

Blood Identification and Typing

A common form of physical evidence found at the scene of crimes involving physical violence is that of blood. Blood may be present in the form of pools, splatters, or stains. Blood should be looked for in burglaries as well. It is difficult for a burglar to break a window or force a door without getting scratched and thereby leaving a small amount of blood at the scene. The first analysis performed on this evidence is the determination of whether or not the stains are blood, and if so, whether they are of human origin. If of nonhuman origin, it may be useful to know from what animal the blood originates. If the stains are of human origin, the analysis is then extended to the determination of blood group and other blood factors that can associate the blood with a particular person.

The fact that there is such a thing as a blood type was discovered in 1900 by Karl Landsteiner, who found that blood from one person would not always mix freely with blood from another person, but would sometimes clump or **agglutinate**.

He identified four types, which he named O, A, B, and AB. It has since been found that approximately 43% of the population has O-type blood, 42% A, 12% B, and 3% AB. If a drop of blood is found at the scene of the crime, identification of its type may serve to screen out several suspects. Leone Lattes was the first to make use of blood groups in the courts of Italy in 1916. It was used in England in 1922 and in the United States in the early 1930s.

In 1927, another system was discovered, the MN system. In this system approximately 30% of the population is M, 22% N, and 48% MN.

In 1940, Alexander Weiner, working with rhesus monkeys, discovered a third system in which 85% of the population had a factor called Rh+; the 15% who did not have the factor were called Rh-. Since that time, other Rh factors have been discovered and divided into six groups, D, d, C, c, E, e. This allows for a further 27 combinations. However, at the time of this writing, we do not yet have an antiserum for d, so there are only 18 usable combinations.

In recent times, many other substances have been found in blood which are becoming increasingly important for the individualization of blood stains; several of these are listed below.

1. Phosphoglucomutase (PGM): PGM-1, 58%; PGM-2, 6%; PCM 2-1, 36%
2. Adenylate kinase (AK): AK 1, 93%; AK 2-1, 7%
3. Adenosine deaminase (ADA): 1, 90%; 2-1, 10%; 2, 0.2%
4. Glucose-6-phosphate dehydrogenase (G-6-PD): B, 62%; BA, 25%; A, 12%
5. 6-phosphogluconate dehydrogenase (6-PGD): A, 92%; AC, 8%; C, 0.2%
6. Erythrocytic acid phosphate (MAP): A, 13%; B, 35%; C, 0.2%; BA, 43%; CA, 3%
7. Esterase D (EsD): 1, 79%; 2-1, 19%; 2, 2%
8. Polymorphic proteins—Group Specific Component (Gc) and haptoglobins (H_p): H_p 1, 14%; H_p 2-1, 53%; H_p 2, 32%

Let us see what value these factors can have for the forensic chemist. Before 1900, blood was just that—blood: and other than the fact that you could tell from an analysis of the blood spatter design that the attacker was wounded and perhaps where, blood could not be used to narrow the list of suspects. That has now changed dramatically.

Procedure

1. Examine the suspected bloodstains. To ^{four} ~~one~~ ^{each} of the stains, add a few drops of hydrogen peroxide. Watch the stain for several seconds and record the results of this test in the box. CAUTION: Handle all chemicals carefully.
2. Collect some of the suspected bloodstain using the scraping technique, or the white thread technique. Place the collected stain on a glass slide.
3. In the 200-ml beaker, mix 0.1 gram of potassium chloride, 0.1 gram of potassium bromide, 0.1 gram of potassium iodide, and 100 milliliter of glacial acetic acid. Stir gently with the stirring rod. CAUTION: Handle all chemicals carefully.
4. Place a drop of the suspected bloodstain on a microscope slide, and add a cover slip.
5. Let one drop of the mixture of potassium salts and acetic acid you just prepared flow under the cover slip and contact the brown stain.
or scraping
6. Carefully hold the slide with tongs and wave the slide slowly over the candle or Bunsen burner flame. Be careful not to let the slide get very hot. Stop heating the slide when the brown stain begins to bubble.
7. Place the slide on the microscope stage, and examine it under low, medium, and high powers. In the chart below, sketch what you see. The presence of hemoglobin crystals under the cover slip is a positive test for blood.

PART B: Blood-Drop Analysis

The patterns left by falling or projected drops of blood can help investigators determine where a crime took place. Therefore, blood drops and stains should be examined closely before the evidence is collected.

Shapely Drops

The shape of a blood drop can indicate the distance from which the blood fell and the angle of its impact. However, very few studies have been done on the patterns produced when blood impacts a surface. Therefore, a thorough forensic scientist will carry out his or her own experiments on the shape of blood droplets. To be accurate, this scientist will conduct the tests under conditions very much like those found at the crime scene.

While a droplet is falling, it is primarily spherical in shape. This is surprising to some people, who may have visualized droplets as tear shaped, as cartoonists often draw them. The smaller a drop, the more spherical its shape during a fall.

Drop Acceleration

As a drop falls through the air, it accelerates until it reaches a constant or terminal velocity. Measurements have shown that a blood drop, resulting from dripping at a height of 15 feet, has a volume of about 0.05 ml and falls at a velocity of about 25 feet per second. Smaller drops have a terminal velocity that is less than 25 feet per second, and larger drops have a terminal velocity that is greater than 25 feet per second. Therefore, an individual blood droplet can give an investigator the following useful information:

- a. the droplet's speed at time of impact,
- b. the direction of the droplet's travel, and
- c. the approximate size of the blood drop.

Round Drops

If you examine a blood droplet that struck a surface straight-on (at a 90-degree angle from the surface), the droplet is generally round. Straight-on impacts on hard, smooth surfaces produce round droplets with smooth edges. Higher velocities and rougher surfaces produce drops with more ragged edges (see Figure 1).

Figure 1 Drops that have ragged edges have fallen at high velocities



Elongated Drops

The angle of impact of a droplet affects the droplet's shape. As we have said, when the angle of impact is 90 degrees, the droplet is round. However, droplets that fall on surfaces at an angle that is greater than 90 degrees have elongated shapes. The larger the angle, the more ellipucal the droplet (see Figure 2).

Figure 2 Droplets that fall on surfaces at angles greater than 90 degrees form ellipucal patterns.



Dripping and Spraying

Blood drops can be produced in several ways. A droplet that forms slowly, as in a dripping wound, has a volume of about 0.05 ml. However, smaller droplets are produced during acuve situations, such as fights and beatings. Blood droplets as small as an aerosol spray indicate that the wound was produced by a powerful force, such as a gunshot or an explosion.

Procedure, Part 1:

Create a set of bloodstain patterns

Materials

Sample of "blood" in 100 ml beaker
Lab apron
Newspapers
micropipette

Protractor

Yard or meter stick

Stake

Small board or string

Pen or marker

Sample of "blood droplet" on newspaper from crime scene

1. Go outdoors. Spread four sheets of newspaper across level ground. Put on your lab apron.
2. ^{Fill the pipette with} Dip the ~~plastic waste~~ ^{it} in the "blood." Holding ~~the waste~~ ^{it} at a height of 12 inches, walk across one of the newspapers, allowing six or eight drops of "blood" to fall on the paper. Label these as "12-inch blood drops at 90 degrees."
3. Again, ^{fill the pipette with} ~~by the waste~~ ^{it} the "blood." Holding ~~the waste~~ ^{it} at a height of 24 inches, walk across another one of the newspapers to create six or eight drops of "blood." Label these as "24-inch drops at 90 degrees."
4. Repeat this process at 36 inches and at 48 inches.
5. Place another four sheets of newspaper on a gentle slope or hillside.
6. Repeat steps 2 through 4 on the newspapers that are spread on the gentle slope.
7. Place another four sheets of newspaper on a steep slope or hillside.
8. Repeat steps 2 through 4 on the newspapers that are spread on the steep slope.

Procedure, Part 2:

Comparing a bloodstain from a crime scene with your bloodstain patterns

1. Your teacher will give you a piece of blood-stained newspaper from the crime scene.
2. Analyze the stain by comparing it with the work you did in Part A.
3. Complete the

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 Data Table.

PART C: INCOMPATIBILITY RELATIONSHIPS IN THE ABO SYSTEM

Table 1 shows the relationship between different blood groups in the ABO system. Whole blood can be separated into serum and red blood cells. Mixing serum or whole blood from donors of one blood type with red blood cells or whole blood from an individual of a different type may result in an incompatibility reaction. For instance, when serum or whole blood from a type A individual is mixed with red blood cells or whole blood cells from a type B individual, the red blood cells clump together, or agglutinate. This reaction occurs since type A blood contains anti-B antibodies. These antibodies combine with B antigens on the red blood cells to cause agglutination. This is shown in Figure 1.

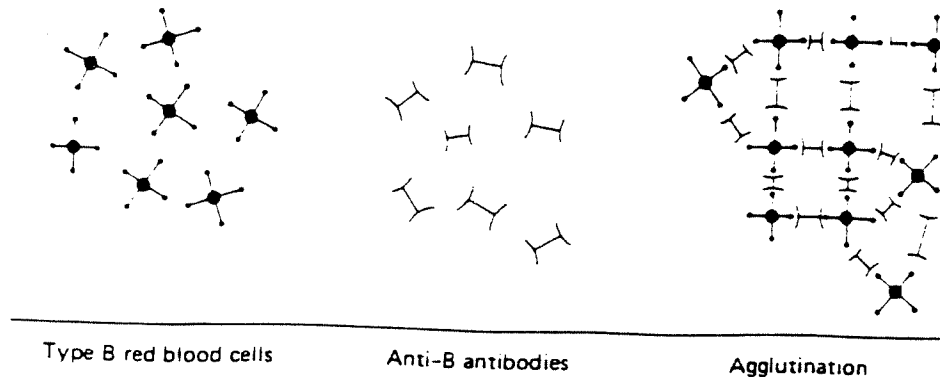
The agglutination takes place because the type-A serum contains substances known as anti-B antibodies, which react with materials called type-B antigens present on the surface of type-B blood cells. This reaction between antigen and antibody causes the red blood cells to stick to each other, as shown in Figure 1. Determinations of blood groups of the ABO system are rapid and simple. The type is readily determined by adding a known commercial antiserum (antibodies) to the blood and looking for the presence or absence of agglutination.

This section deals with the typing of blood by use of the ABO system.

for the Blood Group

Blood Groups	Antigens on Red Blood Cells	Antibodies in Serums
A	A	Anti-B
B	B	Anti-A
AB	A and B	Neither anti-A or anti-B
O	Neither A nor B	Anti-A and anti-B

FIGURE 1
Schematic diagram
of agglutination.



DEFINITIONS

Agglutination—the clumping together of blood cells.

Agglutinin—an antibody in plasma that promotes agglutination.

Agglutinogen—a substance in red blood cells that acts as an antigen and incites the production of agglutinin.

Antibody—a substance in blood that reacts with a specific antigen, causing blood cells to clump together.

Antigen—a substance that incites the formation of antibodies.

Hemoglobin—the oxygen carrying coloring matter of red blood cells.

Plasma—the colorless fluid of the blood.

Serum—the clear yellowish liquid part of blood after the fibrin and corpuscles have been removed.

PROCEDURE:

The procedure that follows is very similar to actual blood typing.

1. Obtain a ~~glass~~ **well plate slide** and a grease pencil.
2. Label the three sections A, B, and D respectively, from left to right (D is for Rh+ or Rh-).
3. Obtain a ~~test~~ tube with a labeled blood sample in it, and record the number of the sample on the data sheet.
4. Place 1 small drop of the blood sample on each of ~~the~~ three sections of the ~~slide~~ **well plate**.
5. Spread out the drop with a wooden splint or tooth pick.
6. Take the small bottles of antisera and add 1 drop of antiserum-A to the drop of blood in the section of the slide labeled A, 1 drop of antiserum B to the section B drop of blood, and 1 drop of antiserum D to the section D drop of blood.

All materials used in this lab activity are SIMULATED. No human or animal blood products are used. These materials

7. Repeat procedure with unknown. Be sure to indicate which unknown you test, on your data table.

PART D: Identifying Blood

Blood is a complex fluid tissue that transports materials throughout the body. Blood has both liquid and solid parts. The liquid portion of blood, plasma, consists mainly of water and proteins. It transports foods, wastes, salts, and hormones. The solid portion of blood consists of red blood cells, white blood cells, and platelets. Red blood cells carry oxygen to the body cells. White blood cells help the body fight infections. Platelets aid in the clotting of blood.

Objective

In this activity you will:

1. Identify blood cells on a prepared slide.

Materials

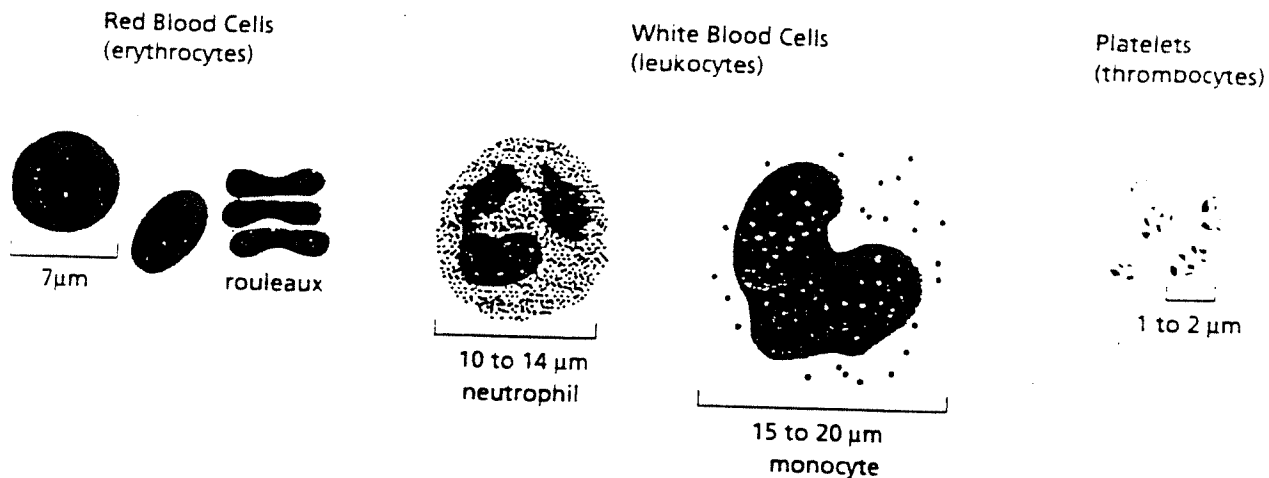
prepared human blood smear
(Wright's stain)
microscope
colored pencils

Procedures

You will be using a prepared slide of a human blood smear. A blood smear is made by spreading a drop of blood thinly across a microscope slide. The smear is then stained with Wright's stain, which makes white blood cells and platelets more visible. Wright's stain darkly stains the nuclei and cytoplasmic granules of cells.

1. Obtain a prepared slide of a blood smear. Observe the slide under low power on your microscope, then switch to high power. Compare what you see in the microscope to Figure 1.

Figure 1



By far the most numerous cells that you see on the slide are red blood cells. You may see them in stacks of cells called *rouleaux*. Look for individual cells to observe their shape.

Platelets are fragments of larger cells called megakaryocytes, which are found in bone marrow. Platelets are smaller than red blood cells, they are disk-shaped, and they stain a pale-blue color.

2. Locate some platelets in the blood smear.

White blood cells are larger and less numerous than red blood cells. There are several types of white blood cells that can be recognized by their size, the shape of their nuclei, and the staining of their cytoplasm.

3. Search the smear for white blood cells. See Figure 1.

Analysis

1. What is hemoglobin?
2. Why does hydrogen peroxide give a positive test for hemoglobin?
3. What are some ways of collecting suspicious stains at a crime scene?
4. Was your hydrogen peroxide test on the brown stain positive or negative for blood?
5. The test you performed in lab today could not tell you whether a blood sample belonged to a human or to some other animal. Name 2 living things that have red blood.
6. The formation of hemoglobin crystals with potassium salts and acetic acid mixture only tells you that the sample contains a component of hemoglobin. It does not indicate whether the hemoglobin came from an animal or from a human. Is this important to know? Why?
7. Was the bloodstain from the crime scene similar to any of the bloodstains you created in steps 1 through 5? If so, which ones?
8. Based on your work, was the crime committed on a flat area, a gently sloping area, or a steeply sloping area?
9. What information can be determined by examining a blood drop?
10. The ABO blood typing was quite easily performed on the volume of blood used in this exercise. Do you feel that it could be done as easily on a very small volume by use of a microscope? What difficulties can you foresee?
11. How do you suppose one would go about typing a dried blood stain?
12. Describe the size, appearance, and relative number of red blood cells in the blood smear.
13. Describe the size, appearance, and relative abundance of white blood cells in the blood smear.
14. Describe the size, appearance, and relative abundance of platelets in the blood smear.