased heart rate and breathing). The remaining participants appeared slightly above or near the trendline. This provides forensic bloodstain analysts with an estimated trend among individuals with physiologically altered blood in which they can estimate BPA based on hematocrit and viscosity of the blood sample.

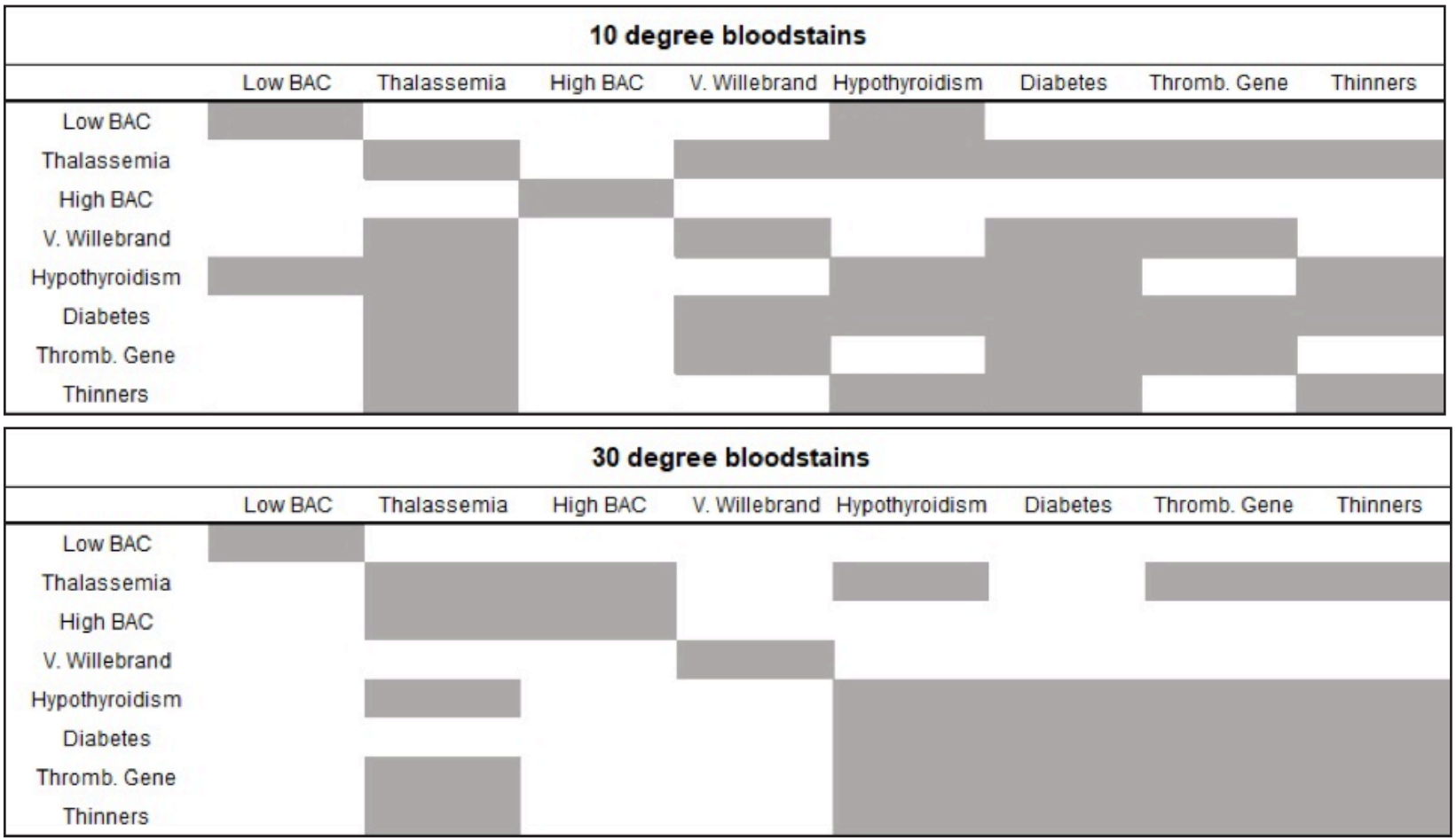
## Variability Among Blood Alteration Relationships

For each experimental angle, five stains were measured and averaged for each participant. These averages were then analyzed through ANOVA statistics for each tested angle. For the 10°, 30°, and 60° stains, participants were found to produce significantly different stain measurements ($p = 9 \times 10^{-14}$, $p = 9.92 \times 10^{-16}$, and $p = 0.0006$, respectively). In 90° stains, all participants produced similar bloodstains ($p = 0.8929$), and therefore could not be used for individualism. To determine which participant(s) cause variability among the sample, a Tukey HSD comparison test was completed. In this test, 28 relationships among the eight blood-altered participants were cross-examined (one vs. two, one vs. three, one vs. four, etc.). For each relationship, a p-value ($\alpha = 0.05$) was assigned to determine the significance between the two cross-examined participants. A Tukey Post Hoc chart was created, in which the grey boxes signify relationships that were significantly similar to each other and white areas represent those that were different (Table 1). In the 10° and 30° samples, over 50 percent of participant relationships were found to be different, which signifies individualism and can be used by BPA experts for reconstruction. In 60° stains, only three of the 28 relationships were significantly different from each other showing little individualism among this population in 60° stains. The 90° ANOVA showed that none of the samples were significantly different and all relationships in the Tukey HSD test were deemed similar. Most of the relationships showing 10° and 30° variability came from the relationships paired with the low and high BAC participant. The chart shows similarity in all relationships of the 90° stains, which is due to their spherical nature and less variability among the circular stains. Individualistic properties in bloodstains appear as the angle of impact becomes smaller; subjectivity increases as an analyst must distinguish between the stain body and tail in elongated stains. Spines, satellite drops, and individualistic formations of each bloodstain were analyzed, but no specific pattern was recognized, and therefore these features are not included in the data between blood alterations. Through this analysis, it can be concluded that smaller angles of impact appear to be more individualistic among a population with physiologically altered blood.

## Deviations From Expected Angles of Impact

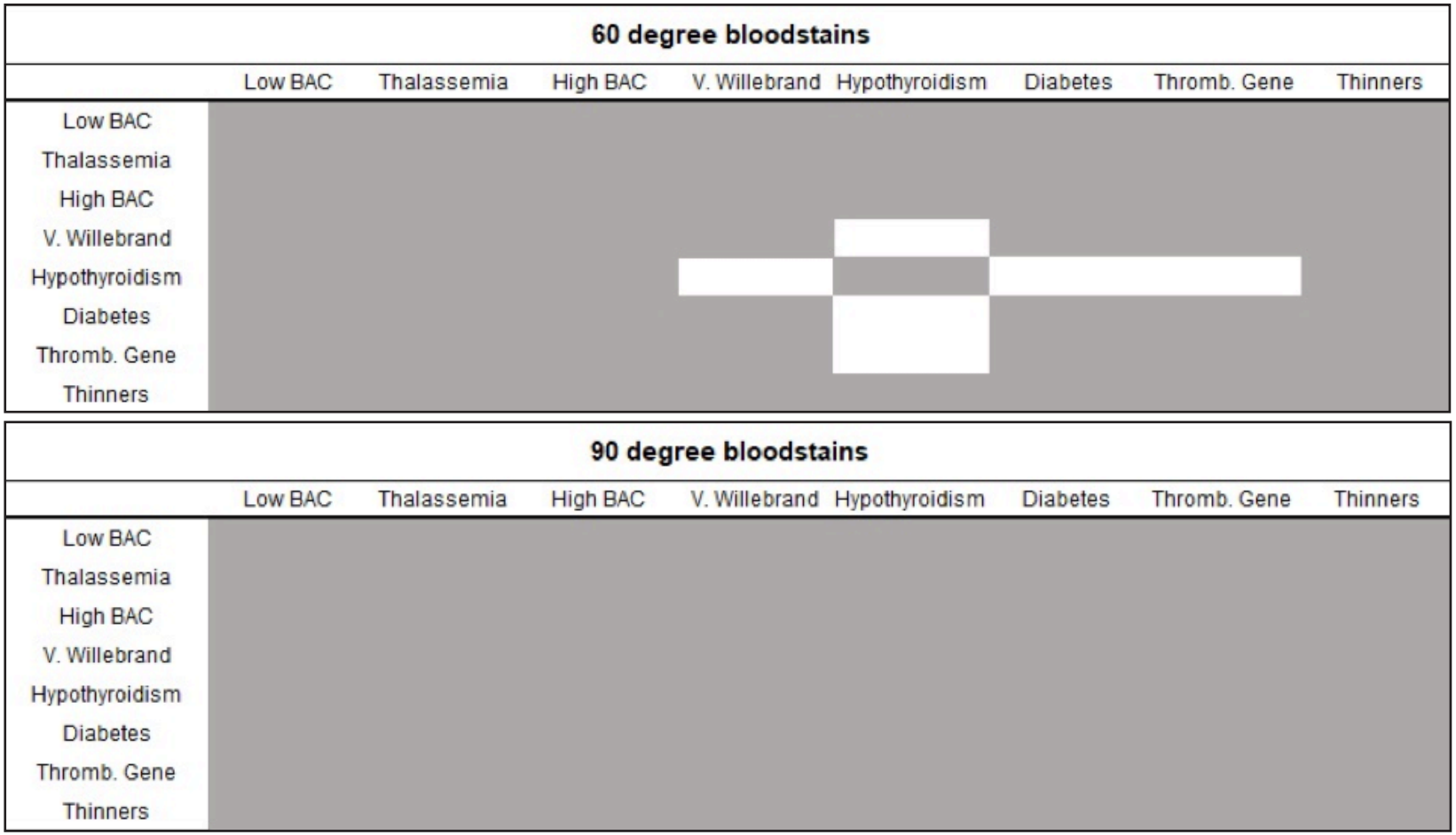
For each experimental angle, the average angle of impact was compared to the angle that each stain was dropped at to test for BPA accuracy among participant blood samples. For example, if a stain was formed by dropping the blood at a 10° angle, the experimental angle that is calculated upon measuring the stain should be significantly close to 10°. A one sample *t*-test ($\alpha$=0.05) was used to compare the average experimental angle of impact to each known expected angle. The control sample was removed from this analysis because

Table 1. Tukey Post Hoc plot showing significant differences existing between physiological blood alterations at measured angles ($10^0$, $30^0$, $60^0$ and $90^0$.)*




* Dark gray boxes indicate similarities between physiological blood alterations at four traditional BPA angles. Tukey Post Hoc analysis shows more significant differences between blood alterations as the angle of impact decreases or becomes more parallel to the surface it impacts.

Table 1. (continued)




* Dark gray boxes indicate similarities between physiological blood alterations at four traditional BPA angles. Tukey Post Hoc analysis shows more significant differences between blood alterations as the angle of impact decreases or becomes more parallel to the surface it impacts.

it was proven to be significantly similar to all expected angles. Using the remaining eight participants, a stacked bar graph was produced to display the significance between experimental and expected angles of impact for each participant (Fig. 6). The black bars signify experimental angles that were significantly different than expected angles, while the gray bars show when experimental and expected angles of impact were significantly similar. Figure 6 shows that, for each tested angle, over 50 percent of participants produced bloodstains that were significantly different from the angle that the stain was dropped. Interestingly, seven of the eight blood-altered participants deviated significantly from the 30° angle of impact. The hypothyroid and diabetic blood samples deviated significantly from almost every expected angle of impact, showing that BPA may be negatively influenced among these patients. Conversely, the low and high BAC bloodstains showed significant similarity to expected angles of impact, suggesting that BAC may not influence BPA. Therefore, the results among the eight tested individuals may be used to assist BPA or challenge its techniques.

## Discussion

BPA has historically been used as an efficient method for crime scene reconstruction. Various BPA methods have been introduced to the forensic science field, but few analyze the relationships between angles of impact and hemodynamic factors among physiologically altered blood. Theoretically, it was expected that patients with blood alterations may cause inaccuracies in BPA. Hence, in the present study, BPA was evaluated for accuracy when individual hematocrit levels and blood viscosity change between physiological alterations. According to the results presented above, hemodynamic factors have been shown to cause inaccuracy in BPA among the tested population. In ≥ 50 percent of the tested population, hemodynamics caused significant deviations from four angles of impact (Fig. 6). As provided in (Table 2), a one sample *t*-test demonstrates the cases where hemodynamics may play a

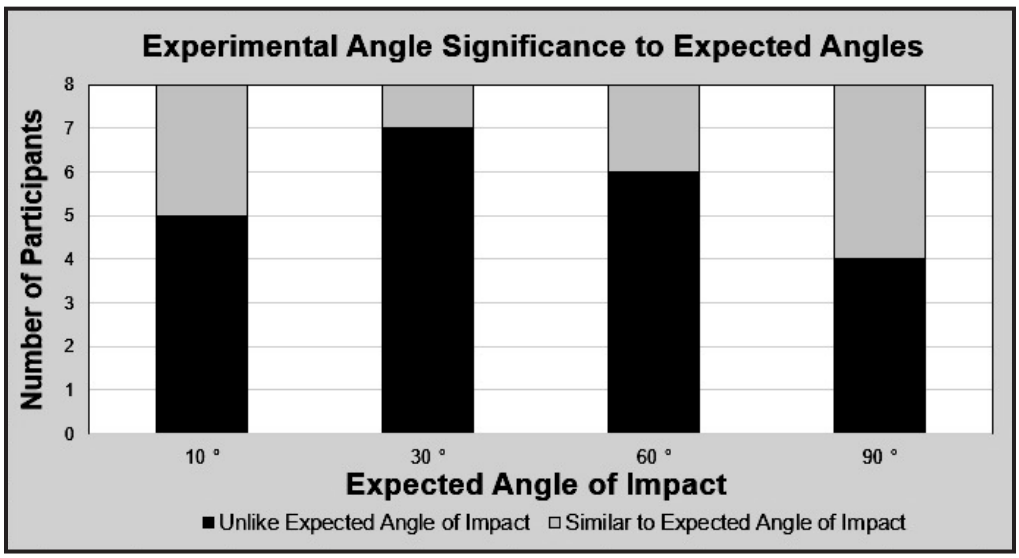



Figure 6. One sample t-test omitting the control sample and placed into a staked bar graph. Black bars show significant differences between the experimentally measured angles of impact and expected angles that blood samples were dropped. Gray bars signify when expected and experimental angles of impact were significantly similar to each other. In almost all expected angles, the diabetic and hypothyroid patient showed significant deviations from expected angles, showing individualism among these participants from the population.

Table 2. One sample t-Test for accuracy of experimental angles of impact among blood-altered participants.

| Participant | 10° *p*-value | 30° *p*-value | 60° *p*-value | 90° *p*-value |
|---|---|---|---|---|
| Low Alcohol Intake (BAC=0.02) | 0.0006 | 0.56 | 0.0285 | 0.0705 |
| High Alcohol Intake (BAC=0.11) | 0.0006 | 0.0131 | 0.219 | 0.0758 |
| Thalassemia | 0.8985 | 0.0002 | 0.0251 | 0.0192 |
| Diabetes (Type 1) | 0.7971 | 0.0001 | 0.0003 | 0.0193 |
| Hypothyroidism | 0.0106 | 0.0435 | 0.4753 | 0.0219 |
| Von Willebrand's | 0.1112 | 0.0001 | 0.0202 | 0.0194 |
| Prothrombin Gene Mutation | 0.0398 | 0.0001 | 0.0087 | 0.2001 |
| Regular Blood Thinners | 0.0395 | 0.0002 | 0.0357 | 0.0721 |

crucial role in BPA. Those with a significant *p*-value (<0.05) displayed inaccurate BPA and should be noted for future research. Individuals that produced erroneous bloodstain measurements may compromise area of origin measurements or the overall crime scene reconstruction.

Additionally, participant individualism was found to increase as angle of impact became more parallel to the surface that they strike. As seen in Table 1, the trend appears to follow previous research which shows inaccuracies in measuring stains with long tails. Consequently, BPA techniques and protocols must compensate for this observation. This is particularly important in forensic science because crime scene reconstruction relies heavily on BPA accuracy. If an analyst is aware that a suspect or victim may have a blood disorder/disease or has consumed alcohol, they can use this to more accurately measure bloodstains. Inaccurately measured bloodstains can three-dimensionally alter the area of origin measurement for all measured bloodstains and produce invalid crime scene reconstruction.

Findings of this study show that hemodynamic factors, such as viscosity and hematocrit levels have the potential to increase the accuracy of BPA. Therefore, it is recommended that the efficiency of this finding is displayed in larger populations of patients with altered-blood. All participants were female, and therefore this finding must be tested among a male population, where hematocrit levels may be higher. Samples should also be cross-examined among a smaller age range. In this survey, a wide variety of ages were tested, in which hematocrit levels could have been altered based on stage of development.

Calibrated BPA equipment should also be used for future research to provide stable conditions for blood depositing. Blood samples dropped for BPA should be of a similar volume and angles of impact should be accurately determined with power-driven mechanisms. Intravenous viscometers would additionally prevent blood from decreasing in temperature once outside of the body, contributing to the results found in this study without chilling all of the samples. Finally, viscometer standards should be closer to that of blood, to create an accurate standard curve. The results obtained in this study must be reinforced with additional testing to determine how the area of origin can be individualistically influenced by physiological blood disorders/diseases. Based on the findings of this study and by considering new BPA variables, it

is suggested that a standard BPA protocol/ technique, that considers hemodynamics, may be useful in forensic science and could potentially reinforce expert witnesses in a court of law.

## Conclusion

The results of this study emphasize that forensic analysts may have to consider hemodynamic factors, such as viscosity and hematocrit levels, when attempting crime scene reconstruction. Altogether, this survey has begun to produce a standard method for taking these factors into consideration, especially when bloodstains have originated from an individual with a known physiological blood alteration. This could be of interest for bloodstain pattern analysts in which BPA can be used more effectively by an expert witness and more importantly to individualize suspects and victims.

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