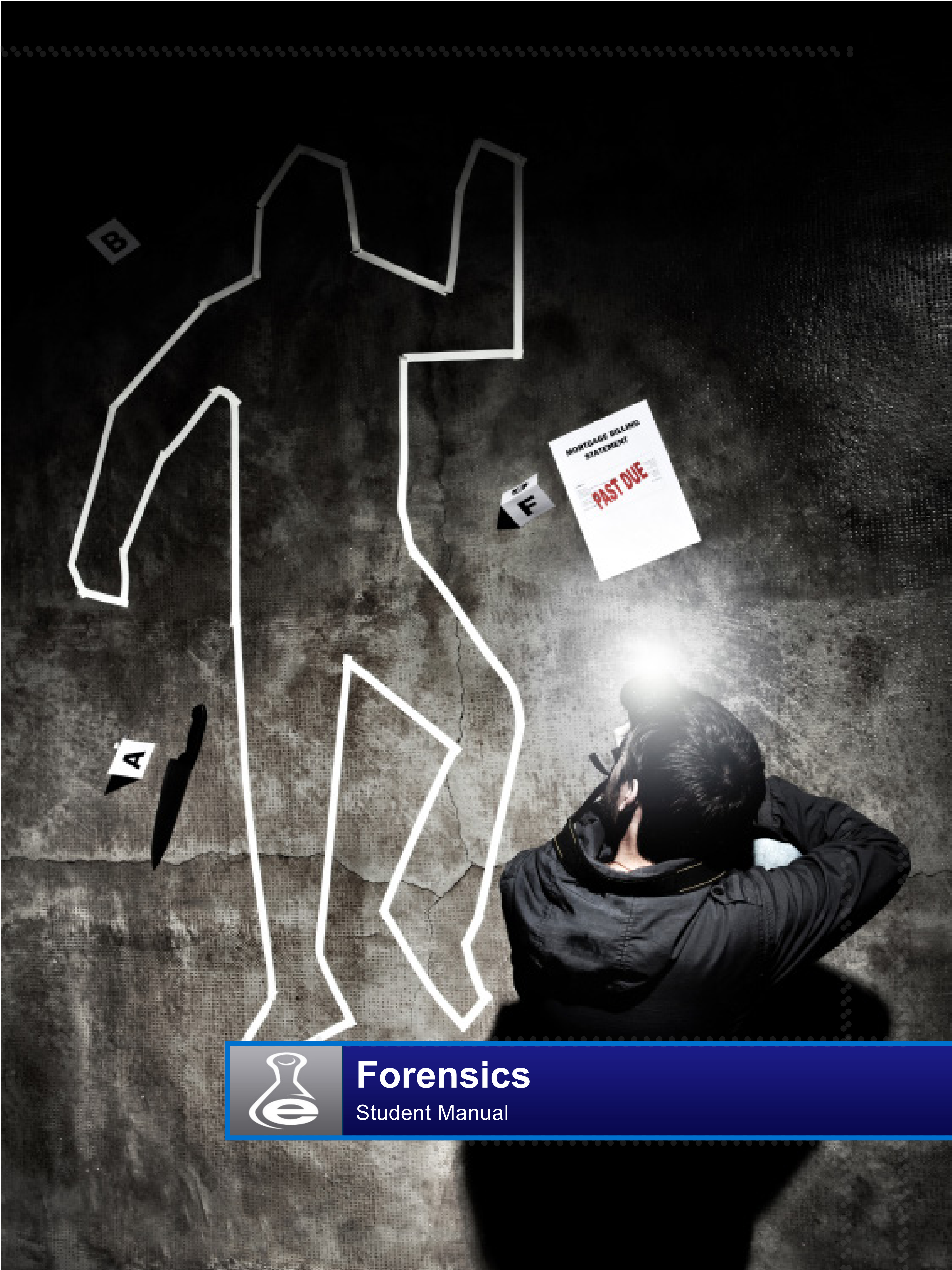

Forensics

Student Manual

The experiments included within this lab manual are suitable for supervised or unsupervised learning environments. eScience Labs assumes full liability for the safety and techniques employed within this manual provided that all users adhere to the safety guidelines outlined in the mandatory eScience Labs Safety Video, Preface, and Appendix. **All users must understand and agree to the eScience Labs safety guidelines prior to beginning their lab experiments**. eScience Labs does not condone use of the lab materials provided in its lab kits for any use outside of the curriculum expressly outlined within the lab manual.

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Lab 4

Blood

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LAB 4
Blood

Learning Objectives

- **Explain the role of blood and bloodstain patterns in forensics science**
- **Analyze and identify bloodstain patterns by performing bloodstain analyses**

INTRODUCTION

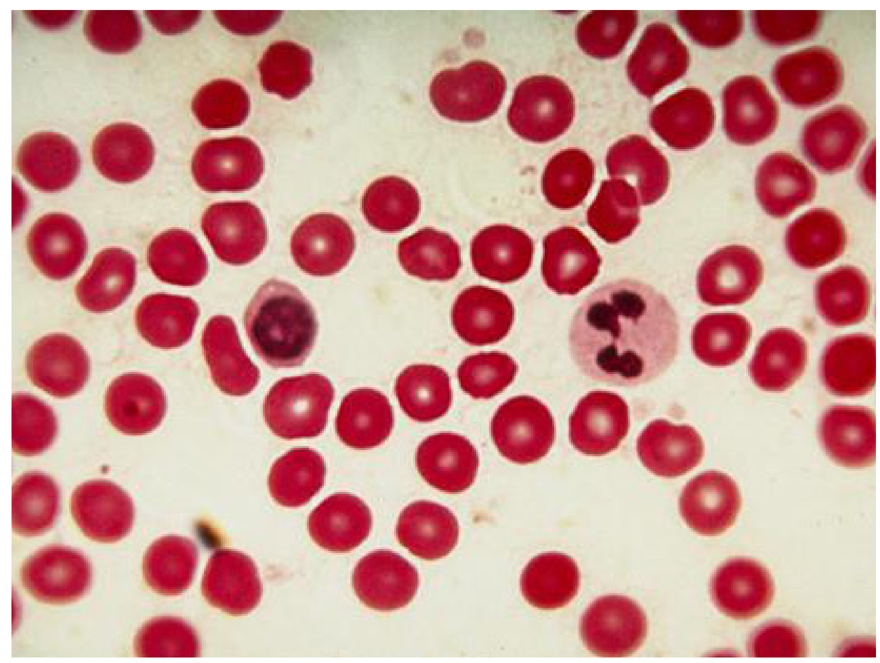


Figure 1. Red blood cells (bright red) and white blood cells (light pink with a dark nucleus) are suspended in plasma.

Blood, a type of biological evidence, consists of red blood cells, white blood cells, and a liquid called plasma (Figure 1). Blood is fundamental to crime scene investigation because it can provide a wealth of information about a crime. Many aspects of the crime, including the height of the blood source, how much blood was spilled, the angle of impact, and the force at which the blood was spattered, can be determined using blood. It can also aid in identification through blood typing and genetic (DNA) analysis.

BLOOD

To fully understand the power of blood in forensics, one must understand the molecular components of blood cells. The adult human body contains approximately five liters of blood, accounting for nearly 8% of a person's body weight. The majority of blood consists of **plasma**, an aqueous solution composed of over 90% water that contains proteins, minerals, blood cells, and cell fragments. Plasma contributes to approximately 55% of blood. Among the cells suspended in the plasma, red blood cells, also called erythrocytes, are predominant, composing 45% of blood. Additionally, white blood cells and platelets are also suspended in plasma.

TESTING FOR BLOOD

The presence of blood at a crime scene often indicates a physical struggle and provides clues about the criminal event. However, the first step to using blood in forensic analysis is to determine if an unknown sample is actually blood. The following tests can be performed to determine the presence of blood in an unknown substance and if a blood sample is from a human or other animal:

- **Kastle-Meyer blood test**: Used in preliminary crime scene investigations due to the simplicity and low cost of the test. Phenolphthalein (a chemical indicator), followed by a drop of hydrogen peroxide, are added to the unknown sample. If blood is present, the iron in the blood cell protein hemoglobin will oxidize phenolphthalein, turning it a vivid pink color.

- **Luminol test**: A potential bloodstain is sprayed with luminol reagent. If the unknown sample produces a light blue glow after contact, the sample is likely blood. This method is accurate, but not always practical, because the glow is very faint and requires a dark environment to clearly view the results.

- **Bluestar test**: Similar to the luminol test, but produces clearer results that do not require a dark environment.

- **A precipitin test**: After a sample is confirmed to be blood, this test determines the presence of human blood versus another animals' blood.

Blood

BLOODSTAIN ANALYSIS

The patterns of bloodstains found at the crime scene can indicate what occurred during a crime. Like all other matter, blood follows the laws of physics as it is projected and hits a surface. It may provide information about the type of weapon, the time since the crime occurred, the injuries involved, or even the handedness of the criminal.

ANGLE OF IMPACT

The characteristics of a bloodstain depend on the angle at which blood spatter hits a wall. For example, a drop of blood that hits a surface head-on at a 90º angle will have a fairly circular shape. As the angle of impact becomes smaller, a bloodstain usually takes on a more elongated, elliptical shape. This makes sense because, the more horizontal the path taken by the spatter, the more horizontal motion it will have when it hits the surface.

ORIGIN AND DIRECTION OF TRAVEL

The direction from which blood spatter came can also be determined by analyzing bloodstain patterns. If blood spatter hits a surface from left to right, the right side (or the direction in which the drop traveled) will generally come to a point and the left side of the stain will be rounded (Figure 2). If multiple spatters come from the same origin, their direction of travel can be traced to where they intercept to determine where the origin was. This can be useful to determine if a body has been moved.

PARENT DROP

The original source from which the rest of the spatter was created is referred to as the **parent drop**. It is usually characterized by a larger bloodstain with smaller spatter emanating from it.

SATELLITE SPATTER

Smaller drops of blood that originate from a parent drop and rest in a position that surrounds the parent drop are called **satellite spatter**. They may take on a circular shape or, with enough velocity, take on an elongated shape. Satellite bloodstains typically have a more pointed end oriented opposite the direction of travel (Figure 3).

SPINE

Spines are pointed or curved lines that extend out from the parent drop.

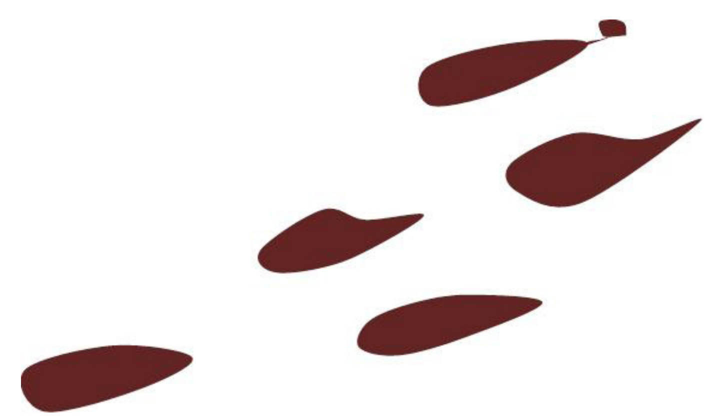


Figure 2. Direction of travel from left to right.

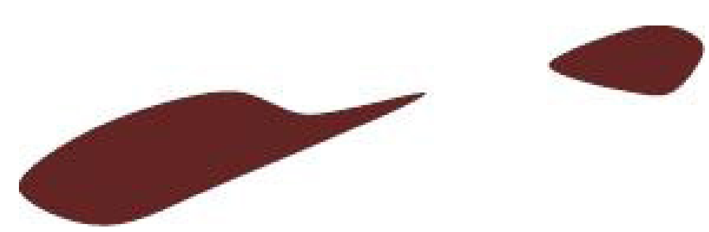
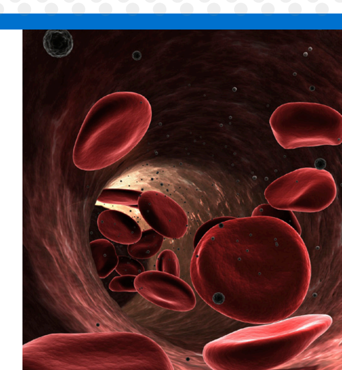


Figure 3. A parent drop on the left and a satellite drop on the right. Note how the satellite drop has a pointed end in the opposite direction that the blood was traveling.

Did You Know?

There are several different types of blood cells, although erythrocytes are predominant. These cells are responsible for transporting oxygen from the lungs to tissues and cells throughout the body. They are able to do this highly specialized function because of a special iron-containing protein called hemoglobin. Hemoglobin possesses a special binding site for oxygen, which can be released into tissues. Hemoglobin also gives blood its characteristic scarlet color; however, it emits a purple-blue hue when deoxygenated. This is why veins appear purple. Other types of blood cells are white blood cells, including macrophages, neutrophils, and basophils, which perform specialized functions during an immune response. Finally, platelets function to stop bleeding by forming clots at the sight of wounds.



Blood

Figures in Forensics

Paul Theodar Uhlenhuth 1870-1957

Paul Theodor Uhlenhuth, a German bacteriologist, experimented with blood serum. When he injected a chicken's blood into a rabbit, and then mixed the serum from the rabbit blood with an egg white, the egg proteins separated from the mix. This act of separation led to his 1901 discovery of a test to distinguish animal blood from human blood, termed the species precipitin test. Until that time, animal blood and human blood could not be distinguished. Image Source: © U.S. National Library of Medicine, History of Medicine Division, http://ihm.nlm.nih.gov/luna/servlet/view/search?q=B025272

Lab 4
Bloodstain Analysis - Height and Volume

Experiment Inventory

Materials

Simulated Blood

(3) Pieces of Cardstock

Masking Tape

(1) Permanent Marker

(1) Pair of Disposable Gloves

(1) Pair of Safety Glasses

*Adjacent Floor and Wall

(1) *Camera / Smart Phone

(1) *Pencil

Labware

(1) Ruler

(1) Transfer Pipette

(1) Tape Measure

*Paper Towels

Note: You must provide the materials listed in *red.

EXPERIMENT 1: BLOODSTAIN ANALYSIS - HEIGHT AND VOLUME

While blood samples are often used as evidence of who was involved in a crime, the bloodstain itself is also useful to learn about what happened during a crime. In this experiment, you will simulate bloodstains and distinguish between the effect of height and volume on the resulting patterns.

Lab Safety: The simulated blood sample may cause stains to cloth or other materials. Small amounts of simulated blood will get on the walls and floor where the experiment is performed. Complete this experiment in an area where blood splatters will not cause concern or protect the wall and floor with paper towels or other absorbent material.

PROCEDURE

Part 1: Single Drop from Different Distances

☐ 1. Put on a pair of disposable gloves and safety glasses.

☐ 2. Use a ruler and permanent marker to divide one piece of cardstock into six areas of approximately equal size. Label the center of the cardstock “Part 1: Single Drop from Different Distances.”

☐ 3. Use the permanent marker to label one edge of the top left area “15 cm” (Figure 4). Label the remaining areas “30 cm,” “45 cm,” “60 cm,” “75 cm,” and “100 cm,” as shown

☐ 4. Use two pieces of masking tape to secure the tape measure to a wall adjacent to a clear floor space so that the 0 cm mark is touching the ground (Figure 5).

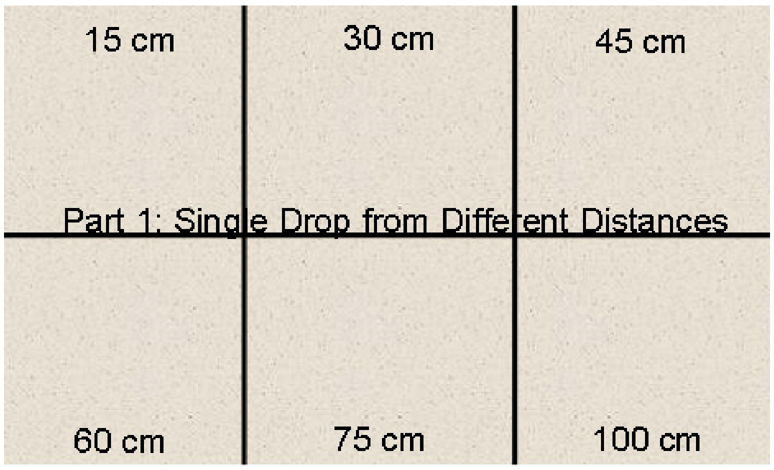


Figure 4. Cardstock setup for Part 1.

- [ ] 5. Place the cardstock on the floor so that the 15 cm area is directly in front of the tape measure (Figure 5).
- [ ] 6. Remove the cap from the simulated blood sample bottle and use a transfer pipette to remove several drops of blood from the bottle.
- [ ] 7. Hold the ruler perpendicular to the 15 cm mark in one hand.
- [ ] 8. Holding the transfer pipette vertically, line up the tip of the pipette with the 15 cm mark. Use the ruler as a guide to ensure the pipette is 15 cm above the cardstock and positioned over the cardstock, so that when it is dropped, it hits the targeted area (Figure 6).
- [ ] 9. Carefully squeeze one drop of simulated blood from the pipette onto the cardstock so that it lands on the 15 cm area.
- [ ] 10. Repeat five more times at the following heights, making sure to adjust the position of the cardstock for each height: 30 cm, 45 cm, 60 cm, 75 cm, and 100 cm.

Note: You may want to wait 1 - 2 minutes between each drop to allow the bloodstain time to dry.

- [ ] 11. Wait approximately 5 minutes or until the bloodstains have completely dried. You may wish to continue to Part 2 while you wait for the bloodstains to dry.
- [ ] 12. Use the ruler to measure the diameter of each bloodstain. Record the diameters in Table 1.
- [ ] 13. Record observations about the bloodstain patterns for each distance in Table 1.
- [ ] 14. Use a camera to take a photograph of the cardstock after it is dry.

Note: You will need to download, scan, or print the photographs to be included with your lab report.

- [ ] 15. Set the Part 1 cardstock aside.

Part 2: Multiple Drops from One Distance

- [ ] 16. Use a ruler and permanent marker to divide one piece of cardstock into six areas of approximately equal size. Label the center of the cardstock "Part 2: Multiple Drops from One Distance."
- [ ] 17. Use the permanent marker to label one edge of the top left area "1 Drop." Label the remaining areas "2 Drops," "3 Drops," "4 Drops," "5 Drops," and "6 Drops," as shown in Figure 7.
- [ ] 18. Place the cardstock on the floor so that the 1 Drop area is directly in front of the tape measure.
- [ ] 19. Hold the ruler perpendicular to the 15 cm mark in one hand.

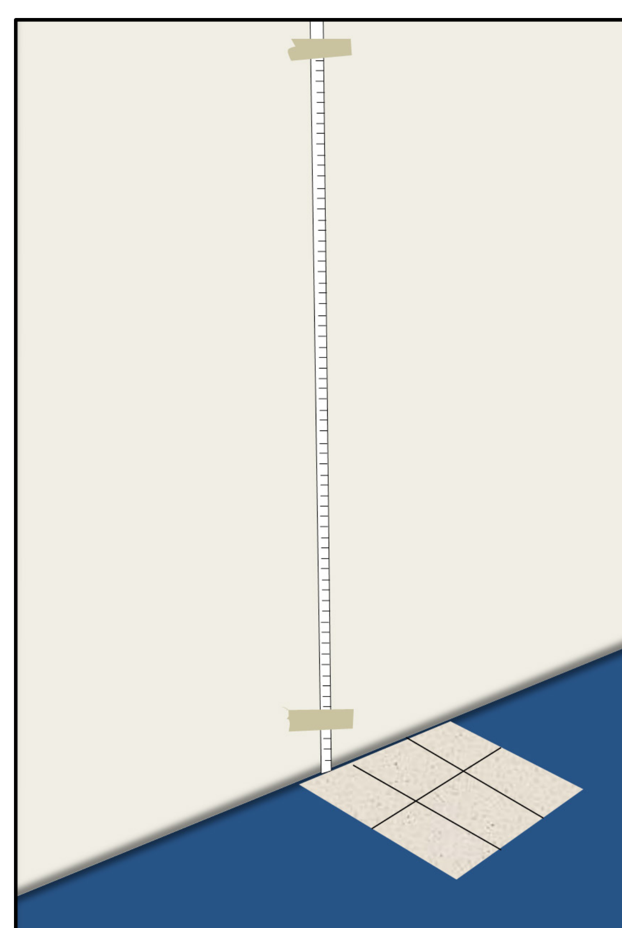


Figure 5. Setup for the tape measure and cardstock.

☐ 20. Use the transfer pipette to remove the simulated blood sample from the bottle. Holding the transfer pipette vertically, line up the tip of the pipette with the 15 cm mark. Use the ruler as a guide to ensure the dropper is 15 cm above the cardstock and positioned over the cardstock so that when it is dropped it hits the targeted area (Figure 6).

☐ 21. Carefully squeeze one drop of blood from the pipette onto the cardstock so that it lands on the 1 Drop area. Observe the splatter pattern and record your observations about the spatter pattern in Table 2.

☐ 22. Repeat for 2, 3, 4, 5, and 6 drops of simulated blood, making sure to adjust the position of the card stock for each set of drops. Be careful to squeeze the pipette slowly so one drop leaves the pipette at a time. Observe how the blood spatter's pattern changes with each additional drop.

> **Note:** You may want to wait 1 - 2 minutes between each drop to allow the splatter time to dry.

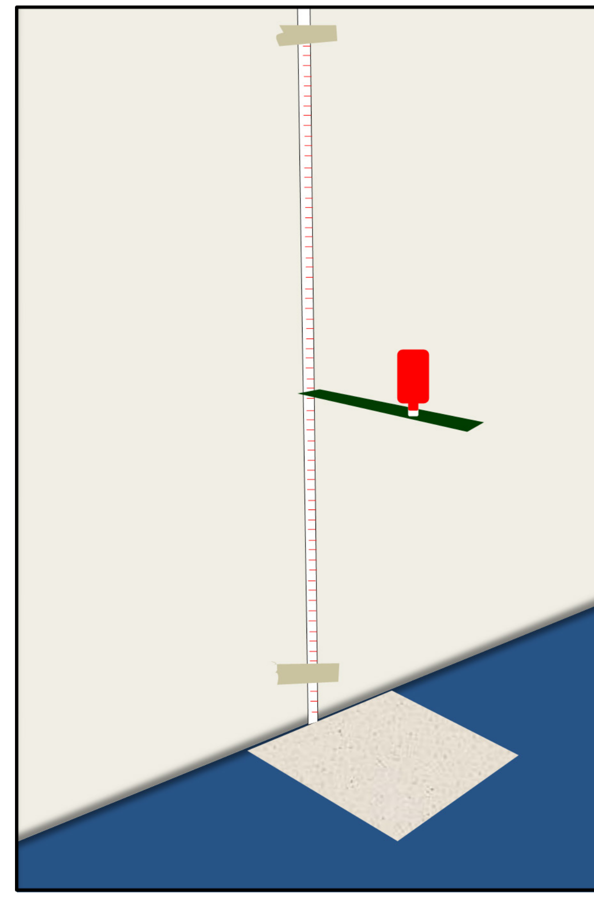


Figure 6. Set up for the ruler and transfer pipette.

☐ 23. Allow the spatters to dry for approximately 10 minutes. You may wish to continue to Part 3 while you wait for the bloodstains to dry.

☐ 24. Use the ruler to measure the diameter of each bloodstain. Record the diameters in Table 2.

☐ 25. Record the bloodstain patterns for each distance in Table 2.

☐ 26. Use a camera to take a photograph of the cardstock after it is dry.

> **Note:** You will need to download, scan, or print the photographs to be included with your lab report.

☐ 27. Set the Part 2 cardstock aside.

Part 3: Bloodstain with Force

☐ 28. Use the permanent marker to label the top of the third piece of cardstock as “Part 3: Bloodstain with Force.”

☐ 29. Place the cardstock on the floor so that the center of the cardstock is directly in front of the tape measure.

☐ 30. Use a transfer pipette to extract 2 mL of simulated blood from the bottle.

☐ 31. Hold the ruler perpendicular to the 15 cm mark in one hand.

☐ 32. With your other hand, hold the transfer pipette vertically, such that the tip of the pipette is 15 cm above the cardstock. Use the ruler as a guide to ensure the dropper is 15 cm above the cardstock and positioned over the center of the cardstock so that when it is dropped it hits the targeted area (Figure 6).

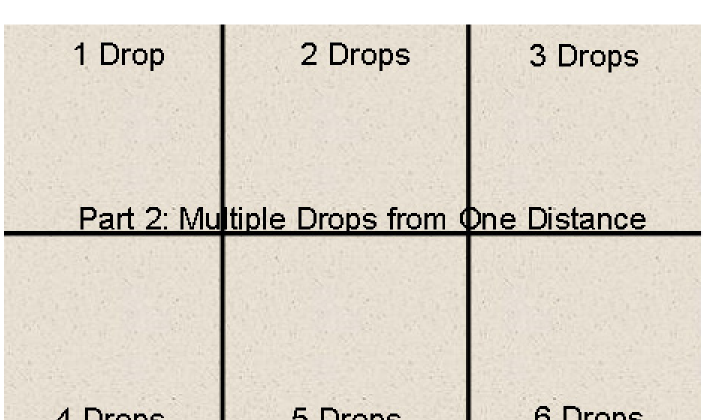


Figure 7. Cardstock setup for Part 2.

☐ 33. Quickly squeeze the simulated blood sample onto the center of the card.

☐ 34. Record your observations about the bloodstain pattern in Table 3.

☐ 35. Allow the cardstock to dry for approximately 10 minutes.

☐ 36. Use the ruler to measure the diameter of the bloodstain. Record the diameter in Table 3.

☐ 37. Use a camera to take a photograph of the cardstock after it is dry.

Note: You will need to download, scan, or print the photographs to be included with your lab report.

☐ 38. Replace the lid on the bottle of simulated blood. You will need the simulated blood for the next experiment.

Lab 4
Experiment 1 Data Sheet

Table 1: Bloodstain Diameter of a Single Drop at Varying Distances

Distance from Impact (cm)	Bloodstain Diameter (mm)	Observations
15		
30		
45		
60		
75		
100		

Table 2: Bloodstain Diameter of Varying Drops at the Same Distance

Number of Drops	Bloodstain Diameter (mm)	Observations
1		
2		
3		
4		
5		
6		

Table 3: 2 mL Bloodstain Diameter with Force

Height (cm)	Bloodstain Diameter (mm)	Observations
15		

Experiment Inventory

Materials

Simulated Blood

(2) Pieces of Cardstock

(1) Clipboard

Masking Tape

(1) Permanent Marker

(1) Pair of Disposable Gloves

(1) Safety Glasses

*Flat Surface

*Camera / Smart Phone

(1) *Pencil

Labware

(1) Ruler

(1) Protractor

(1) Transfer Pipette

*Paper Towels

Note: You must provide the materials listed in *red.

EXPERIMENT 2: BLOODSTAIN ANALYSIS - ANGLE OF IMPACT

In this experiment, you will simulate and analyze bloodstain patterns caused by different angles of impact.

PROCEDURE

☐ 1. Use the masking tape to secure a protractor to the edge of a flat surface, such as a table top (Figure 8). To do this, line the origin (the hole on the flat side) up to the top edge of the flat surface. Secure the flat side of the protractor with a piece of masking tape. To keep the protractor from falling towards you, place a second piece of masking tape perpendicular to and over the first piece, through the protractor, and onto the top of the flat surface.

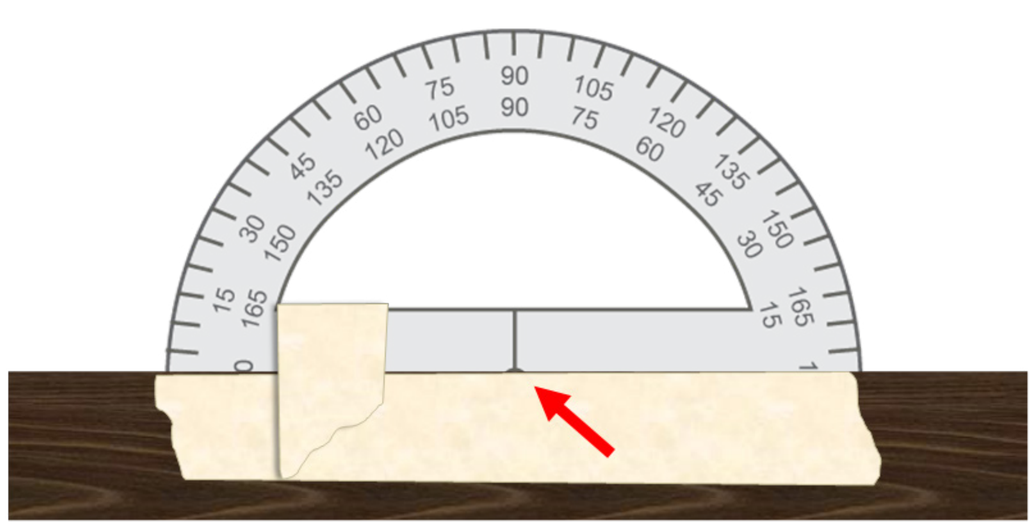


Figure 8. Protractor setup. The red arrow indicates the protractor origin.

- [ ] 2. Use a permanent marker to label the top of a piece of cardstock "60º angle." Under the label, write "Trial 1," "Trial 2," and "Trial 3" evenly spaced out (Figure 9).

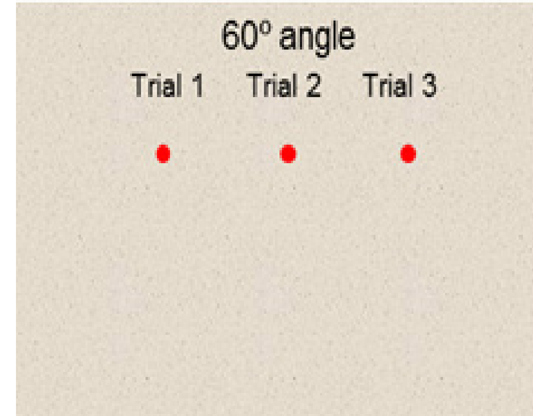


Figure 9. Cardstock setup. The red circles indicate the targeted area to hit with the crime scene blood.

- [ ] 3. Cut the cardstock such that the piece of paper fits within the area of the clipboard (e.g. no edges are overhanging).
- [ ] 4. Line up the bottom edge of the clipboard (the side opposite the clip) with the origin of the protractor. Use masking tape to secure it to the flat surface. This will create a "hinge" that allows the clipboard to rotate and create different angles with the flat surface. See Figure 10 for reference.
- [ ] 5. Remove the cap from the simulated sample and use a transfer pipette to obtain several drops of simulated blood sample.
- [ ] 6. Lift up the free edge of the clipboard (edge with the clip) until a 30° angle is created between the clipboard and the flat surface. This creates an angle of impact of 60°. See Figure 10 for reference.
- [ ] 7. While holding the clipboard in place with one hand, orient the transfer pipette containing the simulated blood vertically, approximately 30 cm above the center of the cardstock.
- [ ] 8. Gently squeeze one drop of simulated blood from the transfer pipette onto the cardstock under the Trial 1 label.

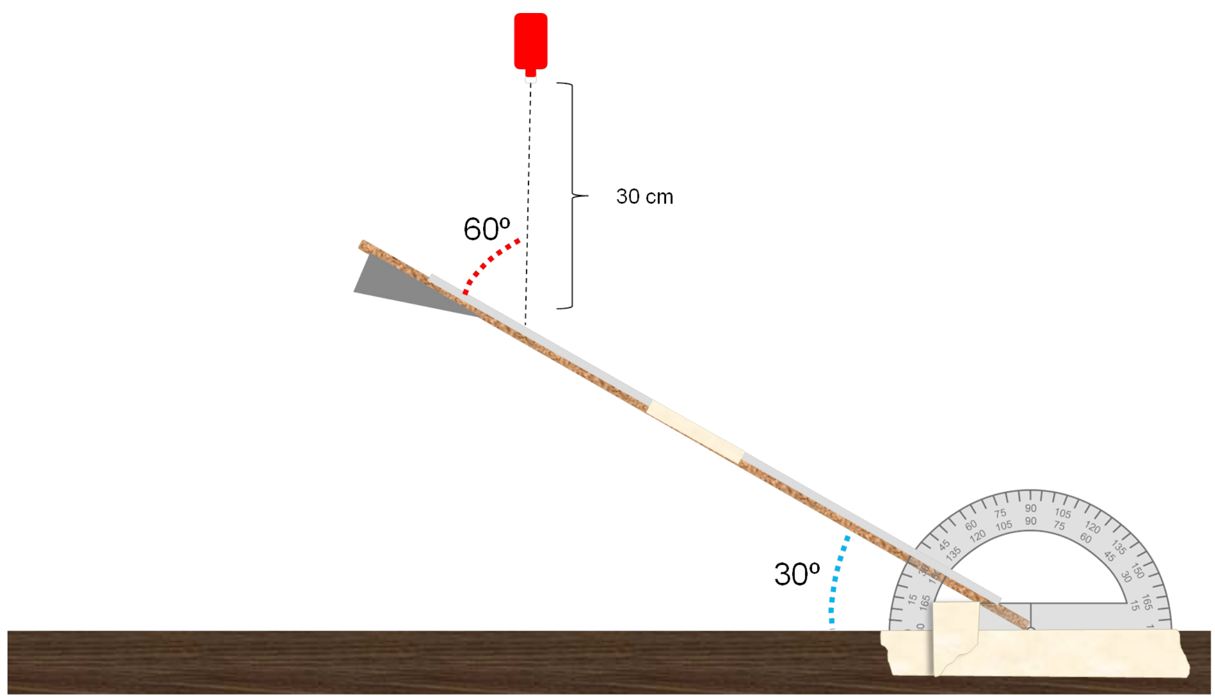


Figure 10. Experimental setup. The blue, dotted line indicates the angle at which you should hold the clipboard to the flat surface. The angle created by the clipboard and drop of blood is the angle of impact, denoted by a red, dotted line. The arrow indicates the protractor origin. The crime scene blood sample is oriented approximately 30 cm above the clipboard.

- [ ] 9. Repeat two more times, dropping the blood under the Trial 2 and Trial 3 labels on the cardstock. Performing three trials helps to visualize the natural variances which occur in bloodstains.
- [ ] 10. Hold the clipboard at a 30° angle (60° angle of impact) until the majority of the simulated blood has been absorbed by the paper. As it dries, examine the three bloodstains. Record observations about the shape and any visible bloodstain components in Table 4.
- [ ] 11. Use a ruler to measure the length and width of each bloodstain. Record the lengths and widths in Table 4.
- [ ] 12. Calculate the average length and width, and record the average length in Table 4.
- [ ] 13. Repeat the procedure using a 60° between the clipboard and the flat surface (30° angle of impact). Record your data in Table 5.
- [ ] 14. Use a camera to take a photograph of the cardstock after it is dry.

Note: You will need to download, scan, or print the photographs to be included with your lab report.

Table 4: Average Bloodstain Length and Width at 60 Degree Angle of Impact
(From a Height of 30 cm)

Bloodstain Dimension	Trial 1	Trial 2	Trial 3	Average	Observations
Length (mm)					
Width (mm)					

Table 5: Average Bloodstain Length and Width at 30 Degree Angle of Impact
(From a Height of 30 cm)

Bloodstain Dimension	Trial 1	Trial 2	Trial 3	Average	Observations
Length (mm)					
Width (mm)					