

Contents

1. HEMODYNAMICS.....	2
Q Outline cascade mechanisms involved in blood clotting process, including blood clot initiation and amplification.....	2
Q A person is found dead wearing a blood soaked shirt yet there is no blood found in the surrounding area. What are a couple possible reasons for this?	4
Q Compare and contrast the three categories of gunshot wounds of entrance. ...	5
2. MOTION AND DIRECTIONALITY	6
Q When determining the angle of impact of a blood drop, an assumption is that the blood drop hitting the target surface is spherical. Since a blood drop is oscillating while falling how would the angle of impact be affected if the blood drop was actually not spherical upon impact?	6
Q A victim has already been removed during the inspection of a crime scene. You note there is a drip trail in the kitchen and a large amount on the floor in the adjoining dining room. What can you deduce about the chain of events based on this information?	7
Q What is the difference between a wipe and a swipe? Give an example of how each could be created. Why are these stains important to an investigation?	7
Q What is a butterfly pattern and how is it created?	8
3. IMPACT SPATTER AND ALTERED BLOODSTAINS	8
Q Impact spatter with upward directionality was found at the homicide scene. What does this indicate regarding the victim's position during the attack?.....	8
Q Police apprehend a suspect hours after he has reportedly attacked the victim. What can the absence of blood spatter on the suspect's clothing tell you about the incident?	9
Q Describe blood spatter patterns you may expect to see after venous breach and arterial breach. How might each type of injury affect the amount of blood loss that you would see at a crime scene?.....	10
4. TRIGONOMETRY: ANGLE OF IMPACT, CONVERGENCE AND ORIGIN	11
Q Calculate the angle of impact for each stain. Determine the height of origin (inches) for each stain.	11
Q Based on the height of origin of each stain, how many different events occurred?	11
Q From this information (above), develop a scenario that may describe what happened. Given the fact that the attack entailed blows to the victim's head and abdomen, what might this data indicate about the victim's positioning?.....	12
5. BLOODSTAIN PATTERN EVALUATION AND DOCUMENTATION.....	13

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Q | Compare and contrast indoor scenes versus outdoor scenes. How are these scenes processed differently? 13

6. **BLOOD DETECTION** 14

Q | Are automated blood pattern analysis and crime scene reconstructions superior to traditional approaches? Discuss the various automated processes used to investigate bloodstain patterns and crime scene reconstruction. 14

Q | Luminol versus BlueStar? 16

7. **COURTROOM TESTIMONY** 19

Q | How do you expect us, the legal counsel, to consider you as an expert? 19

Q | Bloodstains are 1-3 mm in diameter and you described this blood spatter pattern being consistent with medium velocity spatter. The defense examiner states that medium velocity spatter stains are 1-4 mm in diameter. How might you explain this discrepancy?" 19

Q | Chain of custody. Since you did not collect the evidence, how do you know the stain was not contaminated or altered before arrival to your laboratory? 20

8. **REFERENCES**..... 21

Note: Information within this document is certainly not inclusive of bloodstain pattern analysis (BPA), nor is that my intent. Rather, exemplary question and answers are presented to acquaint the reader with BPA. The questions are loosely paraphrased from *Blood Distribution and Spatter* online graduate course (University of Florida), which I completed spring 2018 (refer to references). I am the sole author and all answers, figures, tables, calculations, research, etc. are my own; cite accordingly (refer to footer). Please contact me should you have further specific questions or comments. AD

1. HEMODYNAMICS

Q | Outline cascade mechanisms involved in blood clotting process, including blood clot initiation and amplification.

The hemostasis process | When the body is wounded externally and/or internally, vasculature (arteries, arterioles, veins, venules, etc.) may be cut, crushed or severed. The body's first physiological response is to stop the blood loss by vasoconstriction, fluid retention and recruiting platelets. Platelets (thrombocytes) are the smallest component of plasma, essential to protecting the body from hypotensive shock via traumatic blood loss. The ultimate outcome is polymerization of fibrin and activation of platelets to form a blood clot. The coagulation cascade is a chain reaction, each step a precursor to the next enzymatic protein (clotting factor) catalyzing the next step. Clotting factors are

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designated by Roman numerals I - XIII (outlined below). In summary, the entire hemostatic process: vasoconstriction → platelet activation → clot formation → coagulation.

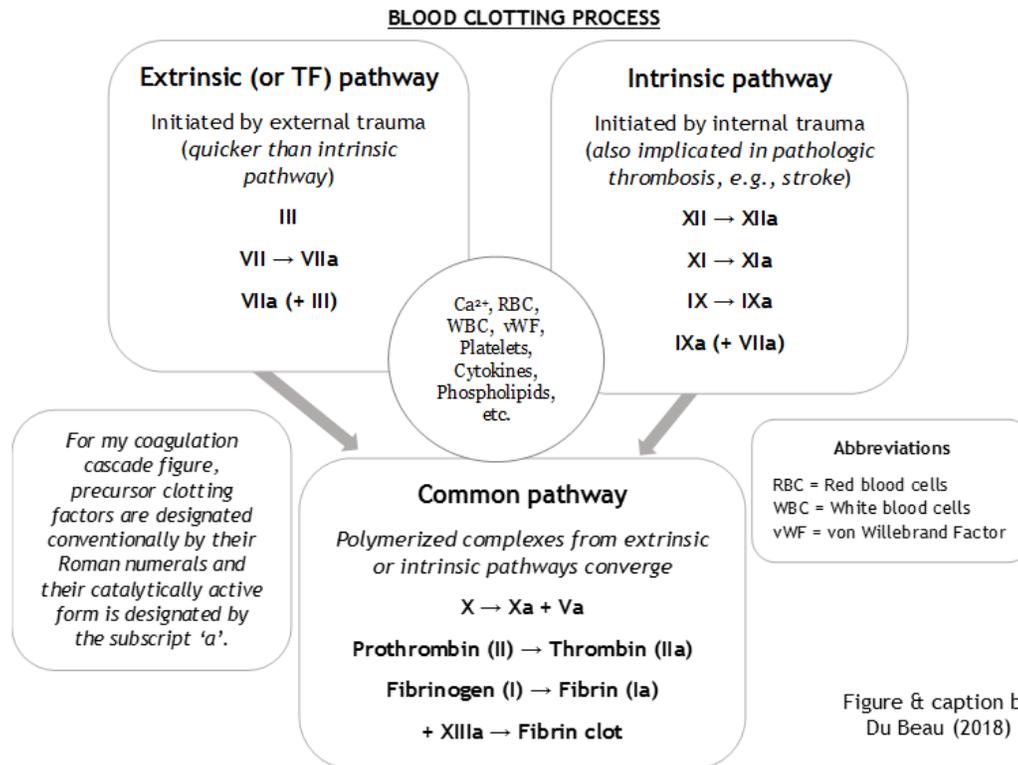
Platelets are irregularly shaped cell fragments, 2 - 4 μm in diameter, produced by megakaryocytes (large bone marrow cells). As the vascular lumen constricts to staunch traumatic blood flow, platelets aggregate on site and release cytokines (small proteins associated with cell signalling). Coagulation occurs when platelets become chemically activated by exposure to collagen and tissue factor (TF) from the physically damaged vasculature in the presence of cofactors such as calcium (Ca^{2+}), vitamin K and phospholipids (platelet membrane constituent). Platelets are dimerized to collagen by blood glycoproteins (von Willebrand Factor). Activated platelets change shape, projecting dendritic-like sticky spines to form an insoluble fibrin mesh covering the vascular breach, trapping more platelets and erythrocytes (red blood cells) to form a plug. In summary (common pathway):

Prothrombin activator (protein complex) → prothrombin → thrombin (proteolytic enzyme)
→ fibrinogen (soluble protein polymer) → fibrin (insoluble protein polymer)

Blood clot initiation and amplification | A clot needs to form at the wound since platelets alone are not enough to plug the damaged vasculature: secondary hemostasis. Two distinct processes can initiate clotting, the extrinsic pathway and intrinsic pathway. These two pathways converge into the common pathway. The clotting process has 'positive feedback loops' to amplify clotting. When platelets stick to the inner wall of the damaged vessel, cytoplasmic granules carrying adenine diphosphate (ADP), thromboxane A₂ and serotonin are released into the lumen to further accelerate platelet aggregation. So what begins as localized clotting is amplified into a cascade of plasma clotting activity.

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Coagulation Factors | Factor I = Fibrinogen; II = Prothrombin; III = Tissue Factor (TF) or Thromboplastin; IV = Ca²⁺; V = Proaccelerin; VI = unassigned (nomenclature no longer used, later found to be activated Factor V); VII = Proconvertin; VIII = Anti-hemophilic Factor; IX = Christmas Factor; X = Stuart Prower Factor; XI = Plasma Thromboplastin Factor; XII = Hageman Factor; XIII = Fibrin Stabilizing Factor.

For healthy individuals, blood usually clots within 3 to 15 minutes. Wounds can range from minor cuts, a skinned knee, etc. to stabbings and gunshots involving both external and internal clotting. When the tissue is finally repaired, assuming the individual survives their traumatic wound, the scabbed blood clot is removed via fibrinolysis, then further broken down enzymatically. Serum is the liquid fraction from clotted blood, often visible exuding from external clots. Angiogenesis (neovascularization) is essential to wound healing too, propagating new blood vessels from pre-existing vessels.

Q | A person is found dead wearing a blood soaked shirt yet there is no blood found in the surrounding area. What are a couple possible reasons for this?

The most likely reason may be that the victim bleed primarily internally, perhaps from an abdominal stab wound. Vasoconstriction constrains blood flow. Compared to the arterial system, the venous system is relatively less elastic, shunting blood flow from venules into larger veins towards the heart. If the wound was anatomically situated such that blood flow was directed towards the heart, then bleeding would be mostly within the internal cavity. Stab wounds are characterized by being deeper than their entry impact on the

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surface of the body. External bleeding from abdominal stab wounds is known to be often relatively minimal.

Pharmacological factors and/or an underlying medical condition may account for why there was no blood found in the area around the body. Physiological pH is ≈ 7.4 . Under alkaline conditions (pH greater than 7.4), the blood clotting process can become abnormally activated, causing excessive clot formation. Alkalosis is induced by prolonged vomiting or diarrhea. Diuretics and laxatives both work by increasing HCO_3 (bicarbonate) levels and, in excess, alkalosis can be a side effect. Such a hyper-clotting cascade could account for, or at least contribute to, why there was relatively less external bleeding than expected from this wound.

Q | Compare and contrast the three categories of gunshot wounds of entrance.

Gunshot wounds can be characterized by the distance the firearm was discharged from the victim, contact (near-contact), intermediate, or distant (long-range). Perforating shots have both an entry and exit wound whereas penetrating shots stay lodged in the body with only an entry wound. Forward spatter (same direction as source of force) is mostly associated with exit wounds. Conversely, back spatter is associated with entrance wounds.

Contact (or near-contact) gunshot wounds | When a firearm is discharged at close range, soot might be expected to blacken the area surrounding the resultant wound as well as bright red discoloration caused by carboxyhemoglobin. From close contact, star-shaped stellate wounds can occur when bone is impacted directly beneath the skin (like the skull). Such distinguishing stellate wounds are caused by hot gases expelled from the firearm that expand differentially between tissue and bone. In some cases, the muzzle of the firearm can even imprint or bruise the skin. For traumatic head wounds, brain matter can be forcefully ejected. The entry wound for near-contact gunshots is often much bigger than the corresponding exit wound. Substantial back spatter of blood and tissue typify contact gunshot wounds.

Intermediate gunshot wounds | Intermediate range gunshots span distances up to 3 to 4 feet (≈ 1 meter) from their target. When a firearm is discharged from this range, gases and soot particles are further dispersed as compared to close range discharge. Stippling around the wound area might be expected, depending on the type of firearm used and ammunition.

Distant gunshot wounds | Long-range gunshot wounds are defined to be about 15 feet (≈ 4.5 meters) from their target. Such wounds lack soot discoloration or stippling since the firearm is discharged from relatively further distances. If the shot is perforating, the exit wounds are expected to be larger than their corresponding entry wound. Substantial forward spatter of blood is expected from long-range gunshots and the resultant exit wound may be lacerated.

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2. MOTION AND DIRECTIONALITY

Q | When determining the angle of impact of a blood drop, an assumption is that the blood drop hitting the target surface is spherical. Since a blood drop is oscillating while falling how would the angle of impact be affected if the blood drop was actually not spherical upon impact?

Free-falling blood drops keep their spherical shape and cannot break apart into smaller drops until an external force, such as impact, disrupts surface tension, as per Newton's First Law of Motion. Surface tension is the force that constrains the viscous drop into its smallest possible volume. As a blood drop forms, such as on a knife blade, gravity exerts a downward force while the opposing surface tension preserves the coherency of drop with an upward force. As blood accumulates on, e.g., a knife blade, then the mass of the drop becomes sufficiently great to overcome inertia, breaking the molecularly cohesive surface tension, forming a teardrop shape as it releases from the blade.

An average passively free-falling blood drop is approximately 0.05 ml, forming a sphere with a diameter of about 4.5 mm. The quantity of blood necessary to form a passive drop depends on the physical properties of the surface and the surface area of the source. For example, a fine glass capillary tube results in a relatively small stain diameter compared to the blunt head of a hammer (14.5 mm vs 26.9 mm, respectively). A free-falling blood drop accelerates at 9.8 m/sec, as per the downward force of gravity. The drop will keep accelerating until the opposing forces, surface tension and gravity, equalize.

Blood drops oscillate in a downward free-fall, a dynamic process, and any given drop assumes a spheroidal shape, then repeatedly changes back and forth between spheroidal-oblong to spheroidal. If free-falling drops collide, then they can oscillate faster, although such collisions are considered to be fairly rare. The maximal terminal velocity of an average blood drop is about 7.6 m/sec (= 25.1 ft/sec). While free-falling, a blood drop oscillates for just about the first meter, damping within 0.05 seconds or so. How fast any given blood drop oscillates is a function of the size of the drop; bigger drops oscillate more than smaller drops.

Air resistance is inversely proportional to drop size. So larger drops oscillate more while free-falling than smaller drops. And larger drops have a greater terminal velocity. Impact events cause blood drops to be smaller in both volume and diameter. Therefore, drops created upon impact oscillate less when airborne as a result of their smaller diameter.

To finally address the question, how would the angle of impact be affected if a drop was not spherical? The angle of impact refers to the angle formed by a blood drop in relations to the targeted surface. Summarizing cumulative information, blood drops most likely not to be spherical upon impact would be relatively bigger drops that have passively free-fallen within less than a meter.

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Interpreting these findings, the impact source of such a blood drop would be relatively blunt, falling from a relatively near distance. Such a bloodstain could be mistaken as coming from a very acute angle and/or dropping onto a slanted surface.

Q | A victim has already been removed during the inspection of a crime scene. You note there is a drip trail in the kitchen and a large amount on the floor in the adjoining dining room. What can you deduce about the chain of events based on this information?

The victim's first wound(s) most likely would result in blood drops from breached superficial vasculature with relatively lesser amounts of spatter found on the floor and surrounding surfaces. Given this crime scene description, the bloodshed chain of event most likely began in the kitchen.

At this point, the victim would still be able to move about and/or defensively escape to another area. As further wounds from their assailant are inflicted, possibly also exacerbating the initial wound(s), more blood is shed from increasingly damaged vasculature. This scenario accounts for the blood drip trail from the kitchen leading towards the adjoining dining room.

As hypotensive shock ensues, the victim's mobility becomes compromised plus becoming further vulnerable to attack, receiving more wound(s). So the victim stays longer in the dining room where substantial bloodstaining is found, finally losing consciousness.

Alternatively, as a less likely scenario, if the drip trail is considered to be short, then the bloodstaining might be from a bloody object(s) or weapon carried by the assailant from the dining room and diminishing to the kitchen rather than from an active bleeding site, such as if the victim had been removed from the site.

Q | What is the difference between a wipe and a swipe? Give an example of how each could be created. Why are these stains important to an investigation?

Swipes and wipes are both a subset of passive transfer bloodstains. As bloodshed events unfold, objects become bloody and stains are formed as evidenced by, e.g., swipes and wipes, etc. In general, recognizing transfer stains helps determine activity and temporal movement of both the victim and assailant during a bloodshed event.

By definition, swipes are characteristic of any given moving wet, bloody object making contact with a non-bloody surface. A swipe pattern could be created, for example, by a victim's bloody clothing making contact against a wall as they are falling to the floor. Hair swipes are often distinguished as bifurcating V-shaped transfer bloodstains.

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When swipes patterns are formed, right/left directionality may be determined by 'feathering' that is the thinning, diminishing terminal edge as blood is incrementally deposited onto a surface. However, if feathering striations are evident on both leading and trailing edges, then directionality may be impossible to determine.

Based on research by R. M. Gardner (2004), motion directionality may be ascertained from swipe bloodstain patterns based on at least four distinctive features: striations, diminished volume, welling and feathering. Striations are considered to be the least valid feature whereas diminished volume may be the most valid.

Conversely, wipes are the result of an object moving through an already existing wet bloodstain, disturbing spatter boundaries. For example, a wipe could be created by fingers moving through a bloodstain, altering the pattern. Or a broom 'wiping' through a wet, bloodstained hardwood floor. Identifying features and directionality of an object making a wipe pattern may be ascertained through careful investigative observation.

Q | What is a butterfly pattern and how is it created?

Butterfly patterns are a special subset of transfer bloodstain, identified as a mirror image of the original surface. For context, transfer bloodstain patterns are passive bloodstains that result from contact with any given wet, bloody object with a non-bloody surface or an alteration of any existing bloodstain by a non-bloody object. Recognizing transfer bloodstains helps establish the activities and movement that occurred during a bloodshed event. Examples of repetitive pattern transfers include bloody shoeprints, socks or handprints, etc. with individualizing features, often conferring directionality.

For butterfly patterns, if a flexible surface folds when immediately contacted by a wet, bloody object, or folds before the original bloody image is dry, then a mirrored 'butterfly' image of the original stain is created.

3. IMPACT SPATTER AND ALTERED BLOODSTAINS

Q | Impact spatter with upward directionality was found at the homicide scene. What does this indicate regarding the victim's position during the attack?

Impact spatter can be observed when any given object, such as a weapon, makes direct, forceful contact with wet blood during an attack. Spatter directed upwards indicates that the victim was on the floor during the bloodshed event. Projected at uniform speed, blood drops show varying flight parabolas where bigger drops disperse further away than smaller drops. And upwardly directed elongated stains can be found relatively close to the source of the force as compared to circular stains resulting from perpendicular force.

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While the size of the whole bloodstain pattern may not be conclusive, we can know that greater separation between radiating bloodstains indicates further distance from the floor to the impact surface(s). So examining individual bloodstains within the pattern could help better characterize the victim's position on the floor.

In this instance, spatter shown on the walls and/or other nearby objects or surfaces may make streaking patterns radiating away from the area of impact. When spatter is intercepted by a weapon/object(s) and/or by the victim or assailant, a distinct void in this radial pattern can happen, including instances where the victim might be crouching on the floor or defensively shielding from the attack. Further analysis would be necessary to correlate this upwardly directed spatter pattern with the mechanism of impact, e.g., beating/stabbing, gunshot, etc.

Q | Police apprehend a suspect hours after he has reportedly attacked the victim. What can the absence of blood spatter on the suspect's clothing tell you about the incident?

While blood spatter on the suspect's clothes might be expected, the absence of blood does not necessarily exonerate this suspect. Blood spattering on the assailant's dominant hand and lower legs would most likely be evident from forceful overhead blows when a victim is lying on the ground, for example. But if such blows struck the victim from an angle directed away from the assailant, such as swings from the side, spatter might not be found on the assailant at all. Kish and MacDonnell (1996) reported that conclusions cannot be drawn based on the presence of bloodstains on a suspect's clothing, and, the absence of bloodstains neither exonerates nor implicates any given suspect.

The directionality of strikes, plus the force of their delivery, ultimately determines the distribution of spatter on the assailant's clothes and surrounding surfaces. Other factors include specifics about the weapon, the quantity, amount, direction and force of impact(s), location of wound(s) in relation to the assailant, volume of dispersed blood and movement of both victim and assailant.

Lack of blood spatter could be due to manifold factors, such as if the assailant is beyond the range of the radiating pattern where dispersed blood drops are relatively small, e.g., high velocity penetrating gunshot exhibiting mostly forward spatter directed away from the assailant. Experimental modelling by Petricevic & Elliot (2013) demonstrated scant spatter transfer onto the clothing of a mock assailant as a hammer struck a blood-soaked plastic head, suggesting that direct contact with blood, either on the victim or from the bloodshed scene, is necessary for blood to be transferred to the assailant's clothing in certain cases.

Anecdotally, I know that native Alaskan subsistence moose or caribou hunters use high caliber rifles, e.g., 30-06, aiming from a far distance, and oftentimes can fatally shoot a moose with one penetrating full metal jacket bullet without any blood on themselves, especially if they are upwind from their game.

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Trace spatter may not be perceptible without microscopic examination. Case studies have demonstrated that other less obvious objects worn by an assailant during the attack might be spattered with blood, such as jewelry, glasses or even socks. Certain dark, thick and/or patterned fabrics, such as denim, can mask spatter too. Further, assailants might change clothes, wash or discard protective clothes after the attack occurs.

Q | Describe blood spatter patterns you may expect to see after venous breach and arterial breach. How might each type of injury affect the amount of blood loss that you would see at a crime scene?

Arterial breach | Arterial vasculature conveys oxygenated blood from the heart to the body, contracting in cadence with the beating heart. If an artery is wounded, blood projects outward in all directions with force, often creating bloodstains having a distinctive undulating/pulsating pattern on surfaces surrounding the victim, even described as looking ‘electrocardiogram-like’ with a downward flow pattern. During the bloodshed event, the height of the resultant fluctuating peaks diminish as the victim’s blood pressure begins to fall. Each individual stain within the pattern can be aligned in parallel according to their direction of impact. Misting spatter (atomized) with a 0.1 mm or less can also characterize arterial bloodstains, e.g., penetrating high velocity gunshot exit wounds, etc.

The size of stains from spraying arterial blood can range from less than 1 mm to 1 cm or greater in diameter. While the shape of central stains can vary substantially, from circular to oval to irregular, satellite spatter with jagged, elongated spines are often found. Arterial blood is richly oxygenated, so their fresh stains are especially bright red compared to venous blood. Certain medical conditions, e.g., stomach ulcers or lung disease, etc. can cause expiratory arterial blood projection too, so medical exam findings can contextualize bloodshed events.

By definition, arterial spurting refers to a series of individual bloodstains whereas gushing usually implies an especially large volume bloodstain (although these definitions can sometimes be used interchangeably by analysts).

Vascular bloodstains are dependent upon the extent of the penetrating damage, the victim’s position, which vessel is breached, and even clothing (or hair, etc.) that can absorb, deflect or inhibit bloodshed. Further, just because vascular bloodstains are absent at the scene does not necessarily mean that damage did not occur.

Venous breach | Venous vasculature circulates oxygen depleted blood from organs back towards the heart. Considerably less muscular than arteries, blood pressure within veins is negligibly detectable. However, if blood pools within engorged veins, such as in certain medical conditions, e.g., varicose veins, venous insufficiency syndrome, the resulting bloodletting stains from breached veins can mimic arterial blood spatter. However, blood from breached veins is typically classified as low velocity spatter with a diameter of about 4 mm or greater.

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Projected bloodstain patterns can be a result of ruptured varicosities from the esophagus associated with liver cirrhosis or even hemorrhoidal tissues. Such scenarios may not be fatal, necessitating review of medical history to confirm circumstances of the bloodshed event. Acutely ulcerated varicose veins causing hemorrhaging in lower legs, for example, can spray blood onto the floor or low levels surfaces.

Pooled blood and/or projected stains typify venous stain patterns. Compared to arterial stains that can be forcefully projected far distances, darker venous stains may be situated closer to the victim who may be ambulatory. To note, both arterial and venous wounds might be expected in serious bloodshed events with massive penetrating wounds, creating a combination of arterial and venous bloodstain patterns.

4. TRIGONOMETRY: ANGLE OF IMPACT, CONVERGENCE AND ORIGIN

Q | Calculate the angle of impact for each stain. Determine the height of origin (inches) for each stain.

Table 1 (Excel spreadsheet) | Blood spatter data from crime scene

Stain no.	Width (mm)	Length (mm)	W/L Ratio	Angle of Impact = Arc Sin (degrees) = I	Distance from Point of Convergence (in) = D	Tan I	Height of Origin (in) = H
1	7.2	14.4	0.500	30.000	37.5	0.577	21.7
2	7.0	9.9	0.707	44.997	12.9	1.000	12.9
3	6.8	9.5	0.716	45.708	19.8	1.025	20.3
4	6.9	17.6	0.392	23.082	31.2	0.426	13.3
5	7.3	8.0	0.913	65.853	9.5	2.231	21.2
6	6.9	13.6	0.507	30.488	22.0	0.589	13.0
<i>Units in millimeters (mm) and inches (in) as indicated</i>							

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Q | Based on the height of origin of each stain, how many different events occurred?

Based on the data (Table 1), two distinct events seem to have occurred at this crime scene. Bloodstains 2, 4 and 6 have an average height of origin of 13.05 inches (standard deviation ± 0.2) whereas bloodstains 1, 3 and 5 have an average height of origin of 21.05 inches (standard deviation ± 0.6). The height of origin is a measure of the direct distance

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from the ground/floor to the area of origin. The angle of impact in conjunction with the 2-dimensional point of convergence defines the 3-dimensional point/area of convergence and origin for each stain, spatially representing where the victim was located and their position (e.g., standing, crouching, etc.), respectively, during the bloodshed event.

While this group of six total bloodstains do have single point of convergence, they may still represent stochastic events. If bloodstain paths are caused by distinct events, then the point/area of convergence may be coincidental and have no investigational relevance. If more intersecting paths were examined, i.e., more than three per pattern in this instance, then the more likely the point/area of convergence is significantly meaningful.

In sum, patterns formed by bloodstains 2, 4, 6 and 1, 3, 5, respectively, may represent the occurrence of different events within this crime scene.

Q | From this information (above), develop a scenario that may describe what happened. Given the fact that the attack entailed blows to the victim's head and abdomen, what might this data indicate about the victim's positioning?

Data from Table 1 (above) and information from this case study (presented in previous modules) indicates that two events may have occurred, corroborating probative fatal blunt force trauma to the head and abdomen. The average width of all 6 bloodstains is 7.0 mm, categorized as large impact spatter, corroborating beating or bludgeoning caused by hammer blows.

Blood drops make a parabolic arc projecting from the source of impact onto the target surface, especially from further distances, and the corresponding calculated area of origin approximates the victim's relative position. Impact spatter is often distributed radially from the wound(s) and the victim received multiple blows. The inset photograph from this module's data set shows blood spatter on the floor and baseboard. The actual 'real life' origin would be less than or equal to the maximal height of origin.

The maximal height of origin is 21.7 inches up from the floor (stain 1), which is only slightly lower than the head of an adult (male) figure seated on the floor, slouching or defensively crouching, perhaps hanging or cradling their head. The data rules out that the victim was standing or sitting on furniture.

If the assailant was swinging a hammer from above, blood from the resultant head wound(s) could be deflected downwards, accounting for the pattern formed by stains 1, 3 and 5. The impact angles for these stains are 30°, 46° and 66° (average 47.2°) which are comparatively greater overall than the pattern formed by the other stains. That is, the head is higher up than the abdomen when the victim was sitting/slumping on the floor, so the standing assailant's blows delivered from above would be less acute, further supporting this development of crime scene events.

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Overcome, the victim slumps flat onto the floor where blows to the abdomen occurred. The lowest height of origin is 12.09 inches (stain 2), corresponding to the victim lying on the floor, either supine or from the side, accounting for the pattern formed by stains 2, 4 and 6. Comparatively, the angles of impact for these stains are 45°, 23° and 30° (average 32.9°) which are relatively more acute than the pattern formed by the other stains as blood accumulates from the wounds, given that blows were delivered from overhead.

The victim was found prone at the scene, implying he defensively rolled over before succumbing to hypotensive shock or was turned over by the assailant. Blood had ultimately fallen passively, pooling onto the floor where the victim had fallen, matching this probable scenario.

5. BLOODSTAIN PATTERN EVALUATION AND DOCUMENTATION

Q | Compare and contrast indoor scenes versus outdoor scenes. How are these scenes processed differently?

Both indoor and outdoor crime scenes generally follow the same systematic processes of documentation, preservation, collection and examination of evidentiary samples and observations. The premise of documentation is to develop a realistic, accurate, and complete record of the discovered crime scene. Effective documentation and analysis characterizes the crime scene, facilitates outside analysis of evidentiary samples, provides for objective evaluation by the defense and ultimately allows for cogent courtroom presentation.

Indoors or out, directionality and orientation of the crime scene (north, south, east, west) with marked reference features (e.g., numbered placards) needs to be documented. Photographic evidence must be appropriately scaled, aligned in the direction of the bloodstain if feasibly possible.

Indoor crime scenes | Indoor crime scenes are comparatively easier to process and preserve since they are protected from weather conditions. Because indoor scenes are not exposed to the elements, they can typically be processed without time limitations (with the exception of some public places). The secured scene is relatively well-defined indoors.

Before any alterations are made (with the notable exception of first responding emergency medical intervention), the secured scene is objectively documented using photography, videography, sketching and descriptive written accounting. Lighting and temperature can be controlled indoors, facilitating documentation processes.

Wet bloodstains and spatter will dry over time, dependent on various impact surfaces and ambient indoor conditions such as heaters or fans. Blood at indoor crime scenes can become dislodged, flake away or be otherwise disturbed as investigators process the scene. First responders can inadvertently create artefactual stains and spatter. Recognizing and accounting for such potential alterations is inherent to processing the crime scene.

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Outdoor crime scenes | Outdoor crime scenes are subject to weather conditions and terrain that can substantially alter evidentiary samples and obscure their recognition. Bloodstains and patterns can melt or be covered in snow, washed away by rain, or absorbed into soil/sand, for example. Target surfaces can be uneven or roughly textured, even drastically so, such as steep terrains. Assessing the directionality and impact angles can be compromised on foliage or irregular surfaces. Bloodstains diluted by water, fog or humidity will appear diffuse and patterns spread over ice can artificially make the volume appear greater. Extreme temperatures can affect blood's physical qualities too (on the upside, bloodstains and spatter can be well preserved when frozen). Predation or insect spatter can occur. Familiarity with the climate and terrain of the locale helps to contextualize the outdoor crime scene. While outdoor crime scenes are less likely to be altered by the assailant, all these various confounding factors need to be taken into account during processing.

Consequently, photographic documentation of outdoor crime scenes needs to be done as soon as possible since conditions are apt to change. Document weather conditions, temperature and scale terrain features. If bloodstains are found or suspected on foliage, brush, soils, etc. then these samples must be collected, packaged and transported expediently. If photographs or videos must be taken at night or in shady areas, auxiliary light sources may be used. In some instances, overall scenes can be captured photographically from a ladder or scaffold. Inconsistent lividity patterns, changes in blood directionality or voids in bloodstain patterns might suggest that the victim was repositioned, moved or dumped outside after the occurrence of fatal bloodshed events.

6. BLOOD DETECTION

Q | Are automated blood pattern analysis and crime scene reconstructions superior to traditional approaches? Discuss the various automated processes used to investigate bloodstain patterns and crime scene reconstruction.

Blood spatter analysis can reveal information pivotal to realistic crime scenes reconstruction. The interaction of various physical and temporal factors such as speed, properties of impact surface, hemodynamics, weapon, relative position of actors at the bloodshed event, room layout, etc. determine the shape and size of two-dimensional blood drops and overall spatter patterning.

The traditional 'string method' entails connecting elliptical bloodstains individually to their corresponding area of origin along major directional axes within a three-dimensional room space, repeating the process to elucidate spatter patterning. Angles (impact, α and glancing, γ) and flight path data are resultantly calculated using trigonometry. Obvious drawbacks to traditional stringing include that methods can be arduously time consuming and tedious. Even experienced analysts are prone to mistakes. Cumbersome stringing activities can inadvertently contaminate evidentiary samples at the crime scene. Most

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crime scenes are released after investigation, constraining repeat analysis if findings are inconclusive or later contested in legal proceedings.

Based on original stringing method principles, automated approaches to blood pattern analysis have emerged with advantageous applications. Specialized virtual interfaces and computer software programs such as BackTrack Suite, HemoSpat and HemoVision use sophisticated visualization technology and experimental manipulation capabilities for reliable interpretation of patterns.

General process of automated blood pattern analysis and application:

- Digital photography, markers and calibration | Digital photographic documentation includes an overview encompassing the whole spatter patterning plus successive zoomed-in images capturing individual spatter detail. To establish consistent, coordinated perspective, black and white checkboard patterned “fiduciary markers” (Joris et al., 2015) are embedded within the scene as a means of fixed comparison, effectively calibrating images. Fiduciary markers serve to eliminate perspective distortion (from tilted images), establish horizontal/vertical frames of reference and scale images so pixels can be converted to metric units. Fiduciary markers also link overviews with zoomed-in images and keep track of sequential photographs. Digital images are uploaded into the program interface for analysis.
- Perspective correction and shape distortion | Accurate bloodstain analysis is contingent upon precise elliptical measurements, which can be especially problematic for manual stringing methods. From an oblique angle, any given bloodstain’s length to width ratios are skewed. Under or overestimating ellipse length, for example, results in errors when calculating the distance of impact from the wall. Fiduciary markers correct such distortions and applications can virtually transform images in space. For accurate evaluation, any given bloodstain needs to be visually distinct from the background impact surface. Automated applications include techniques such as gray-scale conversions, digital contrasting, and filling techniques to optimize bloodstain images.
- Bloodstain analysis and flight path determination | For BackTrack Suite and other applications, only individual blood stain measurements, directionality and shape gleaned from digital photographs are necessary for analysis. Eliminating assumptions about the speed and parabolic flight path of blood drops not only streamlines the process, but also reduces cumulative error inherent to original manual methods. Viewed from above, strings represent actual flight paths. Directional analysis entails two or more blood spatter events. Virtual strings can identify and isolate separate bloodshed events for independent evaluation (such as by using different colored strings to designate different room areas). Using robust mathematical modelling, algorithms and statistical regression linking individual shapes to their corresponding impact angles, spatter data and trajectories can be summarized onto a spreadsheet.

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- Crime scene reconstruction | Automated analysis can be applied with confident certainty to locate the blood source, physicality of actors involved and correlate velocity impacts to spatter patterns to extrapolate detailed crime scene events. The calibrated overview image serves as a contextual frame of reference so that flight paths from corresponding stains can be evaluated. Results from mock crime scenes analyzed virtually using fiducial markers and algorithms revealed that reconstruction errors were minimal. Further, processes were much faster compared to manual stringing methods.

Automated methods provide consistency for blood pattern analysis and scene reconstructions, which is critical for standardization and defending results presented in legal settings. When considering the applicability of novel forensic technologies, the analyst must first confidently understand basic underlying principles to direct the analysis in accordance with aims and avoid the risk of wasting time and valuable resources.

Regardless of which scientific discipline, presenting statistical error is de rigeur to establish the scientific rigor of the methodology and define the scope of the findings. Statistical parameters are built into such automated programs, which is another major advantage over traditional methods. Optimized images and concise data generated from such programs are far superior to traditional methods for courtroom presentation. Overarchingly, automated applications are comparably advantageous.

Q | *Luminol versus BlueStar?*

Chemiluminescent agents, e.g., luminol (3-aminophthalhydrazide) or *BlueStar Forensic*® (Roc Imports), can detect trace amounts of blood which might not be overtly visible. Forensically, these blood visualizing agents are useful because they not only reveal the presumptive presence of blood, but also effectively demarcate bloodstain distributions otherwise hidden at the crime scene. Refer to my table (below): *Compare/contrast forensic blood visualizing agents*.

When faint/dilute bloodstains and/or suspected affected areas are treated with these blood detecting agents, luminescence ranging in color from blue-green, blueish white or yellow-green (depending on the reagent preparation) appear. At the molecular level, when excited photons fall back to their resting configuration, visible light is emitted. Unlike fluorescence, which absorbs/emits only at a specifically defined wavelength, chemiluminescence requires a catalyst.

Luminol is a crystalline solid that chemiluminesces when oxidized (usually via H₂O₂, hydrogen peroxide) in alkaline solutions. In the presence of a catalyst, specifically Fe²⁺ found in hemoglobin (blood), the oxidant H₂O₂ ultimately degrades to form O₂ and H₂O: $2 \text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2 \text{H}_2\text{O}$. Luminol forms a dianion with hemoglobin, visibly detecting blood. *BlueStar* is luminol-based with the same basic formula and functional properties as luminol.

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Assailants often attempt to clean-up bloodstains at the crime scene, such as wiping/mopping using bleach or 'hiding' stains by flipping or covering cushions, for example. *BlueStar* is relatively recent substitute for luminol and more commonly used at crime scenes (according to literature) given its advantageous properties (refer to my table below). Also, these agents are useful for chemical enhancement of latent bloodstain impressions such as footwear and fingerprints, although with different application methods, etc.

Chemiluminescent agents are considered presumptive for blood detection, necessitating further confirmatory testing such as tetramethylbenzidine, o-tolidine, etc. Advantageously, because blood visualizing agents are typically sprayed onto areas where bloodstains might be expected, large regions can be covered quickly and conveniently. Luminol's remarkable sensitivity reportedly range from a maximal 1:1,000,000 (upper limit even reportedly reaching 1:1,000,000,000) or less dilutions.

For both these agents, formula need not be applied to fresh bloodstains at the crime scene. In fact, results are actually better suited for dried or even decomposed bloodstains, provided stains are still viably intact. These blood visualizing agents work really well on dark, patterned soft or textured surfaces (e.g., textiles, upholstery), even soils or foliage, etc. where bloodstaining may be otherwise obscured. These water-based agents can drip when sprayed onto hard or smooth/shiny vertical surfaces. Too much saturation can obviate samples, such as if over-spraying occurs or using too highly concentrated formulations beyond the level of detection.

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Matanuska Forensic Science, LLC | Bloodstain Pattern Analysis
Dr Amy Du Beau | Question & Answer

Compare/Contrast Forensic Blood Visualizing Agents | By AD

Properties	Luminol	BlueStar®
Historical discovery	Firstly synthesized in 1853, then Albrecht later discovered catalytic blood (heme) detection properties in 1928.	Based on luminal formula, Grodsky mixed luminol, Na ² CO ³ (sodium carbonate) & NaBO ³ ·nH ₂ O (sodium perborate) in 1951. <i>BlueStar</i> is a patented formula by <i>Roc Imports</i> .
Photographic documentation Timed exposure	Needs complete darkness	Low-light conditions OK, brighter luminescence!
Chemiluminescence	Diminishes after 2 nd application. However, other literature (e.g., Proescher & Moody, 1939) ¹ reports that luminescence can be reproducible (especially for older stains).	OK to re-apply (although typically fades after 2 nd or more applications). Brighter luminescence overall.
Application Typically sprayed onto probative bloodstained areas from ≈ 15-18” distance.	Reagents should optimally be prepped & mixed on-site (or immediately prior). Cannot be stored over time.	OK to use for days after mixing. Store at ≈ 4° C (refrigerator), protected from light.
Stock solution preparation Photosensitive. Other standard preparations may be used. Store stock at 4° C (refrigerator).	<ul style="list-style-type: none"> ● 8 grams NaOH (sodium hydroxide) + 500 mL distilled H₂O (0.4 N) ● 10 mL 30% H₂O₂ + 490 mL distilled H₂O ● 0.35 grams luminol + 62.5 mL 0.4 N NaOH, dilute to 500 mL 	<i>BlueStar</i> is a patented formula, so use their prep method: blue & white tablets from sealed pouches dissolved in distilled H ₂ O + glycine. Easier prep for non-scientists, e.g., police officers.
Cross-reactivity	Reacts with plant peroxidases (e.g., horseradish), metals & cleaning solutions containing NaOCl (bleach), high concentrations of cigarette smoke.	Less cross-reactivity than luminol. Better & brighter detection of blood with interference from bleach, especially with glycine prep.
DNA² analysis & ABO blood typing	No DNA degradation, does not affect PCR ² or STR ² analysis. May interfere with enzyme & protein genetic marker systems.	No DNA degradation. However, original formulation (for hunters) with pH 12.6 is not suited for DNA analysis.
Safety/biohazard, refer to MSDS³ Aerosol application, so protective equipment recommended. H ₂ O ₂ is never stored in a sealed container! Mix gently.	<p>Eye, skin & respiratory irritant</p> <p>Probable but not known carcinogenic, mutagenic effects unknown.</p>	Refer to luminol formula

1/ Reference from text (James, Kish, Sutton *et al.*), Page 370

2/ DNA, Deoxyribose Nucleic Acid; PCR, Polymerase Chain Reaction; STR, Short Tandem Repeat

3/ MSDS, Materials Safety Data Sheet

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7. COURTROOM TESTIMONY

Q | How do you expect us, the legal counsel, to consider you as an expert?

To qualify as an expert witness, courts consider credentials, academic degrees, certifications, licensures, publications, public engagements, membership affiliations, experience, etc. At the minimum, qualifying experts need more knowledge than any given layperson. Renowned bloodstain expert, Herbert MacDonell, purported that expert witnesses should hold (at least) a bachelor's of science degree. For blood spatter analysts, there is no consensual agreement as to what constitutes expert qualifications.

As per the Daubert decision (1993), the US Supreme Court stipulates that the trial judge's own discretion determines whether to admit expert evidence into their court. If a scientific expert uses credible scientific principles and methods for their analysis, then their knowledgeable expertise is deemed admissible, as per Rule 702 (e.g., Chesbro, 1994, in Kish). Importantly, courts evaluate only the scientific validity used by an expert, not the persuasiveness of their conclusions.

Regardless of prior witness experience, the expert's testimony must be grounded in valid scientific methodology. As per Daubert, the scientific evidence presented is defined through process, not its product. Courts, in accordance with their 'gatekeeping' role, have admitted testimony of expert witnesses from various backgrounds in the area of bloodstain analysis, such as law enforcement agents, criminalists, forensic consultants, scientists, medical pathologists, and more. As per the federal rules of evidence, expert testimony needs to be helpful, clarifying, relevant and reliable to the trier and impactful to jurors.

Q | Bloodstains are 1-3 mm in diameter and you described this blood spatter pattern being consistent with medium velocity spatter. The defense examiner states that medium velocity spatter stains are 1-4 mm in diameter. How might you explain this discrepancy?"

Medium velocity spatter stains associated with beating/stabbing are, by definition, greater than 1 mm but less than 3 mm in diameter. However, this parameter represents a variable range. While stain diameter is a good indicator, it is not the deciding factor in drawing a conclusion.

Statistically, the difference between 1-3 mm and 1-4 mm is insignificant. So, mathematically, there is no discrepancy. This range describes the population of medium velocity impact spatter, so overlap is naturally expected within this group. There is considerably greater variability between groups, effectively ruling out large impact spatter (greater than 6 mm diameter) and misting (less than 0.1 mm diameter), for instance. (By analogy, clovers are botanically defined as having 3 leaves. But some clovers do have 4 leaves).

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Identification of impact is contingent upon circumstances of the individual case. Many variables can affect the size of impact spatter associated with beating mechanisms, e.g., physical characteristics of the weapon (shape, weight, length), amount and direction of the applied force, movement of the victim and assailant during the attack, location of the wound(s), etc. Even that the victim's blood may plausibly contain more clotting factors. Further, some mechanisms are nonviolent, not necessarily directly associated with the actual infliction of injury. That is, some blood stains may have fallen passively, artefactually skewing the reported range.

Q | Chain of custody. Since you did not collect the evidence, how do you know the stain was not contaminated or altered before arrival to your laboratory?

The chain of custody documentation ensures the unbroken path of evidence starting from collection at the crime scene to final presentation at the courthouse. Intermediate custody within laboratory settings must be rigorously maintained, recording transport, handling, analysis, storage and preservation of evidentiary samples, also accounting for aliquoted samples and their archival. Without such documentation, findings from evidentiary samples can be rendered inadmissible in court.

The chain of custody is maintained using a communal spreadsheet or software programs (e.g., Laboratory Information Management System), recording sample identifiers, dates, who had the samples when and where, including any physical/chemical alterations. During discovery and cross-examination, opposing counsel will check for gaps in the chain of custody, questioning for possible misidentification, mishandling, contamination, alteration or tampering of evidentiary samples.

If questioned about bloodstain evidence that I did not personally collect, I would refer to the chain of custody documentation. To answer, in effect, 'Based on the reported chain of custody, I can ensure the sample's security and whereabouts since collection.'

Alternatively, if there was no established chain of custody until my personal receipt of the sample, or it came from an unknown source, then answer 'I do not know,' acknowledging the possibility that the stain could have been contaminated or altered beforehand.

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