



ACTIVITY 8-1 A PRESUMPTIVE TEST FOR BLOOD

Objective:

By the end of this activity, you will be able to:
Use the Kastle-Meyer Presumptive Blood Test to determine if a given stain contains blood.

Scenario:

At dusk, a young man was seen riding his bicycle along the narrow, winding road that encircled a lake. On that same quiet road, an animated young couple was seen speeding around the many turns in the road. They were in a hurry to arrive at a friend's party at the lake. In his haste, the driver of the car lost control of the car while trying to swerve to avoid the young man on the bike. Unfortunately, the biker was hit from behind, causing him to be knocked off his bike and to fall down the hill toward the lake. The young driver of the car panicked and, without looking back, fled the scene of the crime. Although somewhat injured, the biker was able to provide a description of the car to the police.

Later that evening, the police arrived at the party on the lake to question the owner of the car. Although there was no visible blood on the car, the police did find a red stain on the car's bumper. At first the young driver said it was red paint or perhaps the blood from a squirrel he had struck the day before. Is the stain blood or paint? If it is blood, is it possible to distinguish human blood from animal blood? In this activity you will perform tests that confirm the presence of blood.

Time Required to Complete Activity: 45 minutes

Materials:

Activity Sheets for Activity 8-1
ketchup (1 oz or 10 ml)
blood from animal source (1 oz or 10 ml)
cloth or shirt with dime-sized blood stain
cloth or shirt with dime-sized ketchup stain
20 mL 3% hydrogen peroxide solution in dropper bottle
20 mL 95% ethyl alcohol in dropper bottle
20 mL distilled water in dropper bottle
20 mL 2% phenolphthalein solution in dropper bottle
biohazard container
latex or nitrile gloves

Safety Precautions:

Wear protective gloves.
Dispose of all samples in a biohazard container provided.
Assume that all red solutions are blood and handle according to safety regulations.

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Later that evening, the police arrived at the party on the lake to question the owner of the car. Although there was no visible blood on the car, the police did find a red stain on the car's bumper. At first the young driver said it was red paint or perhaps the blood from a squirrel he had struck the day before. Is the stain blood or paint? If it is blood, is it possible to distinguish human blood from animal blood? In this activity you will perform tests that confirm the presence of blood.

Time Required to Complete Activity:

45 minutes

Materials: Activity Sheets for Activity 8-1 ketchup (1 oz or 10 ml) blood from animal source (1 oz or 10 ml) cloth or shirt with dime-sized blood stain cloth or shirt with dime-sized ketchup stain 20 mL 3% hydrogen peroxide solution in dropper bottle 20 mL 95% ethyl alcohol in dropper bottle 20 mL distilled water in dropper bottle 20 mL 2% phenolphthalein solution in dropper bottle biohazard container latex or nitrile gloves

Safety Precautions: Wear protective gloves. Dispose of all samples in a biohazard container provided. Assume that all red solutions are blood and handle according to safety regulations.

All procedures should be done while wearing protective gloves. Do not contaminate any of the reagents. Be sure to drop the solutions onto the cotton swab without touching the cotton swab. Return the caps of all reagent bottles to the correct reagent bottle. Do not switch the caps from one bottle to the other.



Background:

Dried drops of red fluid found on a murder weapon, clothing, or automobile are noted, photographed, and analyzed. Did blood cause the red stain? If the red stain is not blood, then valuable time and money can be saved by not sending the red stain in to the laboratory for further testing. If it is determined that the red stain is blood, then further testing needs to be ordered to identify the source of the blood as human or animal.

A sample of the stain is tested using a presumptive chemical reagent. The Kastle-Meyer test is a catalytic color test that will produce a color change in the presence of blood. When phenolphthalein and hydrogen peroxide react with heme (iron) molecules in hemoglobin, the presence of blood is indicated by a pink color. A negative Kastle-Meyer test indicates the absence of blood. Because animal blood also contains heme molecules, it will also give a positive result. If animal blood is present and pertinent to the case, then additional tests can be performed to determine what type of animal blood is present.

Procedure:

1. Obtain a section of cloth that contains a known bloodstain (positive control). Before testing any unknown stains, it is important to check all reagents on a known sample of blood. If you do not get the expected results on blood, then you know that your reagents were malfunctioning and you need to replace them.
2. Wet a cotton swab with four drops of distilled water and gently rub the wet swab on the known bloodstain.
3. Drop two drops of ethyl alcohol onto the swab (don't allow the dropper to touch the swab).
4. Drop two drops of the phenolphthalein solution onto the swab (don't allow the dropper to touch the swab).
5. Drop two drops of the hydrogen peroxide onto the swab (don't allow the dropper to touch the swab).
6. A positive pink color will appear within seconds if blood is present. Record your results in Data Table 1. (This is your positive control demonstrating what happens when blood is present.)
7. Using a permanent marker, record your initials next to the stain that was just tested.
8. Dispose of all used cotton swabs and bloodstain samples into the Biohazard Waste container.
9. Using clean cotton swabs, repeat steps 1 to 8 using the shirt containing the ketchup stain. (This is your negative control since ketchup is not blood.)
10. Record the appearance of the ketchup test in Data Table 1.

All procedures should be done while wearing protective gloves. Do not contaminate any of the reagents. Be sure to drop the solutions onto the cotton swab without touching the

cotton swab. Return the caps of all reagent bottles to the correct reagent bottle. Do not switch the caps from one bottle to the other.

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wet swab on the known bloodstain. 3. Drop two drops of ethyl alcohol onto the swab (don't allow the drop-

per to touch the swab). 4. Drop two drops of the phenolphthalein solution onto the swab (don't

allow the dropper to touch the swab). 5. Drop two drops of the hydrogen peroxide onto the swab (don't allow

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was just tested. 8. Dispose of all used cotton swabs and bloodstain samples into the

Biohazard Waste container. 9. Using clean cotton swabs, repeat steps 1 to 8 using the shirt containing the ketchup stain. (This is your negative control since ketchup is not blood.) 10. Record the appearance of the ketchup test in Data Table 1.

11. With fresh cotton swabs, repeat steps 1 to 8 on the section of the shirt containing the unknown stain 1.
12. Using fresh cotton swabs, repeat steps 1 to 8 on the section of the shirt containing the unknown stain 2.

Data Table 1: Table of Test Results

Stains	Color Pink or not pink?	Describe Your Observations Is it blood or not blood?
Blood stain (positive control)		
Ketchup (negative control)		
Unknown 1		
Unknown 2		

Questions:

1. Complete the following Data Table indicating the role or function of each of the chemical reagents used in this experiment.

Data Table 2: The Role of Chemical Reagents in Blood Sample Analysis

Chemical	Function
1. Distilled water	
2. Ethyl alcohol	
3. Phenolphthalein	
4. Hydrogen peroxide	

2. Explain why you need to use both a positive and negative control before testing the unknown stains:
 - a. Positive control
 - b. Negative control
3. Should the pink color first be evident:
 - a. when applying the phenolphthalein to the cotton swab?
 - b. when applying the hydrogen peroxide to the cotton swab?
 Explain your answers.

11. With fresh cotton swabs, repeat steps 1 to 8 on the section of the shirt containing the unknown stain 1.
12. Using fresh cotton swabs, repeat steps 1 to 8 on

the section of
the shirt containing the unknown stain 2.

Data Table 1: Table of Test Results

Blood stain (positive control)

Ketchup (negative control)

Unknown 1

Unknown 2

Questions:


1. Complete the following Data Table indicating the role or function of each of the chemical reagents used in this experiment.

Data Table 2: The Role of Chemical Reagents in Blood Sample Analysis

1. Distilled water
2. Ethyl alcohol
3. Phenolphthalein
4. Hydrogen peroxide

2. Explain why you need to use both a positive and negative control before testing the unknown stains: a. Positive control b. Negative control 3. Should the pink color first be evident:

a. when applying the phenolphthalein to the cotton swab? b. when applying the hydrogen peroxide to the cotton swab? Explain your answers.

- 
4. If animal blood is different from human blood, how is it possible to get a positive reaction with the Kastle-Meyer test using dog blood?
 5. List two types of substances that might produce a false-positive test when performing the Kastle-Meyer test for the presence of blood.
 - a. Substance 1 _____
 - b. Substance 2 _____
 6. Why is it important to use a cotton swab when doing this test?
 7. Why aren't the reagents applied directly to the original bloodstain?
 8. Suppose that a red stain was found in a bathtub along with some bathwater. Would it be possible to detect the blood since it might have been diluted? Explain your answer.

Further Research:

1. Research the history of using this method as a presumptive blood test. Investigate the role of each of these scientists:
 - a. Louis-Jacques Thenard
 - b. Christian Freidrich Schonbein
 - c. Dr. Kastle
 - d. Dr. Meyer
2. Research how to distinguish dog blood from human blood.
3. Investigate the presumptive test for the presence of semen. Describe how this test is performed.
4. If a man has had a vasectomy, how will this affect the results of tests to detect semen? Explain.
5. Research each of the following cases. Explain the role of blood analysis in helping to solve the crime.
 - a. Peter Porco case (2006)
 - b. O.J. Simpson case (1994)

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ACTIVITY 8-2 BLOOD TYPING

Objectives:

By the end of this activity, you will be able to:

1. Perform a simulated blood test.
2. Describe the procedure for testing blood.
3. Analyze different blood tests to determine the blood type of a suspect.
4. Describe how blood test results are used to determine if an individual is linked to crime-scene blood evidence.
5. Describe agglutination of red blood cells.
6. Describe the protein-antibody reaction that occurs when typing blood.
7. Describe the role of blood typing in forensics.

Time Required to Complete Activity: 45 minutes

Materials:

(per group of three students)

Activity Sheets for Activity 8-2

Ward's Natural Science Kit 360021 or similar artificial blood-typing kit that includes:

- simulated human blood types
- antibodies for testing type A, B and Rh blood
- plastic blood testing slides each containing three wells
- plastic or paper cup labeled "Biological Waste"
- 10% bleach solution in spray bottle
- paper towels
- latex or nitrile gloves
- marking pen
- red pencil or marker
- toothpicks

Safety Precautions:

Handle artificial blood as if it were actual human blood to practice lab safety techniques. Any blood spills must be cleaned using a 10-percent bleach solution. Dispose of all waste in a container labeled "Biological Waste." Wear disposable gloves. If a student is allergic to latex, substitute a different type of glove, such as nitrile. Students should be careful to avoid spilling any bleach on themselves or on their clothing. Bleach can cause skin and eye irritation and can remove color from fabric immediately.

Background:

Blood typing is a common tool used to solve crimes. It may allow the examiner to match or exclude a suspect from a crime scene. To detect the presence of blood proteins, you will add specific antibodies to individual drops of blood and determine whether clumping (agglutination) occurs.

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1. Perform a simulated blood test. 2. Describe the procedure for testing blood. 3. Analyze different blood tests to determine the blood type of a suspect. 4. Describe how blood test results are used to determine if an individual is linked to crime-scene blood evidence. 5. Describe agglutination of red blood cells. 6. Describe the protein–antibody reaction that occurs when typing blood. 7. Describe the role of blood typing in forensics.

Time Required to Complete Activity:

45 minutes

Materials: (per group of three students) Activity Sheets for Activity 8-2 Ward’s Natural Science Kit 360021 or similar artificial blood-typing kit that includes:

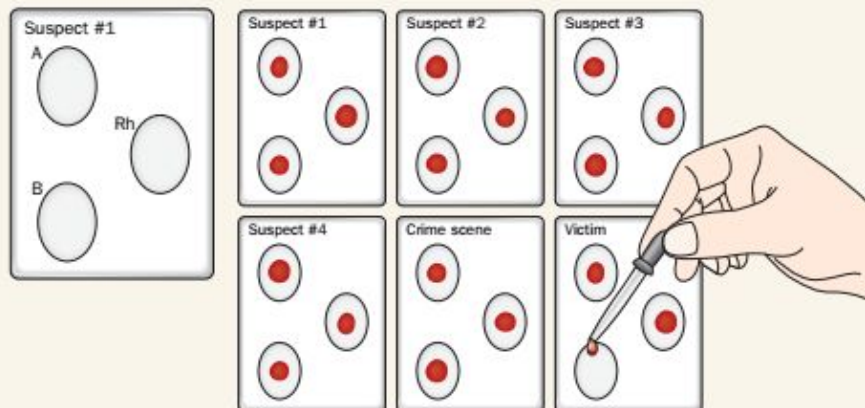
simulated human blood types antibodies for testing type A, B and Rh blood plastic blood testing slides each containing three wells plastic or paper cup labeled “Biological Waste” 10% bleach solution in spray bottle paper towels latex or nitrile gloves marking pen red pencil or marker toothpicks

Safety Precautions: Handle artificial blood as if it were actual human blood to practice lab safety techniques. Any blood spills must be cleaned using a 10-percent bleach solution. Dispose of all waste in a container labeled “Biological Waste.” Wear disposable gloves. If a student is allergic to latex, substitute a different type of glove, such as nitrile. Students should be careful to avoid spilling any bleach on themselves or on their clothing. Bleach can cause skin and eye irritation and can remove color from fabric immediately.

Background: Blood typing is a common tool used to solve crimes. It may allow the examiner to match or exclude a suspect from a crime scene. To detect the presence of blood proteins, you will add specific antibodies to individual drops of blood and determine whether clumping (agglutination) occurs.

Procedure:

1. Obtain six clean plastic three-well slides.
 2. Place the slides on a clean, white sheet of paper.
 3. Write the name of the blood donor in the top left-hand corner of the plastic slide. On the clean white paper, write the name of the blood donor above the slide.
 4. Put on your gloves for the rest of the procedure.
 5. Add two drops of blood to each of the three wells of the slide labeled Suspect 1.
 6. Repeat the process for each of the Suspects 2, 3, 4, Crime Scene, and the Victim slide.
 7. Add two drops of Anti-A serum (blue bottle) to each of the six wells labeled A.
 8. Add two drops of Anti-B serum (yellow bottle) to each of the six wells labeled B.
 9. Add two drops of Anti-Rh serum to each of the six wells labeled Rh.
 10. Gently rock each slide back and forth and up and down. (Do not let the blood from one well contaminate the blood from another well!) You can also use a toothpick to help mix the contents. A new toothpick must be used in each of the wells. You will need 18 toothpicks. Stir gently to avoid scratching the plastic wells.
 11. Wait five minutes to allow reactions to occur.
 12. Observe the blood samples. When using this artificial blood:
 - a. A cloudy, opaque, or goeey mixture is a positive reaction indicating the presence of a blood-type protein.
 - b. A clear mixture is a negative reaction indicating the absence of a blood-type protein.
- Note: Placing each slide on an overhead projector may help in examining the reactions.



Procedure:

1. Obtain six clean plastic three-well slides. 2. Place the slides on a clean, white sheet of paper.

3. Write the name of the blood donor in the top left-hand corner of the plastic slide. On the clean white paper, write the name of the blood donor above the slide. 4. Put on your gloves for the rest of the procedure. 5. Add two drops of blood to each of the three wells of the slide labeled Suspect 1. 6. Repeat the process for each of the Suspects 2, 3, 4, Crime Scene, and the Victim slide. 7. Add two drops of Anti-A serum (blue bottle) to each of the six wells labeled A. 8. Add two drops of Anti-B serum (yellow bottle) to each of the six wells labeled B. 9. Add two drops of Anti-Rh serum to each of the six wells labeled Rh. 10. Gently rock each slide back and forth and up and down. (Do not let the blood from one well contaminate the blood from another well!) You can also use a toothpick to help mix the contents. A new toothpick must be used in each of the wells. You will need 18 toothpicks. Stir gently to avoid scratching the plastic wells. 11. Wait five minutes to allow reactions to occur. 12. Observe the blood samples. When using this artificial blood:

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Suspect #1

A

B

Suspect #1 Suspect #2 Suspect #3

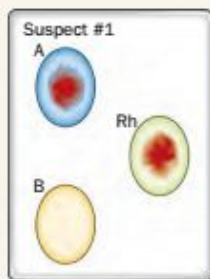
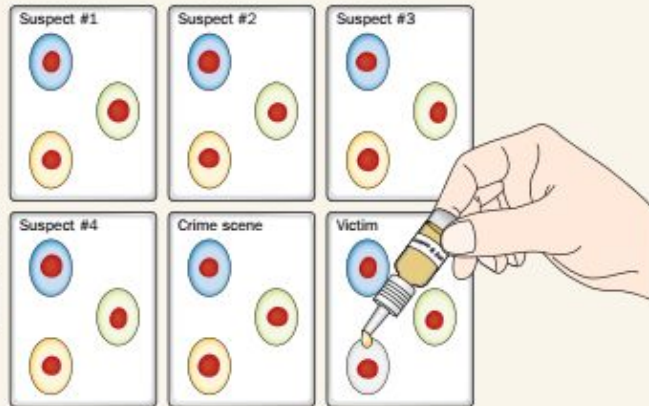
Rh

Suspect #4 Crime scene Victim

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13. Record your data on Figure 1. Include the suspect's name and blood type.



Type A+

14. For a positive reaction (indicating the presence of the blood-type protein), shade in the circle using a red-colored pencil or marker. If the blood test is negative, indicating the absence of blood-type protein, leave the circle empty.
15. Dispose of all typing materials in the "Biological Waste" container provided by your instructor.

Blood Results:

1. To indicate a positive reaction (protein present), use a red pencil or marker to shade in the well.
2. To indicate a negative reaction (no protein), leave the well blank.
3. Label each reaction slide with the blood type.

anti A serum

13. Record your data on Figure 1. Include the suspect's name and blood type.

Suspect #1 Suspect #2 Suspect #3

Suspect #4 Crime scene Victim

m

anti B serum

a

anti Rh serum

a

A B Rh

14. For a positive reaction (indicating the presence of the blood-type protein), shade in the circle using a red- colored pencil or marker. If the blood test is negative, indicating the absence of blood-type protein, leave the circle empty. 15. Dispose of all typing materials in the "Biological

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ru

e

s

B

i t n

Suspect #1

A

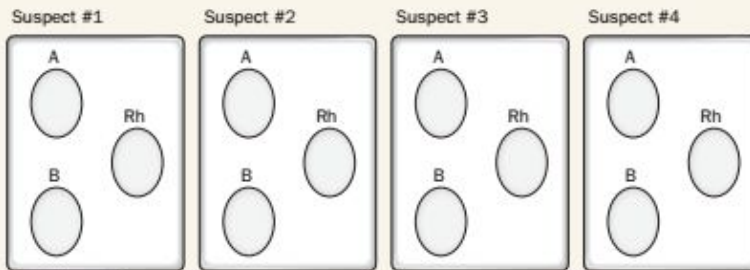
Rh

B

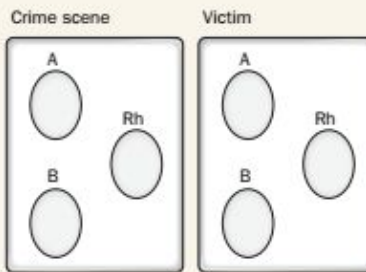
_____ Type A+

Blood Results: 1. To indicate a positive reaction (protein present), use a red pencil or

marker to shade in the well. 2. To indicate a negative reaction (no protein), leave the well blank. 3. Label each reaction slide with the blood type.



Blood type of:
 Suspect #1____ Suspect #2____ Suspect #3____ Suspect #4____



Crime scene____ Victim____

Questions:

1. Why is simulated (man-made) blood instead of real human blood used in this activity?
2. Explain why it is necessary to type the victim's blood when trying to determine if any of the blood found at the crime scene belongs to a particular suspect.
3. In this lab activity, how many different blood-type proteins were examined?
4. List all of the blood-type proteins examined in this activity.
5. Is it possible to exclude any of the suspects based on blood types? Explain your answer.
6. Based on your results, does the crime-scene blood match the blood type of any of the four suspects? Explain your answer.
7. If blood from one of the suspects matches the crime-scene blood, does that prove that the suspect is guilty? Explain your answer.
8. Poor laboratory techniques may lead to erroneous results that could impact the outcome of a trial. Describe some examples of poor laboratory techniques involved in a blood-typing analysis that might produce erroneous results.
9. Explain why identifying the blood type found in both the suspect and at a crime scene as AB- provides a higher degree of probability of a match than if the blood type found in the suspect and at the crime scene is O+.
10. Explain why white blood cells are not used in blood typing.

Suspect #1 Suspect #2 Suspect #3 Suspect #4

A

A

A

A

Rh

Rh

Rh

Rh

B

B

B

B

Blood type of: Suspect #1 _____ Suspect #2 _____ Suspect #3 _____ Suspect #4 _____

Crime scene Victim

A

A

Rh

Rh

B

B

Crime scene _____ Victim _____ Questions:

1. Why is simulated (man-made) blood instead of real human blood used

in this activity? 2. Explain why it is necessary to type the victim's blood when trying to determine if any of the blood found at the crime scene belongs to a particular suspect. 3. In this lab activity, how many different blood-type proteins were exam-

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Explain your answer. 6. Based on your results, does the crime-scene blood match the blood type of any of the four suspects? Explain your answer. 7. If blood from one of the suspects matches the crime-scene blood,

does that prove that the suspect is guilty? Explain your answer. 8. Poor laboratory techniques may lead to erroneous results that could impact the outcome of a trial. Describe some examples of poor laboratory techniques involved in a blood-typing analysis that might produce erroneous results. 9. Explain why identifying the blood type found in both the suspect and at a crime scene as AB- provides a higher degree of probability of a match than if the blood type found in the suspect and at the crime scene is O+. 10. Explain why white blood cells are not used in blood typing.



Further Research:

1. Research cases of innocent people who were convicted as a result of laboratory errors. (Refer to The Innocence Project, www.innocenceproject.org)
2. Research what other blood-type proteins are used in blood typing in addition to the A, B, and Rh proteins.
3. Research information on protein and antibodies. Explain why the red blood cells clump, or agglutinate, when mixed with certain antibodies.
4. Investigate the predominant blood type found in each ethnic group:
 - a. European
 - b. Asian
 - c. African

Math Connection:

1. Explain how the laws of probability are used in determining the probability that a particular person's blood will match the blood found at a crime scene.
2. If blood is found at a crime scene, describe what testing is done to determine if the blood is human blood or not human blood.
3. Research how it is possible to determine blood types from other body cells such as cheek cells or skin cells. (Hint: research secretors and non-secretors)
4. Paternity cases: If a man is the biological father of a child, he has a moral and a legal obligation to provide child support for at least the first 18 years of the child's life. Explain how blood typing can exonerate a man from paternity, but explain why it alone cannot determine paternity.
5. Investigate the role Rhesus monkeys played in the research involving the Rh protein.

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the Rh protein.

ACTIVITY 8-3

BLOOD-SPATTER ANALYSIS: EFFECT OF HEIGHT ON BLOOD DROPS



Scenario:

The police examined the blood spatter at a crime scene. From the size of the droplets, it appeared that the blood had passively dripped as the injured person walked across the floor. The person may have experienced a second injury, because two different patterns of blood spatter appeared halfway across the room. The second injury seemed to be from a source higher up on the person's body.

By examining the size and shape of blood spatter, forensic scientists are able to reconstruct a crime. A partial story of the crime emerges as the blood-spatter analysis starts to "tell the story."

In this activity, you will experiment with dropping artificial blood from different heights, and you will make observations about the effect of height on blood spatter.

Objectives:

By the end of this activity, you will be able to:

1. Prepare reference cards of blood spatter produced from varying heights.
2. Compare and contrast the blood spatter produced from different heights.
3. Distinguish between the blood spatter formed at the point of contact with satellite blood droplets.
4. Distinguish between satellite droplets and spike-like formations of blood droplets.
5. Form a hypothesis about the effect of height on the size and shape of blood-spatter droplets.

Time Required to Complete Activity:

Two 45-minute class periods (one period to do the blood drop, the second period to measure the blood drops)

Materials:

(per group of four students)
2 dropper bottles of simulated blood
12 five-by-eight-inch index cards
4 meter sticks
4 six-inch rulers showing cm or four calipers
newspapers

Safety Precautions:

Cover the floor in the work area with newspaper.
Simulated blood may stain clothing.

ACTIVITY 8-3 BLOOD-SPATTER ANALYSIS: EFFECT OF

HEIGHT ON BLOOD DROPS

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4. Distinguish between satellite droplets and spike-like formations of blood droplets.
5. Form a hypothesis about the effect of height on the size and shape of blood-spatter droplets.

Time Required to Complete Activity: Two 45-minute class periods (one period to do the blood drop, the second period to measure the blood drops)

Materials: (per group of four students) 2 dropper bottles of simulated blood 12 five-by-eight-inch index cards 4 meter sticks 4 six-inch rulers showing cm or four calipers newspapers

Safety Precautions: Cover the floor in the work area with newspaper. Simulated blood may stain clothing.

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