

## RDT&E IWG Bloodstain Pattern Analysis Questions

1. **What literature exists describing any correlation between the experience/training of an individual and an individual's performance on proficiency testing?**

No literature addressing this question has been found.

2. **What literature exists that provides a basis for reliably inferring directionality of a bloodstain pattern?**

**Carter, A. L. (2001). "The Directional Analysis of Bloodstain Patterns: Theory and Experimental Validation." Journal of the Canadian Society of Forensic Science 34(4): 173-189.**

Directional Analysis of bloodstain patterns is a mathematical procedure developed by the author that is based on the string method. It finds the directions in space (virtual strings) that point from the bloodstains to a spot directly above the location of the blood source. When viewed from above, the virtual strings are seen to converge onto the source position. When viewed from the side, the virtual strings provide an upper limit for the probable height of the blood source. The theory relies only on the well-known physical laws of motion, the resolution of a velocity into its three components and simple trigonometry, and does not depend on unknown quantities such as droplet sizes and droplet speeds. This procedure has a solid basis in physics and mathematics, and satisfies the criterion of sound scientific methodology for bloodstain evidence specified by the courts. Digital cameras are used for gathering the evidence and the analysis is carried out with a computer. The second part of this paper will describe a laboratory experiment that validates "Directional Analysis" both in theory and in practice.

**Gardner, R. M. (2002). "Directionality in Swipe Patterns." Journal of Forensic Identification 52(5): 579-593.**

The author indicates that swipe patterns are very common patterns found within crime scenes. The method for determining direction of motion for swipe patterns in bloodstain pattern analysis is outlined in numerous references. However these methods have not been previously studied or validated. This study identifies five physical characteristics that appear in swipe patterns and their orientation in relation to direction of motion. The study suggests that the presence of an irregular demarcation in conjunction with any of the other four characteristics in the opposite boundary is a valid indicator of direction of motion for the pattern.

**Gross, H. (1924). Traces of Blood. Criminal Investigation: A Practical textbook for Magistrates, Police Officers and Lawyers. H. G. J. C. Adam. London, Sweet & Maxwell Ltd. 374-402.**

This is a historical document in which the author (Hans Gross) writes about the importance of bloodstain letting at a scene. Gross indicates how directionality of blood droplets can be determined and explains simple observational experiments that provide understanding of the concept.

**Illes, M. B., A. L. Carter, et al. (2005). "Use of the BackTrack™ Computer Program for Bloodstain Pattern Analysis of Stains from Downward-Moving Drops." Journal of the Canadian Society of Forensic Science 38(4): 213-218.**

Using Directional Analysis, the BackTrack™ suite of computer programs can be used to analyze a crime scene in which bloodstains only from downward-moving drops are available. Only two of three Cartesian coordinates for the blood source location can be accurately determined, but this is still significantly better than the stringing and tangent methods, which cannot accommodate the stains from downward-moving drops without great difficulty. Crime scene investigators with access to this computer program should be aware of the program's ability to use data that cannot be used easily in other methods of analysis.

**Karger, B., R. Nusse, et al. (2002). "Backspatter on the Firearm and Hand in Experimental Close-Range Gunshots to the Head." The American Journal of Forensic Medicine and Pathology 23(3): 211-213.**

The researchers explain the forensic significance of backspatter which is defined as the "ejection of biological material from the entrance wound in a retrograde direction to the line of fire". This research is based on the reconstruction of back spatter events, providing

information on the directional deposition of biological material (blood) on nearby objects, the firearm, hand and arm.

**Karger, B., R. Nusse, et al. (1996). "Backspatter from Experimental Close-Range Shots to the Head I-Macrobackspatter." International Journal of Legal Medicine 109: 66-74.**

This paper explains the phenomenon of a back spatter event. The authors confirm - by observational research - that back spatter exists and has directionality. A blood source was shot and the results were documented on white sheets of paper.

**Karger, B., R. Nusse, et al. (1997). "Backspatter from Experimental Close-Range Shots to the Head II- Microbackspatter and the Morphology of Bloodstains." International Journal of Legal Medicine 110: 27-30.**

This paper explains the phenomenon of a back spatter event. The authors confirm - by observational research - that back spatter exists and has directionality. This research explores the morphology of bloodstains and the distribution of microstains. Microstains are reported to be stains with a diameter less than 0.5 mm.

**Knock, C. and M. Davison (2007). "Predicting the Position of the Source of Blood Stains for Angled Impacts." Journal of Forensic Sciences 52(5): 1044-1049.**

Droplets of pig's blood were dropped onto paper at different angles to the horizontal to produce blood stains. Impact velocities varied from 1.82 to 5.76 m/sec, drop size from 3.7 to 5.0 mm in diameter, and the surface sloped at angles between 22.7° and 90° to the horizontal. From the data a single equation relating stain size to drop size and velocity for all impact angles was produced;  $ab = 111.74 (Re^{1/2} We^{1/4})^{0.75} DoDo + 0.00084$  with  $R^2 = 0.88$ , where a is the stain width, b the stain length, Re the Reynolds number, and We the Weber number. A second equation related the number of spines, N, to drop size, velocity, and surface slope for all impact angles as  $N = 0.76 We^{0.5} \sin^3 h$  with  $R^2 = 0.9$ , where h is the impact angle. By combining these equations the impact velocity can be determined and hence the position of the stain's source.

**Laber, T. L., B. P. Epstein, et al. (2008). "High Speed Digital Video Analysis of Bloodstain Pattern Formation From Common Bloodletting Mechanisms." International Association of Bloodstain Pattern Analysts News 24 (2): 4-12.**

Working on the premise that an understanding of the dynamics of a bloodletting event is critical to the sound interpretation of the resultant bloodstain pattern, a set of over 500

individual high speed digital video clips of common bloodletting mechanisms has been assembled. These include single blood drop formation, impact spatter, gunshot spatter, expiration, cast-off, projected and contact blood staining. These video sequences were evaluated to better understand the basic dynamics of bloodletting events. This set of high speed video sequences is available for distribution to the forensic science community via the Midwest Forensics Resource Center (MFRC) website. The slow motion sequences provide evidence of the direction of motion of blood volumes and drops during pattern formation.

**MacDonell, H. L. and L. F. Bialousz (1971). "Flight Characteristics and Stain Patterns of Human Blood." Law Enforcement Assistance Administration, National Institute of Law Enforcement and Criminal Justice: 1-77.**

This treatise is one of the first modern day explorations into bloodstain pattern analysis. Mr. MacDonell believed that there was a deficiency in bloodstain research and available literature. With funding support from the National Institute of Law Enforcement and Criminal Justice he researched and reported on the basics of bloodstain pattern analysis. Several chapters were dedicated to impact pattern analysis which included target surface characteristics, impact angle considerations and spattered blood. This report was one of the first to correlate impact patterns, the force applied to the blood source and stain size within the pattern. Furthermore, MacDonell advises on the directionality of various blood stain patterns.

### **3. What is the literature on correction factors for gravity and air resistance when determining fluid's trajectory?**

There are hundreds of articles and text books on fluid dynamics. The following articles are a few that may help explain Stokes' Law.

**Hart, F. X. and C. A. Little lii (1976). "Student investigation of models for the drag force." American Journal of Physics 44(9): 872-878.**

An experiment is described that allows students to compare the predictions of several models for the drag force with observations made in the laboratory. The students can ~~determine~~ determine the ranges of validity of the Stokes' law and Newton's law expressions for the drag force as well as discover the good agreement with experiment obtained by the numerical solution of the exact equation of motion. The computer program devised for this purpose can be used to simulate the motion of falling objects in situations not directly covered in the laboratory.

**Luxford, G., D. W. Hammond, et al. (2005). Modelling, Imaging and Measurement of Distortion, Drag and Break-up of Aircraft-icing Droplets. 43 rd AIAA Aerospace Sciences Meeting and Exhibit Reno, Nevada, American Institute of Aeronautics and Astronautics 1-12.**

The distortion, drag and break-up of supercooled drizzle droplets in aircraft icing was investigated in ambient conditions. An effective efficient computer procedure was developed for the distortion and drag of small droplets, < 1mm, at low Reynolds numbers, < 1000, and high Weber numbers, > 10. High-speed videos and photographs were obtained with an improved high-intensity LED strobe. Experimental measurements validated the drag model for droplets distorted by a rapidly accelerating airflow in a convergent wind tunnel. To prevent droplet coalescence, due to wake interactions, droplets were generated with a steady jet impinging of a rotating slotted disk.

**Wierzbna, A. (1990). "Deformation and breakup of liquid drops in at nearly critical Weber numbers." Experiments in Fluids 9: 59-64.**

Experimentally determined values of the critical Weber number available from the literature are scattered over a very wide range of  $We_c$  from 2.2 to 99.6. To study one possible source of these discrepancies an experimental investigation was made of the deformation and breakup of water droplets at nearly critical Weber numbers. Experiments were conducted in a small horizontal wind tunnel. A continuous stream of uniform water droplets was allowed to fall perpendicularly to the continuous stream of air. The time histories of water droplets were

recorded by using a high speed camera. Five different basic behaviours of water droplets were recorded in the range of  $We = 11$  to  $14$ . It was found that an increase in the Weber number in this region resulted in an increased percentage of droplets with regular bag type breakup.

**4. What is the literature (from physics or other relevant sciences) that forms the basis for knowledge regarding creation of bloodstain patterns?**

There are hundreds of articles that are relevant here. A few are presented.

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**Pizzola, P. A., S. Roth, et al. (1986). "Blood Droplet Dynamics- II." Journal of Forensic Sciences 31(1): 50-64.**

An earlier companion paper to the present one dealt with a literature review as well as blood droplet formation and impacts to stationary target surfaces. The present paper discusses the results of experiments with moving target surfaces. These are discussed in the context of their correlation with blood droplet impacts to inclined stationary targets and with respect to the interpretation of bloodstain patterns at crime scenes where the target surface is capable of its own movement. A special belt device was designed and constructed for the experiments reported here. This motorized apparatus was used to drive paper belt target surfaces at various controlled speeds.

**Pizzola, P. A., S. Roth, et al. (1986). "Blood Droplet Dynamics-I." Journal of Forensic Sciences 31(1): 36-49.**

The interpretation of bloodstain patterns at crime scenes has received increased attention in recent years. Important to an understanding of this is knowledge of the fundamentals of blood droplet formation and impact dynamics. A review of the literature reveals that a considerable amount of work has been done with aqueous drop dynamics. Workers in the forensic science area seem to have been unaware of this. In addition, some of the most important and comprehensive early work with blood droplet dynamics seems to have been forgotten. It is not cited in more recent publications dealing with bloodstain pattern interpretation. This literature is reviewed and discussed as well. The present study presents results of experiments with blood

droplet dynamics and high-speed photographs of blood droplet impacts on stationary target surfaces. Some longstanding misconceptions of importance to forensic scientists engaged in crime scene reconstruction are discussed.

**Raymond, M., E. Smith, et al. (1996). "Oscillating Blood Droplets - Implications for Crime Scene Reconstruction." Science & Justice 36(3): 161-171.**

Traditionally, the analysis of blood spatter on surfaces in the reconstruction of crime scenes relies on the assumption that blood droplets are spherical when they strike the surface. This paper explores the effects of their shape on the reconstruction of trajectories from their impact pattern, and reports a theoretical analysis of the lifetime of droplet oscillations. Oscillations damp quickly in blood droplets due to the viscosity. The analysis provides ranges of velocities and distances from the point of droplet projection within which it is unreliable to assume the droplets are spherical when they stain a surface. Non-spherical droplet stains predict incorrect positioning of the droplet projection point. Experimental data are presented to show that the estimates apply in practice.

**Raymond, M. A., E. R. Smith, et al. (1996). "The Physical Properties of Blood - Forensic Considerations." Science & Justice 36(3): 153-160.**

This paper provides a comprehensive description of the usefulness of bloodstain pattern analysis at a crime scene. The authors describe possible scenarios where this expertise can be applied and the type of information that could be gathered to assist with the reconstruction of a crime scene. This includes information such as, the origin of a bloodstain, movement, type of pattern, minimum number of blows, order of deposition, damage to victim, deliberate removal of bloodstains and direction.

**Piotrowski, E. (1895). Ueber Entstehung, Form, Richtung und Ausbreitung der Blutspuren nach Hiebwunden des Kopfes. Elmira, NY (1992), Golos Printing.**

In his paper Piotrowski explains the results of his experiments about the origin, shape, direction and distribution of bloodstains after inflicting blunt force trauma. This is a historic scientific paper that contains conclusions on Dr. Piotrowski's experiments and hand drawn color diagrams of the results.

**Raymond, T. (1997). "Crime Scene Reconstruction from Bloodstains." Australian Journal of Forensic Sciences 29(2): 69-78.**

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**Stephens, B. G. and T. B. Allen (1983). "Back Spatter of Blood from Gunshot Wounds - Observations and Experimental Simulation." Journal of Forensic Sciences 28(2): 437-439.**

It is well known that gunshot wounding can produce fine droplets of blood spatter in a forward direction. Under certain circumstances blood droplets can also be propelled backwards in a direction against the line of fire. Although the phenomenon of back spatter of blood is most commonly seen in contact gunshot wounds of the head, its occurrence is not well recognized. In this article the authors summarize investigative and experimental observations concerning back spatter writing extensively on directionality.

**5. What literature exists describing the effects of viscosity differences on bloodstain pattern analysis conclusions?**

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damp quickly in blood droplets due to the viscosity. The analysis provides ranges of velocities and distances from the point of droplet projection within which it is unreliable to assume the droplets are spherical when they stain a surface. Non-spherical droplet stains predict incorrect positioning of the droplet projection point. Experimental data are presented to show that the estimates apply in practice.

**Raymond, M. A., E. R. Smith, et al. (1996). "The Physical Properties of Blood - Forensic Considerations." Science & Justice 36(3): 153-160.**

This paper presents a comparison of the physical parameters (surface tension, viscosity and density) of aging pig and fresh human blood, under a variety of conditions, as well as measurements of droplet trajectories and stain patterns. The results support the use of porcine blood (up to two weeks old) in representing the behavior of fresh human blood. The study concentrated on blood that was leaving the human body as the viscosity of blood can change once outside the human body. Therefore there are limitations as the blood begins to dry or clot.

**Thurston, G. B. (1972). "Viscoelasticity of human blood." Biophysical Journal 12.**

Measurements made for oscillatory flow of blood in circular tubes show that blood possesses elastic properties which make consideration of its viscous properties alone inadequate. Results are for a frequency of 10 Hz while varying the amplitude of the velocity gradient for red blood cells in plasma at concentrations ranging from 0 to 100% apparent hematocrit. For velocity gradients less than 1-2 sec both the viscous and elastic components of the shearing stress are linearly related to the gradient. For hematocrits above 20% the elastic component of the complex coefficient of viscosity increases with hematocrit approximately to the third power while the viscous component increases exponentially. Oscillatory flow measurements at very low hematocrits, when extrapolated to zero cell concentration, give the intrinsic viscosity of the average individual isolated red cell. The viscous part of this is found to be 1.7 which is compared with theoretical values from the rigid ellipsoid model for which the minimum possible value is 2.5. This difference is attributed to cell deformability. With increasing velocity gradient nonlinear properties develop. The viscous component of the complex viscosity becomes of the order of the steady flow viscosity at high gradients while the elastic component tends to decrease in inverse proportion to the gradient. Thus, the elastic component of the oscillatory stress tends to saturate, this tendency appearing at the approximate level of the yield stress.

6. What is the literature that demonstrates that certain situational or contextual factors can lead to specific “common” patterns of bloodstains and do these hold true across various surfaces and textures?

Hulse-Smith, L. and M. Illes (2007). "A Blind Trial Evaluation of a Crime Scene Methodology for Deducing Impact Velocity and Droplet Size from Circular Bloodstains." Journal of Forensic Sciences 52(1): 65-69.

In a previous study, mechanical engineering models were utilized to deduce impact velocity and droplet volume of circular bloodstains by measuring stain diameter and counting spines radiating from their outer edge. A blind trial study was subsequently undertaken to evaluate the accuracy of this technique, using an applied, crime scene methodology. Calculations from bloodstains produced on paper, drywall, and wood were used to derive surface-specific equations to predict 39 unknown mock crime scene bloodstains created over a range of impact velocities (2.2–5.7 m/sec) and droplet volumes (12–45  $\mu\text{L}$ ). Strong correlations were found between expected and observed results, with correlation coefficients ranging between 0.83 and 0.99. The 95% confidence limit associated with predictions of impact velocity and droplet volume was calculated for paper (0.28 m/sec, 1.7  $\mu\text{L}$ ), drywall (0.37 m/sec, 1.7  $\mu\text{L}$ ), and wood (0.65 m/sec, 5.2  $\mu\text{L}$ ).

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Laber, T. (1985). "Bloodspatter Classification." International Association of Bloodstain Pattern Analysts News 2(4): 44-55.

Laber provides an explanation on the issues surrounding classification of spatter patterns by assigning definitions by velocity. He provides an alternative method of identifying bloodstain patterns by stain size and other detail on classification of spatter patterns.

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Raymond's paper provides a comprehensive description of the usefulness of bloodstain pattern analysis at a crime scene. He describes possible scenarios where this expertise can be applied and the type of information that could be gathered to assist with the reconstruction of a crime scene. This includes information such as, the origin of a bloodstain, movement, type of pattern, minimum number of blows, order of deposition, damage to victim, deliberate removal of bloodstains and direction.

**Saviano, J., A. Allgood, et al. (2010). "Using multiple void patterns at crime scenes to estimate the area of origin in bloodstain cases." Journal of the Association for Crime Scene Reconstruction 16(3): 19-26.**

Void patterns in bloodstain pattern analysis are often used to determine the absence or movement of an intermediate object from its original position during or after a bloodletting event. This article examines the use of void patterns as a method of estimating the area of

origin of events that produce bloodstains. Employment of this method, which the authors have termed the Void Pattern Shadow Matching (VPSM) method, can help the analyst or investigator at the scene to quickly determine an approximate area of origin, which can later be confirmed by mathematical analysis.

**White, B. (1986). "Bloodstain Patterns on Fabrics: The Effect of Drop Volume, Dropping Height and Impact Angle." Journal of the Canadian Society of Forensic Science 19(1): 3-36.**

The authors attempted to determine the relevance of bloodstain patterns on clothing. Two different drop volumes were used to passively deposit blood drops on sixteen fabrics at five angles and from two different heights. The results indicated if it may in some cases be possible to conclude if a bloodstain is consistent with impact spatter or passively falling blood, the orientation of the fabric or clothing when blood was deposited and an indication of the dropping height. Calculations to determine the angle of impact on most fabrics are not reliable and should not be done. In casework applications, blood dropping experiments should be conducted on a piece of the fabric being examined when bloodstain pattern interpretation is requested.

#### **7. What literature exists describing the range of patterns possible with expired blood?**

**Clark, K. (2006). "Differentiating High Velocity Blood Spatter Patterns, Expired Bloodstains, and Insect Activity." International Association of Bloodstain Pattern Analysis News (September).**

This project examines a difficult area in bloodstain pattern interpretation involving three visually and chemically similar stains: high velocity bloodstains due to injury, expired bloodstains, and bloodstains caused by insect activity at the scene. Available literature was researched to determine whether each stain type had characteristics with sufficient uniqueness to allow differentiation. Articles and textbooks published in bloodstain pattern analysis and forensic entomology were utilized to determine the potential for individualizing these stains. The project was undertaken to collect relevant data and allow bloodstain pattern analysts confronted with stains consistent with high velocity to make the best possible classification, and further assist in crime scene reconstruction. By understanding the unique characteristics of each bloodstain pattern and the mechanism with which they are created, this project seeks to provide a focused collection of the research to date that has attempted to differentiate similar stains.

**Denison, D., A. Porter, et al. (2011). "Forensic implications of respiratory derived blood spatter distributions." Forensic Science International 204: 144-155.**

The nature of blood aerosols produced in physiological studies of an upright subject expiring small volumes through straws, spitting and mouthing sounds, and a semi-prone subject spitting through a bloody mouth or snorting through a single nasal orifice and by a simplified physical model of the respiratory system were investigated. Each manoeuvre produced many hundreds of droplets of a range of size, the vast majority being less than 1 mm diameter. Droplets under 1 mm dia. travelled over 1 m – much further than could be expected if their flight was ballistic, like that of impact spatter. Respirated blood aerosol properties are explained in terms of established mechanics of airflow shear induced aerosol production and the fluid mechanics of exhaled air movement.

**Donaldson, A.E., N.K. Walker, I.L. Lamont, S.J. Cordiner, and M.C. Taylor, Characterising the dynamics of expired bloodstain pattern formation using high-speed digital video imaging. Int J Legal Med 2010.**

In this study, high-speed digital video imaging has been used to investigate the formation of expired bloodstain patterns generated by breathing, spitting and coughing mechanisms. Bloodstain patterns from all three expiration mechanisms were dominated by the presence of stains less than 0.5 mm in diameter. Video analysis showed that in the process of coughing blood, high-velocity, very small blood droplets were ejected first. These were followed by lower velocity, larger droplets, strands and plumes of liquid held together in part by saliva. The video images showed the formation of bubble rings and beaded stains, traditional markers for classifying expired patterns. However, the expulsion mechanism, the distance travelled by the blood droplets, and the type of surface the blood was deposited on were all factors determining whether beaded stains were generated.

**Emes, A. (2001). "Expired Blood - A Review." Journal of the Canadian Society of Forensic Science 34(4): 197-203.**

This paper provides a brief review of the literature along with a summary of the properties of expired blood, the mechanisms of production, and the characteristics that may be seen in patterns. In 2001, the researcher found little published work on expired blood. However he indicates that there are specific characteristics, such as, dilution, blood mixed with saliva, mucus or lung surfactant and air bubbles. The patterns may vary significantly in appearance.

**Kettner, M., F. Ramsthaler, et al. (2010). ""Bubbles"-A spot diagnosis." Journal of Forensic Sciences 55(3): 842-844.**

Aspiration of blood is a phenomenon observed in violent and natural death scenarios. Bloodstain patterns evolving from expectoration of aspirated blood may look suspicious of a violent genesis and thus mislead crime scene investigators. In the present case, a woman was found lying in a pool of blood on the kitchen floor. Furthermore, bloodstains covered her face, clothing, and surrounding furniture and walls. Bloodstain pattern analysis and medicolegal inspection of the suspected scene of crime were carried out and revealed dispersed stains with enclosed gas bubbles in the absence of signs of physical violence leading to the assessment of a natural manner of death. The bloodstains were attributed to expiration of blood because of an internal bleeding. Medicolegal autopsy confirmed the on-site diagnosis as a fatal esophageal varix rupture was found.

#### **8. What literature exists that quantifies classification of bloodstain patterns?**

**Karger, B., Rand, S.P., & Brinkman, B. (1998). Experimental Bloodstains on Fabric from Contact and from Droplets. International Journal of Legal Medicine 111:17-21.**

The authors studied the appearance of similar-size bloodstains on three different types of fabrics (bed sheeting, cambric and terry cloth) produced by two different types of mechanisms: contact and dynamic or blood dropping. The volume of blood drops used ranged between 0.1  $\mu\text{l}$  and 10.0  $\mu\text{l}$ . The authors identify four common criteria found for a contact stain which reliably allow for classification in most cases (contact versus dynamic) of bloodstains in the studied size range. Differentiation between these mechanisms "may be difficult if the fabric has a rough and irregular surface structure." Also, as the stain size decreases, "the differences between contact and 'dynamic' stains" diminish, so the authors recommend experimentation and microscopic comparison of known and unknown bloodstains.

**Clark, K. (2006). Differentiating High Velocity Blood Spatter Patterns, Expirated Bloodstains, and Insect Activity. International Association of Bloodstain Pattern Analysts News 22 (3), 8-20.**

This article, a literature review of the three types of patterns included in the title, was condensed from a thesis in partial fulfillment for a Master of Forensic Science degree in 2006. "The project was undertaken to collect relevant data and allow bloodstain pattern analysts confronted with stains consistent with high velocity to make the best possible classification, and further assist in crime scene reconstruction." "This article [examines] the current literature

and research that has been conducted to attempt to differentiate these types of stains and what examinations may provide enough detail to correctly classify them.”

**Emes, A. (2001). Expired Blood – A Review. Journal of the Canadian Society of Forensic Science 34 (4): 197-203.**

“The basis of this article was prepared in response to a Quality Assurance trial in the UK, during which a number of participating laboratories incorrectly identified a bloodstain pattern that had been prepared by coughing blood.” Experiments were done to produce known expired bloodstains patterns demonstrating observed characteristics of expired blood. Emes offered a list of characteristics that could help in the identification of expired blood. The bloodstains under examination must be considered with other circumstances in the case including the nature of the surface with the stains and the reported injuries to the subjects involved.

**9. What is the literature on the relationship between scene or event bloodstain patterns and the accuracy of reconstruction attempts in the laboratory (reproducibility studies)?**

**Pex, J.O. and Vaughan, C.H. (1987). Observations of High Velocity Bloodspatter on Adjacent Objects. Journal of Forensic Sciences 32, No. 6, 1587-1594.**

This study was triggered by the presence of bloodstains on the sleeve cuff of a suspect. The authors’ objective was to determine if back spatter on material could be differentiated from blood transferred to a material through a light contact mechanism. They reported that microscopic examination of the surface receiving the back spatter or light transfer could provide the answer to the mechanistic question. They also stated, “[T]his information was found to be useful in reconstruction of events in suicides as well as possible homicides.” Also, “[A]lthough it (back spatter) may be found as far up the sleeve as the shoulder, it is usually limited to the cuff or lower sleeve area . . .”

**Pizzola, P.A., Roth, S., and De Forest, P.R. (1986). Blood Droplet Dynamics – II. Journal of Forensic Sciences 31, No. 1, 50-64.**

This study involved experiments involving moving target surfaces to examine any correlation with blood drops impacting “inclined stationary targets and with respect to the interpretation of bloodstain patterns at crime scenes where the target surface is capable of its own movement.” The production of bloodstains created at known angles of impact with an inclined target surface, a staple in basic bloodstain pattern courses, correlated with an effective angle of incidence reported in this study.

**Petricevic, S. & Elliot, D. (2005). Bloodstain Pattern Reconstruction – A Hammer Attack. Journal of the Canadian Society of Forensic Science 38 (1) 9-19.**

In this homicide case from New Zealand, the victim was struck “at least six times in the back of the head with a hammer-like implement.” The defense contended that the assailant’s “clothing would have been soaked in blood.” Research was designed and conducted to study the reproducibility of impact spatter being present on the assailant’s clothing for the reconstruction efforts. Three relative positions of victim and assailant were investigated. The experimental results showed that the assailant may have had “very little blood on their clothing” and a lack of blood “is not proof of non-involvement.”

**Karger, B., Rand, S.P., & Brinkman, B. (1998). Experimental Bloodstains on Fabric from Contact and from Droplets. International Journal of Legal Medicine 111:17-21.**

The authors studied the appearance of similar-size bloodstains on three different types of fabrics (bed sheeting, cambric and terry cloth) produced by two different types of mechanisms: contact and dynamic or blood dropping. The volume of blood drops used ranged between 0.1  $\mu\text{l}$  and 10.0  $\mu\text{l}$ . The authors identify four common criteria found for a contact stain which reliably allow for classification in most cases (contact versus dynamic) of bloodstains in the studied size range. Differentiation between these mechanisms “may be difficult if the fabric has a rough and irregular surface structure.” Also, as the stain size decreases, “the differences between contact and ‘dynamic’ stains” diminish, so the authors recommend experimentation and microscopic comparison of known and unknown bloodstains.

**Slemko, J.A. (2003). Bloodstains on Fabric: The Effects of Droplet Velocity and Fabric Composition. International Association of Bloodstain Pattern Analysts News 19 (4): 3-11.**

The author reports the results of experiments conducted on different types of fabrics, different fabric compositions, and condition (new, starched, fabric treatment) with respect to the nature of events or mechanisms (contact of liquid blood with the fabric surface with the blood source immediately above the fabric and different mechanisms to project blood onto the fabric) and the appearance of the resulting stains. Among the reported findings were that both the fabric texture and fabric’s ability to absorb blood may distort the appearance of the stain; washing affects the stain’s appearance, and fabric treatments need to be considered.

**White, B. (1986). Bloodstain Patterns on Fabrics: The Effect of Drop Volume, Dropping Height and Impact Angle. Journal of the Canadian Society of Forensic Science 19 (1), 3-36.**

“The information in this paper has identified some of the limiting factors in the interpretation of bloodstains on clothing or fabric items.” “The information gained from [the] experiments may determine whether an opinion on such aspects as the mechanism of production of the bloodstains, the position of the fabric when the blood was deposited and the dropping height can be expressed.” The author studied a wide variety of fabric types and compositions. “The data indicate that calculations of angles of impact on most fabric surfaces examined are not reliable and should not be reported.”

**Gardner, R.M. (2006). Defining the Diameter of the Smallest Parent Stain Produced by a Drip. Journal of Forensic Identification 56 (2), 210-221.**

The parameters of this study were three different objects (knife tip, plastic fingernail tip, and hypodermic needle) and four surfaces (linoleum, jeans, tile, and carpet) to determine if a minimum diameter stain could be determined for passive drop formation. The author concluded that “[E]xcluding carpet surfaces, gravity-induced stains (drips) were always greater than 4.0 mm. Including the carpet target, the smallest stain was always greater than 3.0 mm.”

**Benecke, M., & Barksdale, L. (2003). Distinction of Bloodstain Patterns from Fly Artifacts. Forensic Science International 137: 152-159.**

Insect activity at scenes may produce small stains, often referred to as fly specks or fly spots, which may introduce confusion into the bloodstain pattern analysis. A laboratory study was conducted to examine the stains produced by adult blow flies on vertical paper surfaces. The resulting shapes and sizes in conjunction with scene context provided information to help differentiate the fly specks from bloodstains at three different scenes (one in the United States and two in Germany). Suggestions by the authors to help differentiate stains resulting from insect activity and bloodstains related to blood-letting events included documenting the presence of flies; noting the locations of small stains with proximity to light sources; documenting windows and rooms without a body or blood source; and noting the presence or absence of bloodstain pattern characteristics.

**Fujikawa, A., Barksdale, L., & Carter, D.O. (2009). *Calliphora vicina* (Diptera: Calliphoridae) and Their Ability to Alter the Morphology and Presumptive Chemistry of Bloodstain Patterns. Journal of Forensic Identification 59 (5) 502-512.**

For this study, wooden boxes with two glass walls and Plexiglas ceilings were built to observe and photograph the activities of flies with respect to their activities involving bloodstains. “Experiments were conducted with six contrasting surfaces: painted wall, paneling, wallpaper, wood laminate, linoleum, and carpet.” Each experiment was replicated four times. Impact spatter and small pools were created using human blood. Flies fed from the edges of the pools and the centers of the impact stains. “Feeding activity [of European blue bottle flies] altered the shape of many stains, whereas other stains were completely eliminated. Regurgitation and defecation [of blood] resulted in the deposition of multiple new artifacts on all surfaces.” “Defecatory artifacts were easily identified by their tailed shape and fluorescence under blue-green light (465 nm) . . .” Presumptive tests for blood did not differentiate the impact spatter from the fly specks.

**Striman, B., Fujikawa, A., et al. (2011). Alteration of Expired Bloodstain Patterns by *Calliphora vicina* and *Lucilia sericata* (Diptera: Calliphoridae) Through Ingestion and Deposition of Artifacts. Journal of Forensic Sciences 56 (S1), S123-S127.**

This study appears to be a continuation of the aforementioned study by Fujikawa, Barksdale, & Carter (2009) in the *Journal of Forensic Identification*, 59 (5) 502-512. Expired blood instead of impact spatter was projected into the boxes. “[T]wo controlled laboratory experiments were conducted using various common household wall and floor coverings and two species of flies commonly found at scenes of crime.” “Not previously observed was that *C. vicina* constructed artifacts via translocation of expired bloodstains.” The authors report that “there are no firm rules [See Benecke, M., & Barksdale, L. (2003). Distinction of Bloodstain Patterns from Fly Artifacts. *Forensic Science International*, 137: 152-159.] that can be used to differentiate artifacts from bloodstain patterns, and it is important not to include or dismiss a stain simply because it does not follow dogma. The context of the bloodstain pattern and crime scene must be used in analysis.”

**Denison, D., Porter, A., Mills, M. & Schroter, R.C. (2011). Forensic Implications of Respiratory Derived Blood Spatter Distributions. Forensic Science International 204: 144-155.**

Multiple experiments were conducted to study the “blood aerosols” produced by a variety of events with a subject being both upright and semi-prone. The resulting bloodstains, numbering in the hundreds, were counted. “Respired blood aerosol properties are explained in terms of

established mechanics of airflow shear induced aerosol production and the fluid mechanics of exhaled air movement.” “The flight characteristics of aerosols such as respired blood, entrained in the airflow by which they are generated, are fundamentally different from those whose ballistic flight is caused by impact or cast off.” “The droplet flight paths cannot be predicted in the same way as droplets – such as impact spatter – ejected into still air.”

**Emes, A. (2001). Expired Blood – A Review. Journal of the Canadian Society of Forensic Science 34 (4): 197-203.**

“The basis of this article was prepared in response to a Quality Assurance trial in the UK, during which a number of participating laboratories incorrectly identified a bloodstain pattern that had been prepared by coughing blood.” Experiments were done to produce known expired bloodstain patterns demonstrating observed characteristics of expired blood. Emes offered a list of characteristics that could help in the identification of expired blood. The bloodstains under examination must be considered with other circumstances in the case including the nature of the surface with the stains and the reported injuries to the subjects involved.

**Karger, B., Nüsse, R., & Bajanowski, T. (2002). Backspatter on the Firearm and Hand in Experimental Close-Range Gunshots to the Head. The American Journal of Forensic Medicine and Pathology 23 (3), 211-213.**

This backspatter study involved the use of a 9mm pistol and two kinds of ammunition to kill nine calves, destined for slaughter, with shooting distances from 0 cm to 10 cm. The firearm, surgical gloves and sleeve of the shooter were examined. Backspatter was found on the firearm in five of the shots with the number of circular to elongated “droplets” ranging from 2 to 52. In four of these five instances, a “fine spray of tiny blood droplets was additionally present.” Between 1 and 12 droplets were observed in the six instances where droplets were found on either or both of the gloves worn at the time of the shooting. In three of these shootings, the spray of blood was also reported. Blood was found on the sleeve of the shooting hand in four of the cases, with the number of droplets between 1 and 24, with the spray of blood in all four instances. Most of these “droplets” were 1 mm to 3 mm in size. The authors conclude that “[t]he number and size of the stains can be very small.” Two of the references cited were published in 1996 and 1997 by some of the authors.

**Hulse-Smith, L. & Illes, M. (2007). A Blind Trial Evaluation of a Crime Scene Methodology for Deducing Impact Velocity and Droplet Size from Circular Bloodstains. Journal of Forensic Sciences 52 (1), 65-69.**

The authors derived surface-specific equations for impact velocities (2.2-5.7 m/sec) and droplet volumes (12-45 $\mu$ L) for bloodstains on paper, drywall and wood based on stain diameter and the number of radiating spines. "This protocol was then subjected to a blind trial evaluation under mock crime scene conditions." "Strong correlations were found between expected and observed results, with correlation coefficients ranging between 0.83 and 0.99."

**Illes, M. & Boue, M. (2011). Investigation of a Model for Stain Selection in Bloodstain Pattern Analysis. Journal of the Canadian Society of Forensic Science 44 (1), 1-12.**

The authors present selection criteria for bloodstains to be used to determine an area of origin for an impact pattern. The statistical model used for the selection criteria were based on impact patterns on vertical surfaces created in the laboratory. They report "zone locations" of 30° from which impact stains may be selected for measurement and improving the accuracy of the area of origin estimation. The results of a validation study are included. While the true horizontal distance from a blood source to the vertical surface is not a known on a scene, "it is included in the model to account for the increase in error as the distance travelled by the blood droplets increases."

**Carter, A.L. (2001). The Directional Analysis of Bloodstain Patterns Theory and Experimental Validation. Journal of the Canadian Society of Forensic Science 34 (4), 173-189.**

"Directional Analysis of bloodstain patterns is a mathematical procedure developed by the author based on the string method." It allows the bloodstain pattern analyst with this software to determine the "virtual strings" that denote the convergences of the flight paths of the bloodstains used to a spot directly above the blood source location. The side view shows an upper limit for the blood source's probable height. Experimental values resulting from the computation of the area of origin are in agreement with the known origin.

**Illes, M.B., Carter, A.L., et al. (2005). Use of the BackTrack™ Computer Program for Bloodstain Pattern Analysis of Stains from Downward-Moving Drops. Journal of the Canadian Society of Forensic Science 38 (4), 213-218.**

While bloodstain pattern analysts are taught to use stains created by upward-moving blood drops to determine an area of origin for bloodstains on vertical surfaces, this study reports how

stains created by downward-moving drops may be used. The two-dimensional or horizontal components of the area of the blood source can still be estimated.

**Knock, C. & Davison, M. (2007). Predicting the Position of the Source of Blood Stains for Angled Impacts. Journal of Forensic Sciences 52 (5), 1044-1049.**

“The experimental work presented in this paper shows that it is possible to use a single equation to take into account the impact angle when calculating a blood stain’s size and number of spines.” Other data used included the stain’s width and length, the Reynolds number, and the Weber number. The parameters in this study were known impact angles from 22.7° to 90°, drop sizes between 3.7 and 5.0 mm in diameter, and impact velocities between 1.82 and 5.76 m/sec.

**Maloney, A., Nicloux, C., et al. (2011). One Sided Impact Spatter and Area-of-Origin Calculations. Journal of Forensic Identification 61 (2), 123-135.**

Bloodstain pattern analysts are taught to use the full range of bloodstains (180° area above the horizontal plane) for the determination of areas of origin. “This study demonstrates that at least some incomplete impact patterns – “one-sided” patterns – need not be eliminated from the analysis of the scene because they can still provide an acceptable calculation of the area of origin. This study used HemoSpat software for their calculations.

**10. What literature exists that describes computer simulations involved in bloodstain pattern modeling experiments?**

No literature addressing this question has been found.

**11. What literature exists regarding the potential for cognitive bias in bloodstain pattern analysis including but not limited to confirmatory hypothesis testing (i.e., testing only the hypotheses that confirm one’s theory of the event) and the effects of “blinding” procedures on examiner conclusions?**

No literature addressing this question has been found.

**12. What is the literature that describes research performed to determine the minimum number or threshold of stains required to be present to determine the presence of back spatter?**

The literature contained references reporting either quantitatively the number of backspatter stains found or qualitatively reported ~~quantitatively~~ their presence. None of the literature reviewed offered a minimum number or threshold of stains required for determining the presence of back spatter.

**Pex, J.O. and Vaughan, C.H. (1987). Observations of High Velocity Bloodspatter on Adjacent Objects. Journal of Forensic Sciences 32, No. 6, 1587-1594.**

This study was triggered by bloodstains on the sleeve cuff of a suspect. The authors' objective was to determine if back spatter on material could be differentiated from blood transferred to a material through a light contact mechanism. They reported that microscopic examination of the surface receiving the back spatter or light transfer could provide the answer to the mechanistic question. They also stated, ". . . because of the elastic nature of the skin, they (*back spatter bloodstains*) may fall from most areas of the shooting hand within minutes." Also, "[T]he observed pattern (*backspatter*) must not be discounted based on a limited number of droplets."

**Betz, P., Peschel, O., Stiefel, D., Eisenmenger, W. (1995). Frequency of blood spatters on the shooting hand and of conjunctival petechiae following suicidal gunshot wounds to the head. Forensic Science International 76 (1995) 47-53.**

The authors studied 103 suicidal gunshot fatalities and 29 homicidal gunshot fatalities breaking them down into categories of type and caliber of firearm. They qualitatively reported the percentage of times back spatter was found but did not quantify the data in terms of the amount of back spatter bloodstains found. [*Their references 1, 3, 11, and 12 were cited for the presence of tissue particles or blood spatters on or in the barrel of the weapon as well as on the hands of the deceased.*]

**Stephens, B.G., and Allen, T.B. (1983). Back Spatter of Blood from Gunshot Wounds – Observations and Experimental Simulation. Journal of Forensic Sciences 28, No. 2, 437-439.**

The authors, both medical doctors working in the office of the San Francisco Medical Examiners-Coroner, conducted the study to support the presence of back spatter. Up to that time, there was little in the literature on the topic and some experts had claimed that back

spatter did not exist. The authors reported qualitative results supporting the existence of back spatter but did not report results quantitatively (amount of bloodstains).

**Burnett, B.R. (1991). Detection of Bone and Bone-Plus-Bullet Particles in Backspatter from Close-Range Shots to Heads. Journal of Forensic Science 36 (6), 1745-1752.**

The author conducted experiments involving the shooting of seven live pigs destined for slaughter. He suggests the inclusion of scanning electron microscopy (SEM) coupled with energy-dispersive X-ray analysis to identify the elements phosphorus and calcium which were present in bone fragments as components of backspatter in addition to blood.

**Karger, B., Nüsse, R., & Bajanowski, T. (2002). Backspatter on the Firearm and Hand in Experimental Close-Range Gunshots to the Head. The American Journal of Forensic Medicine and Pathology 23 (3), 211-213.**

This backspatter study involved the use of a 9mm pistol and two kinds of ammunition to kill nine calves, destined for slaughter, with shooting distances from 0 cm to 10 cm. The firearm, surgical gloves and sleeve of the shooter were examined. Backspatter was found on the firearm in five of the shots with the number of circular to elongated “droplets” ranging from 2 to 52. In four of these five instances, a “fine spray of tiny blood droplets was additionally present.” Between 1 and 12 droplets were observed in the six instances where droplets were found on either or both of the gloves worn at the time of the shooting. In three of these shootings, the spray of blood was also reported. Blood was found on the sleeve of the shooting hand in four of the cases, with the number of droplets between 1 and 24, with the spray of blood in all four instances. Most of these “droplets” were 1 mm to 3 mm in size. The authors conclude that “[t]he number and size of the stains can be very small.” Two of the references cited were published in 1996 and 1997 by some of the authors. [See below.]

**Karger, B., Nüsse, R., et al. (1996). Backspatter from Experimental Close-range Shots to the Head I. Macrobackspatter. International Journal of Legal Medicine 109: 66-74.**

This article appears to be associated with the preceding article published in 2002. This one reported the results of examining bloodstains present “on white paper placed horizontally 60 cm below the [shooting] impact site. In this report the analysis was restricted to stains with a diameter > 0.5 mm.” “The number of stains varied from 31-324 per gunshot.” “The maximum distance droplets travelled varied from 72-119 cm.”

**Karger, B., Nüsse, R., et al. (1997). Backspatter from Experimental Close-range Shots to the Head II. Microbackspatter and the Morphology of Bloodstains. International Journal of Legal Medicine 110: 27-30.**

This article appears to be associated with the preceding article published in 2002. This one reported the results of examining bloodstains present “on white paper placed horizontally 60 cm below the [shooting] impact site. The morphology of bloodstains and the distribution of microstains (diameter < 0.5 mm) is reported.” “The number of microbackspatter stains per gunshot varied between 39 and 262 and the maximum travelling distance was 69 cm.”

**13. What is the literature that describes the basis for measurement error in directionality conclusions?**

Directionality for bloodstain pattern analysis falls into at least two categories: the direction of travel of blood drops impacting a surface and the examination of the resulting bloodstain to ascertain the direction of travel of the drop; second is the direction of motion for events such as contacts involving wipes and swipes. No literature addressing this question was found. Perhaps reviewing the results of proficiency testing such as that provided by Collaborative Testing Services might provide insight.

**14. What literature exists regarding the uncertainty of measurement associated with ground truth stains (i.e., create elliptic stains for 30° to 60° impact angles stepwise 0.5° at a time and determine when one can differentiate the stains)?**

**Laturnus, P. (1994), “Measurement Survey”. International Association of Bloodstain Pattern Analysts News 10(3): p. 14-32.**

The author surveyed 27 IABPA members to determine the accuracy of measuring length and widths of 10 individual bloodstains for the purposes of estimating angles of impact. He showed that many analysts were overestimating angles of impact by overestimating the length of the stain. This was particularly a problem for acute angled stains that had tails.

**Willis, C., A. K. Piranian, J. R. Donaggio, R. J. Barnett and W. F. Rowe (2001). "Errors in the Estimation of the Distance of Fall and Angles of Impact Blood Drops." Forensic Science International 123(1): 1-4.**

This paper presents derived equations for the estimation of the variances in the estimated distance of fall and the estimated angle of impact for blood droplets. The derived equation for

the variance of the estimated distance of fall predicts that as the blood drop size approaches that of a blood droplet falling at terminal velocity, the variance of the estimate grows without limit. The derived equation for the variance in the estimated angle of impact shows that as the angle of impact approaches 90°, the variance grows without limit. The validity of the equation for the estimated variance of the angle of impact was tested for five angles of impact (15, 30, 45, 60 and 75°); the equation correctly predicted the observed variance up to an angle of 60°.

**Pace, A. (2005). "The Relationship between Errors in Ellipse Fitting and the Increasing Degree of Error in Angle of Impact Calculations." International Association of Bloodstain Pattern Analysts News 21(3): 12-14.**

This paper considered the error in angle of impact calculation which is rendered by a 1 millimeter error in the measurement of bloodstain lengths. This error was practically negligible up to impact angle of around 50°, thereafter it increased rapidly. Similar calculations made with other bloodstain width/length ratios will yield different degrees of error, however the general trend will be the same – the greater the impact angle of a bloodstain, the greater will be the result of a measurement error.

**Reynolds, M., D. Franklin, M. A. Raymond and I. Dadour (2008). "Bloodstain measurement using computer-fitted theoretical ellipses: A study in accuracy and precision." Journal of Forensic Identification 58(4): 469-484.**

This paper describes an accuracy and precision study conducted on two methods of computer-assisted ellipse fitting (BackTrack™ Images and the newly developed Microsoft Office Excel 2003 AutoShapes) for bloodstain measurement purposes. The study, conducted in known and blind environments, demonstrated both methods of computer-assisted ellipse fitting to be robust and reliable bloodstain measurement techniques, particularly for those stains that have obliquely impacted a planar surface.

**15. What is the literature that demonstrates the reliability (intra- and inter-examiner) of pattern classification? What is the literature that demonstrates the reliability (intra- and inter-examiner) of determining the cause of a "void"?**

No literature addressing this question has been found.

**16. What is the literature regarding the establishment of error rates associated with conclusions offered by bloodstain pattern analysts?**

**Liesegang, J. (2004). "Bloodstain Pattern Analysis - Blood Source Location." Journal of the Canadian Society of Forensic Science 37(4): 215-222.**

This paper focuses on the useful, well-known paper by Carter and Podworny which describes a procedure for calculating the horizontal planar position (taken as the X-Y plane) or plan position of the source as a point (X, Y), these being the plan co-ordinates of the vertical line of intersection of the vertical trajectory planes associated with two droplet stains. Elementary calculus is applied to equivalent expressions to those of Carter and Podworny for X and Y, allowing in a well-defined way, the calculation of quotable uncertainties or errors in X and Y; i.e., the positive and negative values of which specify the precision limits of the calculated X and Y values. To illustrate the way in which these uncertainties may vary with distance and with angular position with respect to selected stains, a visualization of this precision is also presented.

**Carter, A. L., et al. (2005). "Further Validation of the BackTrack™ Computer Program for Bloodstain Pattern Analysis - Precision and Accuracy." International Association of Bloodstain Pattern Analysts News 21(3): 15-22.**

In this work, 18 bloodstain targets were created, and then analyzed independently by a number of trained bloodstain pattern analysts (N = 8-11) using the BackTrack™ programs. The results indicate high precision, demonstrating that a number of investigators, analyzing the same scene, will arrive at approximately the same result with the program. The standard deviations for the X-, Y-, and Z-values for all 18 patterns were very small, ranging from 0.46 to 7.02 cm, with the majority (44/54) being less than 3 cm.

**Carter, A. L., J. Forsythe-Erman, V. Hawkes, M. Illes, P. Laternus, G. Lefebvre, C. Stewart and B. Yamashita (2006). "Validation of the BackTrack™ Suite of Programs for Bloodstain Pattern Analysis." Journal of Forensic Identification 56(2): 242-254.**

122 impact spatter patterns were analyzed by students on various bloodstain pattern courses using the BackTrack™ software to determine blood source location. The average difference from the true source location was 4.23 cm, 3.15 cm and 6.25 cm for X, Y, Z coordinates respectively.

**Rowe, W. F. (2006). "Errors in the Determination of the Point of Origin of Bloodstains." Forensic Science International 161(1): 47-51.**

This paper extends the derivations in the Liesegang study work by expressing the uncertainties in the coordinates of the point of origin of two bloodstains in terms of the uncertainties in the length and width measurements from which the angles of impact of the bloodstains are calculated.

**Reynolds, M. E., M. A. Raymond and I. Dadour (2009). "The use of small bloodstains in blood source area of origin determinations." Journal of the Canadian Society of Forensic Science 42(2): 133-146.**

Due to the increased likelihood of manual measurement error, small bloodstains ( $\leq 3.0\text{mm}$  long) have rarely been used in three-dimensional blood source area of origin determinations. The advent of computer assisted measurement methods, offering improved levels of accuracy and precision, broadens the range of bloodstain sizes available for selection to determine a blood source area of origin. With inertia, viscosity and surface tension playing important, yet competing, roles in bloodstain formation, the power law relationships that exist between droplet volumes, droplet diameter, and bloodstain width suggest possible non-agreement between experimentally calculated angle of impact values when compared against those values theoretically expected. In order to compare experimental angle of impact calculation trends with angle of impact calculation theory, this study examined small bloodstains created by blood drops falling vertically onto surfaces offset from the vertical and small bloodstains generated by impact events subsequently deposited on adjacent vertical surfaces. This study shows that an apparent power law relationship may exist between the size of a parent blood droplet and the role of viscous and surface tension forces on subsequent bloodstain formation, particularly on those bloodstains caused by small droplets impacting obliquely with planar surfaces.

**17. What is the literature that describes any divergence of interpretations offered by bloodstain pattern analysts?**

No literature addressing this question has been found.

**18. What is the literature describing any research focused on developing techniques for aging bloodstains or developing bloodstains on dark surfaces?**

**18.1 Aging of blood**

**Funao T., Maeda I (1959) Studies on the determination of the age of bloodstains. Jpn J Leg Med 13: 735–740.**

no abstract available

**Kind, S. S., D. Patterson and G. W. Owen (1972). "Estimation of the age of dried blood stains by a spectrophotometric method." Forensic Science 1(1): 27-54.**

The visible absorption spectra of dried blood samples were examined and a time and temperature dependent quantity called the "a ratio" derived. This parameter is independent of the amount of blood present and its determination can provide useful assistance in estimating the age of a bloodstain.

**Kind, S. S. and M. Watson (1973). "The estimation of blood stain age from the spectrophotometric properties of ammoniacal blood stain extracts." Forensic Science 2(0): 325-332.**

The visible absorption spectra of a series of blood stains up to 15 years old were examined and found to vary in a regular manner with age. A quantity called the " $\alpha_s$  ratio" has been derived and calculated for all bloodstains examined. It was suggested that this parameter can give a useful indication of blood stain age up to periods of 10–15 years after stain formation.

**Rajamannar, K. (1977). "Determination of the age of bloodstains using immunoelectrophoresis." Journal of Forensic Sciences 22(1): 159-164.**

The technique of immunoelectrophoresis was used to determine the age of bloodstains. The immunoelectrophoretic patterns (IEP) of bloodstains ranging from 15 days to one year old were obtained by the use of high titer anti-whole human serum. The IEPs revealed gradual disappearance of beta-globulins and gamma-globulin with increase in the age of bloodstains. A comparative study of the IEP of normal human serum with those of the experimental bloodstains showed the absence of some of the corresponding proteins. The absence of a particular serum protein in the IEP of a given bloodstain will indicate the age of that bloodstain.

**Sakurai, H., K. Tsuchiya, Y. Fujita and K. Okada (1989). "Dating of human blood by electron spin resonance spectroscopy." Naturwissenschaften 76(1): 24-25.**

no abstract available

**Inoue, H., F. Takabe, M. Iwasa and Y. Maeno (1991). "Identification of fetal hemoglobin and simultaneous estimation of bloodstain age by high-performance liquid chromatography." International Journal of Legal Medicine 104(3): 127-131.**

A method using reverse-phase high-performance liquid chromatography (HPLC) for the identification of fetal hemoglobin (Hb F) and the simultaneous estimation of bloodstain age is described. Umbilical cord and neonatal bloodstains can be differentiated from adult stains by the presence of  $\alpha$ -globin chains which are characteristic of Hb F. With this method, cord and neonatal blood could be distinguished from adult blood in stains up to 32 weeks old. The age of the stain was estimated from the ratio of the peak area of the  $\alpha$ -globin chain to that of heme on the same chromatogram. The ratio decreased gradually with an increase in the age of the stain up to 20 weeks old. Studies performed at each time period revealed no significant difference in the ratios of cord and neonatal bloodstains or in the ratios of cord and adult stains. The regression equation calculated from the ratios (  $y$  ) and the ages of stains in weeks (  $x$  ) expressed logarithmically is  $y = 2.5758 - 0.2497 \ln(x)$  and the coefficient of correlation is - 0.7491 (  $n = 252$ ,  $P < 0.001$ ).

**Inoue, H., F. Takabe, M. Iwasa, Y. Maeno and Y. Seko (1992). "A new marker for estimation of bloodstain age by high performance liquid chromatography." Forensic Science International 57(1): 17-27**

The applicability of a marker for estimation of bloodstain age by reverse-phase high performance liquid chromatography (HPLC) is described. Using a  $\mu$ Bondasphere C18 column with a two step linear gradient of 10.5–46.25% acetonitrile in 0.1% trifluoroacetic acid, an intriguing peak (unidentified) at a retention time of about 5 min was observed on chromatograms from human adult bloodstains and designated as 'X'. The area of this peak, which could be detected in extracts of bloodstains, but not in their fresh whole blood, increased with time. The ratios of the X area to heme area in bloodstains stored at room temperature and 4°C for up to 52 weeks old linearly correlated with stain age by plotting on a double logarithmic scale. In bloodstains exposed to fluorescent light at room temperature, the regression equation calculated from the ratios (Rx) and the ages of stains in weeks (W) is  $\ln(1000 \cdot Rx) = 1.1084 +$

$0.3937 \cdot \ln(7 \cdot W)$ , and the coefficient of correlation ( $r$ ) is 0.9776 ( $n = 144$ ,  $P < 0.001$ ). When stains were stored at 37°C, the ratio transformed into logarithms correlated linearly with stain age. The regression equation describing the relationship in bloodstains exposed to fluorescent light at 37°C is  $\ln(1000 \cdot Rx) = 2.4477 + 0.0866 \cdot W$  ( $r = 0.9826$ ,  $n = 144$ ,  $P < 0.001$ ). The results suggest that the HPLC method may be applicable to the estimation of bloodstain age.

**Kumagai, R. (1993). "Analysis of hemoglobin in bloodstains using high-performance liquid chromatography." Nihon Hoigaku Zasshi 47(3): 213-219**

Hemoglobin (Hb) extracted from bloodstains was investigated using cation-exchange high-performance liquid chromatography (HPLC). Among the HbA1 fractions, the amount of HbA1d increased linearly as the ages of the bloodstains increased, although the amount of HbA1c remained virtually unchanged. As HbA1 fractions are Hb modified with glucose or glycolytic intermediates, the total Hb were separated into glycosylated and non-glycosylated Hb fractions using affinity chromatography to investigate the effects of glucose on the age-induced Hb changes. The amounts of glycosylated Hb increased in proportion to the blood glucose level, and the increased glycosylated Hbs were eluted at retention times that corresponded to those of HbA1d and HbA0, although HbA1c peak area was virtually unchanged. The amounts of both glycosylated and non-glycosylated HbA1d increased with bloodstain aging, and the proportion of non-glycosylated HbA1d relative to the total HbA1d was greater than that of glycosylated HbA1d. These findings showed that glucose has little effects on the elevation of HbA1d in bloodstains that occurs as they age. Furthermore, measuring HbA1d levels in bloodstains using HPLC may prove useful for the determination of their ages.

**Matsuoka, T., T. Taguchi and J. Okuda (1995). "Estimation of bloodstain age by rapid determinations of oxyhemoglobin by use of oxygen electrode and total hemoglobin." Biol Pharm Bull 18(8): 1031-1035.**

Bloodstain age was estimated from the ratio of oxyhemoglobin/total hemoglobin (fractional oxyhemoglobin) in the bloodstain if the temperature at which the bloodstain had been kept was known. The oxyhemoglobin was determined with an oxygen electrode immersed in water in which the oxygen had been depleted, and the total hemoglobin was determined by conventional colorimetry (cyanomethemoglobin method). Ages of prepared bloodstain samples (within 24 h after bleeding) were estimated by this present method, which requires only 20 microliters of bloodstain and only 5 min for the whole analysis.

**Andrasko, J. (1997). "The estimation of age of bloodstains by HPLC analysis." Journal of Forensic Sciences 42(4): 601-607.**

This paper describes an HPLC system for estimating the age of bloodstains deposited on clothing. In addition to the decomposition peak designated as "X", several other ageing processes were studied and found suitable for estimation of age of the stains. The various processes can be used independently of each other for estimation of bloodstain age when the storing temperature is known. Moreover, the ratio between the different peaks formed by ageing is practically independent of temperature between 0 degree C and 37 degrees C.

**Bauer, M., S. Polzin and D. Patzelt (2003). "Quantification of RNA degradation by semi-quantitative duplex and competitive RT-PCR: a possible indicator of the age of bloodstains?" Forensic Science International 138(1-3): 94-103**

In vitro RNA degradation is a complex and non-linear process which can serve as indicator for the quality and age of stains. The authors report a semi-quantitative duplex reverse transcription-polymerase chain reaction (RT-PCR) assay which, in combination with competitive RT-PCR using an external standard, allows quantification of RNA degradation levels. Using this method, 106 bloodstains stored up to 15 years were investigated. The distribution of the peak area quotients of standard and target messenger-RNA (mRNA) as measured by laser-induced fluorescence capillary electrophoresis was closely correlated with the age of the samples. Further statistical analysis showed that bloodstains with age differences of 5 years and more exhibit statistical significant variances in peak area quotients of both housekeeping genes included in this study,  $\beta$ -actin and cyclophilin. Although RNA continues to be degraded in dried bloodstains, mRNA suitable for RT-PCR can be isolated from samples stored for at least 15 years.

**Anderson, S., B. Howard, G. R. Hobbs and C. P. Bishop (2005). "A Method for Determining the Age of a Bloodstain." Forensic Science International 148: 37-45.**

The authors report the use of a real-time reverse transcriptase PCR to show that the ratio between different types of RNA (mRNA versus rRNA) changes over time in a linear fashion when dried human blood from eight individuals was examined over the course of 150 days. This approach reportedly offers the advantages of enhanced detectability of small samples, simultaneous isolation of DNA and RNA from the same sample, species-specific probes, and an increased time window of usefulness.

**Fujita, Y., K. Tsuchiya, S. Abe, Y. Takiguchi, S. Kubo and H. Sakurai (2005). "Estimation of the Age of Human Bloodstains by Electron Paramagnetic Resonance Spectroscopy: Long-term Controlled Experiment on the Effects of Environmental Factors." Forensic Science International 152: 39-43.**

In this study, the efficacy and limitations of electron paramagnetic resonance (EPR) for estimating the age of human bloodstains was investigated. At 77 K, human bloodstains give four striking EPR signals in the  $g = 6.2$  (g6), 4.3 (g4), 2.27 (H) and 2.005 (R) regions due to ferric high-spin, ferric non-heme, ferric low-spin and free radical species, respectively. Plotting double logarithms of the EPR intensity ratio of H/g4 versus days past bleeding gave a linear correlation up to 432 days with an error range within 25% of the actual number of days under controlled conditions. However, environmental factors such as differences of absorbent, light exposure and fluctuations of storage temperature affected the changes of these EPR-active compounds, which result in miss-estimation of the time since bleeding occurred.

**Strasser, S., A. Zink, G. Kada, P. Hinterdorfer, O. Peschel, W. M. Heckl, A. G. Nerlich and S. Thalhammer (2007). "Age determination of blood spots in forensic medicine by force spectroscopy." Forensic Science International 170(1): 8-14.**

The authors report a method for the estimation of the age of bloodstains using atomic force microscopy (AFM) for high-resolution imaging of erythrocytes in a blood sample and the detection of elasticity changes on a nanometer scale. In the analytic procedure a fresh blood spot was applied to a glass slide and AFM detection is started after drying of the blood drop. In a first step, an overview image was generated showing the presence of several red blood cells, which could easily be detected due to their typical "doughnut-like" appearance. The consecutively morphological investigations in a timeframe of 4 weeks could not show any alterations. Secondly, AFM was used to test the elasticity by recording force-distance curves. The measurements were performed immediately after drying, 1.5 h, 30 h and 31 days. The conditions were kept constant at room temperature (20°C) and a humidity of 30%. The obtained elasticity parameters were plotted against a timeline and repeated several times. The elasticity pattern showed a decrease over time, which are probably influenced by the alteration of the bloodstain during the drying and coagulation process. The preliminary data demonstrated the capacity of this method to use it for development of calibration curves, which can be used for estimation of bloodstain ages during forensic investigations.

**Hanson, E. K. and J. Ballantyne (2010). "A blue spectral shift of the hemoglobin soret band correlates with the age (time since deposition) of dried bloodstains." PLoS One 5(9): e12830.**

The authors report a method for the estimation of the time since deposition (TSD) of dried bloodstains using UV-VIS spectrophotometric analysis of hemoglobin (Hb) that is based upon its characteristic oxidation chemistry. A detailed study of the Hb Soret band ( $\lambda_{\text{max}}=412 \text{ nm}$ ) in aged bloodstains revealed a blue shift (shift to shorter wavelength) as the age of the stain increases. The extent of this shift permits, for the first time, a distinction to be made between bloodstains that were deposited minutes, hours, days and weeks prior to recovery and analysis. The extent of the blue shift was found to be a function of ambient relative humidity and temperature. The method is reportedly extremely sensitive, requiring as little as a 1 microliter of dried bloodstain for analysis. The authors demonstrate that it might be possible to perform TSD measurements at the crime scene using a portable low-sample-volume spectrophotometer.

**Anderson, S. E., G. R. Hobbs and C. P. Bishop (2011). "Multivariate Analysis for Estimating the Age of a Bloodstain\*." Journal of Forensic Sciences 56(1): 186-193.**

The authors report a multiplexed, real-time RT-PCR (or qPCR) method to determine the relative stability of different-sized segments of the same RNA species as well as differences in stability between two different RNAs' change over time in bloodstains. Their results indicate that a multivariate analysis of the changing ratio of the different RNA segments can be used to differentiate between samples of different ages in the defined population. Bloodstains from 29 of 30 donors were partitioned into different ages using this technique. Although further improvements will be required before this approach can be implemented in crime laboratories, the multivariate analysis holds promise of providing a reliable approach for temporally linking a bloodstain to the commission of a crime or excluding a bloodstain as being irrelevant to the case in question.

**Bremmer, R. H., K. G. de Bruin, M. J. C. van Gemert, T. G. van Leeuwen and M. C. G. Aalders "Forensic quest for age determination of bloodstains." Forensic Science International (in press)**

This review gives an overview of an extensive search in scientific literature of techniques that address the quest for age determination of bloodstains. The authors report that most techniques are complementary to each other, in short as well as long term age determination. Techniques are compared concerning their sensitivity for short and long term ageing of bloodstains and concerning their possible applicability to be used on a crime scene. In addition,

experimental challenges like substrate variation, interdonor variation and environmental influences are addressed. Comparison of these techniques contributes to our knowledge of the physics and biochemistry in an ageing bloodstain. Further improvement and incorporation of environmental factors are necessary to enable age determination of bloodstains to be acceptable in court.

## **18.2 Dark Surfaces**

**Bergeron, J. (2003). "Development of Bloody Prints on Dark Surfaces with Titanium Dioxide and Methanol." Journal of Forensic Identification 53(2): 149-161.**

no abstract available

**19. What new technologies and areas of research should be pursued with regard to bloodstain pattern analysis? (Note- this question does not require a list of references, it is for informational purposes only.)**

The following list represents the current research needs developed and agreed to by participants of the SWGSTAIN. The document is intended to focus research efforts on the most pertinent open questions within the discipline. It is neither exclusive nor prescriptive but rather indicative of current needs. No specific priority is intended by the order of this list.

### **1. Research that would minimize ambiguity in the characterization of small stain blood spatter patterns**

Blood spatter patterns consisting of small stains created by different mechanisms can appear similar. Research is needed to develop methods that provide more discrimination between these patterns.

### **2. Research to evaluate the behavior of blood on fabric surfaces**

BPA investigations frequently involve the examination of bloodstained clothing. Research is needed to develop an understanding of the complex problems of how blood transfers to, interacts with and persists on fabric surfaces.

### **3. Research to develop new methods to assist with the visualization of bloodstains on difficult surfaces**

Without the use of enhancement techniques bloodstains can be difficult to visualize, especially on dark surfaces. Research is needed to develop and validate specialized techniques to locate, enhance and record bloodstains.

### **4. Research to develop new automated methods to record bloodstain patterns at crime scenes**

The current manual methods of recording bloodstain data at crime scenes are time consuming, resource intensive and potentially subjective. Research is needed to implement new technologies and methods that would minimize subjectivity and maximize the amount of data that can be collected.

### **5. Research to determine the extent of bias in bloodstain pattern analysis**

It is acknowledged that bias exists within forensic science, including bloodstain pattern analysis. Research is needed to determine what biases exist. Methods to identify, quantify and minimize such biases are required.

### **6. Research to develop methods to determine the age of bloodstains**

Currently there are no validated methods to determine the age of a bloodstain. Research is needed to determine the factors that are relevant to the aging of a bloodstain and to develop reliable methods to determine a bloodstain's age.

### **7. Research to correlate bloodstain patterns at a crime scene with those on involved parties**

Arguments in court often centre on the quantity of blood on relevant individuals, in particular defendants. It is known that the extent of bloodshed at a scene may not obviously correlate with the blood deposited on involved parties, their clothing and other items. Research is needed to develop an understanding of the distribution of blood during bloodletting incidents to assist the court with determining the extent of involvement of relevant individuals.

## **8. Research to develop new methods to simulate human blood, tissue and body parts to assist with reconstruction experiments**

Experiments to investigate bloodstain pattern formation are limited by difficulties in simulating bloodletting events using human subjects. Research is needed to develop standard simulant materials to assist with physical and numerical modeling experiments.

## **9. Research to develop quantitative computer models for bloodstain pattern reconstruction**

Experiments to test specific crime scene hypotheses and fundamental research into the complexity of bloodstain pattern formation are frequently expensive, time consuming, have health and safety implications and require ethical approval. Research is required to assess current numerical models and develop new quantitative computer models for bloodstain pattern reconstruction. These will require robust data sets derived from evidence examination and laboratory experimentation.

## **10. Research to develop reliable means of sequencing overlapping patterns.**

Determining the sequence in which bloodstains were deposited can be difficult. Currently there are no established, validated methods by which this can be done. Research is required to develop methods to address this problem.

## **11. Research to determine the effect of the variation in blood composition on bloodstain pattern formation**

Currently little is known about the effect of the variation in blood composition on the formation and appearance of bloodstain patterns. Research is required to determine the influence of drugs, medical conditions, age, body fluid mixtures and other factors on the properties of blood and bloodstain patterns produced from this blood.

## **12. Research to improve methods for presenting bloodstain pattern evidence within the legal environment**

Bloodstain pattern evidence presented in the legal environment is not always clearly communicated or understood. Research is required to develop new methods to clearly present this type of evidence. This could include the use of visual aids to illustrate evidence, statistical techniques for the expression of likelihood and novel communication techniques.