

# **Objectives**

After reading this chapter, you will understand:

- That an antibody and an antigen of different types will agglutinate, or clump, when mixed together.
- That blood evidence's significance depends on a characteristic's relative occurrence in the population.

## You will be able to:

- Determine whether a stain is blood.
- Determine whether a bloodstain is human or animal blood.
- Determine the blood type of a simulated bloodstain using the ABO/Rh system.
- Explore bloodstain patterns as a function of velocity, direction, and height of fall.
- Design and conduct scientific investigations.
- Use technology and mathematics to improve investigations and communications.
- Identify questions and concepts that guide scientific investigations.



"Out, damned spot! Out, I say! Here's the smell of the blood still: All the perfumes of Arabia will not Sweeten this little hand. Oh! Oh! Oh!"

-William Shakespeare's Lady Macbeth, in Macbeth



# Blood at the Scene of the Crime

#### **Teacher Note**

The TRCD for this chapter includes a PowerPoint presentation, which is an overview of the chapter. It can be used as introductory material or at the end as a review. Investigators often find blood at the scenes of violent crimes. They can use the location, distribution, and pattern of blood and bloodstains to help

### Reminder

*Class evidence* can be associated only with a group of items that share properties or characteristics. Individual evidence, like DNA, can be related to a single source; blood type cannot.

*Presumptive* screening tests presume the presence of a substance but are not confirmatory. A positive benzidine test may also be caused by hypochlorite bleach. A negative test, however, verifies the absence of the substance under test. reconstruct the crime. Blood's general biological characteristics can be used as *class evidence* if there is a transfer of blood between the victim and suspect or crime site. With proper conditions, time, and equipment, investigators can individualize blood evidence by comparing DNA. In this chapter's activities, you will (1) learn how to determine whether a stain is blood, (2) characterize simulated blood according to the ABO/Rh system, and (3) learn what information investigators can obtain from bloodstain patterns. *No human blood is used in any of the activities*.

#### **Teacher Note**

In this chapter, you deal with blood as evidence. You will not need human blood to complete the activities described. You can judiciously scale back the extent of the tests and still cover the important forensic concepts of false positives, statistical evaluation of the evidence, and trigonometric reconstruction from blood patterns. This chapter can be incorporated in a biology course where human and animal blood is microscopically examined, blood enzymes determined, and genetics explored, leading into the study of genes, chromosomes, and DNA.



## Laboratory Activity 11.1

#### **Advance Preparation**

Place a few drops or smudges of some or all of the following substances each on a 1-inchsquare piece of white cotton cloth:

red food coloring or fake blood

# **Detection of Blood**

When confronted by a stain that looks like blood at a crime scene, the investigator's first question should be, "Is it really blood?"

Observe the different samples of possible bloodstains on a piece of fabric. In your notebook record the appearance of each, what you think each stain is, and why. Include color, shape, smell, texture, and so on.

There are a number of *presumptive* chemical color tests that can be used to detect the presence of blood.



## Procedure

Do not write in your textbook. Take notes in your science notebook.

1. Using a commercial blood testing reagent: The heme in hemoglobin catalytically breaks down peroxides with the production of oxygen. Oxygen reacts with the benzidine product in the Hematest tablet or Hemastix test strip to turn it blue.

Press a piece of wet filter paper on the sample of stained cloth. Break a Hematest tablet into quarters. Put one portion of the tablet in the center of the transferred stain and add a drop of water *onto the tablet*. Make sure the water flows down the side of the tablet onto the stain. A blue-green ring spreading out on the filter paper from the tablet indicates that the spot is blood. You may be able to rinse and reuse the tablet for the next stain. A Hemastix strip can be rubbed on the wet stain; a green to blue color means that blood is present. Substances other than blood, such as dry bleach residues and some plastics, can cause similar results. Run a blank test on an unstained area of the filter paper. Make a data table for your results from the stained samples. Record your observations.

2. The Kastle-Meyer color test, like the Hematest above, is based on the catalytic breakdown of peroxides by hemoglobin. The contact of reduced phenolphthalein reagent and hydrogen peroxide with a bloodstain produces a deep pink color. If a pink color develops before adding hydrogen peroxide, you have a false positive—it is not blood. Unfortunately, there are a number of substances that give a *false positive*, such as potatoes and horseradish.

Press a piece of wet filter paper on a sample of stained cloth. Add a drop of K-M reagent to the paper where it touched the stain. Then add a drop of 3 percent hydrogen peroxide. Repeat this procedure for the other stains. You may also use a cotton swab instead of the filter paper. Repeat this test on an unstained area as a control. Record your results.

#### Advance Preparation, continued

tea/coffee ferric nitrate rust ketchup cherry furniture stain dark red or brown paint dirt red magic marker blood

You can get blood from a friendly veterinarian or simply from raw beef or liver. Code each sample and be sure they are dry before distributing them to the class. You may want each investigative group to perform blood tests on all the stained samples or

#### hemoglobin: the

oxygen carrier that gives red blood cells their color. It is composed of four globin (protein) chains, each with a heme group, as shown below:



#### Reminder

*False positive* is a test result that comes out positive when it is not.

# just provide a known sample of blood.

Hematest tablets or Hemastix strips are Bayer products used for determining occult blood in urine. The active chemical is a benzidine derivative, allegedly a carcinogen but perfectly harmless under these

### Laboratory Activity 11.1, *continued*

#### chemiluminescence:

the emission of light from a chemical reaction. Within an organism such as a firefly, it is called bioluminescence.

 $A + B \rightarrow [I]^* \rightarrow$ products + light [I]\* is an excited state

-

#### Advance Preparation, continued

circumstances of use. These materials should be available at a good pharmacy or on the Web. They are expensive, so subdivide them for each test. In the quantity used, they can be flushed down the drain.

For the Kastle-Meyer reagent, dissolve 1 to 2 g of phenolphthalein in 100 ml of 25 percent KOH solution prepared by adding 34 g KOH to 100 ml H20. Add 1 g of zinc dust. Boil this mixture until it turns light yellow or colorless; it may take an hour or so. Decant. Dilute with an equal volume of ethanol (95 percent or 100 percent). Methanol will work, but not as well. Store in a tightly capped bottle. The solution is not stable; if a pink color develops, just reduce it

3. The luminol test is a very sensitive indicator for dried and even washed blood. You can quickly spray a suspected area and make old bloodstains glow (chemiluminesce). The area must be very dark and your eyes conditioned to the darkness to see the luminescence. Certain metals (Cu, Fe, Co), bleach, and sometimes even plaster walls can cause false positives. Describe what you observed.

Other screening tests for blood may use leucomalachite green, which, as in the previous tests, forms a distinctive color when oxidized. Takayama and Teichmann tests involve adding a solution of potassium halides in acetic acid to bloodstains, forming characteristic crystals with hemoglobin, which are observed with a microscope.

# Human or Animal?

**precipitin test**: test that distinguishes between human and animal blood

**serum**: a liquid that separates from clotted blood

**antigens**: foreign substances in the body that are capable of causing disease. The presence of antigens triggers an immune response, usually the production of antibodies.

**agglutinate**: an allergic reaction where red blood cells clump together, usually in response to a particular antibody

#### Reminder

*antibodies:* proteins in the blood or secretory fluids that tag, destroy, or neutralize bacteria, viruses, or other harmful toxins, producing an immune response After an investigator has determined that a stain is blood, the next step is to learn whether it is human or animal blood. The **precipitin test**, the standard method, uses an animal **serum** that contains antibodies specific to human **antigens**; therefore, it reacts to **agglutinate** human blood. The test is so sensitive that the blood can be greatly diluted. Also, bloodstains many years old can test positive. You will learn more about antibodies in the next section.

#### Advance Preparation, continued

dust produces gases which can ignite spontaneously. Dispose in accordance with federal, state, and local requirements.

To create luminol solution, dissolve 0.1 g luminol and 0.5 g  $Na_2CO_3$  in 100 ml of water. Add 0.7 g of sodium perborate just before use. The final solution has a limited shelf life. Both reagents are available from Flinn Scientific.

You can have a spectacular demonstration of luminol detection by using a colored T-shirt or fabric that has been obviously stained with a nonblood material and also with animal blood. Cut it in half and wash one piece. Take both pieces into a darkened room, wait until your audience has become accustomed to the dark, and spray the fabric with luminol. (A bloody handprint is really awesome!)

again. Be sure the zinc residue is dry before storage or disposal. On contact with water, zinc

## Human versus Animal Blood

## Laboratory Activity 11.2

This activity will give you practice in testing bloodstains to determine whether their source is animal or human.



- 1. Add about 1 ml of simulated human **antiserum** to a small test tube.
- 2. Carefully pour the same amount of a diluted simulated blood sample down the sides of the test tube without disturbing the bottom layer.
- 3. There will be agglutination where the two liquids meet if the blood is human. Be patient; sometimes it takes a while.
- 4. Repeat with the simulated animal blood.
- 5. Record your observations. You can discard the contents of the test tube down the drain.

# Serology

The study of bodily fluids is called **serology**. Approximately one-twelfth of our body is blood, which is basically made up of cells suspended in a

liquid. The fluid part is called **plasma**, which makes up 55 percent of the blood. Plasma is composed of 90 percent water and 10 percent metabolites and waste, salts and ions (mostly Na<sup>+</sup>, Cl<sup>-</sup>, HCO<sub>3</sub><sup>-</sup>), and proteins. The solid portion of blood is made up of three principal types of cells.

**serology**: the laboratory study of body fluids using specific antigen and serum antibody reactions

**plasma**: the fluid portion of blood, obtained by centrifuging a sample of whole blood

#### **Advance Preparation**

For the human antiserum, dilute sodium silicate with water and a drop or two of yellow food coloring. Sodium silicate solution can be obtained, for example, from Flinn. The diluted blood sample is merely 1 M HCl colored very light red. Experiment until you get the desired white coagulation at the interface of the two liquids. The simulated animal blood is water colored very light red.

> When a rabbit is injected with human blood, antibodies are produced to fight the foreign invader. The rabbit serum containing these antibodies is used in the precipitin test. The "fight" results in *agglutination*, much like a chemical precipitation.

**antiserum**: human or animal serum containing antibodies that are specific for one or more antigens

#### Reminder

*metabolites:* organic molecules involved in the process of metabolism, which is defined as the sum of all chemical processes occurring in an organism. Metabolites can either form more complex molecules or result from the degradation of compounds.

erythrocytes: red blood cells leukocytes: white blood cells

**ABO**: a basic classification system for blood types based upon the reaction of antigens and antibodies

**Rh factor**: a basic blood factor, independent of ABO types

**blood factors**: a specific combination of antigens, enzymes, and proteins in the blood

**secretors**: people whose blood type antigens are also found in other body fluids



Karl Landsteiner

- 1. Red cells, or **erythrocytes**, which contain hemoglobin. They transport oxygen from the lungs to the cells and then carry carbon dioxide back to the lungs, where it is exhaled.
- **2.** White cells, or **leukocytes**, which are the primary cells of the immune system. They produce antibodies.
- **3.** Platelets, which start the clotting process by initiating the formation of fibrin to form a clot. Removing the solid clotting material leaves a pale yellow, watery fluid called serum.

In 1901 Austrian biologist Karl Landsteiner recognized that all human blood was not the same and worked out the **ABO** classification system to describe the differences. This was important because so many blood transfusions had resulted in immediate death of the patient for no apparent reason. In 1940, after he retired, Landsteiner discovered the rhesus factor (**Rh**) in blood. Now more than 100 different factors are known. Theoretically, no two people, except identical twins, have the same

combination of **blood factors**; practically, however, the complete identification is difficult, time-consuming, and expensive. Also, many factors break down as blood dries and ages. In the forensic science world, this type of categorizing is not yet practical as a means of individualization. DNA analysis offers individualization, but it also is time-consuming and expensive. Nevertheless, ABO and Rh blood characterization is important to forensic serology because it can be done on whole blood as well as dried bloodstains, quickly and without expensive apparatus. Additionally, about 80 percent of the population are **secretors**; that is, their blood type antigens are found in body fluids other than just blood.

On the surface of each red blood cell (RBC), millions of characteristic chemical structures called antigens can be found. These proteins are responsible for the different blood types. For the ABO system, there

RBC

Гуре О







Collecting possible bloodstain

are two types of antigens, A and B. Type A blood cells have A antigens, type B blood cells have B antigens, type AB blood cells have both A and B antigens, and type O blood cells have neither antigen (see Figure 11.1).

Some white blood cells manufacture proteins called antibodies, which are found in the serum. These antibodies are produced to attack invaders that enter the bloodstream, that is, antigens that do not belong in your system (such as snake venom,

bacteria, or someone else's blood). For example, when viruses responsible for mumps enter the blood, the body recognizes them as foreign and begins making antibodies that combine only with the specific antigens on the virus. White blood cells destroy the antibody-coated viruses. If someone is exposed to mumps for a second time, the existing antibodies prevent him or her from getting the illness again. This is the basis of vaccines.

A person with type A blood has A antigens on his or her red blood cells. That person will produce specific antibodies, B antibodies, in his or her serum to attack and destroy type B blood cells as they are introduced into the body, including type AB blood cells.



Testing stain with a Hemastix

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# CRUCIAL EVIDENCE IN X MURDER CASE WITHHELD

Charges were made that evidence was suppressed in a 1981 murder trial that ended with a death sentence.

According to a 1997 ruling by a federal district judge, "neither police nor the prosecution informed the defense team before trial that a Hemastix test had been done or that the chair back tested positive for the presence of blood."

—excerpt from sfbg.com/news story dated November 24, 1999 An allele is one member of a pair of genes occupying a specific spot on a chromosome that controls the same trait. For example, a pair of alleles may control the same trait such as eye color: One codes for blue eyes, another for brown eyes.



Table 11.1: ABO Blood Types					
Blood Type	Antigens on RBC	Antibodies in Serum			
А	А	В			
В	В	А			
AB	A and B	none			
0	none	A and B			

According to the National Institute of Health, as many as 1 in 12,000 units of blood cells are mistakenly given to the wrong recipient. Luckily, only about 1 in 100,000 people who receive a transfusion dies from this type of mistake.

Mothers must be concerned about the *Rh factor* during pregnancies for second and subsequent children, because agglutination can occur in an Rh + embryo. This is called *erythroblastosis fetalis* and can kill the fetus. The problem occurs in 200 to 300 births in the United States each year. Fortunately, it is now easy to counteract with a simple injection.



Likewise, a person with type B blood will have A antibodies in his or her serum and react against type A or AB blood. Type AB blood has both A and B antigens on the red blood cells (RBC) and, therefore, no antibodies in the serum. Type O blood, which has neither A nor B antigens, has both A and B antibodies in its serum. (See Figure 11.2.)

### See Table 11.1 for a summary.

Antibodies are bivalent, which means that they can attach to two antigen sites, thus causing agglutination or clumping, like cross-linking in polymers. If a person with type B blood gets a transfusion of type A blood, the antibodies in the B blood will attach to the donor's A red blood cells and cause agglutination, as shown in Figure 11.3.

The result can be fatal. This is why ABO typing is necessary before undergoing a blood transfusion. The B antibodies received from the donor are diluted by the

> larger volume of the recipient's blood, so the transfused antibodies do not cause a problem. Because antibodies are present in the blood plasma, plasma transfusions as well as whole blood transfusions must be matched to avoid causing an immune reaction. Table 11.2 shows safe blood donor and recipient combinations.

The *Rh factor* is another important means of classifying blood type for forensic serology. It is sometimes referred to as the D antigen. People who have it are called Rh positive (Rh+); those without it, Rh negative (Rh-). Approximately 85 percent of the population is Rh+.

The distribution of blood type varies with both location and race throughout the

Table 11.2: Donor Blood Factors							
Blood Type	Antigen	Antibody	Donor for	<b>Recipient for</b>			
А	А	В	A, AB	A, 0			
В	В	А	B, AB	В, О			
AB	A and B	none	AB	all			
0	none	A and B	all	0			

world (see www.bloodbook. com/world-abo.html). A typical ABO distribution in the United States is shown in Table 11.3.

Blood typing can be important in forensic science because it can show that two samples had different origins; this is how typing evidence can exclude suspects. Also, the distribution of blood

Table 11.3: Distribution of Blood Types				
Blood Type	Percentage			
Туре А	40			
Туре В	11			
Туре АВ	4			
Туре О	45			
Rh+	85			
Rh—	15			

types within a specific population can be used to statistically determine the probability of someone having a particular combination of blood types. For example, what is the probability of a person having type B+ blood?

 $\frac{11}{100} \times \frac{85}{100} = \frac{935}{10,000}$ , or about 1 out of 10 people

What is the probability of a person having type AB – blood?

Blood is tested for type, or "typed," by mixing a drop of the blood to be typed with a drop of serum containing known antibodies. Whether there is agglutination determines the blood type in the ABO system.

## ABO/Rh Blood Typing

A crime: Johnny Appleseed has been mugged, but he managed to slash his attacker with his paring knife. Fresh blood is collected at the scene. The police round up several suspects, but Appleseed cannot positively identify any of them. Blood samples from the crime scene and the suspects are delivered to you with the request for immediate typing because the police can't hold the suspects for very long. Appleseed's blood type has been taken from his blood donor card and is Type A-.

### **Teacher Note**

Probability of a person having AB-:  $\frac{4}{100} \times \frac{15}{100} = \frac{60}{10,000}$ or 6 out of 1,000 people

## Laboratory Activity 11.3

#### **Advance Preparation**

Make up a quart or more of simulated blood; you will need a lot for the blood spatter part of this chapter. Add to clear corn syrup a drop or more of red food coloring and a little blue coloring

#### Advance Preparation, continued

to deepen the red, or use Congo red. You won't need much simulated blood and sera for the typecasting activity, but make up enough for your ultimate crime scene also.

- To make simulated anti-A and anti-Rh sera: Dissolve 16 g of Ca(NO<sub>3</sub>)<sub>2</sub> in 100 ml of water and color it yellow with food dye. Divide in half for each serum.
- To make simulated anti-B serum: Dissolve 5 g Na<sub>2</sub>CO<sub>3</sub> in 50 ml of water and color it yellow with food dye.
- To make simulated blood for Suspect #1 (Type A+): Dissolve 5 g Na<sub>2</sub>CO<sub>3</sub> in 10 ml water, add to 50 ml of the simulated blood, and stir.
- To make simulated blood for Suspect #2 (Type B—): Dissolve
   9 g Ca(NO<sub>3</sub>)<sub>2</sub> in 10 ml water, add to 50 ml of the simulated blood, and stir.
- To make simulated blood for Suspect #3 (Type AB +): Dissolve 7 g Mg(SO<sub>4</sub>)<sub>2</sub> in 10 ml water, add to 50 ml of the simulated blood, and stir.
- To make simulated blood for Suspect #4 (Type 0 – ): Add 10 ml water to 50 ml of the simulated blood and stir.
- To make simulated blood from the crime scene: Pick one of the above.

The chemicals can all be obtained from any scientific supply house.  $MgSO_4$  can be purchased there, but it is a lot cheaper to buy it in a supermarket as Epsom salts.

The  $MgCO_3$  reaction is more of a coagulation with slight cloudiness, whereas the precipitates of  $Ca(CO_3)_2$ and  $CaSO_4$  show a distinct cloudiness. Adding too much serum dilutes the "blood drop" and obscures the coagulation and cloudiness. To be correct,

## Laboratory Activity 11.3, continued

Note: No blood or blood products will be used in this lab. This is merely a simulation of the reactions that happen in the typing of blood, where actual human blood and human antisera are used.



The crime scene

Mate	rials
For each lab group: • stereomicroscope or magnifying glass • glass slides • simulated blood from crime scene • simulated blood from four suspects	<ul> <li>simulated anti-A</li> <li>simulated anti-B</li> <li>simulated anti-Rh</li> <li>pipettes</li> <li>toothpicks or glass stirring rods</li> </ul>
<b>SAFETY ALERT!</b> Always wear goggles and an a	<b>CHEMICALS USED</b> pron when working in the labaratory
<b>SAFETY NOTE</b> Avoid inhalation, in chemicals.	ngestion, and skin contact with

### Procedure

- 1. Place a slide under the stereomicroscope and focus.
- 2. Add three drops of Suspect #1's blood to the slide, side by side, making sure that they do not touch each other.

### Laboratory Activity 11.3, *continued* Advance Preparation, *continued*

- 3. Add one drop of serum with A antibodies (labeled Anti-A) to the first blood drop.
- 4. Add one drop of serum with B antibodies (Anti-B) to the second blood drop.
- 5. Add one drop of the anti-Rh serum to the third blood drop.
- 6. Mix the cells and the sera with a toothpick, watching to see whether agglutination takes place. Be sure to clean the toothpick between drops to avoid contamination.



Agglutinated blood sample

Rather than a stereomicroscope, a good magnifying glass is quite adequate. Place each drop of blood on the microscope slide; placing it over an X on white paper beneath allows observation of developing cloudiness. Sometimes the reaction takes a few minutes; have patience!

7. Use Table 11.4 to determine the blood type and Rh factor of Suspect #1.

Table 11.4: Agglutination Reactions					
If Anti-A	And Anti-B	Then Blood Type Is			
+	—	А			
—	+	В			
+	+	AB			
—	-	0			
*+ means agglutination occurs — means no agglutination					

# controls should be run; that

is, known blood samples are analyzed and results compared to the suspects'.

The simulated blood never really dries; it just gets stickier. Soap and water clean it up quite easily.

#### **Teacher Note**

For an example of indirect typing, refer your students to Ragle, pp. 212–214.

#### **Teacher Note**

#### **Teacher Demonstration: Luminol**

The luminol spiral is a neat teacher demonstration of chemiluminescence. Make up two solutions, each in a 500-ml flask:

#### Solution A

2.0 g of sodium carbonate

0.1 g of luminol

12 g of sodium bicarbonate

0.25 g of ammonium

carbonate monohydrate

0.2 g of cupric sulfate pentahydrate

475 ml of distilled water

Solution B

30 ml of 3 percent hydrogen peroxide

475 ml of distilled water

You can make the luminol spiral from Tygon tubing, five or six clamps, a ring stand, and a 2-liter flask or other receptacle for waste. Assemble it as in the diagram.

Dim the lights and slowly pour the solutions simultaneously into the funnel. You will see a bright blue glow. The product may be disposed of down the sink in these quantities.



#### Answers to Analysis Questions

- **1.** any suspect who has the same blood type as the crime scene
- 2. None of them would work.
- **3.**  $0.15 \times 0.45 = 0.067$ , or 1 in 15

#### Laboratory Activity 11.3, *continued*

8. Repeat the procedure for Suspects #2, #3, and #4 and the sample from the crime scene.

The Rh factor is either present or not present; agglutination indicates its presence, and no reaction indicates its absence.

In your notebook fill out a table like Table 11.5 below:

Table 11.5: Blood Typing Data, Appleseed Case							
Suspect #	Anti-A	Blood Type					
1							
2							
3							
4							
Crime scene							

# **Analysis Questions**

- 1. According to your results, which suspects would you recommend the police investigate further?
- 2. On the way to the police department for questioning, Suspect #4 was in a severe car accident and needed a blood transfusion. Which of the other suspects could be donors for Suspect #4?
- 3. How many people in the general population would have the same blood type as Suspect #4?
- 4. Which blood type(s) could receive a transfusion of type AB blood?
- 5. Which blood type is known as the universal donor? Why can this blood type donate to all others?
- 6. Which blood type is known as the universal receiver? Why can this blood type receive all others safely?
- 7. If you have type B+ blood, what type can you receive?
- **4.** AB
- 5. type 0, because it has no antigens
- 6. type AB; other blood groups have either no antigens or compatible A and B antigens
- **7.** B, O

# **Testing Dried Blood**

Real blood that has been dried can still be typed. The red blood cells have ruptured, but the antigens and antibodies are still there. Investigators use indirect methods. In crime scenes you may encounter later, the standard typing procedure can be used.

When packaging and storing blood evidence, do not block out air; a sealed container may trap any moisture present and cause mold and mildew to form. Paper bags or envelopes may be used.

## 11.1: The Sam Sheppard Case

Early on the morning of July 4, 1954, police received a call from Dr. Sam Sheppard. He reported that his wife, Marilyn, was dead in their bedroom. He explained to police that, the night before, Marilyn had left him on the couch and gone to sleep in the twin bed next to Sam's. He fell asleep and awoke some time later,

believing he heard his wife calling his name.

Running upstairs, he saw a "form" struggling with something or someone, and was suddenly struck from behind. When he came to, he was lying on the floor. His wife Marilyn was covered with blood.

He checked for her pulse and found none. Sheppard heard a noise below, ran downstairs, and saw the back door open and "a form progressing rapidly toward the lake." He chased the person across the lawn and down the steps leading to the beach. He struggled with

a man, 6'3", middle-aged, with dark bushy hair and a white shirt. Sheppard was choked to unconsciousness.

Marilyn had 35 wounds to the head, and blood drenched the walls, door, and bed where she lay. Her face was almost unrecognizable.



CASE STUD





Sheppard was convicted of murder on very little evidence. No fingerprints were found, and there were no signs of a break-in and no witnesses. It was rumored that Sam and Marilyn were having marital troubles. He served ten years in prison before the U.S. Supreme Court ruled that his trial had been tainted.



The evidence was reexamined; blood spatters in the bedroom and blood drops throughout the house gave some of the most telling evidence. The expert, Dr. Paul Kirk, concluded that the killer could not have been Sam because the killer was left-handed. Dr. Sheppard was right-handed.

# **Blood Spatter Evidence**

# MURDER ★ CASE MAY HINGE ON STAINS

In the murder trial of a man charged with killing his pregnant wife, the most telling evidence may lie in the pattern of blood found in the tiny ridges on the cuff of the cotton shirt the man was wearing the day his wife died of a gunshot wound to the head. Blood-spatter evidence will be the star witness in the trial.

—abstracted from The Free Press, June 2, 2000 How can it be determined that the Sheppard killer was left-handed and not right-handed? What about the bloodstains indicated this?

The patterns left by falling, projected, or smeared blood can help the forensic investigator interpret and reconstruct what has happened at a crime scene. Blood spatter patterns are often used to prove or disprove the suspect's account of what happened. Careful observation of the position and shape of stain patterns can give information such as the direction of travel, the angle of impact, the position of origin, and the blood droplet's speed at the time of impact.

Key terms that can be used to describe bloodstains in subsequent exercises include:

*Angle of impact:* The acute angle formed between the direction of a blood drop and the plane of the surface it strikes

Arterial spurting (or gushing) pattern: Bloodstain pattern(s) resulting from blood exiting the body under pressure from a breached arteryBack spatter: Blood directed back toward the source of energy or force that caused the spatter





Arterial spurting

Contact stain-footwear

- *Blood spatter analysis:* A field of forensic science that deals with the physical properties of blood and the patterns produced under different conditions as a result of various forces applied to the source of blood
- *Bloodstain:* Evidence that liquid blood has come into contact with a surface
- *Cast-off pattern:* A bloodstain pattern created when blood is released or thrown from a moving blood-bearing object
- *Contact stain:* Blood deposited from direct contact between two surfaces, at least one of which is bloody
- *Direction of flight:* The trajectory of a blood drop, which can be established by its angle of impact and directionality angle
- *Directionality:* The direction the blood was traveling when it hit the target surface; investigators can usually establish directionality of a blood drop's flight from the geometric shape of its bloodstain
- *Directionality angle:* The angle between the long axis of a bloodstain and a predetermined line on the plane of the target surface that represents 0 degrees
- *Draw-back effect:* Blood in the barrel of a firearm that has been drawn backward into the muzzle
- *Drip pattern:* A bloodstain pattern that results from blood dripping into blood
- *Expirated blood:* Blood that is blown out of the nose, mouth, or a wound as a result of air pressure or air flow, which is the propelling force
- *Flight path*: The path of the blood drop as it moves through space, from the impact site to the target



Forward spatter

High-velocity impact spatter

- *Flow pattern:* A change in the shape and direction of a bloodstain due to the influence of gravity or movement of the object
- *Forward spatter*: Blood that travels in the same direction as the source of energy or force that caused the spatter
- *High-velocity impact spatter (HVIS):* A bloodstain pattern caused by a high-speed impact or force to a blood source such as that produced by gunshot; velocity may be 100 ft/sec, generally forming drops ≤1 mm
  - *Impact pattern:* Bloodstain pattern created when blood receives a blow or force resulting in the random dispersion of smaller drops of blood
  - *Impact site*: The point where force meets a blood source
  - *Low-velocity impact spatter (LVIS):* A bloodstain pattern caused by a low-speed impact or force to a blood source; velocity may be up to about 5 ft/sec with drop size of 4 to 6 mm
  - *Medium-velocity impact spatter (MVIS):* A bloodstain pattern caused by a medium-speed impact or force to a blood source; a beating or stabbing typically causes this type of spatter, and velocity may be about 25 ft/sec with a stain generally of 1 to 4 mm



Low-velocity impact spatter

- *Misting:* Blood that has been reduced to a fine spray as a result of the energy or force applied to it
- *Parent drop:* A drop of blood that casts off a wave or satellite spatter
- *Passive drop (bleeding):* Bloodstain drop(s) created or formed by the force of gravity acting alone
- *Point (area) of convergence:* The common point (area), on a two-dimensional surface, over which the directionality of several blood drops can be retraced



Medium-velocity impact spatter

- *Point (area) of origin*: The common point (area) in a three-dimensional space to which the trajectories of several blood drops can be retraced
- *Projected blood pattern*: A bloodstain pattern produced by blood released under pressure, such as arterial spurting, as opposed to by an impact
- *Satellite spatter:* Small droplets of blood distributed around a drop or pool of blood as a result of the blood hitting the target surface
- *Spatter:* Blood that has been dispersed as a result of force applied to its source; a spatter pattern will vary depending on the force that created it
- *Spine:* The pointed or elongated stains that radiate from the central area of a bloodstain





Passive drop



#### Wipe pattern

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- *Swipe pattern:* The transfer of blood from a moving source onto an unstained surface; the direction of travel may be determined by the feathered edge
- *Target:* The surface on which blood has been deposited
- *Transfer or contact pattern:* A bloodstain pattern created when a wet, bloody surface comes in contact with a second surface; a recognizable image of all or a portion of the original surface may be observed in the pattern
- *Void:* An absence of stains in an otherwise continuous bloodstain pattern, like a reverse shadow

*Wipe pattern:* A bloodstain pattern created when an object moves through an existing stain, removing it or changing its appearance

#### **Removing Real Bloodstains**

Soak or rub washable fabric in cold water until the stain is almost gone, then wash with warm water and a detergent. For old or stubborn stains, apply a few drops of ammonia and wash again with detergent. On nonwashable fabric, sponge the stain with cold or lukewarm water. Sponging with a little hydrogen peroxide usually will remove any final traces of the stain. If not, use chlorine bleach, depending on the material. Bloodstains that have been set by heat are very difficult to remove, but on cottons and linens a warm solution of trisodium phosphate usually will remove such stains.

Laboratory	Blood Pattern Analysis			
Activity 11.4				
	Develop a procedure to analyze and interpret bloodstains. Be sure to make accurate observations, measurements, and sketches and to record all data. The questions you want to answer include:			
	<ul> <li>What effect does release height have on the pattern left by drops of blood?</li> </ul>			
	<ul> <li>What effect does velocity have on impact patterns?</li> </ul>			
	<ul> <li>How does the angle of impact affect the appearance of drops of blood?</li> </ul>			
<ul> <li>How can you determine the direction of travel from a blood patter</li> </ul>				
How can you determine whether an assailant is right- or left-hand				
	<ul> <li>How can you determine the origin of a blood spatter?</li> </ul>			
	Remember, you may be called upon to justify your methods and interpretation in court, subject to cross-examination!			
<b>322</b> Chapter 11				



# Procedure

- 1. *Height:* Work out a method to study the relationship of drop shape and size to the height of origin.
- 2. Velocity: The density of blood is 1.06 g/cc, and its viscosity is 6 times that of water. Its average drop size, because of surface tension and viscosity, is 0.05 ml for a free fall. As with all objects falling freely, a drop of blood accelerates because of gravity, 32 ft/sec<sup>2</sup> (9.8 m/sec<sup>2</sup>). Any object will fall at this rate until it reaches its terminal velocity, which is a direct function of drop size. An average-sized drop reaches the terminal velocity of 25 ft/sec after a fall of 4 feet, so drop size should not change above that. The majority of high-velocity droplets, which tend to be less than 1 mm, usually travel no more than 46 inches in a horizontal direction. Check it out.
- 3. *Angle of impact:* How does the angle of impact of a drop of blood influence its shape?
- 4. *Direction of travel:* By now, you should realize that the distorted end of the drop of blood points in the direction of travel. Verify this by flinging drops of blood off a stirring rod or your finger and note the shape of the stain relative to the direction of travel (see Figure 11.4 on page 324).

This teacher image is provided on the Teacher Resource CD as Blackline Master 11.1 for your convenience.

#### **Advance Preparation**

Make partial blood patterns from different objects, such as a hammer, screwdriver, crowbar, tire iron, knife, letter opener, fork, or brick, and ask students to work out what the object was.

#### **Procedure Note**

This activity is inquiry-based, so the student is given less information than in some previous activities. You can use the teacher's material to guide the class through the steps required to answer the questions posed.

You may want to lead a discussion on the types of information that can be gleaned from the study of blood patterns, for example, the type of weapon used; how many gunshots, stabs, or blows were inflicted; the sequence of injuries; the position of the victim and assailant; whether they were moving during the attack; whether the assailant was right- or left-handed; the degree of force used by the perpetrator; whether there might be blood on the assailant; and whether the victim was moved after the attack. This is a good place to show at least the bloodstain portions of "The Killer's Trail," a 60-minute *Nova* videotape about the Sam Sheppard case.

Use a roll of freezer, butcher, or kraft paper spread out and taped to the floor and walls of the working area. This will protect those surfaces and allow samples of the stains to be cut out.

Use the basic synthetic blood substitute, corn syrup with food coloring, but add 1 Tbsp (15 ml) of dishwasher detergent to each quart (or liter) to allow easy cleanup. The drops will not dry but will remain sticky.

#### Procedure Note, *continued*

You can very effectively use a digital camera to record information.

Student investigation teams should use a Beral or Pasteur pipette to drop the blood substitute vertically from different heights onto a smooth paper surface. They should describe the shape of the drop and its size and note relationships and trends. Perhaps the fastest group could investigate drop shape as a function of surface texture.

Students should place white paper on cardboard and prop it up at an angle. Then, using a pipette, they can direct a drop of blood onto the paper from a fixed height. They should record the shape of the drop as a function of the angle of impact, as measured with a protractor. It is important that they note that the length-to-width ratio increases with decreasing impact angle. You may want to prepare handouts of the figure to guide the students.

### Laboratory Activity 11.4, *continued*



The drop shape can be described trigonometrically by the following relationship (see Figure 11.5):

impact angle, 
$$i = \arcsin\left(\frac{\text{drop width, } w}{\text{drop length, } l}\right)$$

The arc sin can be calculated with a handheld calculator or found in a table such as Table 11.6 on page 327.



Prepare a table such as that below, using your results:

		Impact Angle (°) Calculated Measured		
Width, <i>w</i>	Length, /			

5. Origin of blood source: If there is more than one drop of blood, then you can locate their origin by triangulation. You want to look at drops with a common origin. Try to make some kind of collision, maybe by clapping hands soaked in simulated blood, in front of white paper. Stretch a string through the long axis of each well-formed drop and locate the area of convergence (see Figure 11.6).





Next, place a ring stand or some standing unit on the area of convergence. Then determine the angle of impact for each drop using the trig methods, measuring the length and width of each drop. Write the calculated angle next to each drop. Using string, masking tape, and a protractor, raise the string to the calculated angle and attach it to the ring stand. Convergence indicates location of the point of origin of the blood (see Figure 11.7 on page 326).

6. *Left- or right-handedness:* A right-handed student, standing near a wall covered with paper, should dip a plastic knife, tongue depressor, or other similar object in a container of simulated blood, then rapidly pull back the arm. The spattered blood follows an arc, with the tail of each drop pointing up for an overhead thrust (see Figure 11.8 on page 326).



Now, a left-handed student should try it. How is the cast-off pattern different? Is it important to know where the victim was? Predict the pattern resulting from multiple stabs, then try it.

7. *Simulation of types of stains:* Try to simulate different types of blood spatter. Arterial spatter can be simulated using a syringe. To make an impact pattern, try cupping some blood in the palm of your hand, holding it as nearly vertical as possible, and whacking it with your other hand toward a vertical target. Use an aerosol aspirator to make an expirated blood stain, as may be produced by a bullet through the head.

An assailant is cut and drips blood as he or she walks or runs away. Simulate this on a horizontal target using an eyedropper. Can you estimate the height of the wound, as well as the length of stride, working out whether the subject was walking or running? Can the length of stride be used to estimate the height of the subject?

7181 7420 7420 7536 7649 7976 7976 8080 8181 8281 8377 8471 8563 8652 8738 8821 8902 8980 9056 .9128 .9198 .9265 .9330 .9330 .9449 .9505 .9558 .9608 .9655 .9699 .9740 .9778 .9813 .9813 .9874 .9900 .9923 .9943 .9960 .9974 .9985 .9993 .9998 .9998 6.9 .7749 .7859 .7965 .8070 .8171 .7169 .7290 .7408 .7524 .7538 8271 8368 8462 8554 8643 .8729 .8813 .8894 .8973 .9048 .9121 .9191 .9259 .9323 .9385 .9444 .9500 .9553 .9603 .9650 .9694 .9736 .9774 .9810 .9871 .9898 .9921 .9942 .9973 .9984 .9993 1.000 0.8 7157 7278 7396 7513 7627 7738 7955 8059 8161 8261 8358 8453 8545 8634 8721 8805 88965 9965 9041 9114 9184 9252 9317 9379 9438 9494 9548 9598 9646 9770 9770 9839 9869 9895 9919 9940 .9972 .9983 .9992 .9997 1.000 0.7 .9107 .9178 .9245 .9311 .9373 7145 7727 7837 7944 8049 8151 8251 8348 8443 8536 8625 .8712 .8796 .8878 .8858 .8957 9033 .9432 .9489 .9542 .9593 .9641 17992 19992 19992 19997 1,000 9686 9767 9803 9836 .9866 .9893 .9917 .9938 .9938 9.0 .7716 .7826 .7934 .8039 .8141 .8241 .8339 .8434 .8526 .8616 .8704 .8788 .8870 .8949 .9026 9969 9990 1866 1990 1.000 7133 7373 7373 7373 7373 7504 9171 9239 9239 9304 9367 .9426 .9483 .9537 .9588 .9588 .9681 9724 9763 9799 9799 .9863 .9890 .9914 .9936 0.5 .7120 .7242 .7361 .7478 .7593 .7705 .7815 .7923 .8028 .8131 8231 8425 8517 8607 .8695 .8780 .8862 .8942 .9018 9421 9478 9532 9583 9632 9720 9759 9796 9829 .9860 .9888 .9912 .9934 8966<sup>-</sup> 9666<sup>-</sup> 9666<sup>-</sup> .9092 9164 9232 9238 9361 0.4 .7694 .7804 .7912 .8018 .8121 .7108 .7230 .7349 .7466 .7581 .8221 .8320 .8415 .8508 .8508 .8686 .8771 .8854 .8934 .8934 9085 9157 9225 9291 9354 9415 9472 9527 9528 9578 .9673 .9715 .9755 .9792 .9792 9857 9885 9910 9932 9951 9966 9966 9666 9666 0.3 .7096 .7218 .7337 .7455 .7455 .8211 .8310 .8406 .8499 .8499 .8678 .8763 .8846 .8926 .9003 .9078 .9150 .9219 .9285 .9285 .9409 .9466 .9521 .9573 .9622 .7683 .7793 .7902 .8007 .8111 .9668 9711 9751 9753 9823 .9854 .9882 .9907 .9930 .9965 9978 99955 9995 0.2 .7083 .7206 .7443 .7443 .7559 8202 8300 8490 8581 .8069 .8755 .8838 .8918 .8918 9070 9143 9212 9278 9342 .9403 .9461 .9516 .9568 .9568 .9664 .9707 .9748 .9785 .9785 9851 9880 9905 9928 9947 9963 77999 77999 779999 7672.77891.77891.77997 1.0 .8192 .8290 .8387 .8480 .8480 .8572 .8660 .8746 .8829 .8910 .8988 .9962 .9976 .9986 .9994 7193 7314 7314 7431 7547 77771 177711 177711 17771 9063 9135 9205 9272 9336 .9397 .9455 .9511 .9563 .9613 .9659 .9703 .9744 .9781 .9848 .9877 .9903 .9925 .9925 0 Angle 55556 383383 353350 48474<sup>5</sup> 19 13 13 13 13 13 19 0 88888 69 62 69 882 882

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0.3	.0052 .0227 .0401 .0576	.0924 .1097 .1271 .1444 .1616	.1788 .1959 .2130 .2300 .2470	2639 2807 2974 3140 3305	3469 3633 3633 3955 3955	.4274 .4431 .4586 .4741 .4741	.5045 .5195 .5344 .5490 .5490	5779 5920 16060 16198 16334	.6468 .6600 .6730 .6858 .6858
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Table 11.6: Sine Table

### 11.2: The Ludwig Tessnow Case

In 1901 two boys failed to return home after a day of playing outside in Rugen, Germany. A search the next day turned up the grisly remains of the boys scattered over a field.

A man named Ludwig Tessnow had been seen talking to the boys on the day they disappeared. A subsequent search of his home found freshly laundered clothes with suspicious stains that Tessnow said were wood dyes used in his carpentry work. Strangely, a few weeks before the boys' murder, Tessnow had been caught hacking a neighbor's sheep apart.

As word of this crime spread, it was learned that three years earlier, in a different area of northern Germany, two girls had been killed in the same horrible way. Ludwig Tessnow had been questioned then, too; he had also claimed at that time that the stains on his clothes were wood dyes.

Police had a suspect but no hard evidence. Then they heard about a young biologist who had developed a technique to distinguish blood from other substances, as well as human blood from animal blood. Tessnow's clothing was tested by Professor Uhlenhuth, who found wood dye, sheep blood, and 17 traces of human blood.

This was one of the first cases to use the precipitin test. Ludwig Tessnow was charged with murder, tried, convicted, and executed.



## De La Cruz v. Johnson, 134 F.3d 299 (5th Cir. (Tex.) 1998)

Jose De La Cruz stabbed Domingo Rosas to death. The Friday night before the murder, De La Cruz was a guest in Rosas's home and played a drinking game called "quarters" with Rosas until early Saturday morning. After leaving Rosas's home, De La Cruz returned and killed Rosas in order to steal Rosas's television, VCR, and stereo, a theft that yielded him approximately \$80. De La Cruz's clothing and the knife recovered from his ditched car were both stained with type-A blood, the same blood type as Rosas's. Moreover, blood spatters on De La Cruz's pants indicated that the wearer had forcefully stabbed a seated victim. The deceased victim had been paralyzed and confined to a chair. The defendant was convicted of murder partly because blood spatter on his pants indicated that he had forcibly stabbed the victim who had a blood type corresponding to the one on his pants.

# **Checkpoint Questions**

Answer the following questions. Keep the answers in your notebook, to be turned in to your teacher at the end of the unit.

- 1. What three questions should the investigator answer when examining an apparent dried bloodstain?
- 2. Define a presumptive test.
- 3. What property of blood is used in most presumptive tests?
- 4. A forensic veterinarian may be asked to identify a specific animal's blood. How would this be done?
- 5. What is a secretor?
- 6. Define serology. How is it used in forensic investigations?
- 7. How much blood is there in the average adult human?
- 8. What test can determine whether blood is human or animal? What is the basis for this test? Why is the serum used in this test called human antiserum?
- 9. What are the four major blood types found in humans?
- 10. What happens if a person with type AB blood is given a transfusion of type A blood? Explain.
- 11. To whom can a person with type B blood donate blood and from whom can that person receive blood?

#### Answers

- Is it blood? If so, animal or human? What is the blood type?
- **2.** a screening test that presumes the presence of a substance
- 3. catalytic decomposition to oxygen
- **4.** a precipitin test using serum with antibodies specific to the test animal
- A person whose blood antigens are found in other body fluids. About 80 percent of the population are secretors.
- 6. The study of body fluids using antigen-antibody reactions. Serology can be used to exclude suspects.
- 7. about 5 quarts (students may have to look this up or ask their biology teacher)
- **8.** The precipitin test. Specific antibodies in an animal serum react to human antigens.
- 9. A, B, AB, O
- **10.** There will be no adverse reaction because there are no antibodies to attack antigens.
- **11.** He or she can donate to someone with B or AB blood and receive blood from someone with B or O.

#### Answers, continued

- **12.** Not with serology, but with DNA fingerprinting. Many of the blood factors begin to degrade immediately.
- **13.**  $0.04 \times 0.15 = 0.0060$ , or 1 in 167
- **14.**  $0.11 \times 0.27 \times 0.15 = 0.0045$ , or 1 in 222 persons. Not sufficient odds unless the field of suspects has been narrowed down by other evidence.
- A, 19°; B, 26°; C, 11°; D, 62°; E, 49°; F, 37°. Refer to Blackline Masters 11.2 and 11.3 on the TRCD for copies of illustrations.
- **16.** fingerprints (1880); bite marks (1850); blood (1863); bullet comparison (1835); blood spatter (1514)

Blood typing can be applied to a host of enzymes and proteins that perform specific functions in the body. Their presence or absence varies within the population. More than 150 serum proteins and 250 cellular enzymes have been isolated. Therefore, investigators can use blood typing as individual evidence; however, it is not practical because of the time and techniques involved. Also, most factors degrade with time. Rather, ABO/Rh typing, and often MNS typing, are used as exclusionary tests in forensic science and paternity testing. The typical population in the United States shows an MNS distribution of M = 30 percent, N = 27 percent, S = 48 percent.

- 12. Can a bloodstain be used for individualization?
- **13.** What is the probability of an AB and Rh- blood type combination?
- 14. If a bloodstain found at the scene of a crime is found to be B, N, or Rh-, what is the probability that a suspect would have this combination of antigens? Is this good enough to convince a jury?
- 15. Calculate the angle of impact for the bloodstains below:



16. The precipitin test for human blood was developed in 1901 and is still in use today in forensic investigations, especially those involving the specific identification of animal blood in cases involving poaching and possessing illegal wild game. Name two other forensic tests developed over 100 years ago that are still used in the same way today.



#### Answers, continued

- **17.** A is an arterial spurt; B, high-velocity impact spatter; C, a drop on an earlier drop
- 18. A is a void, possibly the stem of a floor lamp;B, a wipe pattern caused by a boot dragged across drops of fresh blood; C, blood dripping from an object moving and then stopping;D, multiple impacts; E, two overhand thrusts by a right-handed person.

#### Answers, continued

- 19. A is from a brick, could also be a 2 × 4;
  B, crowbar or pry bar; C, hammer; D, fingerprint;
  E, razor blade; F, monkey wrench; G, shirtsleeve with button; H, a fork; I, a knife; J, scissors;
  K, corkscrew; L, high heel from a woman's shoe.
  Consider having students make their own transfer patterns with simulated blood or black waterbased paint.
- **19.** Sometimes a transfer pattern can indicate the weapon used in an assault. Guess what made the following:



# **Additional Projects**

- Investigate the Shroud of Turin from the aspect of whether the imprints were actual blood. What scientific tests were performed? Is there a controversy? What do you think? (There are lots of websites for this topic; a good one is www. shroudstory.com.)
- **2.** How are blood types passed on to offspring? How does this relate to paternity issues?
- **3.** How could the sensitivity of luminol, Kastle-Meyer, and Hemastix tests for blood be determined?
- **4.** What is the effect of age, sunlight, freezing, and heat on blood with respect to the standard presumptive tests?

#### Answers

#### 1. See Web.

- See Web; for example, http://www.biology.arizona. edu/Human\_Bio/problem\_sets/blood\_types/ markers.html.
- **3.** progressive dilution
- 4. Web search



# References

## **Books and Articles**

- Evans, C. *The Casebook of Forensic Detection*. New York: John Wiley, 1996. One hundred real cases.
- Gottfried, S., and M. Sedotti, "Blood Markers," from Mystery Matters in *Chem Matters*, April 1992, pp. 4–6.
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- Ragle, L. Chapter 6 in *Crime Scene*. New York: Avon Books, 1995.

## **Films and Videos**

- "The House That Roared," *Forensic Files*, Court TV, January 11, 2001. Available from Films for the Humanities & Sciences, 24 min (www.films. com). Use of luminol.
- *Nova*, "The Killer's Trail," videotape on the Sam Sheppard murder case with blood spatter evidence. Available from http://main.wgbh.org/ wgbh/shop/products/wg2613.html.
- A&E, "Dead Reckoning: Blood Spatter." Videotape includes the Sam Sheppard murder case with blood spatter evidence, AAE73535. Available from www.aetv.com.

## Websites

- www.physics.carleton.ca/~carter; computerized blood spatter analysis
- http://anthro.palomar.edu/blood/default.htm; excellent account of blood components, typing, and the like
- www.crimelibrary.com; many good stories of crimes and background material. See, for example, Sam Sheppard, O. J. Simpson, and Jeffrey MacDonald.
- www.bloodspatter.com/BPATutorial.htm; good tutorial on bloodstains