chapter 12

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

iso-enzymes

locus

luminol

monoclonal antibodies

oligospermia

phenotype

plasma

polyclonal antibodies

polymorphism

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
- Explain the differences between monoclonal and polyclonal antibodies
- 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 to be properly preserved for laboratory examination
- Describe the proper collection of physical evidence in a rape investigation

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 physi-

cian, 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.

In 1901, Karl Landsteiner announced one of the most significant discoveries of this century—the typing of blood—a finding that twenty-nine years later earned him a Nobel Prize. For years phy-

sicians had attempted to transfuse blood from one individual to another. Their efforts often ended in failure because the transfused blood tended to coagulate 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 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 had opened up a completely 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 was demonstrated, and shortly thereafter, numerous blood factors or groups were discovered. More than a hundred different blood factors have been shown to exist. 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 to the forensic scientist 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 high frequency of occurrence of bloodstains at crime scenes, especially crimes of the most serious nature—that is, 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 evidence of the strongest kind for linking the suspect to the crime scene.

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)**. The individualization of dried blood and other biological evidence, now a reality, has significantly altered the role that crime laboratories play in criminal investigations. As we will learn in the next chapter, the high sensitivity of DNA analysis has even altered the type 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

Antigens and Antibodies

The word *blood* actually refers to a highly complex mixture of cells, enzymes, proteins, and inorganic substances. The fluid portion of blood is called **plasma**. Plasma is composed principally of water and accounts for 55 percent of blood content. Suspended in the plasma are solid materials consisting chiefly of cells—that is, 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 one

were to remove the clotted material, 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.

Functionally, red blood cells transport oxygen from the lungs to the body tissues and in turn 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 blood-type 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 contain both A and B antigens. Type O individuals have neither A nor B antigens on their cells. Hence, the presence or absence of the 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. People with the D antigen are said to be *Rh positive;* 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 for the compatibility of the donor and recipient.

Serum is important because it contains certain proteins known as **antibodies**. **The funda-**

mental 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 immediately 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 12–1).

Let's look a little more closely at this phenomenon. In normal blood, shown in Figure 12–2(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. The occurrence of a specific antigen–antibody reaction immediately causes the red blood cells to link together, or agglutinate, as shown in Figure 12–2(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, transfusing 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** is used to describe 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 12–1 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 12–2 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.

Table 12–1 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 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 route to the determination of 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 12–2.

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%

IMMUNOASSAY TECHNIQUES

The concept of a specific antigen–antibody reaction is finding application in other areas unrelated to the blood typing of individuals. Most significantly, this approach has been extended to the detection of drugs in blood and urine. Antibodies that react with drugs do not naturally exist; 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 12–3). The recovered blood serum of the animal will contain antibodies that are specific or nearly specific to the drug.

A number of 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 the drug being measured. For example, if someone's urine is being checked for the presence of methadone, one would add methadone antibodies to the

urine. Any methadone present in the urine immediately combines with these antibodies. Then enzyme-labeled methadone is added to the urine. Methadone antibodies that did not interact with the methadone now combine with the enzyme-labeled methadone. The quantity of enzyme-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 smokers. In marijuana, THC is considered the primary pharmacologically active agent (see p. 256). To facilitate its elimination, the body converts THC to a series of substances or 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 smokers contains THC-9-carboxylic acid in a very small quantity (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. While smoking marijuana will result in the detection of THC metabolite, it is very difficult to determine when the individual actually used marijuana. In individuals who smoke 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. Though 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 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 anti-

body 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 at the end of Chapter 1.

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 one, an antigen typically has structurally different sites to which an antibody may bind. So when the animal is actively producing attack antibodies it 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**. However, the disadvantage of polyclonal antibodies is that an animal can produce antibodies that 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.

As the technologies associated with forensic science have grown in importance, a need has developed, in some instances, to have access to antibodies that are more uniform in their composition and attack power than the traditional polyclonals. This is best accomplished by adopting a process in which an animal will 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 will 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. The hybridoma cells that bear 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 12–4).

Monoclonal antibodies are being incorporated into commercial forensic test kits with increasing frequency. Many immunoassay test kits for drugs of abuse are being formulated with monoclonal antibodies. Also, a recently introduced test for seminal material that incorporates a monoclonal antibody has found wide popularity in crime laboratories (see pp. 369–370).

As a side note, in 1999 the U.S. Food and Drug Administration approved a monoclonal drug treatment for cancer. Rituxin is a nontoxic monoclonal antibody designed to attack and destroy cancerous white blood cells containing an antigen designated as CD20. Other monoclonal drug treatments are in the pipeline. Monoclonals are finally beginning to fulfill their long-held expectation as medicine's version of the "magic bullet."

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 of human origin, how closely can it be associated with a particular individual?

The determination of blood is best made by means of a preliminary color test. For many years, the most commonly used test for this purpose 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 by peroxides. When a bloodstain, phenolphthalein reagent, and hydrogen peroxide are mixed together, the blood's hemoglobin will cause the formation of 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, it is unlikely that such materials will 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 is indicative of blood.

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. By spraying luminol reagent onto a suspect item, investigators can quickly screen large areas for bloodstains. The sprayed objects must be located in a darkened area while being viewed for the emission of light (luminescence).

The luminol test is extremely sensitive—it is 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 12–5). It is important to note that luminol does not interfere with any subsequent DNA testing.[3]

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 of these depend on the addition of specific chemicals to the blood so that characteristic crystals with hemoglobin derivatives will form. 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.

Once the stain has been characterized as blood, the serologist determines whether the stain is of human or animal origin. For this purpose, the standard test used 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. This serum contains antibodies that specifically react with human 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. Currently, antiserums are commercially available for humans and for a variety of commonly encountered animals—for example, dogs, cats, and deer.

A number of 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 12–6).

Another method, called *gel diffusion*, takes advantage of the fact that antibodies and antigens diffuse or move toward one another on an agar gel–coated plate. The extracted bloodstain and

the human antiserum are placed in separate holes opposite each other on the gel. If the blood is of human origin, a line of precipitation will form 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* (see pp. 142–146), 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 12–7).

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 12–8).

Once it has been determined that the bloodstain is of human origin, 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.

In addition to the A and B antigens discussed earlier, other substances found in the red blood cell were widely investigated until the mid-1990s as forensic scientists sought to individualize bloodstains. These other substances are called **enzymes**. Enzymes are proteins that have important functions in regulating many of the body's chemical reactions. In the past, forensic serologists were particularly interested in enzymes that exist in different forms, or are **polymorphic**. These enzymes can actually be separated into protein components called **iso-enzymes**. Again, the advent of DNA analysis has reduced this approach for characterizing biological stains to one

of historical interest only.

Let's look at one such enzyme, PGM, in order to understand how forensic serologists use this marker to characterize biological evidence. The iso-enzymes of PGM (phosphoglucomutase) can be separated from one another by electrophoresis (see pp. 142–146). What is interesting and most important about this separation is the observation that everyone does not have the same PGM iso-enzymes. Actually, as shown in Figure 12–9, there are three common variations or types of PGM: PGM 1, PGM 2-1, and PGM 2. These variations are distributed unevenly throughout the population: PGM 1 is present in approximately 58 percent of the population; PGM 2-1 in 36 percent; and PGM 2 in 6 percent. Thus, identification of the PGM type in a dried bloodstain provides the forensic serologist with added statistical information with which to reduce the number of possible sources of the bloodstain. Numerous polymorphic enzymes in red blood cells provide potential markers for determining blood origin. Also, a number of polymorphic proteins have been found in blood serum. However, from a practical point of view, only enzymes and proteins that are capable of surviving the drying and aging processes are of any value to the forensic serologist.

Because antigens, enzymes, and proteins occur independently of one another, the probability of a dried bloodstain having a particular combination of these factors is determined by the product of their distribution in the population (see p. 75). For example, if a bloodstain is found to be type A, then such a stain could have originated from approximately 42 percent of the population. Now, if it is also determined that this stain contains PGM 1, then its origin can be narrowed to 24 percent of the population (42% × 58% = 24%). Obviously, the more factors a serologist can find in a stain, the smaller its frequency of occurrence in a population. Hence, forensic researchers have made extensive efforts at uncovering blood factors that are identifiable in bloodstains (see

Table 12–3).

Table 12–3 Blood Enzymes and Proteins Used to Discriminate Bloodstains

Blood Factor	Abbreviation
Adenosine deaminase	ADA
Adenylate kinase	AK
Carbonic anhydrase II	CA II
Erythrocyte acid phosphatase	EAP
Esterase D	EsD
Glucose-6-phosphate dehydrogenase	G6PD
Glyoxylase I	GLO I
Group-specific component	Gc
Haptoglobin	Hp
Peptidase A	Pep A
Phosphoglucomutase	PGM
6-Phosphogluconate dehydrogenase	6PGD
Transferrin	Tf

STAIN PATTERNS OF BLOOD

The crime-scene investigator must not overlook the fact that the location, distribution, and appearance of bloodstains and spatters may be useful for interpreting and reconstructing the events

that must have occurred to have 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 of paramount importance 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 results. The effect of surface is shown in Figure 12–10.

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 12–11, 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 gives rise to a nearly circular stain; as the angle decreases, the stain becomes elongated in shape. This progressive elongation is evident in Figure 12–12.

4. 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

point of convergence of the lines represents the point from which the blood emanated (see Figure 12–13).

An example of the utility of blood spatter formations in performing crime-scene reconstruction is illustrated in Figures 12–14 through 12–16. 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.

PRINCIPLES OF HEREDITY

Transmission of Our Traits

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 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.

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 12–17. 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 only twenty-three unmated chromosomes. During fertilization, a sperm and egg combine so that each contributes 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**; but the sperm cell may contain either a short chromosome, known as the **Y chromosome**, or a long X 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 ultimately determines the nature of the chromosome pair, we can say that the father biologically determines the sex of the child.

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 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 chro-

mosome 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**. Another simple example of allele genes in humans is that of blood types belonging to the A-B-O system. Inheritance of the A-B-O type is best described by a theory that utilizes three genes designated A, B, and O.

A gene pair made up of two similar genes—for example, AA and BB—is said to be **homozygous**; a gene pair made up of two different genes—AO, for example—is said to be **heterozygous**. If the chromosome inherited from the father carries the A gene and the chromosome inherited from the mother carries the same gene, the offspring would have an AA combination. Similarly, if one chromosome contains the A gene and the other has the O gene, the genetic makeup of the offspring would be AO.

When an individual inherits two similar genes from his or her parents, there is no problem in determining the blood type of that person. Hence, an AA combination will always be type A, a BB type B, and an OO type O. However, when two different genes are inherited, one gene will be dominant. It can be said that the A and B genes are *dominant* and that the O gene is always *recessive*—that is, its characteristics remain hidden. For instance, with an AO combination, A is always dominant over O, and the individual will be 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. Hence, the combination OO is typed simply as O.

A pair of allele genes together constitutes the **genotype** of the individual. However, no laboratory test can determine an individual's A-B-O genotype. For example, a person's outward

characteristic, or **phenotype**, may be that of type A, but this does not tell us whether his or her 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 figure this out 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	AO	AO
B	BO	BO

Hence, 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.**

In the same way, the genotypes of parents determine the identity of all blood group systems as well as the polymorphic enzymes and proteins of their offspring. For example, an individual whose blood carries the enzyme EAP-BA has two allelic genes determining this trait. One gene corresponds to EAP-B, the other to EAP-A. When paired, these genes are codominant.

Paternity 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.

Many cases of disputed paternity can be resolved when the suspected parents and the offspring are related according to their blood group systems. For instance, in the previous example, had the child been type AB, the suspected father would have been cleared. A type O father and a type AB mother cannot have a type AB child. On the other hand, if the child had been type A or type B, the most that could be said is that the suspect may have been 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 discrepan-

cies are found between offspring and 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 a suspect cannot be excluded as fathering a child after this test is performed, the chances are better than 90 percent that he is the father. Currently, paternity testing laboratories have implemented DNA test procedures that can raise the odds of establishing paternity beyond 99 percent.

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 normal male releases 2.5 to 6 milliliters of seminal fluid during an ejaculation. Each milliliter contains 100 million or more spermatozoa, the male reproductive cells. 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 outergarments, undergarments, and possible bedclothing submitted for examination, this may prove to be an arduous task. Once located, the stain will have to 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.

Often, seminal stains are readily visible on a fabric because they exhibit a stiff, crusty appearance. However, reliance on such appearance for locating the stain is at best unreliable and is useful only when the stain is present in a rather obvious area. Certainly, if the fabric has been washed or contains only minute quantities of semen, visual examination of the article offers little

chance of detecting the stain. The best way to locate and characterize a seminal stain is to perform the *acid phosphatase color 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 greater than 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) fluoresces 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 numerous garments or large fabric areas for seminal stains. If a filter paper is simply moistened with water and rubbed lightly over the suspect area, acid phosphatase, if present, is transferred to the filter paper. Then, when a drop or two of the sodium alpha naphthylphosphate and Fast Blue B solution are placed on the paper, the appearance of a purple color 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 bedsheet or carpet—the article can be tested in sections, narrowing the location of the stain with each successive test. Alternatively, the garment under investigation 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 meaning the 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 the presence 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 12–18). The criminalist can normally locate them by immersing the stained material in a small volume of water. Rapid stirring of the liquid transfers a small percentage of the spermatozoa present into the 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×.[4]

Considering the extremely large number of spermatozoa found in seminal fluid, one would think the chance of locating one would be very good; however, this is not always true. One reason is that spermatozoa are bound 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 can happen 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 they may involve individuals 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.

Forensic analysts often must 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 an-

tibodies (anti-p30). The sera collected from these immunized rabbits can then be used to test suspected semen stains. As shown in Figure 12–19, 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 in nature.

A more elegant approach to identifying PSA (p30) involves placing an extract of a questioned sample on a porous membrane in the presence of a monoclonal PSA antibody that is linked to a dye. If PSA is present in the extract, a PSA antigen–monoclonal PSA antibody complex forms. This complex then migrates along the membrane, where it interacts with a polyclonal PSA antibody imbedded in the membrane. The antibody– antigen–antibody "sandwich" that forms will be apparent by the presence of a colored line (see Figure 12–20). This monoclonal antibody technique is about a hundred times more sensitive for detecting PSA than the one described in the previous paragraph.[6]

Once the material under examination is proven to be semen, the next task is to attempt to associate the semen as closely as possible with a single individual. As we will learn in Chapter 13, 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.

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 or bleeding tend to confirm that a violent assault did take place. 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 physical evidence will help forge a vital link in the chain of circumstances surrounding a sexual crime.

To protect this kind of evidence, all the outer- and undergarments from the involved parties 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 the items in separate paper bags to avoid cross-contamination of physical evidence. Carefully fold the paper sheet so that all foreign materials are contained inside.

If it is deemed 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 12–21).

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 moistened 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 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 buccal 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 skin line a minimum of five full-length hairs from each of the following scalp locations: center, front, back, left side, and right side. It is recommended that a total of at least fifty hairs be cut and submitted to the laboratory.

9. ***Blood sample.*** Collect at least 20 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 the purpose of conducting a drug toxicological analysis for Rohypnol, GHB, and other substances associated with drug-facilitated sexual assaults (see pp. 263–264).

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 perpetuator'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 which 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. *Penile swab* within twenty-four hours of assault when appropriate to case history.

5. A blood sample or buccal swab (see p. 414) for DNA typing purposes.

The advent of DNA profiling has forced investigators to rethink what items are evidential with respect to a sexual assault. As we will learn in Chapter 13, 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. From a practical point of view, the presence of seminal constituents on a man's underwear had little or no investigative value. Today, the high sensitivity of DNA analysis has created new areas of investigation. Experience now tells us that it is possible to establish a link between a victim and her 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 a suspect's DNA on exhibits recovered from the victim.

Forensic Brief

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 aptly illustrated in a case in which the female victim of a rape had consensual sexual intercourse with a male partner prior to 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 find-

ing male DNA on the same underwear which 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.

The persistence of seminal constituents in the vagina may become a factor when trying to ascertain the time of an alleged sexual attack. While the presence of spermatozoa in the vaginal cavity provides evidence of intercourse, important information regarding the time of sexual activity can be obtained from the knowledge that motile or living sperm generally survive up to four to six hours in the vaginal cavity of a living person. 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 the 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 prior to the sexual assault. This information will be useful for evaluating the significance of finding these seminal constituents in

the female victim. Blood or buccal swabs for DNA analysis are to be taken from any consensual partner having 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]

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 drugs of abuse 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. This collection of antibodies is known as polyclonal antibodies. Alternately, a more uniform and specific collection of antibodies designed to combine with a single antigen site can be manufactured. Such antibodies are known as *monoclonals.*

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 of human origin, how closely can it be associated to 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 search out trace amounts of blood located 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. Prior to the advent of DNA typing, bloodstains were linked to a source by A-B-O typing and the characterization of polymorphic blood enzymes and proteins. This approach has now been supplanted by the newer DNA technology.

The crime-scene investigator must remember that 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 the presence of 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

1. Karl Landsteiner discovered that blood can be classified by its _____.
2. True or False: No two individuals, except for identical twins, can be expected to have the same combination of blood types or antigens. _____
3. _____ is the fluid portion of unclotted blood.

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

5. _____ transport oxygen from the lungs to the body tissues and carry carbon dioxide back to the lungs.

6. On the surface of red blood cells are chemical substances called _____, which impart blood type characteristics to the cells.

7. Type A individuals have _____ antigens on the surface of their red blood cells.

8. Type O individuals have (both, neither) A and B antigens on their red blood cells.

9. The presence or absence of the _____ and _____ antigens on the red blood cells determines a person's blood type in the A-B-O system.

10. The D antigen is also known as the _____ antigen.

11. Serum contains proteins known as _____, which destroy or inactivate antigens.

12. An antibody reacts with (any, only a specific) antigen.

13. True or False: Agglutination describes the clumping together of red blood cells by the action of an antibody. _____

14. Type B blood contains _____ antigens and anti- _____ antibodies.

15. Type AB blood has (both, neither) anti-A (and, nor) anti-B.

16. A drug–protein complex can be injected into an animal to form specific _____ for that drug.

17. The term _____ describes the study of antigen–antibody reactions.

18. Type AB blood (is, is not) agglutinated by both anti-A and anti-B serum.

19. Type B red blood cells agglutinate when added to type (A, B) blood.

20. Type A red blood cells agglutinate when added to type (AB, O) blood.

21. An immunological assay technique used to detect the presence of minute quantities of drugs in blood and urine is _____.

22. The distribution of type A blood in the United States is approximately (42, 15) percent.

23. The distribution of type AB blood in the United States is approximately (12, 3) percent.

24. (All, Most) blood hemoglobin has peroxidase-like activity.

25. For many years, the most commonly used color test for identifying blood was the _____ color test.

26. _____ reagent reacts with blood, causing it to luminesce.

27. Blood can be characterized as being of human origin by the _____ test.

28. Antigens and antibodies (can, cannot) be induced to move toward each other under the influence of an electrical field.

29. Antibodies designed to interact with a specific antigen site are (monoclonal, polyclonal).

30. True or False: Hybridoma cells are used to produce antigens designed to attack one and only one site on an antibody. _____

31. _____ are proteins that have important functions in regulating many of the body's chemical reactions.

32. Enzymes that exist in different forms in a population are (polymorphic, monomorphic).

33. Protein and enzyme components can be separated and typed by the technique of

_____.

34. True or False: The shape of bloodstains may provide useful information regarding the direction, dropping distance, and angle of impact of spattered blood. _____

35. The basic unit of heredity is the _____.

36. Genes are positioned on threadlike bodies called _____.

37. All cells in the human body, except the reproductive cells, have _____ pairs of chromosomes.

38. The sex of an offspring is always determined by the (mother, father).

39. Genes that influence a given characteristic and are aligned with one another on a chromosome pair are known as _____.

40. When a pair of allelic genes is identical, the genes are said to be (homozygous, heterozygous).

41. A (phenotype, genotype) is an observable characteristic of an individual.

42. The combination of genes present in the cells of an individual is called the _____.

43. A gene (will, will not) appear in a child when it is present in one of the parents.

44. A type B individual may have the genotype _____ or the genotype _____.

45. A type AB mother and type AB father will have offspring of what possible genotypes?

46. A type AB mother and type AB father will have offspring of what possible phenotypes?

47. The _____ color test is used to locate and characterize seminal stains.

48. Semen is unequivocally identified by the microscopic appearance of _____.

49. Males with a low sperm count have a condition known as (oligospermia, aspermia).

50. The protein _____ is unique to seminal plasma.

51. True or False: DNA may be transferred to an object through the medium of perspiration. _____

52. True or False: Seminal constituents may remain in the vagina for up to six days after intercourse. _____

Further References

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DNA

Abbreviation for deoxyribonucleic acid—the molecules carrying the body's genetic information.

DNA is double stranded in the shape of a double helix.

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 that destroys or inactivates a specific antigen. Antibodies are found in the blood serum.

Antiserum

Blood serum that contains specific antibodies.

Agglutination

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

Serology

The study of antigen–antibody reactions.

Polyclonal Antibodies

Antibodies produced by injecting animals with a specific antigen. A series of antibodies are pro-

duced responding to a variety of different sites on the antigen.

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.

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.

Enzyme

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

Polymorphism

The existence of more than one form of a genetic trait.

Iso-enzymes

Multiple molecular forms of an enzyme, each having the same or very similar enzyme activities.

Web-Extra 12-1

See How Blood Stain Patterns are Formed

www.prenhall.com/Saferstein

Gene

A unit of inheritance consisting of a DNA segment located on a chromosome.

Chromosome

A rodlike structure in the cell nucleus, along which the genes are located. It is composed of DNA surrounded by other material, mainly proteins.

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.

Web-Extra 12-2

Learn About the Chromosomes Present in Our Cells

www.prenhall.com/Saferstein

Y Chromosome

The male sex chromosome.

Locus

The physical location of a gene on a chromosome.

Allele

Any of several alternative forms of a gene located at the same point on a particular pair of chromosomes. For example, the genes determining the blood types A and B are alleles.

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.

Web-Extra 12-3

Learn About the Structure of Our Genes

www.prenhall.com/Saferstein

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, and blood type.

Web-Extra 12-4

See How Genes Position Themselves on a Chromosome Pair

www.prenhall.com/Saferstein

Web-Extra 12-5

See How Genes Define Our Genetic Makeup

www.prenhall.com/Saferstein

Acid Phosphatase

An enzyme found in high concentration in semen.

Oligospermia

An abnormally low sperm count.

Aspermia

The absence of sperm; sterility in males.

Figure 12–1

Figure 12–2 (a) Microscopic view of normal red blood cells (500×). (b) Microscopic view of agglutinated red blood cells (500×). *Courtesy J.C. Revy, Phototake NYC*

(a)

(b)

Figure 12–3

Figure 12–4 Steps required to produce monoclonal antibodies.

Figure 12–5 (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*

(a)

(b)

Figure 12–6

Figure 12–7

Figure 12–8 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.*

Figure 12–9 Photograph and diagram of the separation of PGM iso-enzymes accomplished by electrophoresis. PGM can be grouped into one of three types—1, 2-1, and 2—according to band patterns. *Reproduced from Harry Harris,* The Principles of Human Biochemical Genetics, *2nd ed. New York: North-Holland, 1975, p. 61*

Figure 12–10 (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*

(a)

(b)

Figure 12–11 Bloodstain pattern produced by droplets of blood that were traveling from left to right. *Courtesy A. Y. Wonder*

Figure 12–12 The higher pattern is of a single drop of human blood that fell 24 inches and struck a hard, smooth cardboard at 50 degrees. The lower pattern is of a single drop of human blood that fell 24 inches and struck a hard, smooth cardboard at 15 degrees. *Courtesy A. Y. Wonder*

Figure 12–13 Illustration of stain convergence on a two-dimensional plane. Convergence represents the point from which the stains emanated. *Courtesy Judith Bunker, J. L. Bunker & Assoc., Ocoee, Fla.*

Figure 12–14a Three-dimensional diagram illustrating bloodstain patterns (A, B, C, E, F, G, and H) that were located, documented, and reconstructed. Also see Figure 12–16 (a–c). *Courtesy Judith Bunker, J.L. Bunker & Assoc., Ocoee, FLa.*

Figure 12–14b Crime-scene photograph of bloodstained areas. *Courtesy Sarasota County (Fla.) Sheriff's Department*

Figure 12–14c Detail photograph of bloodstains designated A, B, and C on the wall adjacent to the interior door. Positions of impact spatter from blows that were inflicted to victim's face are indicated in Figure 12–16(a). Arrow no 1 points to cast-off pattern directed left to right as blood was flung from the perpetrator's fist while inflicting blows. Arrow no. 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 no. 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.*

Figure 12–15 (a) Laboratory test pattern showing impact spatter. Size and shape of stains demonstrate forceful impact 90 degrees to target. (b) Laboratory test pattern illustrating cast-off pattern directed left to right from a right overhead swing. (c) Laboratory test pattern showing repetitive transfer impression pattern produced by a bloodstained hand moving left to right across the target. (d) Laboratory test patterns illustrating vertical flow patterns. Left pattern represents stationary source; right pattern produced by left-to-right motion. *Courtesy Judith Bunker, J.L. Bunker & Assoc., Ocoee, Fla.*

(a)

(b)

(c)

(d)

Figure 12–16 (a) Convergence of impact spatter patterns associated with beating by fist. (b) Convergence of impact spatter associated with victim falling to the floor while bleeding from the nose. (c) Convergence of impact spatter associated with victim while face down at the door, being struck with a cane. *Courtesy Judith Bunker, J.L. Bunker & Assoc., Ocoee, Fla.*

(a) Patterns A, B, C

(b) Patterns E and F

(c) Patterns G and H

Figure 12–17 Computer-enhanced photomicrograph image of human chromosomes. *Courtesy Alfred Pasieka, Science Photo Library, Photo Researchers, Inc.*

Figure 12–18 Photomicrograph of human spermatozoa (300×). *Courtesy John Walsh, Photo Researchers, Inc.*

Figure 12–19

Figure 12–20 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.

Figure 12–21a 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 12–21b 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 12–21c Victim collection rape 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*

[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; S. J. Denison, E. M. Lopes, L. D'Costa, and J. C. Newman, "Positive Prostate-Specific Antigen (PSA) Results in Semen-Free Samples," *Canadian Society of Forensic Science Journal* 37 (2004): 197.

[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.