



The Sam Sheppard Case—A Trail of Blood

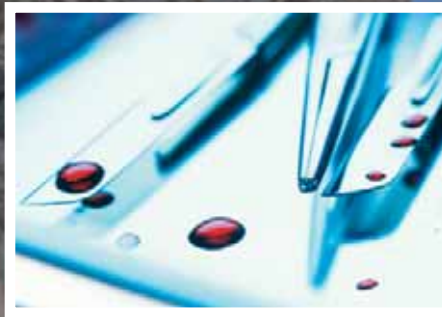
Convicted in 1954 of bludgeoning his wife to death, Dr. Sam Sheppard achieved celebrity status when the storyline of TV's *The Fugitive* was apparently modeled on his efforts to seek vindication for the crime he professed not to have committed. Dr. Sheppard, a physician, claimed he was dozing on his living room couch when his pregnant wife, Marilyn, was attacked. Sheppard's story was that he quickly ran upstairs to stop the carnage, but was knocked unconscious briefly by the intruder. The suspicion that fell on Dr. Sheppard was fueled by the revelation that he was having an adulterous affair. At trial, the local coroner testified that a pool of blood on Marilyn's pillow contained the impression of a "surgical instrument." After Sheppard had been imprisoned for ten years, the U.S Supreme Court set aside his conviction due to the "massive, pervasive, and prejudicial publicity" that had attended his trial.

In 1966, the second Sheppard trial commenced. This time, the same coroner was

forced to back off from his insistence that the bloody outline of a surgical instrument was present on Marilyn's pillow. However, a medical technician from the coroner's office now testified that blood on Dr. Sheppard's watch was from blood spatter, indicating that Dr. Sheppard was wearing the watch in the presence of the battering of his wife. The defense countered with the expert testimony of eminent criminalist Dr. Paul Kirk. Dr. Kirk concluded that blood spatter marks in the bedroom showed the killer to be left-handed. Dr. Sheppard was right-handed.

Dr. Kirk further testified that Sheppard stained his watch while attempting to obtain a pulse reading. After less than twelve hours of deliberation, the jury failed to convict Sheppard. But the ordeal had taken its toll. Four years later Sheppard died, a victim of drug and alcohol abuse.

Forensic Serology



Key Terms

- acid phosphatase
- agglutination
- allele
- antibody
- antigen
- antiserum
- aspermia
- chromosome
- deoxyribonucleic acid (DNA)
- egg
- enzyme
- erythrocyte
- gene
- genotype
- hemoglobin
- heterozygous
- homozygous
- hybridoma cells
- locus
- luminol/monoclonal antibodies
- oligospermia
- phenotype
- plasma
- polyclonal antibodies
- precipitin
- serology
- serum
- sperm
- X chromosome
- Y chromosome
- zygote

Learning Objectives

After studying this chapter you should be able to:

- List the A-B-O antigens and antibodies found in the blood for each of the four blood types: A, B, AB, and O
- Understand and describe how whole blood is typed
- List and describe forensic tests used to characterize a stain as blood
- Understand the concept of antigen–antibody interactions and how it is applied to species identification and drug identification
- Contrast chromosomes and genes
- Learn how the Punnett square is used to determine the genotypes and phenotypes of offspring
- List the laboratory tests necessary to characterize seminal stains
- Explain how suspect blood and semen stains are properly preserved for laboratory examination
- Describe the proper collection of physical evidence in a rape investigation

In 1901, Karl Landsteiner announced one of the most significant discoveries of the twentieth century—the typing of blood—a finding that twenty nine years later earned him a Nobel Prize. For years physicians had attempted to transfuse blood from one individual to another. Their efforts often ended in failure because the transfused blood tended to coagulate, or clot, in the body of the recipient, causing instantaneous death. Landsteiner was the first to recognize that all human blood was not the same; instead, he found that blood is distinguishable by its group or type.

Out of Landsteiner’s work came the classification system that we call the *A-B-O system*. Now physicians had the key for properly matching the blood of a donor to that of a recipient. One blood type cannot be mixed with a different blood type without disastrous consequences. This discovery, of course, had important implications for blood transfusion and the millions of lives it has since saved.

Meanwhile, Landsteiner’s findings opened a new field of research in the biological sciences. Others began to pursue the identification of additional characteristics that could further differentiate blood. By 1937, the Rh factor in blood had been demonstrated and, shortly thereafter, numerous blood factors or groups were discovered. More than 100 different blood factors have been identified. However, the ones in the A-B-O system are still the most important for properly matching a donor and recipient for a transfusion.

Until the early 1990s, forensic scientists focused on blood factors, such as A-B-O, as offering the best means for linking blood to an individual. What made these factors so attractive was that in theory no two individuals, except for identical twins, could be expected to have the same combination of blood factors. In other words, blood factors are controlled genetically and have the potential of being a highly distinctive feature for personal identification. What makes this observation so relevant is the great frequency of bloodstains at crime scenes, especially crimes of the most serious nature—homicides, assaults, and rapes. Consider, for example, a transfer of blood between the victim and assailant during a struggle; that is, the victim’s blood is transferred to the suspect’s garment or vice versa. If the criminalist could individualize human blood by identifying all of its known factors, the result would be strong evidence for linking the suspect to the crime.

The advent of DNA technology has dramatically altered the approach of forensic scientists toward individualization of bloodstains and other biological evidence. The search for genetically controlled blood factors in bloodstains has been abandoned in favor of characterizing biological evidence by select regions of our **deoxyribonucleic acid (DNA)**, which carries the body's genetic information. As a result, the individualization of dried blood and other biological evidence has become a reality and has significantly altered the role that crime laboratories play in criminal investigations. In fact, as we will learn in the next chapter, the high sensitivity of DNA analysis has even altered the types of materials collected from crime scenes in the search for DNA.

The next chapter is devoted to discussing recent breakthroughs in associating blood and semen stains with a single individual through characterization of DNA. This chapter focuses on underlying biological concepts that forensic scientists historically relied on as they sought to characterize and individualize biological evidence prior to the dawning of the age of DNA.

The Nature of Blood

The word *blood* refers to a highly complex mixture of cells, enzymes, proteins, and inorganic substances. The fluid portion of blood is called **plasma**; it is composed principally of water and accounts for 55 percent of blood content. Suspended in the plasma are solid materials consisting chiefly of several types of cells—red blood cells (**erythrocytes**), white blood cells (leukocytes), and platelets. The solid portion of blood accounts for 45 percent of its content. Blood clots when a protein in the plasma known as *fibrin* traps and enmeshes the red blood cells. If the clotted material were removed, a pale yellowish liquid known as **serum** would be left.

Obviously, considering the complexity of blood, any discussion of its function and chemistry would have to be extensive, extending beyond the scope of this text. It is certainly far more relevant at this point to concentrate our discussion on the blood components that are directly pertinent to the forensic aspects of blood identification—the red blood cells and the blood serum.

Antigens and Antibodies

Red blood cells transport oxygen from the lungs to the body tissues and remove carbon dioxide from tissues by transporting it back to the lungs, where it is exhaled. However, for reasons unrelated to the red blood cell's transporting mission, on the surface of each cell are millions of characteristic chemical structures called **antigens**. Antigens impart specific characteristics to the red blood cells. Blood antigens are grouped into systems depending on their relationship to one another. More than fifteen blood antigen systems have been identified to date; of these, the A-B-O and Rh systems are the most important.

If an individual is type A, this simply indicates that each red blood cell has A antigens on its surface; similarly, all type B individuals have B antigens, and the red blood cells of type AB individuals contain both A and B antigens. Type O individuals have neither A nor B antigens on their cells. Hence, the presence or absence of A and B antigens on the red blood cells determines a person's blood type in the A-B-O system.

Another important blood antigen has been designated as the *Rh factor*, or D antigen. Those people having the D antigen are said to be *Rh positive*;

deoxyribonucleic acid (DNA)

The molecules that carry the body's genetic information.

plasma

The fluid portion of unclotted blood.

erythrocyte

A red blood cell.

serum

The liquid that separates from the blood when a clot is formed.

antigen

A substance, usually a protein, that stimulates the body to produce antibodies against it.

antibody

A protein in the blood serum that destroys or inactivates a specific antigen.

antiserum

Blood serum that contains specific antibodies.

agglutination

The clumping together of red blood cells by the action of an antibody.

those without this antigen are *Rh negative*. In routine blood banking, the presence or absence of the three antigens—A, B, and D—must be determined in testing compatibility of the donor and recipient.

Serum is important because it contains proteins known as **antibodies**. The fundamental principle of blood typing is that for every antigen, there exists a specific antibody. Each antibody symbol contains the prefix *anti-*, followed by the name of the antigen for which it is specific. Hence, anti-A is specific only for A antigen, anti-B for B antigen, and anti-D for D antigen. The serum-containing antibody is referred to as the **antiserum**, meaning a serum that reacts against something (antigens).

An antibody reacts only with its specific antigen and no other. Thus, if serum containing anti-B is added to red blood cells carrying the B antigen, the two will combine, causing the antibody to attach itself to the cell. Antibodies are normally *bivalent*—that is, they have two reactive sites. This means that each antibody can simultaneously be attached to antigens located on two different red blood cells. This creates a vast network of cross-linked cells usually seen as clumping or **agglutination** (see Figure 8–1).

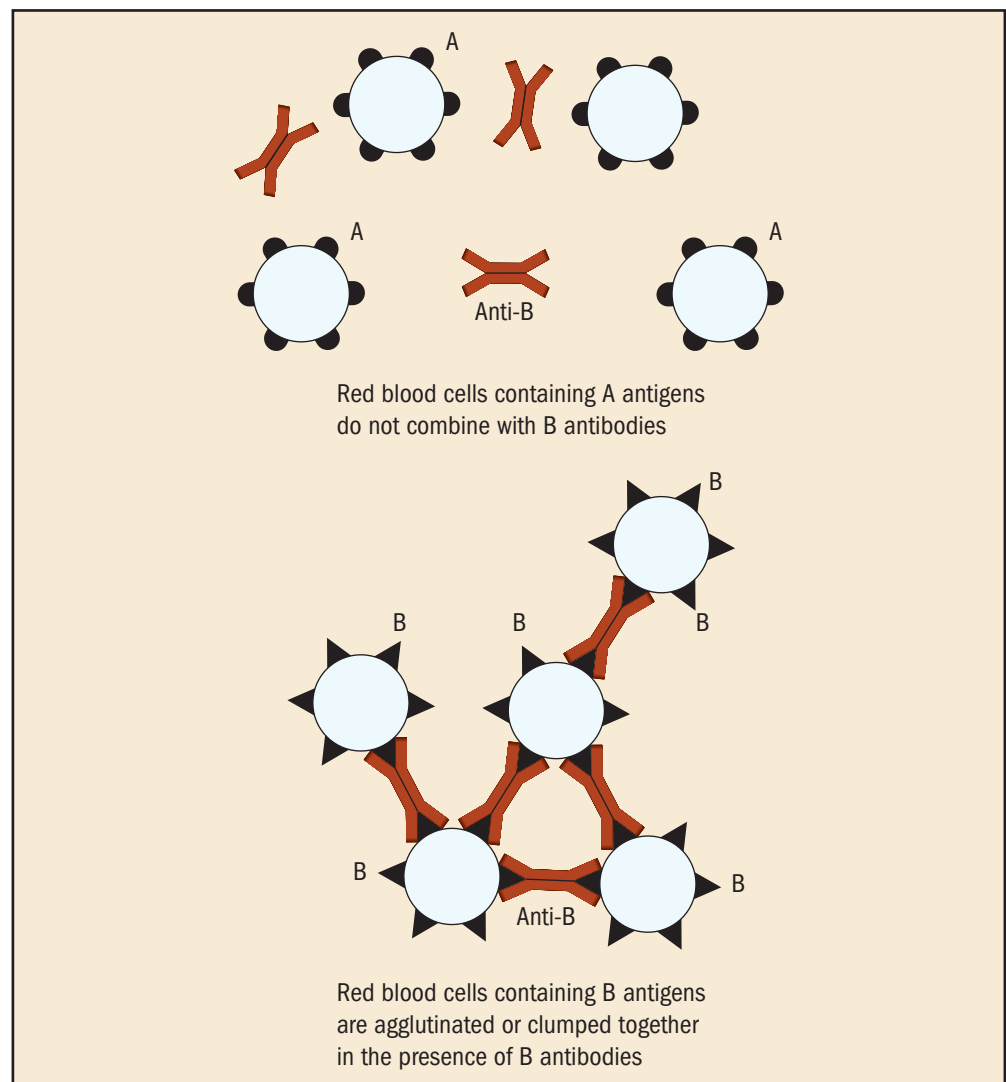


FIGURE 8–1

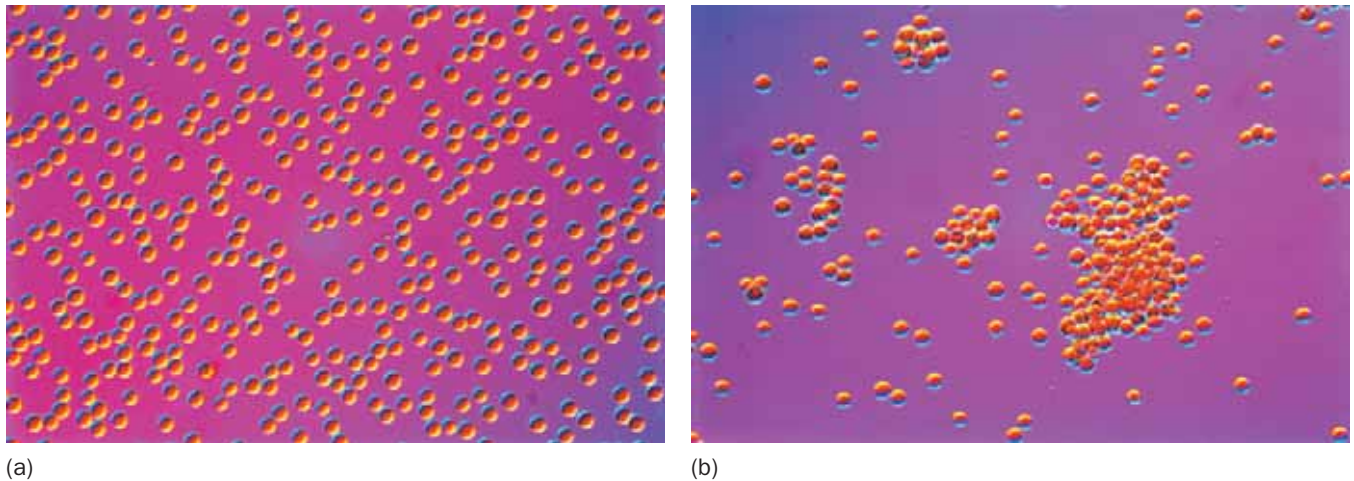


FIGURE 8-2 (a) Microscopic view of normal red blood cells (500 \times). (b) Microscopic view of agglutinated red blood cells (500 \times). Courtesy J. C. Revy, Phototake NYC

Let's look a little more closely at this phenomenon. In normal blood, shown in Figure 8-2(a), antigens on red blood cells and antibodies coexist without destroying each other because the antibodies present are not specific toward any of the antigens. However, suppose a foreign serum added to the blood introduces a new antibody. This results in a specific antigen-antibody reaction that immediately causes the red blood cells to link together, or agglutinate, as shown in Figure 8-2(b).

Evidently, nature has taken this situation into account, for when we examine the serum of type A blood, we find anti-B and no anti-A. Similarly, type B blood contains only anti-A, type O blood has both anti-A and anti-B, and type AB blood contains neither anti-A nor anti-B. The antigen and antibody components of normal blood are summarized in the following table:

Blood Type	Antigens on Red Blood Cells	Antibodies in Serum
A	A	Anti-B
B	B	Anti-A
AB	AB	Neither anti-A nor anti-B
O	Neither A nor B	Both anti-A and anti-B

The reasons for the fatal consequences of mixing incompatible blood during a transfusion should now be quite obvious. For example, the transfusion of type A blood into a type B patient will cause the natural anti-A in the blood of the type B patient to react promptly with the incoming A antigens, resulting in agglutination. In addition, the incoming anti-B of the donor will react with the B antigens of the patient.

Blood Typing

The term **serology** describes a broad scope of laboratory tests that use specific antigen and serum antibody reactions. The most widespread application of serology is the typing of whole blood for its A-B-O identity. In determining the A-B-O blood type, only two antiserums are needed—anti-A and anti-B. For routine blood typing, both of these antiserums are commercially available.

Table 8-1 summarizes how the identity of each of the four blood groups is established when the blood is tested with anti-A and anti-B serum. Type A blood is agglutinated by anti-A serum; type B blood is agglutinated

serology

The study of antigen-antibody reactions.

by anti-B serum; type AB blood is agglutinated by both anti-A and anti-B; and type O blood is not agglutinated by either the anti-A or anti-B serum.

The identification of natural antibodies present in blood offers another way to determine blood type. Testing blood for the presence of anti-A and anti-B requires using red blood cells that have known antigens. Again, these cells are commercially available. Hence, when A cells are added to a blood specimen, agglutination occurs only in the presence of anti-A. Similarly, B cells agglutinate only in the presence of anti-B. All four A-B-O types can be identified in this manner by testing blood with known A and B cells, as summarized in Table 8–2.

The population distribution of blood types varies with location and race throughout the world. In the United States, a typical distribution is as follows:

O	A	B	AB
43%	42%	12%	3%

Key Points

- Serology involves a broad scope of laboratory tests that use specific antigen and serum antibody reactions.
- An antibody reacts or agglutinates only with its specific antigen. The concept of specific antigen–antibody reactions has been applied to techniques for detecting abused drugs in blood and urine.
- Every red blood cell contains either an A antigen, a B antigen, or no antigen (this is called type O). The type of antigen on one’s red blood cells determines one’s A-B-O blood type. People with type A blood have A antigens on their red blood cells, those with type B blood have B antigens, and those with type O blood have no antigens on their red blood cells.

Table 8–1 Identification of Blood with Known Antiserum

Anti-A Serum	Anti-B Serum		
+	+		
Whole Blood	Whole Blood	Antigen Present	Blood Type
+	–	A	A
–	+	B	B
+	+	A and B	AB
–	–	Neither A nor B	O

Note: + shows agglutination; – shows absence of agglutination.

Table 8–2 Identification of Blood with Known Cells

A Cells	B Cells		
+	+		
Blood	Blood	Antibody Present	Blood Type
+	–	Anti-A	B
–	+	Anti-B	A
+	+	Both anti-A and anti-B	O
–	–	Neither anti-A nor anti-B	AB

Note: + shows agglutination; – shows absence of agglutination.

Immunoassay Techniques

The concept of a specific antigen–antibody reaction is finding application in other areas unrelated to blood typing. Most significantly, this approach has been extended to the detection of drugs in blood and urine. Antibodies that react with drugs do not exist naturally; however, they can be produced in animals such as rabbits by first combining the drug with a protein and injecting this combination into the animal. This drug–protein complex acts as an antigen stimulating the animal to produce antibodies (see Figure 8–3). The recovered blood serum of the animal now contains antibodies that are specific or nearly specific to the drug.

Currently, each day, thousands of individuals are voluntarily being subjected to urinalysis tests for the presence of drug-of-abuse. These individuals include military personnel, transportation industry employees, police and correction personnel, and subjects requiring pre-employment drug screening. Immunoassay testing for drugs has proven quite suitable for handling the large volume of specimens that must be rapidly analyzed on a daily basis for drug content. Testing laboratories have available to them a variety of commercially prepared sera arising from animals being injected with any one of a variety of drugs. Once a particular serum is added to a urine specimen, it's designed to interact with either opiates, cannabinoids, cocaine, amphetamines, phencyclidine, barbiturates, methadone, or other drugs. A word of caution: immunoassay is only presumptive in nature and its result must be confirmed by additional testing.

Radioimmunoassay (RIA)

Although EMIT is currently a popular immunoassay technique in forensic laboratories, other immunoassay procedures are commercially available. For example, *radioimmunoassay (RIA)* uses drugs labeled with radioactive

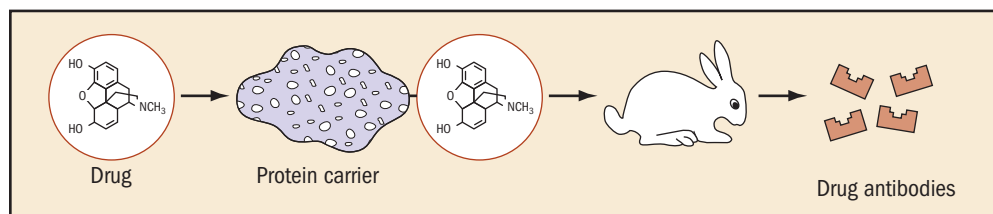


FIGURE 8-3 Stimulating production of drug antibodies.

Closer Analysis

Enzyme-Multiplied Immunoassay Technique (EMIT)

Several immunological assay techniques are commercially available for detecting drugs through an antigen–antibody reaction. One such technique, the *enzyme-multiplied immunoassay technique (EMIT)*, has gained widespread popularity among toxicologists because of its speed and high sensitivity for detecting drugs in urine.

A typical EMIT analysis begins by adding to a subject's urine antibodies that bind to a particular type or class of drug being looked for. This is followed by adding to the urine a chemically-labeled version of the drug. As shown in the figure, a competition will ensue between the labeled and unlabeled drug (if it's present in the subject's urine) to

(continued)

Closer Analysis

Enzyme-Multiplied Immunoassay Technique (EMIT)

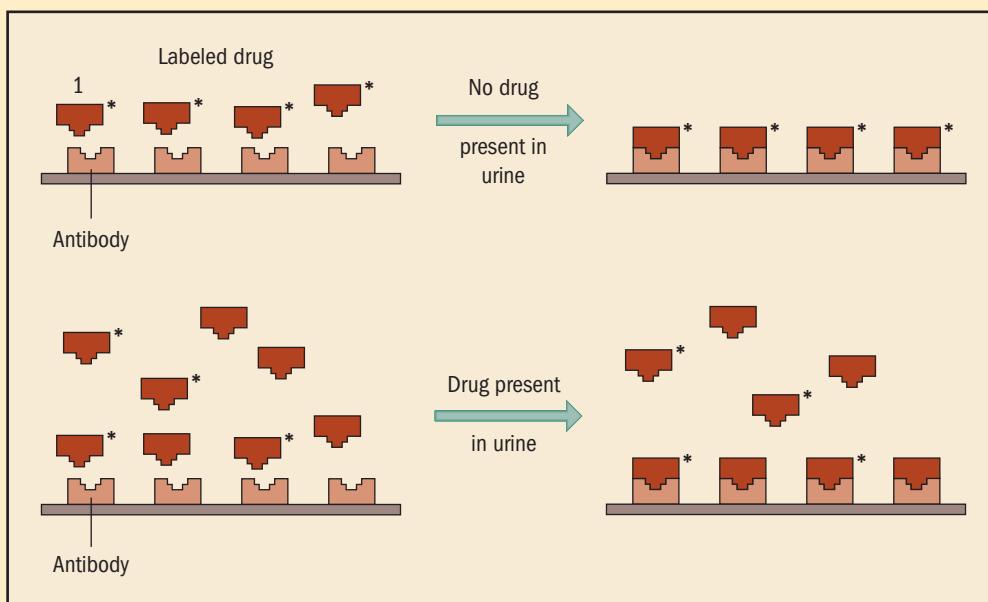
(continued)

bind with the antibody. If this competition does occur in a person's urine, it signifies that the urine screen test was positive for the drug being tested. For example, to check someone's urine for methadone, the analyst would add methadone antibodies and chemically-labeled methadone to the urine. Any methadone present in the urine immediately competes with the labeled methadone to bind with the methadone antibodies. The quantity of chemically-labeled methadone left uncombined is then measured, and this value is related to the concentration of methadone originally present in the urine.

One of the most frequent uses of EMIT in forensic laboratories has been for screening the urine of suspected marijuana users. The primary pharmacologically active agent in marijuana is tetrahydrocannabinol, or THC. To facilitate the elimination of THC, the body converts it to a series of substances called

metabolites that are more readily excreted. The major THC metabolite found in urine is a substance called *THC-9-carboxylic acid*. Antibodies against this metabolite are prepared for EMIT testing. Normally the urine of marijuana users contains a very small quantity of THC-9-carboxylic acid (less than one-millionth of a gram); however, this level is readily detected by EMIT.

The greatest problem with detecting marijuana in urine is interpretation of the test results. Although marijuana use results in the detection of THC metabolites, it is very difficult to determine when the individual actually used marijuana. In individuals who use marijuana frequently, detection is possible within two to five days after the last use of the drug. However, some individuals may yield positive results up to ten days after the last use of marijuana.



In the EMIT assay, a drug that may be present in a urine specimen will compete with added labeled drug for a limited number of antibody binding sites. The labeled drugs are indicated by an asterisk. Once the competition for antibody sites is completed, the number of remaining unbound labeled drug is proportional to the drug's concentration in urine.

tags. Whether using an enzyme tag as in EMIT or a radioactive tag as in RIA, the analyst must be cautious because immunoassay techniques are not totally specific for any drug. Substances with a chemical structure similar to the drug in question may cross-react with the antibody to give a false-positive reaction. Hence, positive immunoassay tests must always be confirmed by another reliable analytical procedure. The issue of specificity, along with other questions relating to the reliability of RIA, was raised during the murder trial of Dr. Mario E. Jascalevich, which is described in detail in the Case Reading at the end of Chapter 1.

Closer Analysis

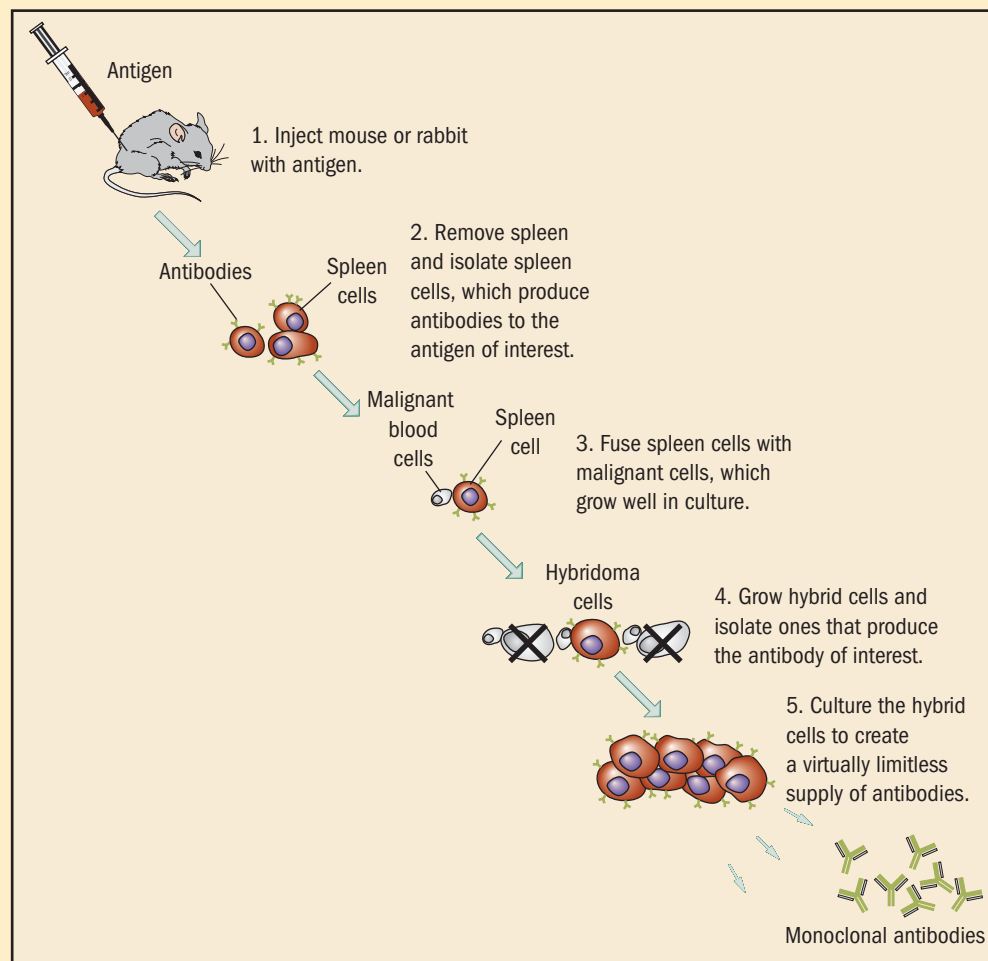
Polyclonal and Monoclonal Antibodies

As we have seen in the previous section, when an animal such as a rabbit or mouse is injected with an antigen, the animal responds by producing antibodies designed to bind to the invading antigen. However, the process of producing antibodies designed to respond to foreign antigens is complex. For instance, an antigen typically has structurally different

sites to which an antibody may bind. Thus, in the presence of a specific antigen, an animal produces many different antibodies, all of which are designed to attack some particular site on the antigen of interest. These antibodies are known as **polyclonal antibodies**. However, the disadvantage of polyclonal antibodies is that an animal can produce antibodies that

polyclonal antibodies

Antibodies produced by injecting animals with a specific antigen. A series of antibodies are produced responding to a variety of different sites on the antigen.



Steps required to produce monoclonal antibodies.

(continued)

Closer Analysis

Polyclonal and Monoclonal Antibodies (*continued*)

vary in composition over time. As a result, different batches of polyclonals may vary in their specificity and their ability to bind to a particular antigen site.

Modern forensic technologies occasionally require antibodies that are more uniform in their composition and attack power than the traditional polyclonals. Forensic scientists thus need a way to produce antibodies designed to attack one and only one site on an antigen. Such antibodies are known as **monoclonal antibodies**. How can such monoclonals be produced?

The process begins by injecting a mouse with the antigen of interest. In response, the mouse's spleen cells produce antibodies to fight off the invading antigen. The spleen cells are removed from the animal and are fused to fast-growing blood

cancer cells to produce **hybridoma cells**.

The hybridoma cells are then allowed to multiply and are screened for their specific antibody activity. Hybridoma cells bearing the antibody activity of interest are then selected and cultured. The rapidly multiplying cancer cells linked to the selected antibody cells produce identical monoclonal antibodies in a limitless supply (see figure).

Monoclonal antibodies are being incorporated into commercial forensic test kits with increasing frequency. Many immunoassay test kits for abused drugs are being formulated with monoclonal antibodies. Also, a recently introduced test for semen that incorporates a monoclonal antibody has found wide popularity in crime laboratories.

monoclonal antibodies

A collection of identical antibodies that interact with a single antigen site.

hybridoma cells

Fused spleen and tumor cells. Used to produce identical monoclonal antibodies in a limitless supply.

Key Points

- To produce antibodies capable of reacting with drugs, the analyst combines a specific drug with a protein and injects this combination into an animal such as a rabbit. This drug-protein complex acts as an antigen, stimulating the animal to produce antibodies. The recovered blood serum of the animal now contains antibodies that are specific or nearly specific to the drug.
- When an animal is injected with an antigen, its body produces a series of different antibodies, all of which are designed to attack some particular site on the antigen of interest. These antibodies are known as polyclonal antibodies.
- A more uniform and specific collection of antibodies designed to combine with a single antigen site can be manufactured. Such antibodies are known as monoclonal antibodies.

Forensic Characterization of Bloodstains

The criminalist must answer the following questions when examining dried blood: (1) Is it blood? (2) From what species did the blood originate? (3) If the blood is human, how closely can it be associated with a particular individual?

Color Tests

The determination of blood is best made by means of a preliminary color test. For many years, the most common test was the *benzidine color test*.

However, because benzidine has been identified as a known carcinogen, its use has generally been discontinued, and the chemical phenolphthalein is usually substituted in its place (this test is also known as the *Kastle-Meyer color test*).¹

Both the benzidine and Kastle-Meyer color tests are based on the observation that blood **hemoglobin** possesses peroxidase-like activity. Peroxidases are enzymes that accelerate the oxidation of several classes of organic compounds when combined with peroxides. For example, when a bloodstain, phenolphthalein reagent, and hydrogen peroxide are mixed together, oxidation of the hemoglobin in the blood produces a deep pink color.

The Kastle-Meyer test is not a specific test for blood; some vegetable materials, for instance, may turn Kastle-Meyer pink. These substances include potatoes and horseradish. However, such materials will probably not be encountered in criminal situations, and thus from a practical point of view, a positive Kastle-Meyer test is highly indicative of blood. Field investigators have found Hemastix strips a useful presumptive field test for blood. Designed as a urine dipstick test for blood, the strip can be moistened with distilled water and placed in contact with a suspect bloodstain. The appearance of a green color indicates blood.

Luminol

Another important presumptive identification test for blood is the **luminol** test.² Unlike the benzidine and Kastle-Meyer tests, the reaction of luminol with blood produces light rather than color. After spraying luminol reagent onto suspect items, agents darken the room; any bloodstains produce a faint blue glow, known as *luminescence*. Using luminol, investigators can quickly screen large areas for bloodstains.

The luminol test is extremely sensitive, capable of detecting bloodstains diluted up to 300,000 times. For this reason, spraying large areas such as carpets, walls, flooring, or the interior of a vehicle may reveal blood traces or patterns that would have gone unnoticed under normal lighting conditions (see Figure 8-4). Luminol does not interfere with any subsequent DNA testing.³

Microcrystalline Tests

The identification of blood can be made more specific if microcrystalline tests are performed on the material. Several tests are available; the two most popular ones are the *Takayama* and *Teichmann tests*. Both depend on the addition of specific chemicals to the blood to form characteristic crystals containing hemoglobin derivatives. Crystal tests are far less sensitive than color tests for blood identification and are more susceptible to interference from contaminants that may be present in the stain.

Precipitin Test

Once the stain has been characterized as blood, the serologist determines whether the blood is of human or animal origin. The standard test is the **precipitin** test. Precipitin tests are based on the fact that when animals (usually rabbits) are injected with human blood, antibodies form that react with the invading human blood to neutralize its presence. The investigator can recover these antibodies by bleeding the animal and isolating the blood serum, which contains antibodies that specifically react with human

hemoglobin

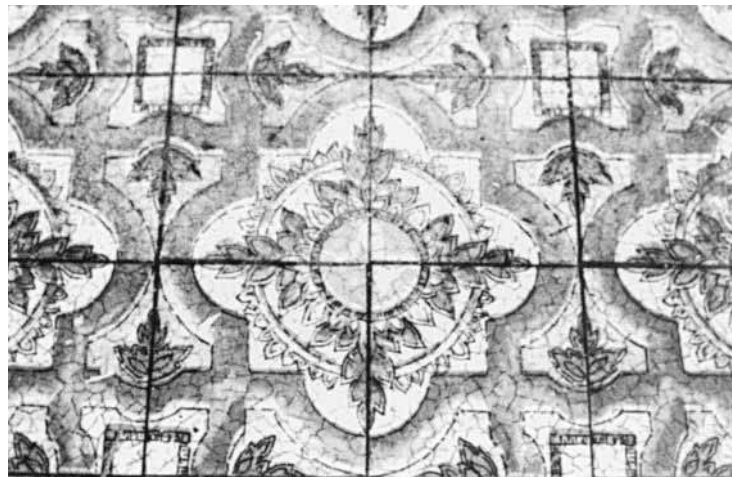
A red blood cell protein that transports oxygen in the bloodstream; it is responsible for the red color of blood.

luminol

The most sensitive chemical test that is capable of presumptively detecting bloodstains diluted up to 300,000 times. Its reaction with blood emits light and thus requires the result to be observed in a darkened area.

precipitin

An antibody that reacts with its corresponding antigen to form a precipitate.



(a)



(b)

FIGURE 8-4 (a) A section of a linoleum floor photographed under normal light. This floor was located in the residence of a missing person. (b) Same section of the floor shown in (a) after spraying with luminol. A circular pattern was revealed. Investigators concluded that the circular blood pattern was left by the bottom of a bucket carried about during the cleaning up of the blood. A small clump of sponge, blood, and hair was found near where this photograph was taken. *Courtesy North Carolina State Bureau of Investigation*

antigens. For this reason, the serum is known as *human antiserum*. In the same manner, by injecting rabbits with the blood of other known animals, virtually any kind of animal antiserum can be produced. Antiserums are commercially available for humans and for a variety of commonly encountered animals—for example, dogs, cats, and deer.

Several techniques have been devised for performing precipitin tests on bloodstains. The classic method is to layer an extract of the bloodstain on top of the human antiserum in a capillary tube. Human blood, or for that matter, any protein of human origin in the extract, reacts specifically with antibodies present in the antiserum, as indicated by the formation of a cloudy ring or band at the interface of the two liquids (see Figure 8-5).

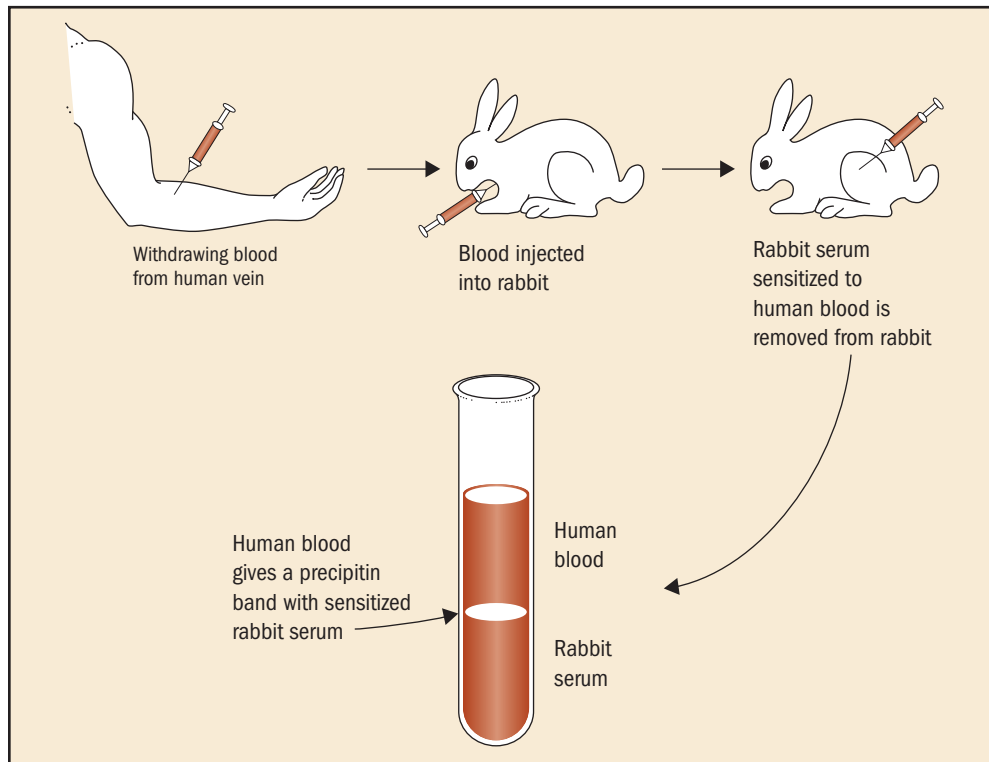


FIGURE 8-5 The precipitin test.

Gel Diffusion

Another method, called *gel diffusion*, takes advantage of the fact that antibodies and antigens diffuse or move toward one another on a plate coated with a gel medium made from a natural polymer called agar. The extracted bloodstain and the human antiserum are placed in separate holes opposite each other on the gel. If the blood is human, a line of precipitation forms where the antigens and antibodies meet.

Similarly, the antigens and antibodies can be induced to move toward one another under the influence of an electrical field. In the *electrophoretic method* (examined in detail in Chapter 9), an electrical potential is applied to the gel medium; a specific antigen–antibody reaction is denoted by a line of precipitation formed between the hole containing the blood extract and the hole containing the human antiserum (see Figure 8-6).

The precipitin test is very sensitive and requires only a small amount of blood for testing. Human bloodstains dried for ten to fifteen years and longer may still give a positive precipitin reaction. Even extracts of tissue from mummies four to five thousand years old have given positive reactions with this test. Furthermore, human bloodstains diluted by washing in water and left with only a faint color may still yield a positive precipitin reaction (see Figure 8-7).

Once it has been determined that the bloodstain is human, an effort must be made to associate or disassociate the stain with a particular individual. Until the mid-1990s, routine characterization of bloodstains included the determination of A-B-O types; however, the widespread use of DNA profiling or typing has relegated this subject to one of historical interest only.

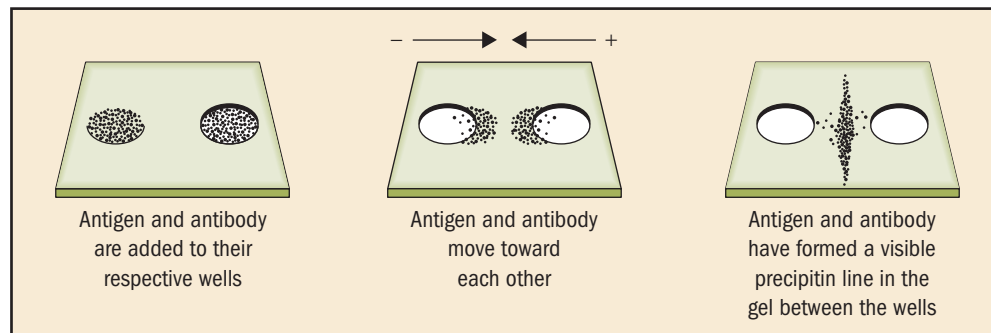


FIGURE 8-6 Gel diffusion.

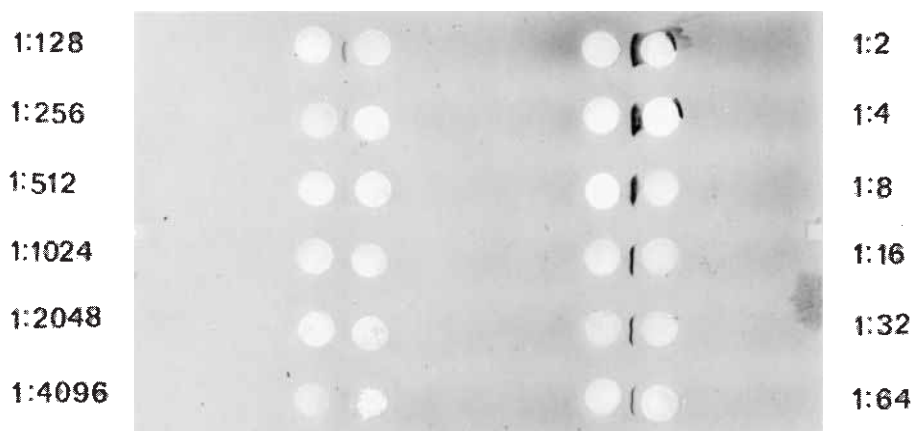


FIGURE 8-7 Results of the precipitin test of dilutions of human serum up to 1 in 4,096 against a human antiserum. A reaction is visible for blood dilutions up to 1 in 256. Courtesy Millipore Biomedica, Acton, Mass

Key Points

- The criminalist must answer the following questions when examining dried blood: (1) Is it blood? (2) From what species did the blood originate? (3) If the blood is human, how closely can it be associated with a particular individual?
- The determination of blood is best made with a preliminary color test. A positive result from the Kastle-Meyer color test is highly indicative of blood.
- The luminol test is used to find trace amounts of blood at crime scenes.
- The precipitin test uses antisera normally derived from rabbits that have been injected with the blood of a known animal to determine the species origin of a questioned bloodstain.
- Materials undergoing electrophoresis are forced to move across a gel-coated plate under the influence of an electrical potential. Antigens and antibodies can be induced to move toward one another under the influence of an electrical field.

Bloodstain Patterns

The location, distribution, and appearance of bloodstains and spatters may be useful for interpreting and reconstructing the events that produced the bleeding. A thorough analysis of the significance of the position and shape of blood patterns with respect to their origin and trajectory is exceedingly complex and requires the services of an examiner who is experienced in such determinations. Most important, the interpretation of bloodstain patterns necessitates carefully planned control experiments using surface materials comparable to those found at the crime scene.

A number of observations and conclusions have important implications for any investigator who seeks to trace the direction, dropping distance, and angle of impact of a bloodstain. Some of them can be summarized as follows:

1. Surface texture is very important in the interpretation of bloodstain patterns, and correlations between standards and unknowns are valid only if identical surfaces are used. In general, the harder and less porous the surface, the less spatter. The effect of surface is shown in Figure 8–8.
2. The direction of travel of blood striking an object may be discerned by the stain's shape. The pointed end of a bloodstain always faces its direction of travel. In Figure 8–9, the bloodstain pattern was produced by several droplets of blood that were traveling from left to right before striking a flat level surface.
3. It is possible to determine the impact angle of blood on a flat surface by measuring the degree of circular distortion of the stain. A drop of blood striking a surface at right angles produces a nearly circular stain; as the angle decreases, the stain elongates. This progressive elongation is evident in Figure 8–10.

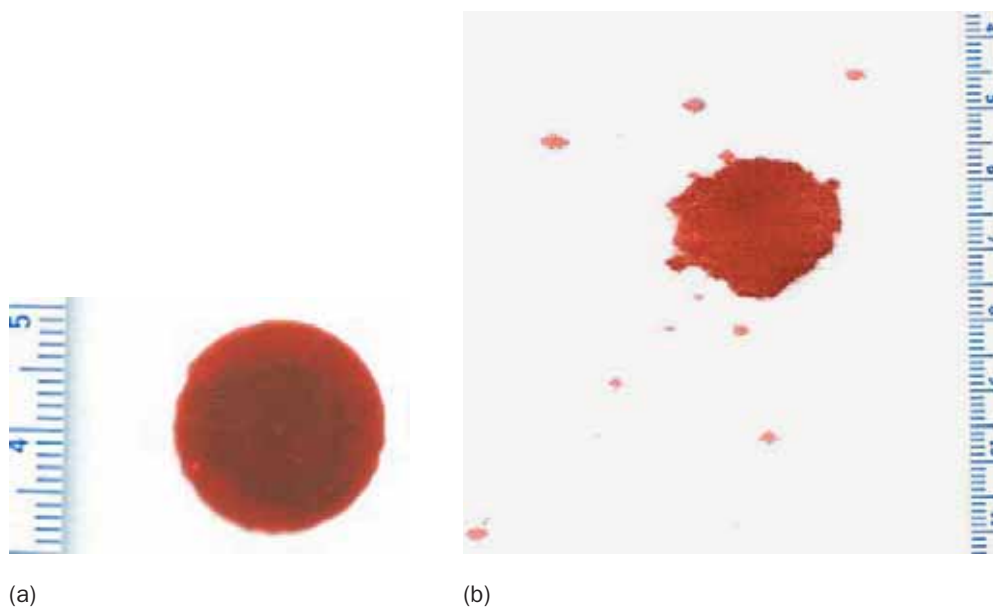
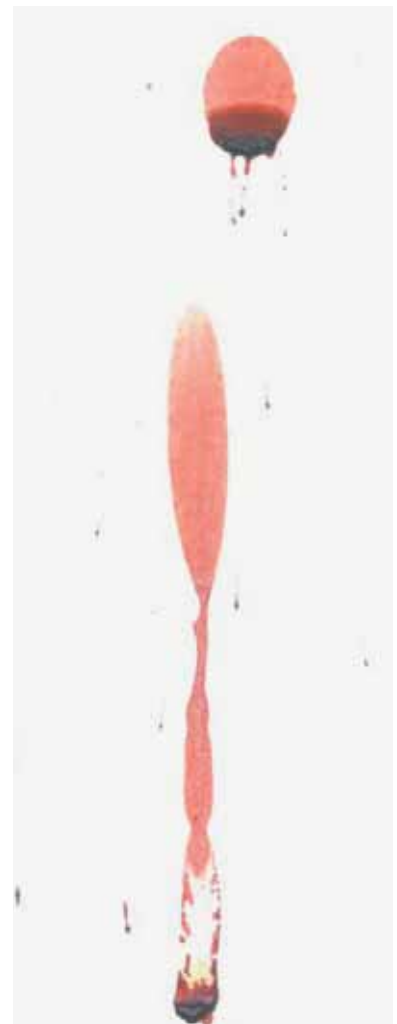


FIGURE 8-8 (a) Bloodstain from a single drop of blood that struck a glass surface after falling 24 inches. (b) Bloodstain from a single drop of blood that struck a cotton muslin sheet after falling 24 inches. *Courtesy A. Y. Wonder*



FIGURE 8-9 Bloodstain pattern produced by droplets of blood that were traveling from left to right. *Courtesy A. Y. Wonder*

FIGURE 8-10 The higher pattern is of a single drop of human blood that fell 24 inches and struck hard, smooth cardboard at 50 degrees. The lower pattern is of a single drop of human blood that fell 24 inches and struck hard, smooth cardboard at 15 degrees. *Courtesy A. Y. Wonder*



- The origin of a blood spatter in a two-dimensional configuration can be established by drawing straight lines through the long axis of several individual bloodstains. The intersection or area of convergence of the lines represents the area from which the blood emanated (see Figure 8–11).

An example of the utility of blood spatter formations in performing crime-scene reconstruction is illustrated in Figures 8–12 through 8–14. This case relates to an elderly male who was found lying dead on his living room floor. He had been beaten about the face and head, then stabbed in the chest and robbed. The reconstruction of bloodstains found on the interior front door and the adjacent wall documented that the victim was beaten about the face with a fist and struck on the back of the head with his cane. A suspect was apprehended three days later, and he was found to have an acute fracture of the right hand. When he was confronted with the bloodstain evidence, the suspect admitted striking the victim, first with his fist, then with a cane, and finally stabbing him with a kitchen knife. The suspect pleaded guilty to three first-degree felonies.

WebExtra 8.1

See How Bloodstain Spatter Patterns Are Formed

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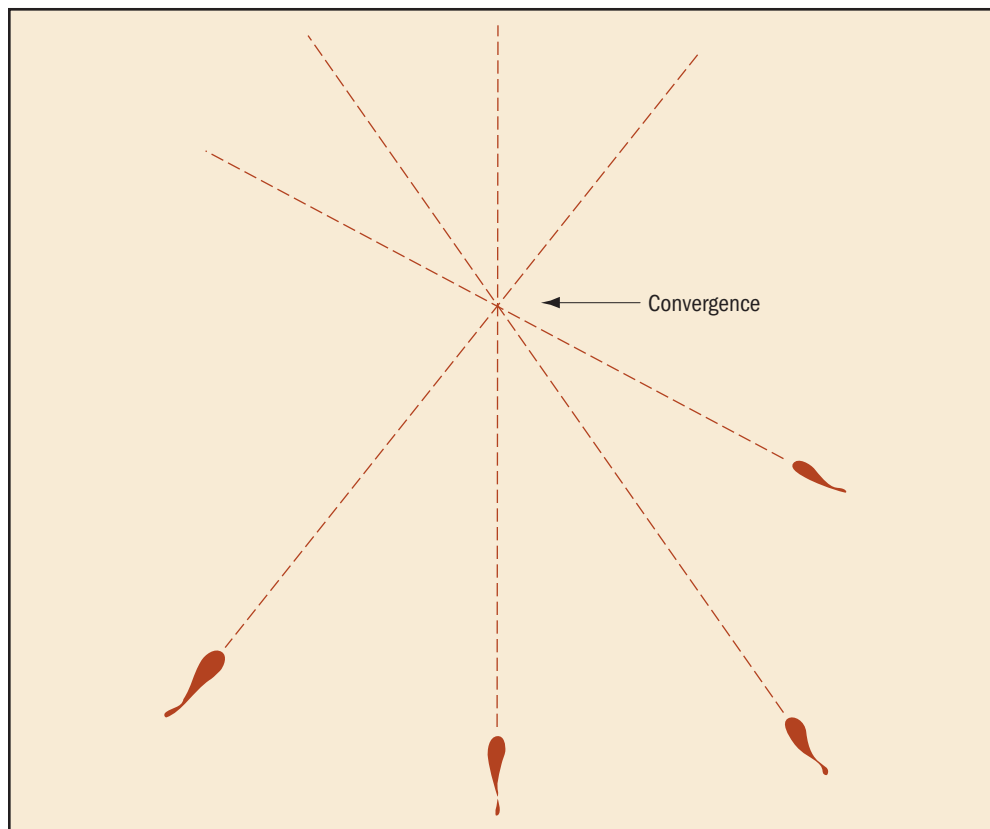


FIGURE 8–11 Illustration of stain convergence on a two-dimensional plane. Convergence represents the area from which the stains emanated. Courtesy Judith Bunker, J. L. Bunker & Assoc., Ocoee, Fla.

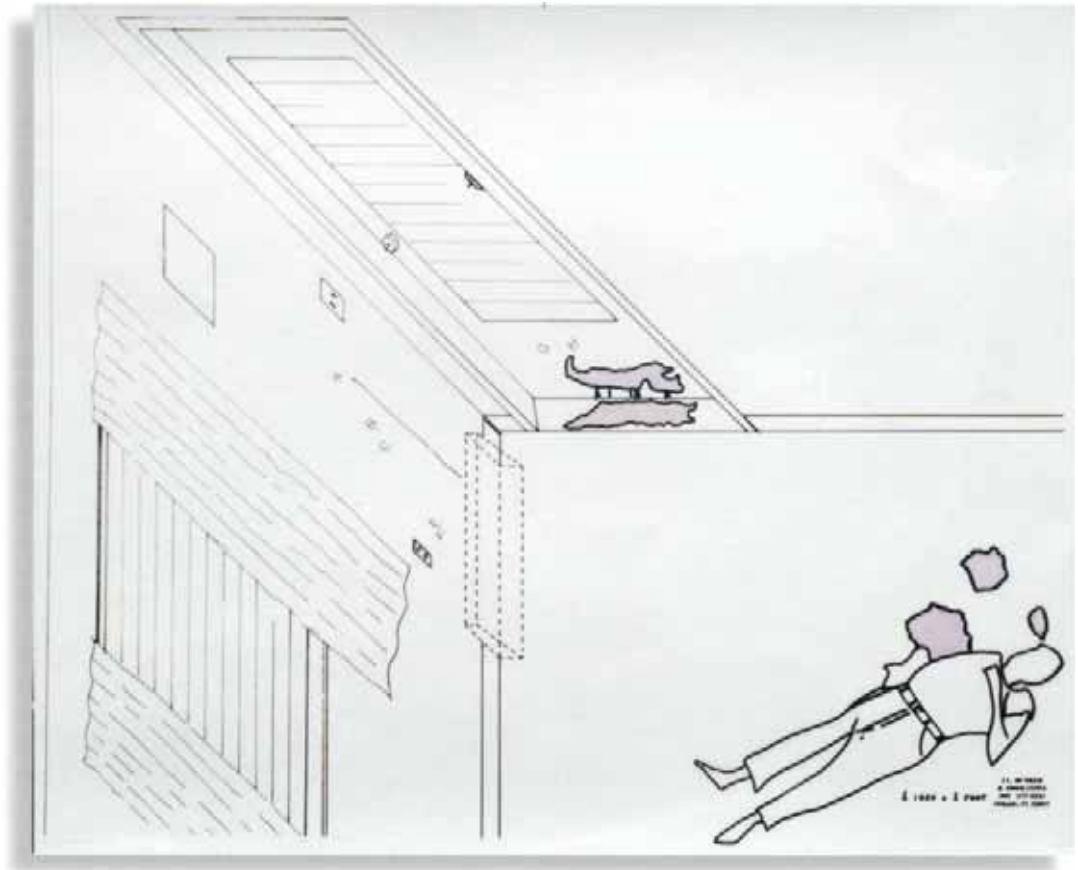


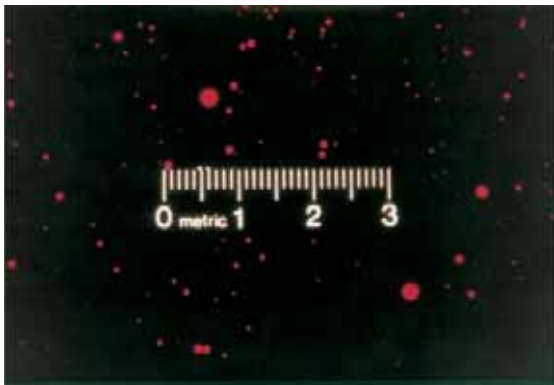
FIGURE 8-12a Three-dimensional diagram illustrating bloodstain patterns that were located, documented, and reconstructed. *Courtesy Judith Bunker, J. L. Bunker & Assoc., Ocoee, Fla.*



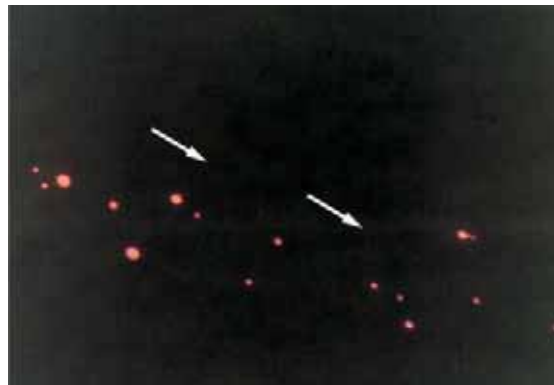
FIGURE 8-12b Crime-scene photograph of bloodstained areas. *Courtesy Sarasota County (Fla.) Sheriff's Department*



FIGURE 8-12c Detail photograph of bloodstains on the wall next to the interior door. Positions of impact spatter from blows that were inflicted to the victim's face are indicated in Figure 8-14(a). Arrow 1 points to the cast-off pattern directed left to right as blood was flung from the perpetrator's fist while inflicting blows. Arrow 2 points to three repetitive transfer impression patterns directed left to right as the perpetrator's bloodstained hand contacted the wall as the fist blows were being inflicted on the victim. Arrow 3 points to blood flow from the victim's wounds as he slumped against the wall. *Courtesy Judith Bunker, J. L. Bunker & Assoc., Ocoee, Fla.*



(a)



(b)



(c)



(d)

FIGURE 8-13 (a) Laboratory test pattern showing an impact spatter. The size and shape of the stains demonstrate a forceful impact 90 degrees to the target. (b) Laboratory test pattern illustrating a cast-off pattern directed left to right from a right overhead swing. (c) Laboratory test pattern showing a repetitive transfer impression pattern produced by a bloodstained hand moving left to right across the target. (d) Laboratory test patterns illustrating vertical flow patterns. The left pattern represents a stationary source; the right pattern was produced by left-to-right motion. *Courtesy Judith Bunker, J. L. Bunker & Assoc., Ocoee, Fla.*

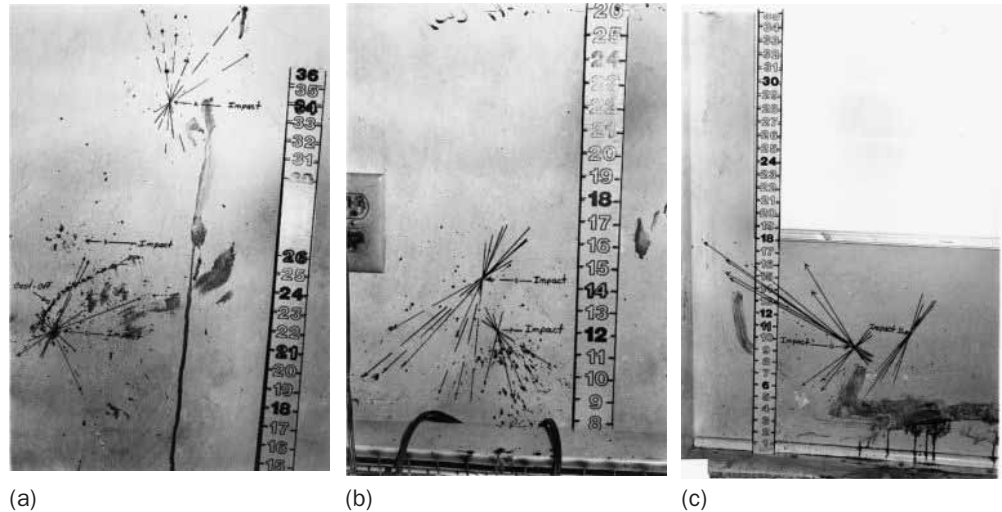


FIGURE 8-14 (a) Convergence of impact spatter patterns associated with beating by fist. (b) Convergence of impact spatter associated with the victim falling to the floor while bleeding from the nose. (c) Convergence of impact spatter associated with the victim while face down at the door, being struck with a cane. Courtesy Judith Bunker, J. L. Bunker & Assoc., Ocoee, Fla.

Forensic Brief

Stephen Scher banged on the door of a cabin in the woods outside Montrose, Pennsylvania. According to Scher, his friend, Marty Dillon, had just shot himself while chasing after a porcupine. The two had been skeet shooting at Scher's cabin, enjoying a friendly sporting weekend, when Dillon spotted a porcupine and took off out of sight. Scher heard a single shot and waited to hear his friend's voice. After a few moments, he chased after Dillon and found him lying on the ground near a tree stump, bleeding from a wound in his chest. Scher administered CPR after locating his dying friend, but he was unable to save Dillon, who later died from his injuries. Police found that Dillon's untied boot had been the cause of his shotgun wound. They determined that he had tripped while running with his loaded gun and shot himself. The grief-stricken Scher aroused no suspicion, so the shooting was ruled an accident.

Shortly thereafter, Scher moved from the area, divorced his wife, and married Dillon's widow. This was too suspicious to be ignored; police reopened the case and decides to reconstruct the crime scene.

The reconstruction provided investigators with several pieces of blood evidence that pointed to Scher as Dillon's murderer.

Police noticed that Scher's boots bore the unmistakable spray of high-velocity impact bloodspatter, evidence that he was standing within an arm's length of Dillon when Dillon was shot. This pattern of bloodstains can not be created while administering CPR, as Scher claimed had happened. The spatter pattern also clearly refuted Scher's claim that he did not witness the incident. In addition, the tree stump near Dillon's body bore the same type of blood spatter, in a pattern that indicated that Dillon was *seated on the stump* and not running when he was shot. Finally, Dillon's ears were free of the high-velocity blood spatter that covered his face, but blood was on his hearing protectors found nearby. This is a clear indication that he was wearing his hearing protectors when he was shot and they were removed before investigators arrived. This and other evidence resulted in Scher's conviction for the murder of his longtime friend Marty Dillon.

Key Points

- The location, distribution, and appearance of bloodstains and spatters may be useful for interpreting and reconstructing the events that produced bleeding.
- Surface texture and the stain's shape, size, and location must be considered when determining the direction, dropping distance, and angle of impact of a bloodstain.
- In general, the harder and less porous the surface, the less spatter.
- The direction of travel of blood striking an object may be discerned by the stain's shape. The pointed end of a bloodstain always faces its direction of travel.

Principles of Heredity

All of the antigens and polymorphic enzymes and proteins that have been described in previous sections are genetically controlled traits. That is, they are inherited from parents and become a permanent feature of a person's biological makeup from the moment he or she is conceived. Determining the identity of these traits, then, not only provides us with a picture of how one individual compares to or differs from another, but also gives us an insight into the basic biological substances that determine our overall makeup as human beings and the mechanism by which those substances are transmitted from one generation to the next.

Genes and Chromosomes

Hereditary material is transmitted via microscopic units called **genes**. The gene is the basic unit of heredity. Each gene by itself or in concert with other genes controls the development of a specific characteristic in the new individual; the genes determine the nature and growth of virtually every body structure.

The genes are positioned on **chromosomes**, threadlike bodies that appear in the nucleus of every body cell (see Figure 8–15). Almost all human cells contain forty six chromosomes, mated in twenty three pairs. The only exceptions are the human reproductive cells, the **egg** and **sperm**, which contain twenty three unmated chromosomes. During fertilization, a sperm and egg combine so that each contributes twenty three chromosomes to form the new cell (**zygote**). Hence, the new individual begins life properly with twenty three mated chromosome pairs. Because the genes are positioned on the chromosomes, the new individual inherits genetic material from each parent.

Actually, two dissimilar chromosomes are involved in the determination of sex. The egg cell always contains a long chromosome known as the **X chromosome**; the sperm cell may contain either a long X chromosome or a short **Y chromosome**. When an X-carrying sperm fertilizes an egg, the new cell is XX and develops into a female. A Y-carrying sperm produces an XY fertilized egg and develops into a male. Because the sperm cell determines the nature of the chromosome pair, we can say that the father biologically determines the sex of the child.

Alleles Just as chromosomes come together in pairs, so do the genes they bear. The position a gene occupies on a chromosome is its **locus**. Genes that

gene

The basic unit of heredity, consisting of a DNA segment located on a chromosome.

chromosome

A threadlike structure in the cell nucleus, along which the genes are located.

egg

The female reproductive cell.

sperm

The male reproductive cell.

zygote

The cell arising from the union of an egg and a sperm cell.

X chromosome

The female sex chromosome.

Y chromosome

The male sex chromosome.

locus

The physical location of a gene on a chromosome.



FIGURE 8-15 Computer-enhanced photomicrograph image of human chromosomes.
 Courtesy Alfred Pasiaka, Science Photo Library

WebExtra 8.2

Learn about the Structure of Our Genes

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allele

Any of several alternative forms of a gene located at the same point on a particular pair of chromosomes.

homozygous

Having two identical allelic genes on two corresponding positions of a pair of chromosomes.

heterozygous

Having two different allelic genes on two corresponding positions of a pair of chromosomes.

WebExtra 8.3

Learn about the Chromosomes Present in Our Cells

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govern a given characteristic are similarly positioned on the chromosomes inherited from the mother and father. Thus, a gene for eye color on the mother's chromosome will be aligned with a gene for eye color on the corresponding chromosome inherited from the father. Alternative forms of genes that influence a given characteristic and are aligned with one another on a chromosome pair are known as **alleles**.

Inheritance of blood type offers a simple example of allele genes in humans. An individual's blood type is determined by three genes, designated A, B, and O. A gene pair made up of two similar alleles—for example, AA and BB—is said to be **homozygous**. For example, if the chromosome inherited from the father carries the A gene and the chromosome inherited from the mother carries the same gene, the offspring will have an AA combination. Thus, when an individual inherits two similar genes from his or her parents, there is no problem in determining the blood type of that person. An individual with an AA combination will always be type A, a BB will be type B, and an OO will be type O.

A gene pair made up of two different alleles—AO, for example—is said to be **heterozygous**. For example, if the chromosome from one parent carries the A gene and the chromosome from the other parent carries the O gene, the genetic makeup of the offspring will be AO. When two different genes are inherited, one gene will be *dominant*—that is, the characteristic coded for by that gene is expressed. The other gene will be *recessive*—that is, its characteristics remain hidden. In the case of blood types, A and B genes are dominant and the O gene is recessive. Thus, with an AO combination, A is always dominant over O, and the individual is typed as A. Similarly, a BO combination is typed as B. In the case of AB, the genes are

codominant, and the individual's blood type will be AB. The recessive characteristics of O appear only when both recessive genes are present in combination OO, which is typed simply as O.

Genotypes and Phenotypes A pair of allele genes together constitutes the **genotype** of the individual. However, no known laboratory test can determine an individual's A-B-O genotype. For example, a person's outward characteristic, or **phenotype**, may be type A, but this does not tell us whether the genotype is AA or AO. The genotype can be determined only by studying the family history of the individual. If the genotypes of both parents are known, that of their possible offspring can be forecast.

An easy way to determine an individual's genotype is to construct a *Punnett square*. To do this, write along a horizontal line the two genes of the male parent, and in the vertical column write the two kinds of female genes present, as shown. In our example, we assume the male parent is type O and therefore has to be an OO genotype; the female parent is type AB and can be only an AB genotype:

		Father's genotype	
		O	O
Mother's genotype	A		
	B		

Next, write in each box the corresponding gene contributed from the female and then from the male. The squares will contain all the possible genotype combinations that the parents can produce in their offspring:

		O	O
A	A	AO	AO
	B	BO	BO

In this case, 50 percent of the offspring are likely to be AO and the other 50 percent BO. These are the only genotypes possible from this combination. Because O is recessive, 50 percent of the offspring will probably be type A and 50 percent type B. From this example, we can see that no blood group gene can appear in a child unless it is present in at least one of the parents.

Paternal Testing

Although the genotyping of blood factors has useful applications for studying the transmission of blood characteristics from one generation to the next, it has no direct relevance to criminal investigations. It does, however, have important implications in disputed-paternity cases, which are normally encountered in civil, not criminal, courts.

WebExtra 8.4

See How Genes Position Themselves on a Chromosome Pair

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genotype

The particular combination of genes present in the cells of an individual.

phenotype

The physical manifestation of a genetic trait such as shape, color, or blood type.

WebExtra 8.5

See How Genes Define Our Genetic Makeup

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Many cases of disputed paternity can be resolved by comparing the blood group genotypes of the suspected parents and offspring. For instance, suppose the male in the preceding Punnett square example is suspected of fathering a child by the female. If the child has type AB blood, the suspected father will be cleared because a type O father and a type AB mother cannot have a type AB child. On the other hand, if the child has type A or type B, the most that can be said is that the suspect may be the father. This does not mean that he *is* the father, just that he is not excluded based on blood typing. Obviously, many other males also have type O blood. Of course, the more blood group systems that are tested, the better the chances of excluding an innocent male from involvement. Conversely, if no discrepancies are found between the offspring and the suspect father, the more certain one can be that the suspect is indeed the father.

In fact, routine paternity testing involves characterizing blood factors other than A-B-O. For example, the HLA (human leukocyte antigen) test relies on identifying a complex system of antigens on white blood cells. If this test cannot exclude a suspect as the father of a child, the chances are better than 90 percent that he is the father. Paternity-testing laboratories have implemented DNA test procedures that can raise the odds of establishing paternity beyond 99 percent.

Key Points

- The gene is the basic unit of heredity. A chromosome is a threadlike structure in the cell nucleus, along which the genes are located.
- Most human cells contain forty six chromosomes, arranged in twenty-three mated pairs. The only exceptions are the human reproductive cells, the egg and sperm, which contain twenty three unmated chromosomes.
- During fertilization, a sperm and egg combine so that each contributes twenty three chromosomes to form the new cell, or zygote, that develops into the offspring.
- An allele is any of several alternative forms of genes that influence a given characteristic and that are aligned with one another on a chromosome pair.
- A heterozygous gene pair is made up of two different alleles; a homozygous gene pair is made up of two similar alleles.
- When two different genes are inherited, the characteristic coded for by a dominant gene is expressed. The characteristic coded for by a recessive gene remains hidden.
- A genotype is the particular combination of genes present in the cells of an individual. A phenotype is the physical manifestation of a genetic trait.

Forensic Characterization of Semen

Many cases received in a forensic laboratory involve sexual offenses, making it necessary to examine exhibits for the presence of seminal stains. The forensic examination of articles for seminal stains can actually

be considered a two-step process. First, before any tests can be conducted, the stain must be located. Considering the number and soiled condition of outer garments, undergarments, and possible bedclothing submitted for examination, this may prove to be an arduous task. Once located, the stain must be subjected to tests that will prove its identity. It may even be tested for the blood type of the individual from whom it originated.

Testing for Seminal Stains

Often seminal stains are visible on a fabric because they exhibit a stiff, crusty appearance. However, reliance on such appearance for locating the stain is unreliable, and is useful only when the stain is in an obvious area. If the fabric has been washed or contains only minute quantities of semen, visual examination offers little chance of detecting the stain. The best way to locate and at the same time characterize a seminal stain is to perform the *acid phosphatase color test*.

Acid Phosphatase Test **Acid phosphatase** is an **enzyme** that is secreted by the prostate gland into seminal fluid. Its concentrations in seminal fluid are up to 400 times those found in any other body fluid. Its presence can easily be detected when it comes in contact with an acidic solution of sodium alpha naphthylphosphate and Fast Blue B dye. Also, 4-methyl umbelliferyl phosphate (MUP) will fluoresces (emit light) under UV light when it comes in contact with acid phosphatase.

The utility of the acid phosphatase test is apparent when it becomes necessary to search many garments or large fabric areas for seminal stains. Simply moistening a filter paper with water and rubbing it lightly over the suspect area transfers any acid phosphatase present to the filter paper. Placing a drop or two of the sodium alpha naphthylphosphate and Fast Blue B solution on the paper produces a purple color that indicates the acid phosphatase enzyme. In this manner, any fabric or surface can be systematically searched for seminal stains.

If it is necessary to search extremely large areas—for example, a bed-sheet or carpet—the article can be tested in sections, narrowing the location of the stain with each successive test. Alternatively, the garment can be pressed against a suitably sized piece of moistened filter paper. The paper is then sprayed with MUP solution. Semen stains appear as strongly fluorescent areas under UV light. A negative reaction can be interpreted as absence of semen. Although some vegetable and fruit juices (such as cauliflower and watermelon), fungi, contraceptive creams, and vaginal secretions give a positive response to the acid phosphatase test, none of these substances normally reacts with the speed of seminal fluid. A reaction time of less than 30 seconds is considered a strong indication of semen.

Microscopic Examination of Semen Semen can be unequivocally identified by the presence of spermatozoa. When spermatozoa are located through a microscope examination, the stain is definitely identified as having been derived from semen. *Spermatozoa* are slender, elongated structures 50–70 microns long, each with a head and a thin flagellate tail (see Figure 8–16). The criminalist can normally locate them by immersing the stained material in a small volume of water. Rapidly stirring the liquid transfers a small percentage of the spermatozoa present into the

acid phosphatase

An enzyme found in high concentration in semen.

enzyme

A protein that acts as a catalyst for certain specific reactions.

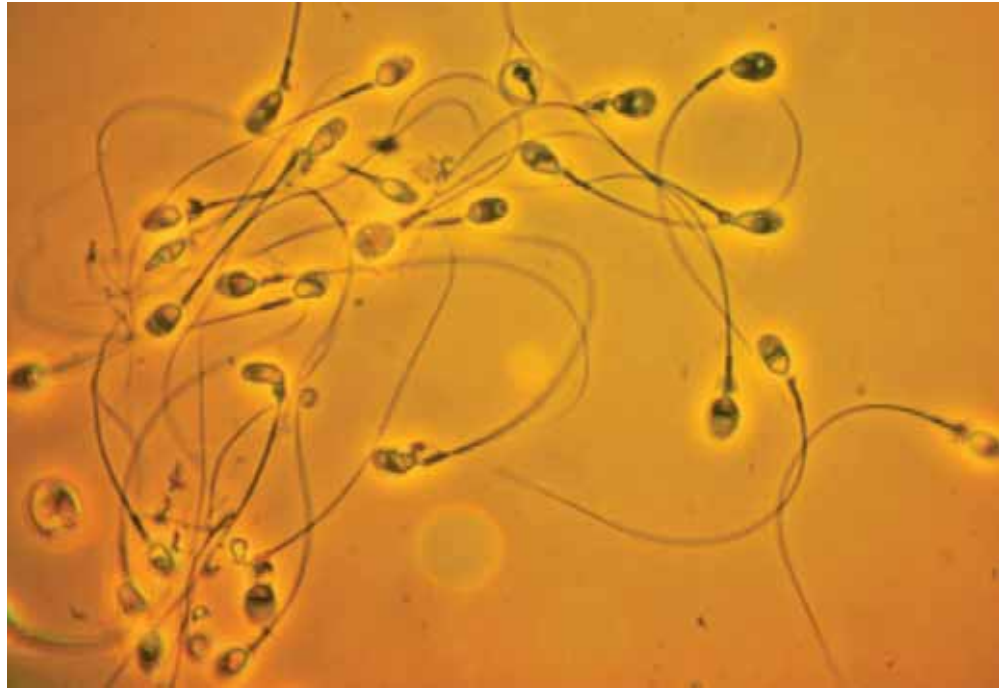


FIGURE 8-16 Photomicrograph of human spermatozoa (300 \times). Courtesy John Walsh

water. A drop of the water is dried onto a microscope slide, then stained and examined under a compound microscope at a magnification of approximately 400 \times .⁴

Considering the extremely large number of spermatozoa found in seminal fluid (the normal male releases 250–600 million spermatozoa during ejaculation), the chance of locating one should be very good; however, this is not always true. One reason is that spermatozoa bind tightly to cloth materials.⁵ Also, spermatozoa are extremely brittle when dry and easily disintegrate if the stain is washed or when the stain is rubbed against another object, as happens frequently in the handling and packaging of this type of evidence. Furthermore, sexual crimes may involve males who have an abnormally low sperm count, a condition known as **oligospermia**, or who have no spermatozoa at all in their seminal fluid (**aspermia**). Significantly, aspermatic individuals are increasing in numbers due to the growing popularity of vasectomies.

oligospermia

An abnormally low sperm count.

aspermia

The absence of sperm; sterility in males.

Prostate Specific Antigen (PSA) Analysts often examine stains or swabs that they suspect contain semen (because of the presence of acid phosphatase), but that yield no detectable spermatozoa. How, then, can one unequivocally prove the presence of semen? The solution to this problem came with the discovery in the 1970s of a protein called *p30* or *prostate specific antigen (PSA)*. Under the analytical conditions employed in forensic laboratories, *p30* is unique to seminal plasma.

When *p30* is isolated and injected into a rabbit, it stimulates the production of polyclonal antibodies (anti-*p30*). The serum collected from these immunized rabbits can then be used to test suspected semen stains. As shown in Figure 8-17, the stain extract is placed in one well of an

electrophoretic plate and the anti-p30 in an opposite well. When an electric potential is applied, the antigens and antibodies move toward each other. The formation of a visible line midway between the two wells shows the presence of p30 in the stain and proves that the stain was seminal.

A more elegant approach to identifying PSA (p30) is shown in Figure 8–18. First, a monoclonal PSA antibody is attached to a dye and placed on a porous membrane. Next, an extract from a sample suspected of containing PSA is placed on the membrane. If PSA is present in the extract, it combines with the monoclonal PSA antibody to form a PSA antigen–monoclonal PSA antibody complex. This complex migrates along the membrane, where it interacts with a polyclonal PSA antibody imbedded in the membrane. The antibody–antigen–antibody “sandwich” that forms is apparent by the presence of a colored line (see Figure 8–18). This monoclonal antibody technique is about 100 times as sensitive as the electrophoretic method for detecting PSA.⁶

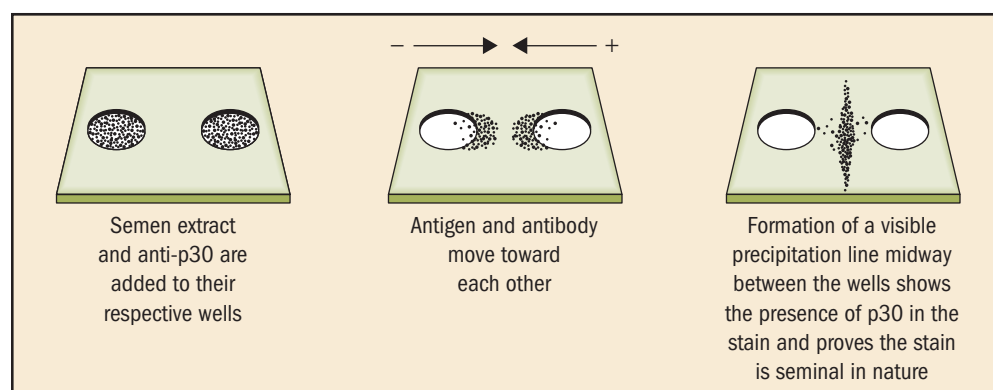


FIGURE 8–17 PSA testing by electrophoresis.

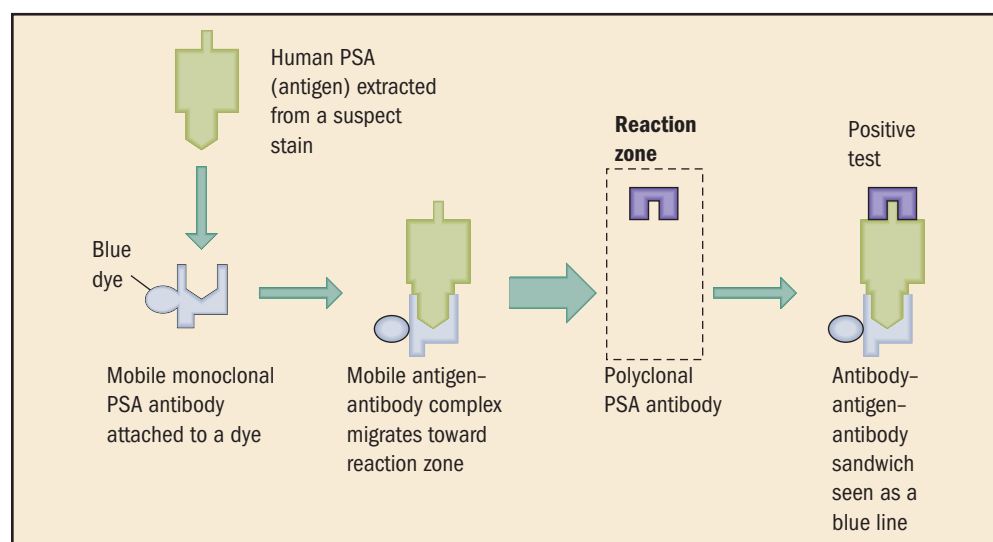


FIGURE 8–18 An antibody–antigen–antibody sandwich or complex is seen as a colored band. This signifies the presence of PSA in the extract of a stain and positively identifies human semen.

Once the material is proven to be semen, the next task is to associate the semen as closely as possible with an individual. As we will learn in Chapter 9, forensic scientists can link seminal material to one individual with DNA technology. Just as important is the knowledge that this technology can exonerate many of those wrongfully accused of sexual assault.

Key Points

- The best way to locate and characterize a seminal stain is to perform the acid phosphatase color test.
- The presence of spermatozoa, or of the protein called prostate specific antigen (PSA), also known as p30, proves that a sample stain contains semen.
- Forensic scientists can link seminal material to an individual by DNA typing.

Collection of Rape Evidence

Seminal constituents on a rape victim are important evidence that sexual intercourse has taken place, but their absence does not necessarily mean that a rape did not occur. Physical injuries such as bruises and bleeding tend to confirm that a violent assault occurred. Furthermore, the forceful physical contact between victim and assailant may result in a transfer of physical evidence—blood, semen, hairs, and fibers. The presence of such evidence helps forge a vital link in the chain of circumstances surrounding a sexual crime.

To protect this kind of evidence, all the outer garments and undergarments from the people involved should be carefully removed and packaged separately in paper (not plastic) bags. Place a clean bedsheet on the floor and lay a clean paper sheet over it. The victim must remove her shoes before standing on the paper. Have the person disrobe while standing on the paper in order to collect any loose foreign material falling from the clothing. Collect each piece of clothing as it is removed and place in separate paper bags to avoid cross-contamination. Carefully fold the paper sheet so that all foreign materials are contained inside. If appropriate, bedding or the object on which the assault took place should be submitted to the laboratory for processing.

Items suspected of containing seminal stains must be handled carefully. Folding an article through the stain may cause it to flake off, as will rubbing the stained area against the surface of the packaging material. If, under unusual circumstances, it is not possible to transport the stained article to the laboratory, the stained area should be cut out and submitted with an unstained piece as a substrate control.

In the laboratory, analysts try to link seminal material to a donor(s) using DNA typing. Because an individual may transfer his or her DNA types to a stain through perspiration, investigators must handle stained articles with care, minimizing direct personal contact. The evidence collector must wear disposable latex gloves when such evidence must be touched.

The rape victim must undergo a medical examination as soon as possible after the assault. At this time, the appropriate items of physical evidence are collected by trained personnel. Evidence collectors should have an evidence-collection kit from the local crime laboratory (see Figure 8–19).

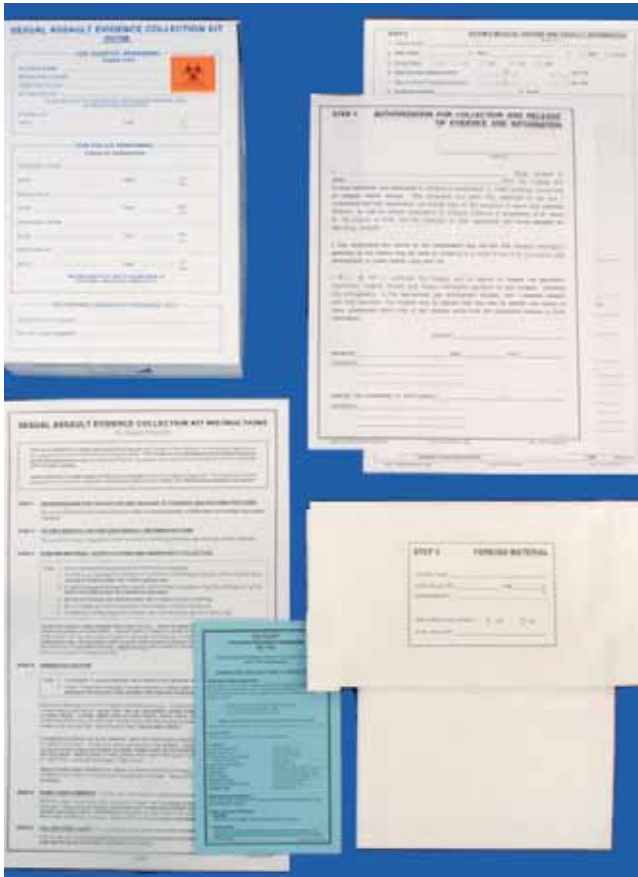
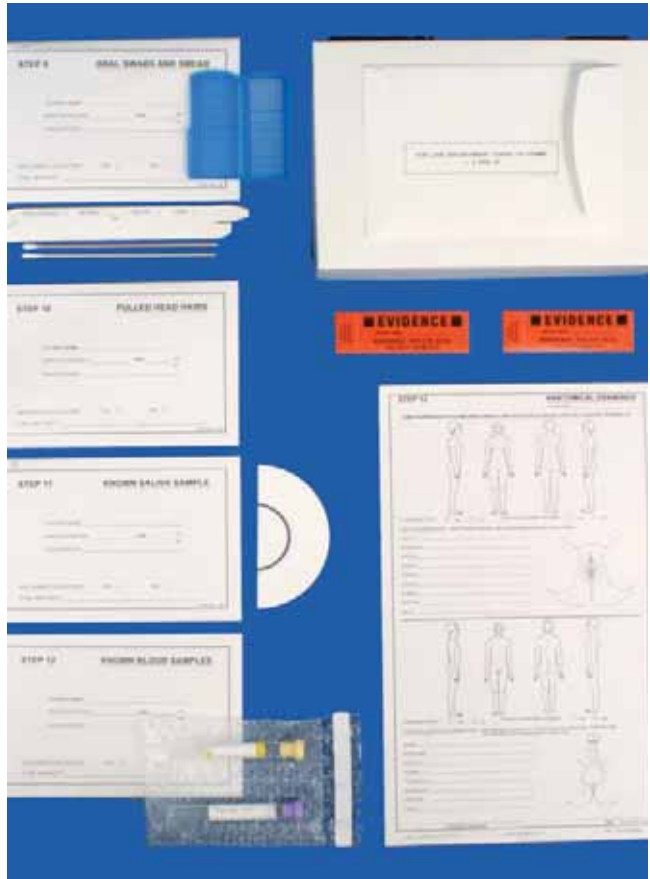


FIGURE 8-19a Victim rape collection kit showing the kit envelope, kit instructions, medical history and assault information forms, and foreign materials collection bag. Courtesy Tri-Tech, Inc., Southport, N.C., www.tritechusa.com



FIGURE 8-19b Victim rape collection kit showing collection bags for outer clothing, underpants, debris, pubic hair combings, pubic hair standard/reference samples, vaginal swabs, and rectal swabs. Courtesy Tri-Tech, Inc., Southport, N.C., www.tritechusa.com

FIGURE 8–19c Victim rape collection kit showing collection bags for oral swabs and smear, pulled head hair standard/reference, known saliva sample, known blood samples, and anatomical drawings. Courtesy Tri-Tech, Inc., Southport, N.C., www.tritechusa.com



The following items of physical evidence are to be collected:

1. **Pubic combings.** Place a paper towel under the buttocks and comb the pubic area for loose or foreign hairs.
2. **Pubic hair standard/reference samples.** Cut fifteen to twenty full-length hairs from the pubic area at the skin line.
3. **External genital dry-skin areas.** Swab with at least one dry swab and one moistening swab.
4. **Vaginal swabs and smear.** Using two swabs simultaneously, carefully swab the vaginal area and let the swabs air-dry before packaging. Using two additional swabs, repeat the swabbing procedure and smear the swabs onto separate microscope slides, allowing them to air-dry before packaging.
5. **Cervix swabs.** Using two swabs simultaneously, carefully swab the cervix area and let the swabs air-dry before packaging.
6. **Rectal swabs and smear.** To be taken when warranted by case history. Using two swabs simultaneously, swab the rectal canal, smearing one of the swabs onto a microscope slide. Allow both samples to air-dry before packaging.
7. **Oral swabs and smear.** To be taken if oral–genital contact occurred. Use two swabs simultaneously to swab the cheek area and gum line. Using both swabs, prepare one smear slide. Allow both swabs and the smear to air-dry before packaging.
8. **Head hairs.** Cut at the skin line a minimum of five full-length hairs from each of the following scalp locations: center, front, back, left side, and

right side. A total of at least fifty hairs should be cut and submitted to the laboratory.

9. **Blood sample.** Collect at least 7 milliliters in a vacuum tube containing the preservative EDTA. The blood sample can be used for DNA typing as well as for toxicological analysis if required.
10. **Fingernail scrapings.** Scrape the undersurface of the nails with a dull object over a piece of clean paper to collect debris. Use separate paper, one for each hand.
11. **All clothing.** Package as described earlier.
12. **Urine specimen.** Collect 30 milliliters or more of urine from the victim for analysis for Rohypnol, GHB, and other substances associated with drug-facilitated sexual assaults (see pp. 166–167).

Often during the investigation of a sexual assault, the victim reports that a perpetrator engaged in biting, sucking, or licking of areas of the victim's body. As we will learn in the next chapter, the tremendous sensitivity associated with DNA technology offers investigators the opportunity to identify a perpetrator's DNA types from saliva residues collected off the skin. The most efficient way to recover saliva residues from the skin is to first swab the suspect area with a rotating motion using a cotton swab moistened with distilled water. A second, dry swab is then rotated over the skin to recover the moist remains on the skin's surface from the wet swab. The swabs are air-dried and packaged together as a single sample.⁷

If a suspect is apprehended, the following items are routinely collected:

1. *All clothing* and any other items believed to have been worn at the time of assault.
2. *Pubic hair combings.*
3. *Pulled head and pubic hair standard/reference samples.*
4. A *penile swab* taken within twenty four hours of the assault, when appropriate to the case history.
5. A blood sample or buccal swab (see p. 341) for DNA typing purposes.

The advent of DNA profiling has forced investigators to rethink what items are evidential in a sexual assault. As we will learn in Chapter 9, DNA levels in the range of one-billionth of a gram are now routinely characterized in crime laboratories. In the past, scant attention was paid to the underwear recovered from a male who was suspected of being involved in a sexual assault; seminal constituents on a man's underwear had little or no investigative value. Today, the sensitivity of DNA analysis has created new areas of investigation. It is possible to link between a victim and an assailant by analyzing biological material recovered from the interior front surface of a male suspect's underwear. This is especially important when investigations have failed to yield the presence of suspect's DNA on exhibits recovered from the victim.

The persistence of seminal constituents in the vagina may help determine the time of an alleged sexual attack. While spermatozoa in the vaginal cavity provide evidence of intercourse, important information regarding the time of sexual activity can be obtained from the knowledge that motile (living) sperm generally survive for up to four to six hours in the vaginal

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A common mode of DNA transfer occurs when skin cells from the walls of the victim's vagina are transferred onto the suspect during intercourse. Subsequent penile contact with the inner surface of the suspect's underwear often leads to the recovery of the female victim's DNA from the underwear's inner surface. The power of DNA is illustrated in a case in which the

female victim of a rape had consensual sexual intercourse with a male partner before being assaulted by a different male. DNA extracted from the inside front area of the suspect's underwear revealed a female DNA profile matching that of the victim. The added bonus in this case was finding male DNA on the same underwear that matched that of the consensual partner.

Source: Gary G. Verret, "Sexual Assault Cases with No Primary Transfer of Biological Material from Suspect to Victim: Evidence of Secondary and Tertiary Transfer of Biological Material from Victim to Suspect's Undergarments," *Proceedings of the Canadian Society of Forensic Science*, Toronto, Ontario, November 2001.

cavity of a living female. However, a successful search for motile sperm requires a microscopic examination of a vaginal smear immediately after it is taken from the victim.

A more extensive examination of vaginal collections is later made at a forensic laboratory. Nonmotile sperm may be found in a living female for up to three days after intercourse and occasionally up to six days later. However, intact sperm (sperm with tails) are not normally found sixteen hours after intercourse but have been found as late as seventy two hours after intercourse. The likelihood of finding seminal acid phosphatase in the vaginal cavity markedly decreases with time following intercourse, with little chance of identifying this substance forty eight hours after intercourse.⁸ Hence, with the possibility of prolonged persistence of both spermatozoa and acid phosphatase in the vaginal cavity after intercourse, investigators should determine when and if voluntary sexual activity last occurred before the sexual assault. This information will help in evaluating the significance of finding these seminal constituents in a female victim. Blood or buccal swabs for DNA analysis should be taken from any consensual partner who had sex with the victim within seventy two hours of the assault.

Another significant indicator of recent sexual activity is p30. This semen marker normally is not detected in the vaginal cavity beyond twenty four hours following intercourse.⁹

Key Points

- A rape victim must undergo a medical examination as soon as possible after the assault. At that time clothing, hairs, and vaginal and rectal swabs can be collected for subsequent laboratory examination.

Chapter Summary

The term *serology* describes a broad scope of laboratory tests that use specific antigen and serum antibody reactions. An antibody reacts or agglutinates only with its specific antigen. The identity of each of the four A-B-O blood groups can be established by testing the blood with anti-A and anti-B sera.

The concept of specific antigen–antibody reactions has been applied to immunoassay techniques for detecting abused drugs in blood and urine. When an animal is injected with an antigen, its body produces a series of different antibodies, all of which are designed to attack some particular site on the antigen of interest. These antibodies are known as polyclonal antibodies. Alternatively, a more uniform and specific collection of antibodies designed to combine with a single antigen site can be manufactured. Such antibodies are known as monoclonal antibodies.

The criminalist must answer the following questions when examining dried blood: (1) Is it blood? (2) From what species did the blood originate? (3) If the blood is human, how closely can it be associated with a particular individual? The determination of blood is best made by means of a preliminary color test. A positive result from the Kastle-Meyer color test is highly indicative of blood. Alternatively, the luminol test is used to find trace amounts of blood at crime scenes. The precipitin test uses antisera normally derived from rabbits that have been injected with the blood of a known animal to determine the species origin of a questioned bloodstain. Before the advent of DNA typing, bloodstains were linked, in part, to a source by A-B-O typing. This approach has now been supplanted by DNA technology.

The location, distribution, and appearance of bloodstains and spatters may be useful for interpreting and reconstructing the events that produced the bleeding. Surface texture and the stain's shape, size, and location must be considered when determining the direction, dropping distance, and angle of impact of a bloodstain.

Many cases sent to a forensic laboratory involve sexual offenses, making it necessary to examine exhibits for seminal stains. The best way to locate and characterize a seminal stain is to perform the acid phosphatase color test. Semen can be unequivocally identified by the presence of either spermatozoa or p30, a protein unique to seminal plasma. Forensic scientists can link seminal material to an individual by DNA typing. The rape victim must undergo a medical examination as soon as possible after the assault. At that time clothing, hairs, and vaginal and rectal swabs can be collected for subsequent laboratory examination. If a suspect is apprehended within twenty four hours of the assault, it may be possible to detect the victim's DNA on the male's underwear or on a penile swab of the suspect.

Review Questions

Facts and Concepts

1. Who discovered that blood is distinguishable by its group or type?
2. What blood factors are the most important for properly matching a donor and recipient for a transfusion?
3. What technique supplanted blood typing for associating bloodstain evidence with a particular individual?
4. What is plasma? What percentage of blood content does plasma account for?
5. Which of the following types of cells are not contained in plasma?
 - a. phagocytes
 - b. leukocytes
 - c. erythrocytes
 - d. platelets
6. What are antigens and antibodies? What part of the blood contains antibodies?
7. Describe how antibodies and antigens determine one's A-B-O blood type.
8. What is the fourth important antigen other than A, B, and O?
9. What happens when serum containing B antibodies is added to red blood cells carrying the B antigen? Will the same thing happen if serum containing B antibodies is added to red blood cells carrying the A antigen? Explain your answer.
10. What is serology and what is its most widespread application? In what other areas related to forensic science is it finding application?
11. Briefly describe how antibodies capable of reacting with drugs are produced in animals.
12. What immunoassay technique has gained widespread popularity among toxicologists because of its speed and high sensitivity for detecting drugs in urine? What drug is this technique frequently used to screen for?
13. What is the greatest problem associated with detecting marijuana in urine?
14. What is the difference between monoclonal and polyclonal antibodies?
15. What three questions must the criminalist answer when examining dried blood?
16. Name the most common color test for blood and briefly describe how it identifies bloodstains.
17. Briefly describe how luminol is used to detect bloodstains.
18. Name two reasons why color tests are superior to microcrystalline tests for identifying blood.
19. What is the standard test used to determine whether blood is of human or animal origin? Briefly state the principle underlying the test.

20. Which technique takes advantage of the fact that antibodies and antigens move toward one another on a plate coated with medium made from a natural polymer called agar?
21. In what technique can antigens and antibodies be induced to move toward one another under the influence of an electrical field?
22. How do the hardness and porosity of a surface affect blood spattering on that surface?
23. How does a blood spatter analyst determine the direction of travel of blood striking an object?
24. A drop of blood striking a surface at right angles produces a stain of what shape? As the angle decreases, what happens to the shape of the stain?
25. When examining a blood spatter in a two-dimensional configuration, how does an analyst determine the area from which the blood emanated?
26. Define *gene* and *chromosome*.
27. How many chromosomes do most human cells contain and how are chromosomes arranged in the cell? What cells are the exception to this rule? How are these cells different from all other cells?
28. Describe how genetic material is transferred from parents to offspring.
29. What is an allele?
30. What is the difference between a heterozygous gene pair and a homozygous gene pair?
31. What is the difference between a dominant and a recessive gene?
32. Define *genotype* and *phenotype*. What is the only way to determine an individual's genotype?
33. In what type of noncriminal cases does blood typing have important implications?
34. The best way to locate and characterize a seminal stain is to perform what test? In what situation is this test particularly useful?
35. Define *ogliospermia* and *aspermia*.
36. The presence of what protein proves that a sample stain contains semen? What two techniques are used to detect this protein?
37. Why must a rape victim stand on a sheet of paper while disrobing for forensic analysis?
38. Besides swabbing for semen constituents, what other bodily fluids should be collected from a rape victim during a medical examination?
39. What items should be collected from the suspected perpetrator of a sexual assault?
40. How long do motile sperm generally survive in the vaginal cavity of a living female? How long do nonmotile sperm generally survive in the vaginal cavity of a living female?

Application and Critical Thinking

1. After looking at the bloodstains in the figure, answer the following questions:
 - a. Which three drops struck the surface closest to a 90-degree angle? Explain your answer.
 - b. Which three drops struck the surface farthest from a 90-degree angle? Explain your answer.
 - c. In what direction were drops 2 and 7 traveling when they struck the surface? Explain your answer.



2. Complete the following Punnett squares and answer the questions that follow.
Subject: Greg

		Father's genotype	
		A	O
Mother's genotype	A		
	B		

Subject: Dale

		Father's genotype	
		A	B
Mother's genotype	A		
	A		

Subject: Maura

		Father's genotype	
		B	O
Mother's genotype	B		
	O		

- a. Among this group of people, which blood type(s) is most likely to be expressed? Which blood type is least likely to be expressed? Explain your answers.
 - b. What blood type is Greg most likely to have? Explain your answer.
 - c. Which person is least likely to have type B blood? Explain your answer.
 - d. In which, if any, of these people may a recessive gene express itself? Explain your answer.
3. Suppose that Greg and Maura have a child. Construct all of the possible Punnett squares for that child and answer the following questions:
 - a. What blood type(s) is the child most likely to have?
 - b. What genotype(s) is the child most likely to have?
 - c. If Maura and Greg have the same genotype, what blood type is the child most likely to have? What are the chances that the child will have type O blood? Explain your answers.
 4. Police investigating the scene of a sexual assault recover a large blanket that they believe may contain useful physical evidence. They take it to the laboratory of forensic serologist Scott Alden, asking him to test it for the presence of semen. Noticing faint pink stains on the blanket, Scott asks the investigating detective if he is aware of anything that might recently have been spilled on the blanket. The detective reports that an overturned bowl of grapes and watermelon was found at the scene, as well as a broken glass that had contained wine. After the detective departs, Scott chooses and administers what he considers the best test for analyzing the piece of evidence in his possession. Three minutes after completion of the test, the blanket shows a positive reaction. What test did Scott choose and what was his conclusion? Explain your answer.
 5. Criminalist Cathy Richards is collecting evidence from the victim of a sexual assault. She places a sheet on the floor, asks the victim to disrobe, and places the clothing in a paper bag. After collecting pubic combings and pubic hair samples, she takes two vaginal swabs, which she allows to air-dry before packaging. Finally, Cathy collects blood, urine, and scalp hair samples from the victim. What mistakes, if any, did she make in collecting this evidence?

WebExtra 8.6**Virtual Crime Scene 8.1**www.prenhall.com/hsforensics**WebExtra 8.7****Virtual Crime Scene 8.2**www.prenhall.com/hsforensics**Virtual Crime Scenes****Crime Scene 8.1**

Step into the role of the first responding officer at a sexual assault scene

www.prenhall.com/hsforensics**Crime Scene 8.2**

Assume the duties of an evidence-collection technician at a sexual assault scene

www.prenhall.com/hsforensics

Web Resources

Animated Blood Types (Discusses blood factors, blood typing, antibodies, and antigens using both text and animated sequences)

waynesword.palomar.edu/aniblood.htm

Blood Typing Game (Interactive application teaches about blood types and factors by having the user analyze a patient's blood and determine the right type of blood to give in a transfusion)

nobelprize.org/educational_games/medicine/landsteiner/

Bloodstains as Investigative Evidence (Discussion of characteristics of blood; shape, persistence, and age of bloodstains; procedures for processing blood evidence; and lab analysis of blood evidence)

www.pimall.com/nais/nl/n.bloodstains.html

Bloodstain Pattern Analysis Tutorial (Illustrated guide to bloodstain analysis includes examples of different types of bloodstains, blood spatter patterns and directionality, and impact angle determination)

www.bloodspatter.com/BPATutorial.htm

Forensic Serology

faculty.ncwc.edu/toconnor/425/425lect13.htm

How Luminol Works

www.howstuffworks.com/luminol.htm

Human Heredity (Online slide show covering basic principles of heredity and gene interactions)

www.bsd405.org/teachers/suttonk/Inheritance/Powerpoint1/humheredity/humanheredity/index.htm

Endnotes

1. M. Cox, "A Study of the Sensitivity and Specificity of Four Presumptive Tests for Blood," *Journal of Forensic Sciences* 36 (1991): 1503.
2. The luminol reagent is prepared by mixing 0.1 grams 3-amino-phthalhydrazide and 5.0 grams sodium carbonate in 100 milliliters distilled water. Before use, 0.7 grams sodium perborate is added to the solution.
3. A. M. Gross et al., "The Effect of Luminol on Presumptive Tests and DNA Analysis Using the Polymerase Chain Reaction," *Journal of Forensic Sciences* 44 (1999): 837.
4. J. P. Allery et al., "Cytological Detection of Spermatozoa: Comparison of Three Staining Methods," *Journal of Forensic Sciences* 46 (2001): 349.
5. In one study, only a maximum of 4 sperm cells out of 1,000 could be extracted from a cotton patch and observed under the microscope. Edwin Jones (Ventura County Sheriff's Department, Ventura, Calif.), personal communication.

6. J. Kearsey, H. Louie, and H. Poon, "Validation Study of the Onestep ABACard® PSA Test Kit for RCMP Casework," *Canadian Society of Forensic Science Journal* 34 (2001): 63.
7. D. Sweet et al., "An Improved Method to Recover Saliva from Human Skin: The Double Swab Technique," *Journal of Forensic Sciences* 42 (1997): 320.
8. Anne Davies and Elizabeth Wilson, "The Persistence of Seminal Constituents in the Human Vagina," *Forensic Science* 3 (1974): 45.
9. J. Kearsey, H. Louie, and H. Poon, "Validation Study of the Onestep ABACard® PSA Test Kit for RCMP Casework," *Canadian Society of Forensic Science Journal* 34 (2001): 63.