Problem-solving in the Crime Lab

The challenge of using scientific analysis of physical evidence to aid in identifying and convicting criminals is a fascinating form of problem-solving. The application of science in criminal investigations is called **forensic science** or **criminalistics**. The first requirement for a career as a criminalist is a degree in a physical or biological science. Frequently, eminent criminalists have been trained as chemists. Much of the work in the crime lab involves chemical analysis, but other scientific disciplines are used also. Perhaps a reason for the success of chemists in the forensic science field is their training and experience in using problem-solving skills.

**Sherlock Holmes: the First Forensic Scientist?**

Surprisingly, most historians conclude that the first forensic scientist was a fictional character, the famous detective residing at 221 Baker Street in London named Sherlock Holmes. Sir Arthur Conan Doyle, the author of the Sherlock Holmes detective stories, was a physician whose training in both medicine and chemistry is evident in his stories. The first time Watson saw Holmes he was bending over a table "which bristled with retorts, test-tubes, and little bunsen lamps," and many of Holmes' cases are solved with the aid of chemical experiments he performs. In one of the earliest Sherlock Holmes stories, "A Study in Scarlet," published in 1887, Holmes discovers a chemical test which identifies bloodstains:

"I've found it. I've found it," he shouted to my companion, running towards us with a test tube in his hand. "I have found a reagent which is precipitated by hemoglobin and by nothing else... Why, man, it is the first practical medico-legal discovery for years. Don't you see that it gives us an infallible test for blood stains?... The old guaiacum test was very clumsy and uncertain. So is the microscopic examination for blood corpuscles. The latter is valueless if the stains are a few hours old. Now, this appears to act as well whether the stains are old or new. Had this test been invented, there are hundreds of men now walking the earth who would long ago have paid the penalty of their crimes...Criminal cases are continually hinging upon that one point. A man is suspected of a crime months perhaps after it has been committed. His linen or clothes are examined and brownish stains are found upon them. Are they blood stains, or rust stains, or fruit stains, or what are they? That is a question which has puzzled many an expert, and why? Because there was no reliable test. Now we have the Sherlock Holmes test, and there will no longer be any difficulty."

At the time of publication of this detective story, the guaiacum test was the standard test for blood, and a simple chemical test for blood was yet to be found. The "Sherlock Holmes" test described might be
any of several chemical tests that have since been developed, and we do not know for certain what chemicals Conan Doyle had in mind. We do know that the Sherlock Holmes stories inspired a generation of scientific investigators who did develop such tests and put them into practice. In 1910 in Lyon, France, the famous criminalist Edmund Locard founded the first forensic science laboratory, made possible in part as a result of the interest in forensic science stimulated by the adventures of Sherlock Holmes.

Testing of Bloodstains

The testing of bloodstains found at the scene of a crime remains one of the most common functions of the crime laboratory. Simple chemical tests like that described in the Sherlock Holmes story are often performed at the crime scene or on garments or other materials removed from the crime scene. These are called "presumptive" tests because they must be confirmed by other tests. Stains with a negative reaction to the presumptive tests are usually assumed not to be blood. Phenolphthalein reacts with hemoglobin in the presence of hydrogen peroxide to give a presumptive test for blood. Phenolphthalein is also familiar in the chemistry laboratory for its use as an acid-base indicator; in an entirely different use, phenolphthalein is the active ingredient in some over-the-counter laxatives. The phenolphthalein reagent forms a bright pink color as a positive test for hemoglobin, and is sensitive to one part in six million. Luminol reagent reacts with blood to form a luminescent compound. Large areas can be sprayed with this reagent to visualize bloodstains, which then glow in the dark. Through these sensitive methods, even areas which have been scrubbed and show no visible residue can be revealed to harbor bloodstains.

Presumptive tests which indicate the presence of hemoglobin indicate the need for further testing. Human blood must be distinguished from the blood of animals like dogs, cats, and deer. The precipitin test uses a substance called human antiserum which reacts only with human blood. An extract from the bloodstain is placed in a capillary tube on top of a layer of antiserum, and the formation of a cloudy ring between the two layers indicates a positive test for human blood. The precipitin test is very sensitive, requiring only a small amount of sample, and has been performed successfully on bloodstains over ten years old, and even on extracts from mummies thousands of years old. Antiserum can be prepared for any type of blood by injecting that type of blood into another animal, which produces antiserum for that blood. For example, human antiserum is produced by injecting human blood into a rabbit or other animal, then collecting the rabbit's blood. The serum, or clear, liquid portion, of the rabbit's blood, which has been sensitized to human blood, is used for the precipitin test. Similarly, if the rabbit were injected with dog's blood, dog antiserum would result.
Precipitin Test

Once identified as human blood, the stain can be further characterized by blood type, whether A, B, AB, or O, by adding different types of antibodies to determine the type of antigen present on the red blood cells. Further tests can be performed as well for specific enzymes and proteins present in the blood. Each of these tests can help to narrow down further the source of the bloodstains.

As in many other types of forensic testing, the testing of bloodstains relies on the use of controls, or samples gathered for comparison purposes. In the case of a homicide, for example, blood is taken from the victim and the suspect, and the results of analysis of these samples are compared with results obtained from bloodstains. Is the blood on the suspect’s shirt has own, for example, or or does it match the blood of the victim? If the victim and suspect have different ABO blood types, and if the blood type matches that of the victim and not the suspect, this information can be useful in constructing a legal case against the suspect. It does not, however, prove that the bloodstain is from the victim, because a given blood type is not a characteristic limited to one person, but belongs to a many persons. This type of evidence, which is associated with a group of persons but not a single source, is said to have class characteristics. The distribution of the various blood types in the population varies with race and with location over the world. Blood types are typically distributed among the population of the United States as indicated in Table 20-1.

Table 20-1. A Typical Distribution of Blood Types in the United States.

<table>
<thead>
<tr>
<th>Blood Type</th>
<th>O</th>
<th>A</th>
<th>B</th>
<th>AB</th>
</tr>
</thead>
</table>
Statistical evidence like that of Table 20-1 is extremely useful to the criminalist in describing the probability that a given sample is associated with a particular person. If the victim has type B blood, for example, it can be stated that only about 12% of the U.S. population would have that same type blood. This information becomes even more useful when combined with the results of other laboratory tests.

**Using probabilities of class characteristics**

The blood protein PGM-1, for example, occurs in 24% of the population. If the blood sample tests positive for both type B and PGM-1, the total probability of a person having both those blood characteristics is 12% x 24%, or 2.9%. Thus, the probabilities associated with class characteristics can be combined to give a very high degree of probable association of two samples. The more types of test results are available, the higher is the probability than can be obtained, linking a given person to one or more pieces of evidence.

**Hairs and Fibers as Evidence**

The microscopic examination of hairs and fibers often gives important evidence in a criminal case. Both hairs and fibers have only class characteristics, and cannot therefore be linked with certainty to one individual. Variations among samples can be useful in eliminating suspects or helping to identify a criminal by establishing a probability that the person is associated with a piece of evidence. Hair can be identified as to color and racial origin, and often distinguishing characteristics such as dyeing or breakage are present. If the root is intact, indicating possible forcible removal, further information may be obtained from the adhering tissue.

Fibers can vary considerably in color and composition, and can constitute a useful form of class evidence. Synthetic fibers especially can vary, both in the polymer from which they are made and the shape of the holes in the spinneret from which they were formed (See Chapter 13 for a discussion of the formation of synthetic fibers from polymers). In a well-known serial murder case in Atlanta, an important part of the case that led to the conviction of Wayne Williams was the analysis of fibers found on the victims that matched the carpet and bedspread from his home, the carpet from the trunk of his car, and the hair from his German Shepherd dog. Although all these constituted class evidence rather than individual evidence, their combined presence led to a very high probability of connecting the murder victims with Wayne Williams. To gain further evidence about the probability of occurrence, investigators traced green carpet fibers found both in Williams' bedroom and on a number of the
victims to the original manufacturer, West Point Pepperell. This carpet fiber had an unusual trilobal cross section and had been manufactured for only a year before it was discontinued. From the number of square yards of carpet of this type that had been manufactured and the number of housing units in Atlanta, assuming an average room size of twelve by fifteen feet, investigators were able to calculate the probability of finding this kind of carpeting in an Atlanta dwelling as being 1 chance in 7,792. This very low probability showed the unlikelihood that this fiber could have come from any other source than Williams’ apartment.

The Analysis of Drugs

The drug laboratory provides analyses of samples of drugs that have been seized by law enforcement officials. Conviction of those found to be possessing or selling these substances is contingent upon proof that they are indeed the illegal substances they are assumed to be. For that reason chemists must choose analytical methods that provide definite analyses for the specific substances being tested for. Often color tests are performed to give a preliminary test for the drug, analogous to the presumptive tests for blood. A negative test usually means that the sample is not tested further, while a positive test must be corroborated by further testing. A commonly used presumptive tests for drugs is the Marquis test for heroin, morphine, and most opium derivatives, in which 2% formaldehyde in sulfuric acid produces a purple color when it comes into contact with the drug. Further tests performed usually involve one or both of two types of laboratory analysis, chromatography or spectrophotometry.

Chromatography encompasses an entire family of analytical methods for separating mixtures and identifying their components. A simple example of a chromatographic method is paper chromatography, in which a mixture of substances is dissolved in an appropriate solvent, and a spot of the dissolved mixture is placed a short distance from the bottom of a piece of absorbant paper. The paper is placed in a container with enough liquid to barely cover the end of the piece of paper. As the liquid travels up the paper past the position of the spot containing the mixture of substances, a given substance may travel up the paper with the liquid as it rises toward the top by capillary action.
Figure 1. A paper chromatogram
The relative tendencies for a given substance to travel with the moving liquid or remain in place on the paper will depend on the relative strength of the attractive forces between that substance and the moving liquid compared with the attractive forces holding it to the paper (and to any water molecules that may be hydrogen-bonded to the -OH groups of the cellulose that makes up the paper). Since, as we learned in Chapter 7, the attractive forces of a molecule depend upon its structure, chromatography is able to separate molecules of different structures because they will be attracted to differing degrees to the moving and stationary (or non-moving) phases and hence move at different rates up the paper. If the substances in the mixture are colored, as in the case of food dyes or ink dyes, then they can be observed throughout the experiment as the liquid moves up the paper. Some may move almost as far as the moving liquid by the time the liquid reaches the top of the paper, some may remain in the same place where the spot was originally applied, and others may be distributed at varying points along the way. Each spot has a characteristic $r_f$ value depending how far it has risen, calculated as

$$r_f = \frac{\text{distance travelled by the spot}}{\text{distance travelled by the same solute in the solvent}}$$
distance travelled by the moving liquid

Each time a substance undergoes paper chromatography with a particular moving phase, it will have approximately the same $r_f$ value. Often control substances are spotted on the same chromatogram as the unknown mixture for comparison purposes.

These same general principles are found in all types of chromatography: components of a mixture are partitioned between a moving phase and a stationary phase depending on their relative affinities for the two phases

Table 20-1. *Types of Chromatography*

(* indicates instrumental method)

<table>
<thead>
<tr>
<th>Type of chromatography</th>
<th>Moving phase</th>
<th>Stationary phase</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paper chromatography</td>
<td>Liquid</td>
<td>Water adsorbed on paper</td>
<td>Simple, inexpensive</td>
</tr>
<tr>
<td>Thin layer chromatography (TLC)</td>
<td>Liquid</td>
<td>Fine granular layer of material on plastic or glass backing</td>
<td>Used for quick drug screening</td>
</tr>
<tr>
<td><em>Gas chromatography (GC)</em></td>
<td>Gas</td>
<td>Fine granular material in a column</td>
<td>Widely used instrumental method, often in combination with MS</td>
</tr>
<tr>
<td><em>Liquid chromatography</em></td>
<td>Liquid</td>
<td>Fine granular material in a column</td>
<td>HPLC (high performance LC) is an instrumental method similar to gc; done at room temp.</td>
</tr>
</tbody>
</table>

In thin-layer chromatography, the stationary phase is usually a fine, granular solid deposited
uniformly on a flat surface of plastic or glass. As with paper chromatography, the end of the chromatographic plate is immersed in a liquid, and the liquid serves as the moving phase, travelling past a spot where the unknown sample or control sample has been placed, and moving up the plate. Components of a mixture are separated as they are partitioned between the stationary phase and the moving phase, and samples are identified by comparing their $r_f$ values with control samples. An interesting application of thin-layer or paper chromatography to forensic science is the analysis of the ink used to write a document. Inks are usually mixtures of several dyes, and formulations vary among manufacturers; these dye mixtures, like most mixtures of organic compounds, can be separated and identified by chromatographic methods. For example, in a suspected case of scientific fraud conducted by the Secret Service in 1988 and 1989, forensic experts examined laboratory notebooks. Using chromatographic methods to analyze the inks used to write the notebooks, they found pages dated 1984 had been written with a type of ballpoint pen that was not manufactured until 1986.

Thin-layer chromatography is a quick, inexpensive method for the analysis of drug samples. Since most drugs are colorless, their spots do not show up on the chromatography plate unless they are developed by reacting them with a reagent that will cause a colored spot to form. A chromatogram with a mixture of drugs may have to be treated with a succession of developing reagents before all the spots are visualized.

Fig. 20-2. Sample thin layer chromatogram of drugs.

Thin layer chromatography does not constitute a final proof for the identity of a drug; it must
be corroborated by a more specific technique. One such chromatographic technique is **gas chromatography (GC)** combined with **mass spectrometry (MS)**. In gas chromatography, the moving phase is an unreactive gas such as nitrogen or helium, and the stationary phase is inside a narrow, coiled column made of metal or glass. Gas chromatography is an example of **instrumental analysis**, in which a scientific instrument, frequently rather complex and expensive, is used to perform the chemical analysis.

Fig. 20-3. *gas chromatography instrument*
The sample is dissolved in an appropriate solvent, then taken up into a hypodermic needle with a very small capacity (measured in microliters), and injected into a heated area at one end of the column. The vaporized compounds present in the sample travel through the column at rates dependent on their relative degree of attraction toward the moving phase and the stationary phase. The column is enclosed in a temperature-regulated oven, and the temperature is adjusted so that the samples remain vaporized and travel through the column within a few minutes. As chemical compounds reach the end of the column, they pass through a detector, which registers an electrical signal. The chromatogram from a gas chromatography experiment is a graph in which each of the signals registered by the detector appears as a peak. The time at which the compound passes through to the end of the column and enters the detector is its **retention time**. A sample gas chromatogram of a drug sample is shown in Fig. 20-4.

Fig. 20-4. *Gas chromatogram.*

Methamphetamine, Cocaine, Heroin-AT-35ms
Though the gas chromatogram can be compared with data from known drug samples to identify a drug sample, it is not considered to be conclusive evidence unless combined with the more detailed evidence provided by a mass spectrogram. To obtain this information, the GC column is connected to a mass spectrometer; each compound passing through the column enters a high-vacuum chamber, where it is bombarded with a beam of high-energy electrons. The molecules are broken up into fragments, which are positively charged because they have lost electrons. Each chemical substance has a characteristic individual fragmentation pattern, which, when coupled with the gas chromatogram, constitutes conclusive identification of a compound. Fig. 20-5 shows some of these fragmentation patterns, called mass spectra.

Frequently, the GC-MS instrument is integrated with a computer system which can compare the experimental data with a library of stored data for a fast and conclusive identification of all substances present in the sample.
The Toxicology Laboratory

Frequently, an analysis is required for chemical substances present in a human body. The bodies of victims of homicide and suicide are frequently tested for the presence of drugs, alcohol, and poisons. Drug testing and alcohol testing of subjects are required in a variety of circumstances related to criminal law. The forensic toxicology laboratory usually performs these tests.

Like the drug laboratory, the toxicology laboratory makes extensive use of chromatographic methods. Another type of chemical analysis used by both drug labs and toxicology labs is spectrophotometric analysis. In this type of analysis, light absorbed from the electromagnetic spectrum by a chemical compound is measured. Since, as we learned in Chapter 2, the electromagnetic spectrum comprises several different types of radiation, there are different types of spectrophotometric analysis as well. Forensic science labs use visible (VIS) spectrophotometry, ultraviolet (UV) spectrophotometry, and infrared (IR) spectrophotometry.

Chemical compounds appear colored to us when they absorb light from the visible range of the electromagnetic spectrum. When a portion of the visible spectrum is absorbed by a compound, the remainder of the spectrum is reflected to the observer, and the substance is colored (Table 20-2). For example, if the compound absorbs red light, it appears bluish green to our eyes. When all the wavelengths of visible light are present in the light reflected back from a compound, the substance appears white or colorless.

Table 20-2. Light Absorption of Colored Compounds.

<table>
<thead>
<tr>
<th>Wavelength of Light Absorbed (nm)</th>
<th>Color of Light Absorbed</th>
<th>Color of Substance Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Visible light absorption is the basis for a common instrument for alcohol breath-testing, the Breathalyzer. Since beverage alcohol, or ethanol, is a colorless compound, this test involves the reaction of ethanol with another compound which absorbs visible light. The Breathalyzer relies on the fact that ethanol (C\textsubscript{2}H\textsubscript{5}OH) reacts in an oxidation-reduction reaction with the yellow-colored oxidizing agent potassium dichromate (K\textsubscript{2}Cr\textsubscript{2}O\textsubscript{7}). As the reaction proceeds, the potassium dichromate is consumed and the yellow color is seen to disappear gradually. Sulfuric acid is also a reactant in this reaction. Silver nitrate is added to the reaction mixture, but it acts as a catalyst, so that, although it helps the reaction to proceed, it is not itself consumed in the process.

\begin{equation}
2\text{K}_2\text{Cr}_2\text{O}_7 + 3\text{C}_2\text{H}_5\text{OH} + 8\text{H}_2\text{SO}_4 \rightarrow 2\text{Cr}_2(\text{SO}_4)_3 + 2\text{K}_2\text{SO}_4
\end{equation}

\begin{equation}
+ 3\text{CH}_3\text{COOH} + 11\text{H}_2\text{O}
\end{equation}

Since a given amount of potassium dichromate always reacts with a known amount of ethanol as predicted by the chemical equation, the amount of ethanol present can be determined by the amount of potassium dichromate consumed. When the Breathalyzer is used, the subject breathes into a mouthpiece, and the instrument automatically discards the first portion of the breath, collecting the last 56.5 mL of alveolar breath (deep lung air). The sample is bubbled through an ampule containing pre-measured amounts of potassium dichromate, sulfuric acid, and silver nitrate. The spectrophotometer measures the change in absorbance of visible light by potassium dichromate at a wavelength of 420 nanometers after the sample was added, and hence the amount of alcohol that was present. This form of breathalyzer is now being replaced by one that measures the infrared spectrum of the alcohol- this is better because it gives a direct measurement,
Though only a limited number of organic compounds absorb in the visible region, many absorb ultraviolet light. Ultraviolet spectra, or patterns of absorption of ultraviolet light, are often used to help identify drug compounds. Figure 20-6 shows the ultraviolet absorption spectrum of heroin.

Fig. 20-6 Ultraviolet absorption spectrum of heroin (from Saferstein, Criminalistics, 4th Ed., Prentice Hall 1990).

The absorption spectrum of heroin is typical of both ultraviolet and visible spectra in its lack of fine structure. For that reason, UV and VIS spectra cannot be used for the conclusive identification of organic compounds such as drugs. Absorption of infrared light by organic compounds does result in complex spectra, as shown in Fig. 20-7, which shows infrared spectra of heroin and the barbituate secobarbital.
The right-hand side of the infrared spectrum is particularly rich in detail which is characteristic of each different compound; for this reason, it is often called the "fingerprint region" of the infrared spectrum. Like GS-MS data, an IR spectrum which matches that of a known compound is considered to be conclusive proof of identity. Infrared absorption is the basis of a method of alcohol testing which in some states has served as a replacement for the Breathalyzer. Since alcohol, like all molecules, absorbs infrared light, the infrared testing method does not require reaction of the alcohol with other substances, but tests for the presence of alcohol molecules directly.

DNA as Forensic Science Evidence

The technology which has made it possible to learn the structure of the DNA molecule has been applied to crime-solving as well. As we learned in Chapter 15, the double helix structure of DNA is held together by linkages between organic nitrogen-containing base pairs, with adenine (A) invariably bonding with thymine (T) and cytosine (C) bonding with guanine (G). Though 99% of the three billion base pairs in human DNA are identical among all individuals, producing the patterns of heredity and protein synthesis which are common to all human beings, there are parts of a DNA molecule for which the functions are not known by scientists, and which are highly variable from one individual to another. These short DNA sequences, sometimes called "junk DNA" because they have no obvious function, are the basis of DNA typing for forensic purposes. Blood from a crime scene, for example, or semen from a rape victim, can be analyzed and its DNA compared with those of a suspect. DNA comparisons can be made between a known DNA sample and a sample from the crime scene with a very high degree of probability. Depending on the individual sample and the method employed, a DNA sample can be matched to a suspect so that the possibility of another person having the same DNA results is one in several million or even less. For this reason, DNA evidence is said to possess individual
characteristics, as opposed to merely class characteristics. Evidence with individual characteristics is particularly useful as forensic evidence. The most commonly employed type of evidence with individual characteristics is fingerprints. In analogy with fingerprint evidence, DNA typing is sometimes called DNA fingerprinting.

DNA typing is a multistep process. First, DNA is isolated from a sample of blood, semen, or even certain other material such as saliva or the root of a hair. The DNA is cut into fragments using restriction enzymes which act only on certain patterns in the DNA. For example, the restriction enzyme Pst 1 cuts the DNA molecule only when the base sequence CTGCAG occurs. Because the patterns in the "junk DNA" vary, the fragment lengths that result from individuals vary. A short tandem repeat (STR) ranging from 2 to 10 base pairs is produced. The resulting mixture of fragments is separated into its component parts using gel electrophoresis or capillary electrophoresis, chromatographic methods in which an electrical charge is applied while the mixture components migrate. Shorter fragments migrate faster than longer ones, and separation of different-sized components occurs on the gel. When the mixture has been separated into its components, the resulting chromatogram must then be treated so that the bands become visible, a process which involves several more steps. Fluorescent "probes," made of short DNA sequences, are added, and these bind to the DNA fragments wherever they find a matching sequence of complementary bases. These bands, like fingerprints, are always the same for the same individual, but different for different individuals.
Fig. 20-8. DNA typing is a multi-step process
Some interesting applications of DNA evidence have been identifying September 11 victims, identifying Holocaust victims, identifying disappeared children in Argentina, and racing human migration patterns around the world. http://www.ornl.gov/sci/techresources/Human_Genome/elsi/forensics.shtml#4 is an excellent reference for modern DNA applications.

Since its introduction as an evidence tool, new technology has expanded the possibilities for DNA typing. Among the most important is a method for amplifying, or increasing, the amount of available DNA from a sample. This method, called polymerase chain reaction, or PCR, is particularly useful because it can use broken-down DNA as well as DNA in which the whole chain is in intact condition. In one of the more spectacular uses of PCR, the DNA was analyzed from a wooly mammoth whose remains were found frozen in Siberian ice about 40,000 years after it died. PCR uses "primers," or short pieces of DNA with base sequences complementary to base sequences at either end of the target region of the DNA to be analyzed. Fig. 20-10 shows several cycles of a polymerase chain reaction. The DNA sample is heated and the two DNA strands separate. The primers, shown as dark bars in the figure, bind to the two ends of the portion of the DNA molecule that is to be duplicated. In the presence of a DNA polymerase, or polymerizing enzyme, the primers then initiate a process in which a complementary strand is produced for each of the two original DNA strands. Thus, the original DNA, made of two complementary strands, has been duplicated. The process is repeated over and over, each cycle doubling the amount of DNA. Twenty cycles can theoretically amplify the amount of DNA by a factor of about a million, and thirty cycles by about a billion.

http://www.dnalc.org/ddnalc/resources/pcr.html

This DNA amplification technique is being applied with great success in clinical medicine, for example, to speed up the prenatal diagnosis of genetic diseases and the diagnosis of viral infections including AIDS. In forensic science, the fact that tiny samples of body materials can yield usable amounts of DNA means that the amount of saliva obtained from one envelope flap in a ransom or terrorism case, or from one cigarette butt from a crime scene, can yield a positive identification of an individual.

Since 1988, the FBI has been analyzing blood samples from individuals from different population groups to provide estimates of DNA profile frequencies for these groups; the individuals associated with these samples are not identified, since the purpose of these files is to provide a statistical database. Eventually, it is expected that DNA files of criminals may be formed, and used in much in the same way as the extensive files of fingerprints are used in identifying lawbreakers.
**The Criminalist's Job**

The scientific methods employed so successfully to convict criminals are the same as those used in other types of scientific laboratories. Chromatography and spectrophotometry are techniques of **analytical chemistry**, the branch of chemistry in which chemists solve problems which involve finding what is in a given sample of unknown composition (qualitative analysis), and sometimes how much of each substance is present (**quantitative analysis**). DNA analysis is a technique of **biochemistry**, introduced in Chapter 15. Blood typing is a technique of **serology**, a broad category of laboratory tests that utilize specific antigen and serum antibody reactions. **Microscopy** is used extensively in the crime lab, as samples of hair, fiber, and other types of evidence are examined using a variety of microscope types. **Impressions** of various kinds, such as shoe prints, toolmarks, or the marks made on a bullet as it is fired, are analyzed and compared with control samples. Even when the serial number of a gun has been obliterated, the criminalist can often use chemical means to visualize the impressions left in the deeper molecular structure of the metal. In summary, the criminalist must be proficient in a number of these scientific skills, and with their application to physical evidence from the crime scene. Often he or she is called to the crime scene to collect evidence, since the manner in which the evidence is collected and packaged can be of critical importance.

The criminalist employs scientific skills and knowledge in the forensic science laboratory, and then offers expert testimony about the results of the laboratory work, often explaining to a jury untrained in science the basic principles behind the tests that were performed. As in other areas of science, the criminalist must be totally objective and honest about the results of the laboratory work and about any limitations or problems related with the experiments. Often the outcome of a trial, and the fate of others, may depend on the criminalist’s scientific skills, judgment, and testimony.
CONCEPTS TO UNDERSTAND FROM CHAPTER 20

The application of science in criminal investigations is called forensic science or criminalistics.

**Presumptive tests** are quick preliminary tests which must be confirmed by other tests.

Presumptive tests for the presence of blood include the formation of a bright pink color when hemoglobin is exposed to phenolphthalein reagent and the formation of a luminescent compound with luminol reagent. Through these sensitive methods, even areas which have been scrubbed and show no visible residue can be revealed to harbor bloodstains.

Human blood must be distinguished from the blood of animals like dogs, cats, and deer by use of the precipitin test, which uses a substance called human antiserum which reacts only with human blood. An extract from the bloodstain is placed in a capillary tube on top of a layer of antiserum, and the formation of a cloudy ring between the two layers indicates a positive test for human blood.

Human blood can be characterized by blood type, whether A, B, AB, or O, by adding different types of antibodies to determine the type of antigen present on the red blood cells; this is called the ABO typing system. Further tests can be performed as well for specific enzymes and proteins present in the blood.

Frequently controls, or samples gathered for comparison purposes, are used in forensic science. In the case of a homicide, for example, blood is taken from the victim and the suspect, and the results of analysis of these samples are compared with results obtained from bloodstains.

Evidence which is associated with a group of persons but not a single source is said to have class characteristics.

The probability that a given combination of class characteristics will occur can be calculated by multiplying together the product of the frequencies of the individual characteristics. For example, about 12% of the U.S. population have type B blood, and 24% have the blood protein PGM-1. The
probability of having both these characteristics is 12% x 24%, or 2.9%. The more types of class characteristics are known, the stronger becomes the evidence linking a given person to the crime.

The **drug laboratory** provides analyses of samples of drugs that have been seized by law enforcement officials.

The **toxicology laboratory** performs analyses for chemical substances present in the human body.

**Analytical chemistry** is the branch of chemistry in which chemists solve problems which involve finding what is in a given sample of unknown composition (qualitative analysis), and sometimes how much of each substance is present (quantitative analysis). Drug laboratories and toxicology laboratories employ analytical chemistry methods, especially chromatography and spectrophotometry.

**Chromatography** encompasses an entire family of analytical methods for separating mixtures and identifying their components. The same general principles are found in all types of chromatography: components of a mixture are partitioned between a moving phase and a stationary phase depending on their relative affinities for the two phases. *Table 20-1* lists several types of chromatography, along with the stationary and moving phases for each.

Though thin layer chromatography (TLC) and gas chromatography data can be compared with data from known drug samples to identify a drug sample, conclusive evidence requires gas chromatography combined with the more detailed evidence provided by a mass spectrogram (GC/MS). Each chemical substance has a characteristic fragmentation pattern, which, when coupled with the gas chromatogram, constitutes positive identification of a compound.

**Spectrophotometric analysis** is employed extensively in forensic laboratories, especially in the drug laboratory and the toxicology laboratory. In this type of analysis, light absorbed from the electromagnetic spectrum by a chemical compound is measured. Visible (VIS), ultraviolet (UV), and infrared (IR) absorption measurements are all useful, but only infrared has a sufficiently detailed fine structure to constitute conclusive identification of a compound.

Visible light absorption is the basis for a common instrument for alcohol breath-testing, the **Breathalyzer**. The Breathalyzer relies on the fact that beverage alcohol, or ethanol (C₂H₅OH), reacts in an oxidation-reduction reaction with the yellow-colored oxidizing agent potassium dichromate (K₂Cr₂O₇).
\[
2K_2Cr_2O_7 + 3C_2H_5OH + 8H_2SO_4 \rightarrow 2Cr_2(SO_4)_3 + 2K_2SO_4 + 3CH_3COOH + 11H_2O
\]

Since a given amount of potassium dichromate always reacts with a known amount of ethanol as predicted by the chemical equation, the amount of ethanol present can be determined by the amount of potassium dichromate consumed.

**DNA typing**, or **DNA fingerprinting**, makes use of the analysis of parts of the DNA molecule that vary among individuals to compare samples of blood, semen, or other materials obtained from the crime lab with DNA from the blood of suspects and victims. DNA samples obtained in this way can be compared with a very high degree of probability.

Forensic evidence like DNA typing or fingerprints that can be linked to an individual with a very high degree of probability is aid to have **individual characteristics**.

**DNA typing is a multistep process** involving isolation of the DNA, cutting the DNA is cut into fragments using restriction enzymes, separating the resulting mixture of fragments into its component parts using gel electrophoresis, chemically "unzipping" the two helical strands of the DNA fragments from each other, then transferring the material from the soft gel to a more rigid nylon sheet. Radioactive "probes," made of short DNA sequences, are added, and these bind to the DNA fragments wherever they find a matching sequence of complementary bases. The nylon sheet is placed against an x-ray film and left there for several days. As the radiation from the probes develops the film, the radioactive areas on the nylon sheet show up as dark bands on the x-ray film. The band patterns of samples from the crime scene are compared with samples taken from the suspect and the victim. Identical patterns indicate a DNA match, or samples originating from the same person, with a very high degree of probability. An FBI database is being developed so that probabilities of pattern matches in given types of populations can be determined with a high degree of certainty.

Polymerase chain reaction, or PCR, is a method for amplifying, or increasing, the amount of available DNA from a sample. This method can use broken-down DNA as well as DNA in which the whole chain is in intact condition. Each cycle of the chain reaction doubles the amount of DNA. Twenty cycles can theoretically amplify the amount of DNA by a factor of about a million, and thirty cycles by about a billion.
CHAPTER 20 PROBLEMS

1. Why is DNA typing called "DNA fingerprinting"?

2. If hair and fiber evidence has only class characteristics, why was it used as forensic evidence in the Wayne Williams trial?

3. If about 12% of the U.S. population has type B blood, and 24% has the blood enzyme PGM-1, what is the probability that a person will have both?

4. List some of the job skills of a criminalist.

5. What training does a criminalist need?

6. Name three steps in the identification of a reddish-brown stain found on a garment at a crime scene suspected of being human blood.
7. What is the chief function of the drug laboratory?

8. What is the chief function of the crime laboratory?

9. What is the role of Sir Arthur Conan Doyle in the history of forensic science?

10. What is an STR?

11. Give the full name of each of the following terms, and give a brief definition.
   a. GC
   b. TLC
   c. GC/MS
   d. UV spectroscopy
12. What is an $r_f$ value, and how is it used?

13. Which of the following constitute positive proof of the identity of a drug?
   a. VIS spectroscopy
   b. UV spectroscopy
   c. IR spectroscopy
   d. TLC
   e. GC
   f. GC/MS

14. a. The Breathalyzer measures light absorption at 420 nm. What compound in the Breathalyzer absorbs light at this wavelength?

   b. How does light absorption of this compound enable the instrument to determine the amount of ethanol in alveolar breath?

   c. A newer method to measure alcohol in alveolar breath uses infrared absorption measurement to measure IR absorption of the alcohol molecule. Can you think of an advantage of this method?

15. If a molecule absorbs light at 700 nm, what color is the compound?
16. If a molecule absorbs light at 450 nm, what color is the compound?

17. What information is yielded by each of the following tests?
   a. Marquis test
   b. Phenolphthalein test
   c. Precipitin test
   d. Luminol test
   e. Breathalyzer

18. Name some types of information that can be obtained from a sample hair.